Antimicrobial Therapy in Veterinary Medicine

Fifth Edition

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Preface

The field of anti-infective therapy has expanded considerably since the first edition of *Antimicrobial Therapy in Veterinary Medicine* was published in 1988. The fifth edition is a completely updated and considerably expanded version of the previous edition, with the same aim of providing a comprehensive source for this crucial topic in veterinary medicine. Everyone working with antimicrobial drugs is aware of the continuing threat of resistance and of the important role that each of us plays in trying to preserve the efficacy of these drugs.

The book is divided into four sections. The first provides general principles of antimicrobial therapy and includes a new chapter on antimicrobial stewardship. The second section describes each class of antimicrobial agents, revised to include not only the most up-to-date information on antimicrobial agents specific to veterinary species but also newly developed drugs not yet used in veterinary medicine. The third section deals with special considerations. It includes chapters on prophylactic and metaphylactic use of antimicrobial agents, antimicrobial chemotherapy for the neutropenic patient, and approach to therapy of selected bacterial pathogens and organ systems. Chapters on regulations of antibiotic use in animals, performance uses of antimicrobial agents, and antimicrobial drug residues in foods of animal origin have been revised extensively against the background of new regulations and the extensive re-examination in many countries of the use of antimicrobial agents as growth promoters or in the prevention of disease in animals. The final section addresses the specific principles of antimicrobial therapy in multiple veterinary species. A chapter on antimicrobial therapy in zoological animals has been added to this edition to reflect the increase in popularity of these species.

Two members of the previous editorial team (J.D. Baggot and R.D. Walker) have retired. We thank them for their outstanding contributions over the years and we wish them the best in their new endeavors. The fifth edition welcomes 13 new contributors. We are grateful to all the contributors for the care and effort they have put into their chapters. We thank the staff of Wiley Blackwell Publishing, particularly Susan Engelken and Erica Judisch, for their help, patience, and support of this book. We encourage readers to send us comments or suggestions for improvements so that future editions can be improved.

Steeve Giguère, John Prescott, and Patricia Dowling

Important Notice

The indications and dosages of all drugs in this book are the recommendations of the authors and do not always agree with those given on package inserts prepared by pharmaceutical manufacturers in different countries. The medications described do not necessarily have the specific approval of national regulatory authorities, including the U.S. Food and Drug Administration, for use in the diseases and dosages recommended. In addition, while every effort has been made to check the contents of this book, errors may have been missed. The package insert for each drug product should therefore be consulted for use, route of administration, dosage, and (for food animals) withdrawal period, as approved by the reader's national regulatory authorities.

Abbreviations

Abbreviations used in this book include:

MIC	minimum inhibitory concentration
MBC	minimum bactericidal concentration
PO	per os, oral administration
IM	intramuscular administration
IV	intravenous administration
SC	subcutaneous administration
SID	single daily administration
BID	twice-daily administration (every 12 hours)
TID	3 times daily administration (every 8 hours)
QID	4 times daily administration (every 6 hours)
q 6 h, q 8 h, q 12 h, etc.	Every 6, 8, 12 hours, etc.

For example, a dosage of "10 mg/kg TID IM" means 10 milligrams of the drug per kilogram of body weight, administered every 8 hours intramuscularly.

Section I

General Principles of Antimicrobial Therapy

1

Antimicrobial Drug Action and Interaction: An Introduction

Steeve Giguère

Antimicrobial drugs exploit differences in structure or biochemical function between host and parasite. Modern chemotherapy is traced to Paul Ehrlich, a pupil of Robert Koch, who devoted his career to discovering agents that possessed selective toxicity so that they might act as so-called "magic bullets" in the fight against infectious diseases. The remarkable efficacy of modern antimicrobial drugs still retains a sense of the miraculous. Sulfonamides, the first clinically successful broad-spectrum antibacterial agents, were produced in Germany in 1935.

However, it was the discovery of the antibiotic penicillin, a fungal metabolite, by Fleming in 1929, and its subsequent development by Chain and Florey during World War II, that led to the antibiotic revolution. Within a few years of the introduction of penicillin, many other antibiotics were described. This was followed by the development of semisynthetic and synthetic (e.g., sulfonamides and fluoroquinolones) antimicrobial agents, which has resulted in an increasingly powerful and effective array of compounds used to treat infectious diseases. In relation to this, the term *antibiotic* has been defined as a low molecular weight substance produced by a microorganism that at low concentrations inhibits or kills other microorganisms. In contrast, the word *antimicrobial* has a broader definition than antibiotic and includes any substance of natural, semisynthetic, or synthetic origin that kills or inhibits the growth of a microorganism but causes little or no damage to the host. In many instances, *antimicrobial agent* is used synonymously with *antibiotic*.

The marked structural and biochemical differences between prokaryotic and eukaryotic cells give antimicrobial agents greater opportunities for selective toxicity against bacteria than against other microorganisms such as fungi, which are nucleated like mammalian cells, or viruses, which require their host's genetic material for replication. Nevertheless, in recent years increasingly effective antifungal and antiviral drugs have been introduced into clinical practice.

Important milestones in the development of antibacterial drugs are shown in Figure 1.1. The therapeutic use of these agents in veterinary medicine has usually followed their use in human medicine because of the enormous costs of development. However, some antibacterial drugs have been developed specifically for animal health and production (e.g., tylosin, tiamulin, tilmicosin, ceftiofur, tulathromycin, gamithromycin, tildipirosin). Figure 1.1 highlights the relationship between antibiotic use and the development of resistance in many target microorganisms.

Spectrum of Activity of Antimicrobial Drugs

Antimicrobial drugs may be classified in a variety of ways, based on four basic features.

Class of Microorganism

Antiviral and antifungal drugs generally are active only against viruses and fungi, respectively. However, some imidazole antifungal agents have activity against staphylococci

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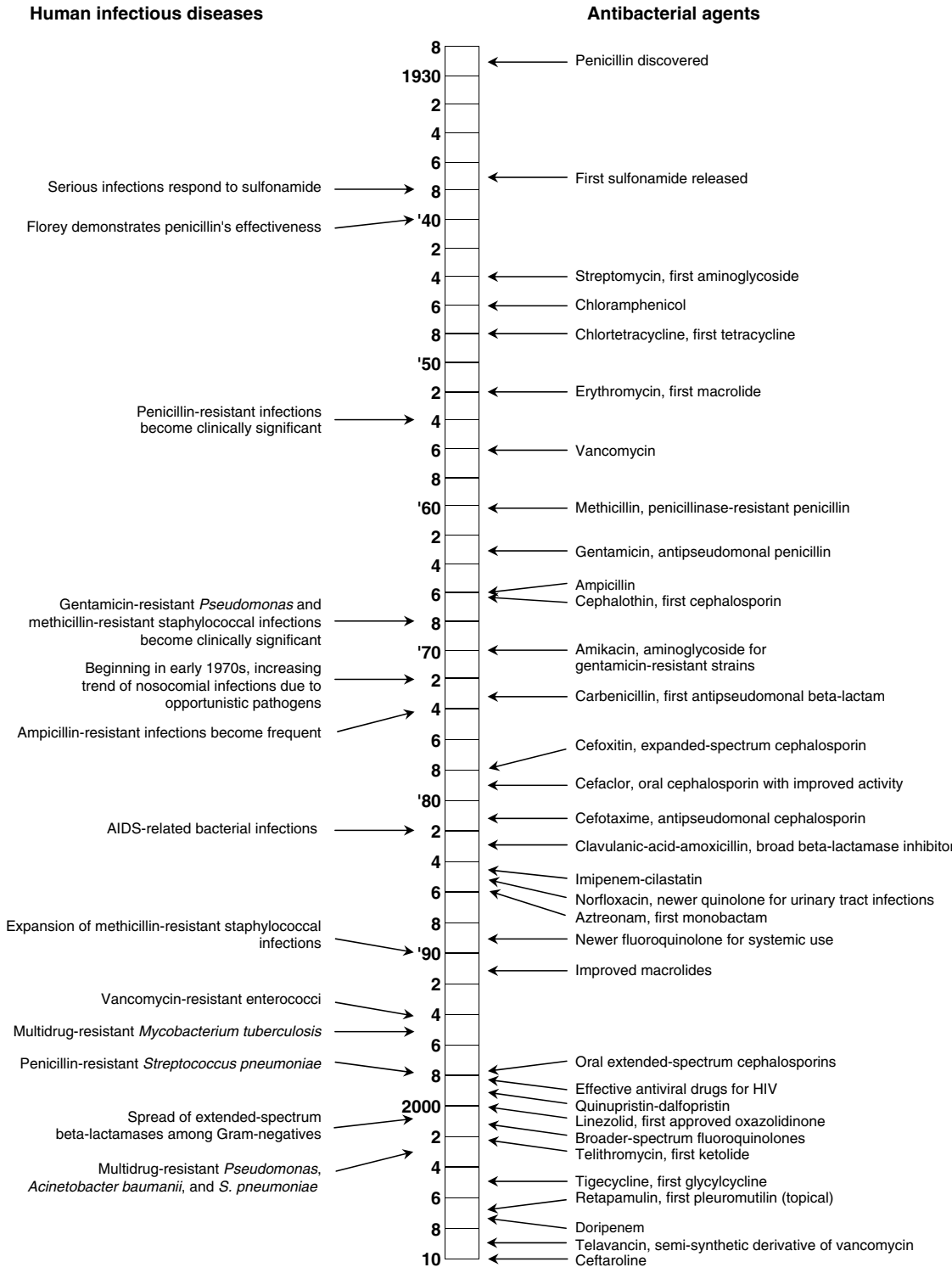

Figure 1.1. Milestones in human infectious disease and their relationship to development of antibacterial drugs. Modified and reproduced with permission from Kammer, 1982.

Table 1.1. Spectrum of activity of common antibacterial drugs.

	Class of Microorganism					
Drug	Bacteria	Fungi	Mycoplasma	Rickettsia	Chlamydia	Protozoa
Aminoglycosides	+	–	+	–	–	–
Beta-lactams	+	–	–	–	–	–
Chloramphenicol	+	–	+	+	+	–
Fluoroquinolones	+	–	+	+	+	–
Glycylcyclines	+		+	+	+	+/–
Lincosamides	+	–	+	–	–	+/–
Macrolides	+	–	+	–	+	+/–
Oxazolidinones	+	–	+	–	–	–
Pleuromutilins	+	–	+	–	+	–
Tetracyclines	+	–	+	+	+	+/–
Streptogramins	+	–	+	–	+	+/–
Sulfonamides	+	–	+	–	+	+
Trimethoprim	+	–	–	–	–	+

+/–: Activity against some protozoa.

and nocardioform bacteria. Antibacterial agents are described as *narrow-spectrum* if they inhibit only bacteria or *broad-spectrum* if they also inhibit mycoplasma, rickettsia, and chlamydia. The spectrum of activity of common antibacterial agents is shown in Table 1.1.

Antibacterial Activity

Some antibacterial drugs are also considered narrow-spectrum in that they inhibit only Gram-positive or Gram-negative bacteria, whereas broad-spectrum drugs inhibit both Gram-positive and Gram-negative bacteria. However, this distinction is not always absolute, as some agents may be primarily active against Gram-positive bacteria but will also inhibit some Gram-negatives (Table 1.2).

Bacteriostatic or Bactericidal Activity

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent required to prevent the growth of the pathogen. In contrast, the minimum bactericidal concentration (MBC) is the lowest concentration of an antimicrobial agent required to kill the pathogen. Antimicrobials are usually regarded as bactericidal if the MBC is no more than 4 times the MIC. Under certain clinical conditions this distinction is important, but it is not absolute. In other words, some drugs are often bactericidal (e.g., beta-lactams, aminoglycosides) and others are usually bacteriostatic (e.g., chloramphenicol, tetracyclines), but this distinction is an approximation, depending on both the drug concentration at the site of infection and the microorganism involved. For example, benzyl penicillin is bactericidal at usual therapeutic concentrations and bacteriostatic at low concentrations.

Time- or Concentration-Dependent Activity

Antimicrobial agents are often classified as exerting either time-dependent or concentration-dependent activity depending on their pharmacodynamic properties. The pharmacodynamic properties of a drug address the relationship between drug concentration and antimicrobial activity (chapter 5). Drug pharmacokinetic features, such as serum concentrations over time and area under the serum concentration-time curve (AUC), when integrated with MIC values, can predict the probability of bacterial eradication and clinical success. These pharmacokinetic and pharmacodynamic relationships are also important in preventing the selection and spread of resistant strains. The most significant factor determining the efficacy of beta-lactams, some macrolides, tetracyclines, trimethoprim-sulfonamide combinations, and chloramphenicol is the length of time that serum concentrations

Table 1.2. Antibacterial activity of selected antibiotics.

	Aerobic Bacteria		Anaerobic Bacteria		
Spectrum	Gram +	Gram –	Gram +	Gram –	Examples
Very broad	+	+	+	+	Carbapenems; chloramphenicol; third-generation fluoroquinolones; glycylcyclines
Intermediately broad	+	+	+	(+)	Third- and fourth-generation cephalosporins
	+	(+)	+	(+)	Second-generation cephalosporins
	(+)	(+)	(+)	(+)	Tetracyclines
Narrow	+	+/–	+	(+)	Ampicillin; amoxicillin; first-generation cephalosporins
	+	–	+	(+)	Penicillin; lincosamides; glycopeptides; streptogramins; oxazolidinones
	+	+/–	+	(+)	Macrolides
	+/–	+	–	–	Monobactams; aminoglycosides
	(+)	+	–	–	Second-generation fluoroquinolones
	(+)	(+)	–	–	Trimethoprim-sulfa
	–	–	+	+	Nitroimidazoles
	+	–	(+)	(+)	Rifamycin

+: Excellent activity.
(+): Moderate activity.
+/–: Limited activity.
–: No or negligible activity.

exceed the MIC of a given pathogen. Increasing the concentration of the drug several-fold above the MIC does not significantly increase the rate of microbial killing. Rather, it is the length of time that bacteria are exposed to concentrations of these drugs above the MIC that dictates their rate of killing. Optimal dosing of such antimicrobial agents involves frequent administration. Other antimicrobial agents such as the aminoglycosides, fluoroquinolones, and metronidazole exert concentration-dependent killing characteristics. Their rate of killing increases as the drug concentration increases above the MIC for the pathogen and it is not necessary or even beneficial to maintain drug levels above the MIC between doses. Thus, optimal dosing of aminoglycosides and fluoroquinolones involves administration of high doses at long dosing intervals. Some drugs exert characteristics of both time- and concentration-dependent activity. The best predictor of efficacy for these drugs is the 24-hour area under the serum concentration versus time curve (AUC)/MIC ratio. Glycopeptides, rifampin, and, to some extent, fluoroquinolones fall within this category (chapter 5).

Mechanisms of Action of Antimicrobial Drugs

Antibacterial Drugs

Figure 1.2 summarizes the diverse sites of action of the antibacterial drugs. Their mechanisms of action fall into four categories: inhibition of cell wall synthesis, damage to cell membrane function, inhibition of nucleic acid synthesis or function, and inhibition of protein synthesis.

Antibacterial drugs that affect cell wall synthesis (beta-lactam antibiotics, bacitracin, glycopeptides) or

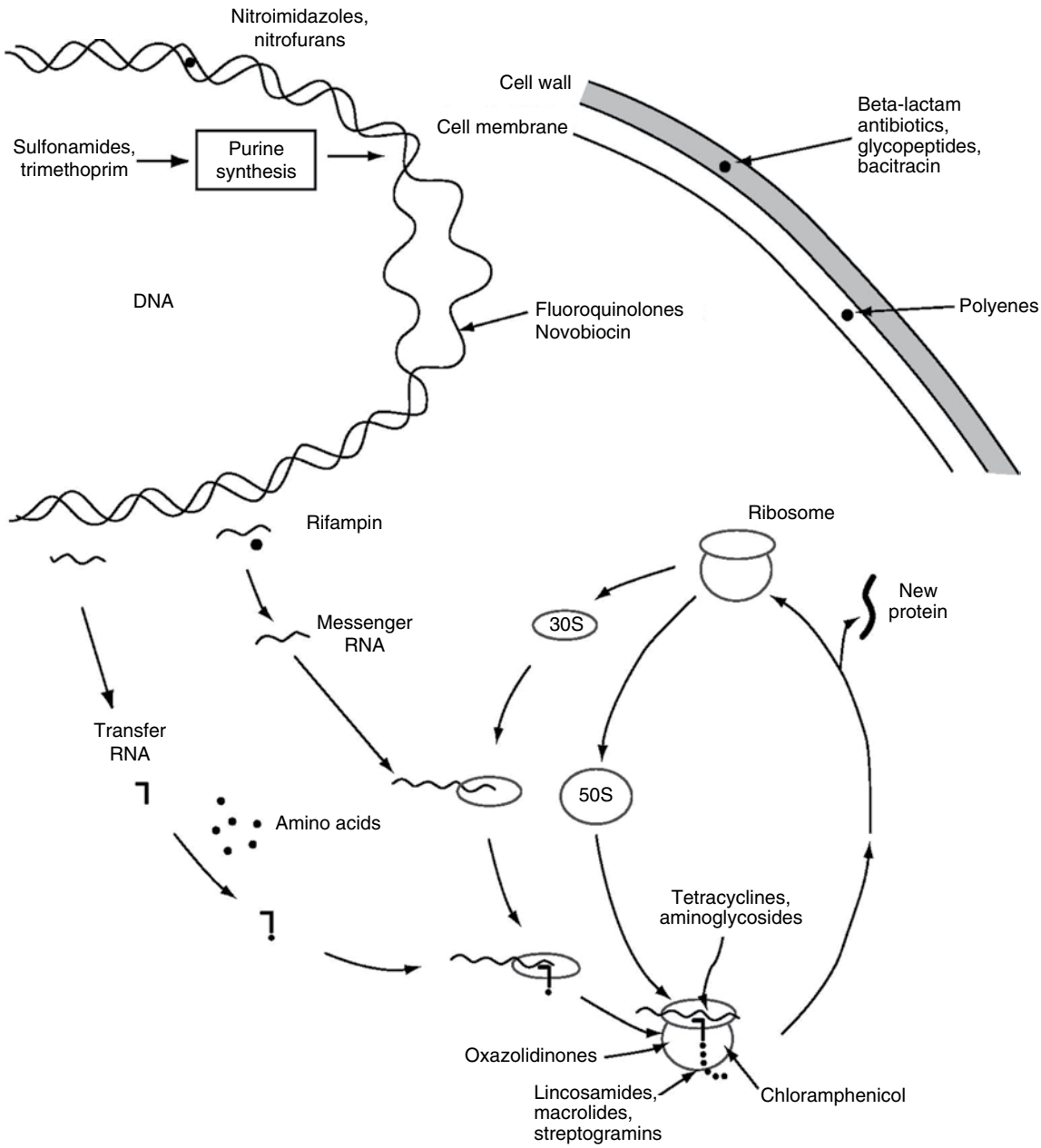

Figure 1.2. Sites of action of commonly used antibacterial drugs that affect virtually all important processes in a bacterial cell. Modified and reproduced with permission after Aharonowitz and Cohen, 1981.

inhibit protein synthesis (aminoglycosides, chloramphenicol, lincosamides, glycylcyclines, macrolides, oxazolidinones, streptogramins, pleuromutilins, tetracyclines) are more numerous than those that affect cell membrane function (polymyxins) or nucleic acid function (fluoroquinolones, nitroimidazoles, nitrofurans, rifampin), although the development of fluoroquinolones has been a major advance in antimicrobial

therapy. Agents that affect intermediate metabolism (sulfonamides, trimethoprim) have greater selective toxicity than those that affect nucleic acid synthesis.

Searching for New Antibacterial Drugs

Infection caused by antibiotic-resistant bacteria has been an increasingly growing concern in the last decade. The speed with which some bacteria develop resistance considerably outpaces the slow development of new antimicrobial drugs. Since 1980, the number of antimicrobial agents approved for use in people in the United States has fallen steadily (Figure 1.3). Several factors such as complex regulatory requirements, challenges in drug discovery, and the high cost of drug development coupled with the low rate of return on investment antibiotics provide compared with drugs for the treatment of chronic conditions all contribute to driving pharmaceutical companies out of the antimicrobial drug market. This has left limited treatment options for infections caused by methicillin-resistant staphylococci and vancomycin-resistant enterococci. The picture is even bleaker for infections cause by some Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and extended-spectrum beta-lactamase (ESBL)-resistant *E. coli*, *Klebsiella* spp., and *Enterobacter* spp., which are occasionally resistant to all the antimicrobial agents on the market. Judicious use of the antibiotics currently available and better infection control practices might help prolong the effectiveness of the drugs that are currently available. However, even if we improve these practices, resistant bacteria will continue to develop and new drugs will be needed.

The approaches in the search for novel antibiotics include further development of analogs of existing agents; identifying novel targets based on a biotechnological approach, including use of information obtained from bacterial genome sequencing and gene cloning; screening of natural products from plants and microorganisms from unusual ecological niches other than soil; development of antibacterial peptide molecules derived from phagocytic cells of many species; screening for novel antimicrobials using combinatorial chemical libraries; development of synthetic antibacterial drugs with novel activities, such as oxazolidinones; development of new antibiotic classes that were abandoned early in the antibiotic revolution because there were existing drug classes with similar activities; development of "chimeramycins" by laboratory recombination of genes encoding antibiotics of different classes; and combination of antibacterial drugs with iron-binding chemicals targeting bacterial iron uptake mechanisms.

Antifungal Drugs

Most currently used systemic antifungal drugs damage cell membrane function by binding ergosterols that are unique to the fungal cell membrane (polyenes, azoles; chapter 20). The increase in the number of

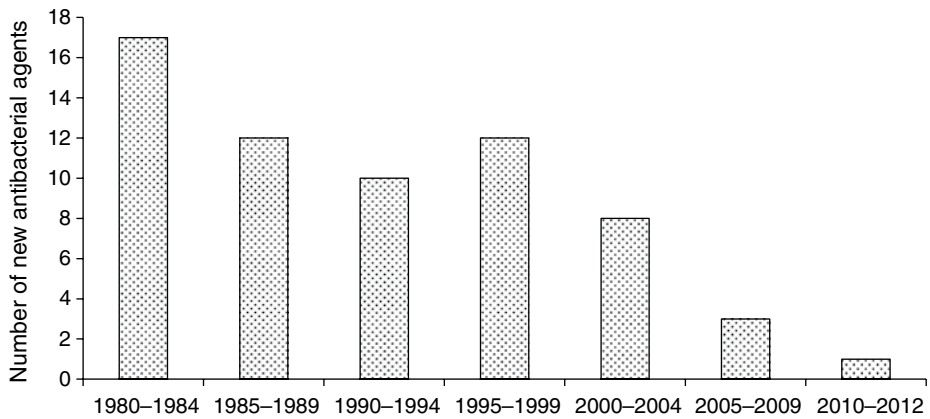

Figure 1.3. New antimicrobial agents approved for use in people in the United States since 1980.

HIV-infected individuals and of people undergoing organ or bone marrow transplants has resulted in increased numbers of immunosuppressed individuals in many societies. The susceptibility of these people to fungal infections has renewed interest in the discovery and development of new antifungal agents. The focus of antifungal drug development has shifted to cell wall structures unique to fungi (1,3-β-D-glucan synthase inhibitors, chitin synthase inhibitors, mannoprotein binders; Figure 20.1).

Antibacterial Drug Interactions: Synergism, Antagonism, and Indifference

Knowledge of the different mechanisms of action of antimicrobials provides some ability to predict their interaction when they are used in combination. It was clear from the early days of their use that combinations of antibacterials might give antagonistic rather than additive or synergistic effects. Concerns regarding combinations include the difficulty in defining synergism and antagonism, particularly their method of determination *in vitro*; the difficulty of predicting the effect of a combination against a particular organism; and the uncertainty of the clinical relevance of *in vitro* findings. The clinical use of antimicrobial drug combinations is described in chapter 6. Antimicrobial combinations are used most frequently to provide broad-spectrum empiric coverage in the treatment of patients that are critically ill. With the availability of broad-spectrum antibacterial drugs, combinations of these drugs are less commonly used, except for specific purposes.

An antibacterial combination is *additive* or *indifferent* if the combined effects of the drugs equal the sum of their independent activities measured separately; *synergistic* if the combined effects are significantly greater than the independent effects; and *antagonistic* if the combined effects are significantly less than their independent effects. Synergism and antagonism are not absolute characteristics. Such interactions are often hard to predict, vary with bacterial species and strains, and may occur only over a narrow range of concentrations or ratios of drug components. Because antimicrobial drugs may interact with each other in many different ways, it is apparent that no single *in vitro* method will detect all such interactions. Although the techniques to quantify and detect interactions are relatively crude, the observed interactions occur clinically.

The two methods commonly used, the *checkerboard* and the *killing curve* methods, measure two different effects (growth inhibition and killing, respectively) and have sometimes shown poor clinical and laboratory correlation. In the absence of simple methods for detecting synergism or antagonism, the following general guidelines may be used.

Synergism of Antibacterial Combinations

Antimicrobial combinations are frequently synergistic if they involve (1) sequential inhibition of successive steps in metabolism (e.g., trimethoprim-sulfonamide); (2) sequential inhibition of cell wall synthesis (e.g., mecillinam-ampicillin); (3) facilitation of drug entry of one antibiotic by another (e.g., beta-lactam-aminoglycoside); (4) inhibition of inactivating enzymes (e.g., amoxicillin-clavulanic acid); and (5) prevention of emergence of resistant populations (e.g., macrolide-rifampin).

Antagonism of Antibacterial Combinations

To some extent the definition of antagonism as it relates to antibacterial combinations reflects a laboratory artifact. However, there have been only a few well-documented clinical situations where antagonism is clinically important. Antagonism may occur if antibacterial combinations involve (1) inhibition of bactericidal activity such as treatment of meningitis in which a bacteriostatic drug prevents the bactericidal activity of another; (2) competition for drug-binding sites such as macrolide-chloramphenicol combinations (of uncertain clinical significance); (3) inhibition of cell permeability mechanisms such as chloramphenicol-aminoglycoside combinations (of uncertain clinical significance); and (4) induction of beta-lactamases by beta-lactam drugs such as imipenem and cefoxitin combined with older beta-lactam drugs that are beta-lactamase unstable.

The impressive complexity of the interactions of antibiotics, the fact that such effects may vary depending of the bacterial species, and the uncertainty of the applicability of *in vitro* findings to clinical settings make predicting the effects of some combinations hazardous. For example, the same combination may cause both antagonism and synergism in different strains of the

same bacterial species. Laboratory determinations are really required but may give conflicting results depending on the test used. Knowledge of the mechanism of action is probably the best approach to predicting the outcome of the interaction in the absence of other guidelines.

In general, the use of combinations should be avoided, because the toxicity of the antibiotics will be at least additive and may be synergistic, because the ready availability of broad-spectrum bactericidal drugs has made their use largely unnecessary, and because they may be more likely to lead to bacterial superinfection. There are, however, well-established circumstances, discussed in chapter 6, in which combinations of drugs are more effective and often less toxic than drugs administered alone.

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2

Antimicrobial Susceptibility Testing Methods and Interpretation of Results

Joseph E. Rubin

The veterinary diagnostic microbiology laboratory plays a key role in the practice of evidence-based antimicrobial therapy by providing culture and susceptibility information to practitioners. Before the introduction of antimicrobials, we were largely powerless to treat invasive infections. The antimicrobial age began with the familiar story of the discovery of penicillin in 1928 by Alexander Fleming. By the early 1940s that *Penicillium notatum* extract was successfully used against infections caused by organisms ranging from *Staphylococcus aureus* to *Neisseria gonorrhoeae* (Aronson, 1992; Bryskier, 2005). Unfortunately, the evolutionary power of bacteria resulted in the rapid emergence of antimicrobial resistance. Susceptibility testing is now vital to effective therapeutic decision making.

Although veterinary laboratories utilize many of the same basic microbiological techniques as human diagnostic labs, they face some unique challenges. These challenges include the difficulty in cultivation of fastidious veterinary-specific organisms, selection of species-customized antimicrobial panels for susceptibility testing, and considerations of drug withdrawal times and food safety.

In the clinical setting, the goal of antimicrobial susceptibility testing is to help clinicians choose optimal antimicrobial therapy. The decision to undertake culture and susceptibility testing depends on the site of infection, state of the patient (otherwise healthy vs. critically ill), prior history of infections and antimicrobial use, co-morbidities and underlying disease, and the predictability of the susceptibility patterns of the most likely pathogen(s). For example, susceptibility testing is not indicated in horses with "strangles," as *S. equi* is uniformly susceptible to penicillin (Erol et al., 2012). Similarly, culture and susceptibility testing is not required for first time, uncomplicated urinary tract infections in dogs, as empiric amoxicillin therapy is advocated (Pressler et al., 2010).

Early methods used to assess the susceptibility of organisms to antimicrobials were developed by individual labs and lacked standardization; the first effort to standardize susceptibility testing was published in 1971 (Ericsson et al., 1971). National standards organizations responsible for guidelines for conducting and interpreting antimicrobial susceptibility tests were subsequently formed. In the United States, the Clinical and Laboratory Standards Institute (CLSI) formed in the late 1960s as the National Committee for Clinical Laboratory Standards (NCCLS) and was tasked with developing a standard for disk diffusion antimicrobial susceptibility testing (Barry, 2007). While standardization of methods yields more comparable data between labs, heterogeneity in interpretive criteria persists (see Table 2.1). In 1997, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) was formed to harmonize both testing methods and interpretive criteria throughout Europe. In North America, the CLSI methodologies are used for both human and veterinary diagnostics. The CLSI standards are available for purchase on their website (www.clsi.org), while

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Table 2.1. Test factors leading to spurious results.

Factor	Artificially Resistant	Artificially Susceptible
Expired reagents	Mueller-Hinton agar used for diffusion-based testing that has dried out, may not be thick enough allowing the drug to diffuse out further leading to larger zones of inhibition	Degraded drugs
Inoculum density	Too dense an inoculum	Too light an inoculum
Incubation period	Prolonged incubation period	Inadequate incubation period
Incubation temperature		Above 35°C methicillin resistance may not be expressed in MRSA
Medium	Decreased divalent cations pH too high or too low	Increased divalent cations pH too high or too low
Incubation atmosphere	Depending on drug, CO_2 atmosphere may increase or decrease zone diameter or MIC	
Endpoint definition	For the sulfonamides, endpoints are defined by 80% reduction in growth compared to control	
Failure to identify	Accurate identification of organism is required to interpret susceptibility test results	
Mixed culture	The phenotype of the more resistant organism may dominate	

EUCAST publishes their guidelines free of charge on their website (www.eucast.org).

Antimicrobial Susceptibility Testing Methods

Antimicrobial susceptibility tests yield either categorical (susceptible, intermediate, or resistant) or quantitative (minimum inhibitory concentration [MIC]) data that can be categorically interpreted. Testing methods can be divided into two distinct categories, diffusion and dilution based.

Diffusion-Based Methods

Two types of diffusion tests are available that yield either categorical (disk diffusion) or quantitative (gradient strip) susceptibility data. These tests are based on the inhibition of bacterial growth by antimicrobial diffusing from a source disk or strip through solid media (Figure 2.2). The size of the inhibitory zone is a function of the rate of drug diffusion, thickness of the media, concentration of drug in the disk, and the susceptibility of the organism, making method standardization necessary for interpretive criteria to be applied (Figure 2.1).

Disk diffusion testing is conducted on 4-mm thick Mueller-Hinton agar plates using antimicrobial impregnated filter paper discs (CLSI, 2006a,b). Room-temperature plates are inoculated with a lawn of bacteria drawn from a McFarland 0.5 (approximately 10^8 CFU/ml) suspension using a sterile swab. Plates are allowed to

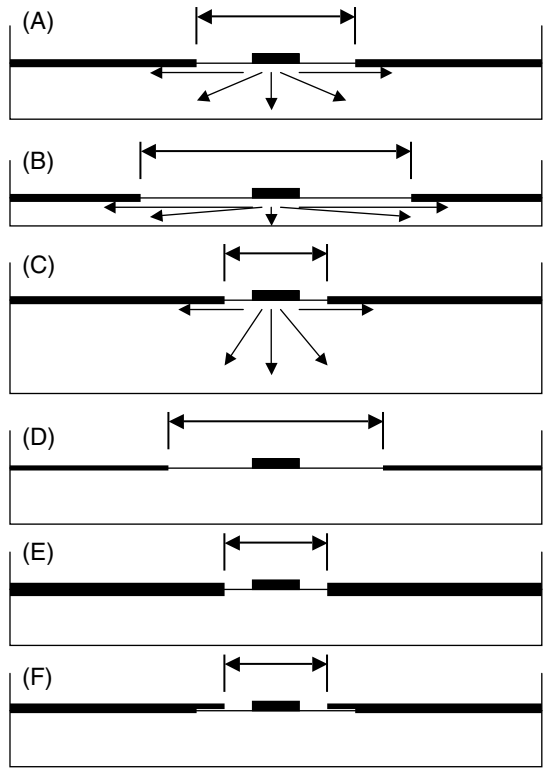

Figure 2.1. Disk diffusion: The results of the disk diffusion test can be influenced by the depth of the medium (A and B, increase in zone of inhibition; C, decrease in zone of inhibition) or the quality of the inoculum (D, false increase in zone of inhibition; E, false decrease in zone of inhibition; F, mixed culture, false decrease in zone of inhibition).

dry for up to 15 minutes before the disk is applied and are then incubated at 35°C at room atmosphere. After up to 24 hours the zone of inhibition is measured (Figure 2.2A). Owing to differences in antimicrobial diffusion rate, amount of drug included in disks, and pharmacodynamic interactions, the size of the inhibitory zone corresponding to resistance breakpoints is unique to each drug organism combination. The relative clinical appropriateness of different antimicrobials can therefore not be determined by simply comparing inhibitory zone diameters.

Gradient tests (e.g., Etest) are conducted in the same way as disk tests. These strips contain a gradient of antimicrobial from low to high concentrations corresponding to printed MIC values on the back of the strip. Following incubation, the apex of the teardrop zone of inhibition indicates the MIC of the organism (Figure 2.2B).

Diffusion-based tests are technically simple to perform and versatile, allowing customization of test panels to bacterial and patient species and type of infection. While disk diffusion tests are less inexpensive than gradient tests, they only provide categorical information (susceptible, intermediate, or resistant).

Dilution-Based Methods

Dilutional susceptibility testing can be done using either broth or agar media and yields quantitative (MIC) data. Doubling dilutions of antimicrobial (. . . 0.12 μg/ml, 0.25 μg/ml, 0.5 μg/ml, 1 μg/ml, 2 μg/ml . . .) are tested. An antimicrobial free control plate or broth must always be included. The lowest concentration without bacterial growth defines the MIC, except for the sulfonamides and trimethoprim, where an 80% reduction in growth compared to the control constitutes inhibition.

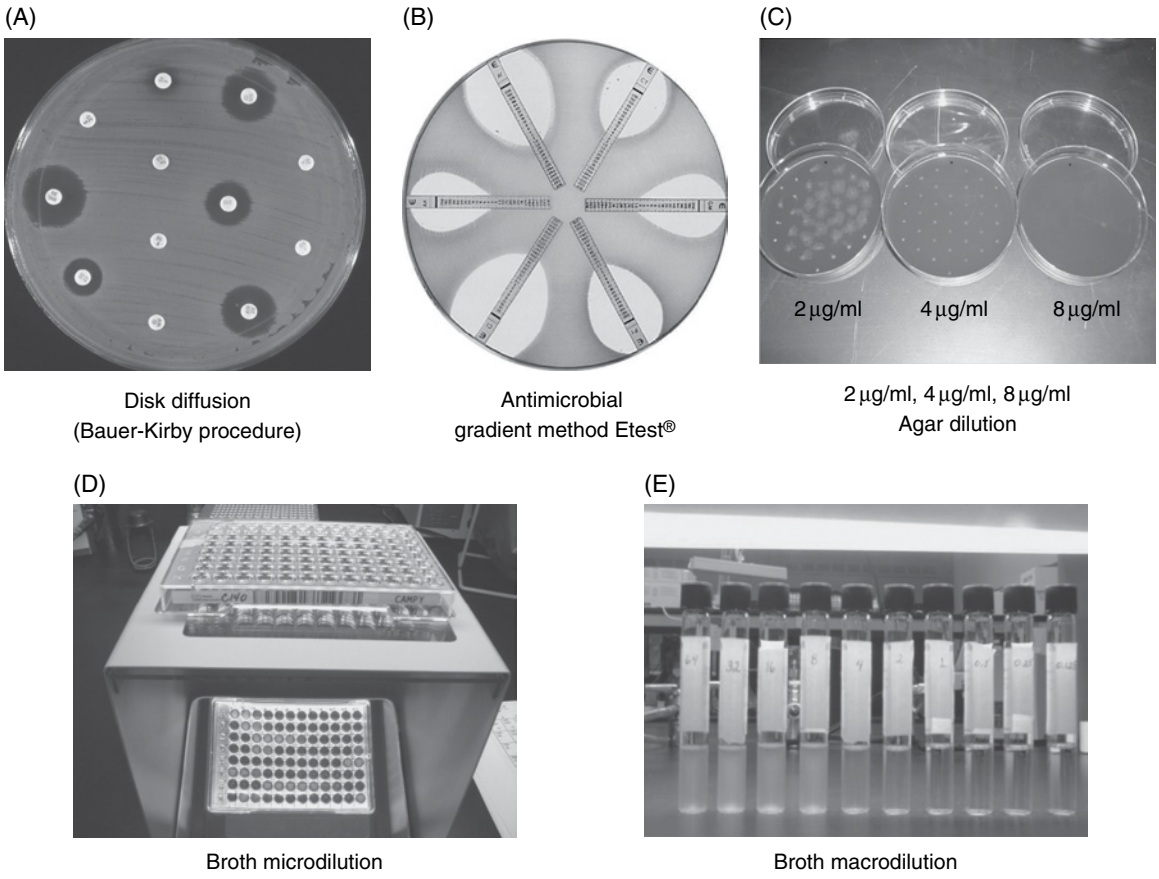

Figure 2.2. Antimicrobial susceptibility testing methods.

For agar media dilution, Mueller-Hinton agar plates are prepared incorporating doubling dilutions of antimicrobial. Antimicrobial stock solutions at 10 times the test concentration are prepared using the solvents and diluents recommended by the CLSI (CLSI, 2006a,b). The mass of antimicrobial required is determined by the following equation:

$$\text{Mass} = [(\text{Volume ml})(\text{Concentration mg / ml})] / \text{potency}$$

To prepare media, antimicrobial stock solution is added in a 1:9 ratio to molten Mueller-Hinton agar no hotter than 50°C, and poured into sterile petri dishes. Separate plates are prepared for each antimicrobial concentration test. Plates must not be stored for more than 7 days prior to use and for some drugs (e.g., imipenem), they must be prepared fresh on the day of use (CLSI, 2006a,b). Room-temperature plates are inoculated with approximately 10^4 CFU using either a multi-spot replicator or manually by pipette. To prevent discrete samples from mixing, plates are left on the bench top for up to 30 minutes for the bacterial spots to be absorbed prior to incubation. Plates are incubated in room air at 35°C for 16–20 hours and examined for growth (Figure 2.2C). Because this technique is very labor intensive, its use is mainly limited to research.

For broth dilution, Mueller-Hinton broths containing doubling dilutions of antimicrobial are prepared. As in agar dilution, antimicrobial stock solutions at 10 times the final concentration are prepared and added to test medium in a 1:9 ratio. Each antimicrobial concentration is dispensed into separate vials and inoculated with bacteria to yield a final concentration of 5×10^5 CFU/mL. A McFarland 0.5 inoculum is typically made in either sterile water or saline and then aliquoted into the Mueller-Hinton broth to yield the final concentration. Growth is evidenced by turbidity and the MIC is defined by the lowest concentration where growth is not seen.

Commercially prepared microdilution plates (Figure 2.2D) allow a large number of bacterial isolates to be tested efficiently without the need to prepare, store, and incubate large volumes of media in house. The efficiency of the microdilution method comes with increased costs for consumables. (Figure 2.2E).

Interpretation of Susceptibility Test Results

Categorical interpretation of antimicrobial susceptibility test results requires the development of clinical resistance breakpoints. Resistance breakpoints are designed to predict clinical outcomes: susceptible = high probability of success following treatment, resistant = low probability of success following treatment. For an antimicrobial to be effective clinically, it must reach a sufficiently high concentration at the site of infection to inhibit growth or kill the organism. Resistance breakpoints are therefore related to achievable drug concentrations in target tissues. Because drug concentrations vary in different body sites or fluids, pharmacokinetic studies are required to determine if therapeutic concentrations are reached in target tissues. Resistance breakpoints are also specific to animal species, dosing regimen (dose, route of administration, and frequency), disease, and target pathogen. When any of these factors are altered (e.g., drug given orally instead of injected), the predictive value of resistance breakpoints for clinical outcomes cannot be relied upon. Veterinary-specific resistance breakpoints are published by the CLSI. The CLSI human guidelines, EUCAST, and the British Society for Antimicrobial Chemotherapy (BSAC) are resources that may be useful when species-specific criteria are not available. However, extrapolation of non-approved breakpoints should be done with extreme caution. The lack of validated veterinary-specific resistance breakpoints is an important limitation for veterinarians trying to practice evidence-based medicine. As an example, there are no validated breakpoints for any pathogens causing enteric disease in veterinary species (Table 2.2).

Furthermore, when antimicrobials are used in food animals, the prescribing veterinarian is responsible for the prevention of violative drug residues. Expert-mediated advice regarding drug withdrawal periods is available from food animal residue avoidance databases. In the United States, practitioners can contact www.farad.org and in Canada, www.cgfarad.usask.ca.

Because it is conceptually simple to think of an isolate's susceptibility categorically (susceptible, intermediate, or resistant), it is tempting to classify an isolate as susceptible or resistant even when no validated breakpoints exist. It is essential to remember that resistance breakpoints are designed to be clinically predictive,

Table 2.2. Drugs with veterinary-specific CLSI resistance breakpoints.

Drug	Animal Species/Pathogens
Gentamicin	Canine (Enterobacteriaceae, *Pseudomonas aeruginosa*)
	Equine (Enterobacteriaceae, *Pseudomonas aeruginosa, Actinobacillus* spp.)
Spectinomycin	Bovine (respiratory disease—*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*)
Ampicillin	Canine (skin and soft tissue infections—*Staphylococcus pseudintermedius, Streptococcus canis;* other infections—*Escherichia coli*)
	Equine (respiratory disease—*Streptococcus equi* subsp. *zooepidemicus*)
Penicillin-novobiocin	Bovine (mastitis—*Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis*)
Cefpodoxime	Canine (wounds and abscesses—*Staphylococcus aureus, Staphylococcus pseudintermedius, Streptococcus canis, Escherichia coli, Pasteurella multocida, Proteus mirabilis*)
Ceftiofur	Bovine (respiratory disease—*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni;* mastitis—*Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli*)
	Porcine (respiratory disease—*Actinobacillus pleuropneumoniae, Pasteurella multocida, Salmonella cholerasuis, Streptococcus suis*)
	Equine (respiratory disease—*Streptococcus equi* subsp. *zooepidemicus*)
Danofloxacin	Bovine (respiratory disease—*Mannheimia haemolytica, Pasteurella multocida*)
Enrofloxacin	Feline (dermal)
	Canine (dermal, respiratory, and UTI—Enterobacteriaceae, *Staphylococcus* spp.)
	Chickens and turkeys (*Pasteurella multocida, Escherichia coli*)
	Bovine (respiratory disease—*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*)
Difloxacin	Canine (dermal and UTI—Enterobacteriaceae, *Staphylococcus* spp.)
Marbofloxacin	Feline (dermal)
	Canine (dermal and UTI—Enterobacteriaceae, *Staphylococcus* spp.)
Orbifloxacin	Feline (dermal)
	Canine (dermal and UTI—Enterobacteriaceae, *Staphylococcus* spp.)
Clindamycin	Canine (skin and soft tissue infections—*Staphylococcus* spp.)
Pirlimycin	Bovine (mastitis—*Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis*)
Tilmicosin	Bovine (respiratory disease—*Mannheimia haemolytica*)
	Porcine (respiratory disease—*Actinobacillus pleuropneumoniae, Pasteurella multocida*)
Tulathromycin	Bovine (respiratory disease—*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*)
Florfenicol	Bovine (respiratory disease—*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*)
	Porcine (respiratory disease—*Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Pasteurella multocida, Streptococcus suis, Salmonella cholerasuis*)
Tiamulin	Porcine (respiratory disease—*Actinobacillus pleuropneumoniae*)
Oxytetracycline	Bovine (respiratory disease—*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*)
	Porcine (respiratory disease—*Actinobacillus pleuropneumoniae, Pasteurella multocida, Streptococcus suis*)

viewing antimicrobial susceptibility through the lens of the patient by incorporating pharmacokinetic information. In contrast, epidemiological cut-offs describe antimicrobial susceptibility from the perspective of the organism. Isolates with MICs above the epidemiological cut-off have acquired resistance mechanisms that make them less susceptible to an antimicrobial than wild-type organisms of the same species. Epidemiological cut-offs are established by evaluating the MIC distributions of large isolate collections. An organism can have an MIC below the epidemiological cut-off for a particular drug and be clinically resistant or have an MIC above the epidemiological cut-off while remaining susceptible (Figure 2.3). While epidemiological cut-offs are invaluable research tools, they do not incorporate pharmacokinetic data and should not be used to guide therapy of patients.

In practice, the application of antimicrobial susceptibility test results is reduced to susceptible = good treatment choice and resistant = bad treatment choice, rather than a thorough analysis of the susceptibility profile. Interpretive reading is a more biological approach that

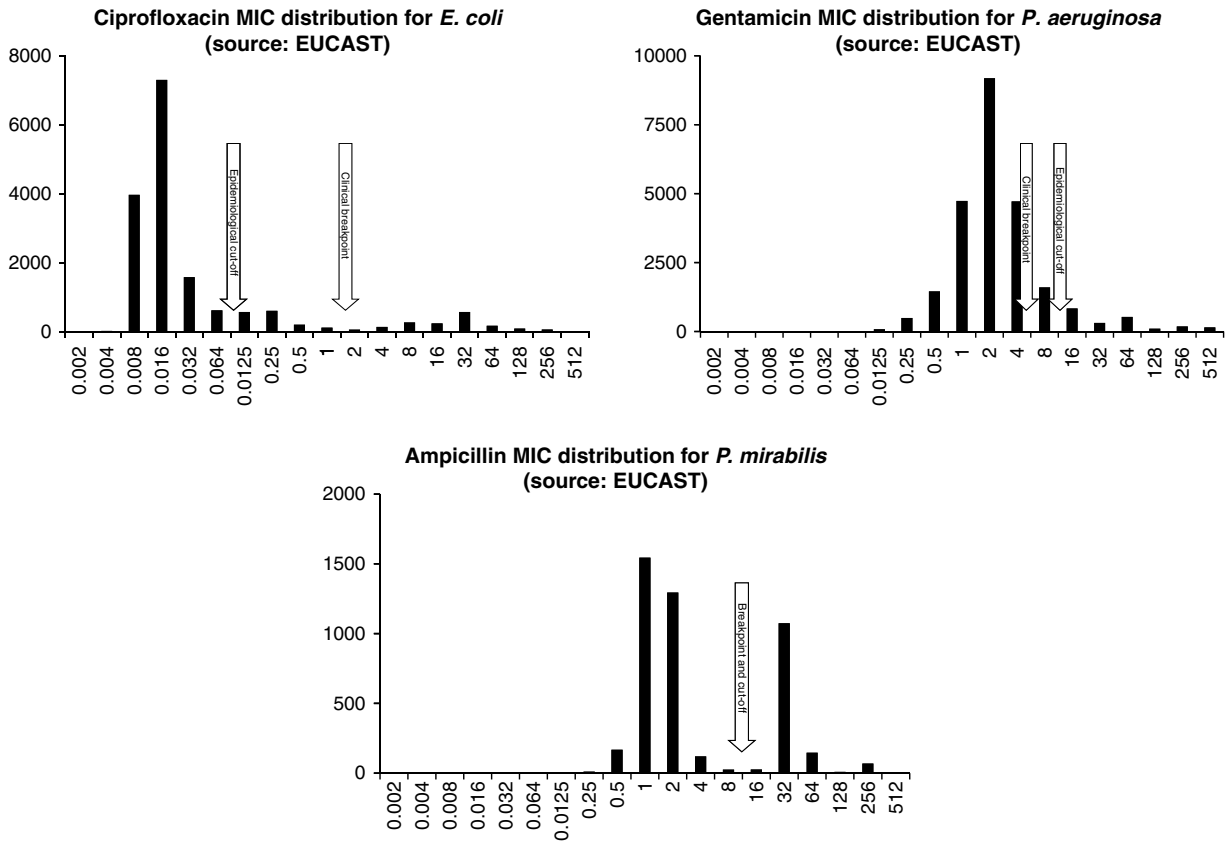

Figure 2.3. Comparison of clinical resistance breakpoints and epidemiological cut-off values from EUCAST databases. Each histogram depicts the number of isolates (y axis) with each MIC (x axis). Epidemiological cut-offs are higher (*E. coli* and ciprofloxacin), lower (*P. aeruginosa* and gentamicin), or the same (*P. mirabilis* and ampicillin) as clinical resistance breakpoints.

incorporates knowledge of intrinsic drug resistance, indicator drugs, exceptional resistance phenotypes, and consideration of antimicrobial selection pressure. For example, "*Enterococcus* spp." may be commonly reported by diagnostic labs, but identification at the species level (e.g., *Enterococcus faecium* vs. *Enterococcus faecalis*) is necessary for interpretive reading. For an excellent review of interpretive reading, see Livermore (2001). Interpretive reading is used to detect specific resistance phenotypes such as methicillin resistance or the production of extended-spectrum beta-lactamases (ESBLs). Some of these tests are organism specific and use across species or genera may not yield reliable results. For example, the CLSI recommends that either cefoxitin or oxacillin resistance may be used as indicators of *mec*A mediated methicillin resistance in *S. aureus*, while only oxacillin resistance reliably predicts *mec*A in *S. pseudintermedius* (CLSI, 2008a,b; Papich, 2010). In Enterobacteriaceae, a combination of ceftazidime and cefotaxime with and without clavulanic acid is used to detect ESBLs; a greater than or equal to eight-fold increase in susceptibility (decrease in the MIC) in the clavulanic acid potentiated cephalosporins indicates the presence of ESBL and therefore clinical resistance to all penicillins, cephalosporins, and aztreonam (CLSI, 2008a,b; Table 2.3).

Knowledge of intrinsic resistance is invaluable when interpreting susceptibility reports. Resistance should always be assumed for certain drug-organism combinations (e.g., cephalosporins and enterococci). Because *in vitro* resistance expression may not be reflective of drug-organism interactions *in vivo*, isolates should be reported

Table 2.3. Failure of *in vitro* tests to predict *in vivo* outcomes.

	Factor	Positive Outcomes	Negative Outcomes
Patient/Disease Factors	Pharmacokinetic	High urine drug concentrations	Failure of drugs to penetrate sequestered sites such as the CNS or prostate Drug interactions decreasing absorption or increasing elimination
	Pharmacodynamic		Failure of aminoglycosides in acidic or anaerobic environments Failure of folate synthesis inhibitors in purulent environments (excessive PABA in environment)
	Disease/pathology	No infection Self-limiting infection	Predisposing disease or underlying pathology such as atopy, diabetes, or neoplasia Indwelling medical device
	Therapeutic	Utilization of localized therapy, high concentrations overcoming low-level resistance Different dose, dosing frequency, route of administration than label	Different dose, dosing frequency, route of administration than label Poor owner compliance
Organism/Test Factors	Resistance		Development of resistance *in vivo*
	Organism lifestyle		Biofilm formation Intracellular infections
	Organism identification	Misidentified organism False positive culture	Misidentified organism Mixed infection
	Antimicrobial susceptibility test	Incorrectly performed or reported test	Incorrectly performed or reported test Inducible resistance

as resistant irrespective of *in vitro* test results where intrinsic resistance is recognized. A detailed description of intrinsic resistance phenotypes is published by EUCAST and is available at www.eucast.org/expert_rules/. Some commonly encountered veterinary pathogens with intrinsic resistance to antimicrobials are included in Table 2.4.

An appreciation of exceptional (unexpected) resistance phenotypes allows unusual isolates or test results to be identified and investigated further. Vancomycin-resistant staphylococci, penicillin-resistant group A streptococci, and metronidazole-resistant anaerobes are all exceptional phenotypes that should be confirmed before starting antimicrobial therapy. While such results can be due to the emergence of resistance, it is more likely that these results reflect errors in reporting, testing, isolate identification, or testing mixed cultures isolation (Livermore et al., 2001). The CLSI M100 document as well as the EUCAST expert rules describe exceptional phenotypes (CLSI, 2008b; Leclerq et al., 2008).

Bacterial resistance mechanisms often predictably confer resistance to multiple antimicrobials such that resistance to one may indicate resistance to others.

Table 2.4. Intrinsic resistance phenotypes of importance to veterinary medicine.

Organism	Intrinsic Resistance Phenotypes
Enterobacteriaceae	Benzylpenicillin, macrolides, lincosamides, streptogramins, and rifampin
Klebsiella spp.	Ampicillin and ticarcillin
Proteus mirabilis	Tetracycline and nitrofurantoin
Proteus vulgaris	Ampicilin, cefazolin, tetracycline, and nitrofurantoin
Acinetobacter baumannii	Ampicillin, amoxicillin + clavulanic acid, cefazolin, and trimethoprim
Pseudomonas aeruginosa	Ampicillin, amoxicillin + clavulanic acid, piperacillin, cefazolin, chloramphenicol, trimethiprim + sulphonamide, and tetracycline
Enterococcus faecalis	Cephalosporins, aminoglycosides (low-level resistance), erythromycin, clindamycin, sulfonamides
Enterococcus faecium	Cephalosporins, aminoglycosides (low-level resistance), erythromycin, sulfonamides
Enterococcus galinarum	Cephalosporins, aminoglycosides (low-level resistance), erythromycin, clindamycin, sulphonamides, and vancomycin

Data from EUCAST expert rules.

By testing indicator drugs, susceptibility test results can be extrapolated to a broader panel of antimicrobials than could practically be tested. For example, oxacillin resistance in staphylococci indicates methicillin resistance and therefore resistance to all beta-lactams without having to specifically test other beta-lactams. For Enterobacteriaceae, cephalothin test results are predictive for cephalexin and cefadroxil but not for ceftiofur or cefovecin. For β-hemolytic streptococci, penicillin susceptibility is predictive of ampicillin, amoxicillin, amoxicillin/clavulanic acid, and a number of cephalosporins. See the CLSI guidelines for other examples.

Minimizing the selective pressure for antimicrobial resistance should always be considered when selecting therapy. While antimicrobial resistance follows usage, certain bug-drug combinations are more likely to select for resistance or promote mutational resistance than others and should be avoided when possible. For example, staphylococci readily develop resistance to rifampin, while the fluoroquinolones and cephalosporins are known to select for methicillin-resistant isolates (Dancer, 2008; Livermore et al., 2001). Among Gram-negative bacteria, there is evidence to suggest that the fluoroquinolones and extended-spectrum cephalosporins are more potent selectors of resistance than the aminoglycosides, and that the third-generation cephalosporins select for resistance more so than beta-lactamase inhibitor potentiated penicillins (Peterson, 2005). See chapter 3 for a discussion of the epidemiology of antimicrobial resistance.

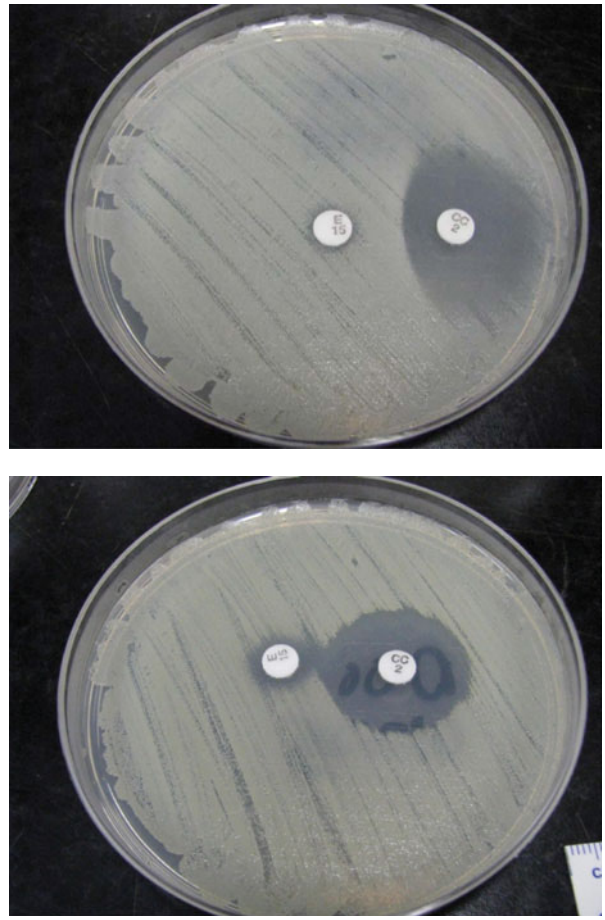

Figure 2.4. Inducible clindamycin resistance *Staphylococcus aureus* displaying typical "D-zone" of inhibition associated with inducible clindamycin resistance (top), and clindamycin susceptibility with erythromycin resistance (bottom).

Other Susceptibility Testing Methods

Inducible resistance phenotypes pose unique diagnostic challenges; standard diffusion or dilution testing methods may fail to detect resistance. Interpretive reading can play a key role in identifying those phenotypes. For example, inducible clindamycin resistance should be suspected in staphylococci and streptococci appearing to be resistant to erythromycin but susceptible to clindamycin. Resistance can be elicited in inducible isolates using the "D-test," a double disk test where erythromycin and clindamycin disks are placed adjacently in an otherwise standard disk diffusion test. Blunting of the inhibitory zone surrounding the clindamycin disk (resulting in a "D" shape) in the presence of erythromycin indicates resistance induction (Figure 2.4). It is recommended that staphylococci and streptococci appearing to be clindamycin susceptible but erythromycin resistant should be tested for inducible clindamycin resistance using the D-test. Inducibly clindamycin resistant isolates should always be reported as resistant, as *in vivo* induction of resistance following clindamycin therapy can lead to treatment failure (Levin et al., 2005). Recent studies have documented inducible clindamycin resistance in both *Staphylcoccus aureus* and *Staphylococcus pseudintermedius* isolated from animals (Rubin et al., 2011a,b).

Selective media have been designed to quickly identify particular antimicrobial-resistant organisms from clinical samples. A detailed description of screening media for extended-spectrum beta-lactamases in Enterobacteriaceae, methicillin (oxacillin) resistance in staphylococci, and high-level aminoglycoside and vancomycin resistance in enterococci is published by the CLSI (CLSI, 2008a,b).

Antimicrobial resistance can also be identified by testing for the products of resistance genes. For example, the nitrocefin test utilizes a cephalosporin (nitrocefin) that turns to red from yellow when hydrolyzed by most beta-lactamases. However, this reaction is non-specific; the susceptibility of nitrocefin to hydrolysis means that narrow- or broad-spectrum beta-lactamases yield the same positive result. Additionally, as the presence or absence of beta-lactamase does not preclude other resistance mechanisms, interpretation of these results in the context of susceptibility testing is therefore essential.

A latex agglutination test targeting PBP2a, the penicillin-binding protein conferring methicillin resistance, is available. This test can be done on primary cultures, identifying methicillin resistance before the complete antimicrobial susceptibility profile can be determined, saving 1 day in the diagnostic process.

For some investigations, MICs insufficiently describe pharmacodynamic interactions. Time kill assays define the effects of antimicrobials on an organism over time, rather than at the single end point with MIC testing. A time kill curve is performed by growing a bacterial culture in broth containing a known concentration of antimicrobial and evaluating changes in the concentration of viable organisms over time (CFU/ml) using colony counts. Although the time points selected depend on the research question, time zero, 4 hours, 8 hours, 12 hours, 24 hours, and 48 hours is a good base model. At time zero, broths are inoculated to a known organism concentration (e.g., 10^5 CFU/ml). Colony counts are performed on serial ten-fold dilutions of 100 μL broth aliquots. The first dilution, 10^{-1}, is made by plating out 100 μL of broth directly. The next dilution, 10^{-2}, is made by diluting 100 μL broth in 900 μL of saline; the third dilution is made by diluting 100 μL of 10^{-2} in 900 μL of saline, and so on. Depending on the organism being tested and the expected concentration of bacteria, dilutions from 10^{-1} to 10^{-8} should be sufficient. Plates are incubated overnight and those plates with between 20 and 200 colonies are counted and recorded; higher or lower counts are not reliable. Preliminary analysis includes visual inspection of bacterial counts plotted on a $\log_{10}$ scale. A ≥ 3 log decrease in counts after 24 hours incubation indicates bactericidal activity (CLSI, 1999). See chapters 4 and 5 for discussions of pharmacokinetics and the selection of antimicrobial therapy.

Summary

Antimicrobials are some of the most commonly used drugs in veterinary medicine and have improved the health of food and companion animals alike. When properly performed and carefully analyzed, antimicrobial susceptibility testing is an invaluable component of evidence-based treatment of infectious disease. In the clinical setting, results should always be interpreted in the context of the patient. By considering the pharmacokinetic/pharmacodynamic properties of the antimicrobials in conjunction with interpretive reading of *in vitro* susceptibility test results, clinical success can be maximized.

While categorical susceptibility data can provide vital information to clinicians, MIC data are superior for allowing pharmacokinetic principles to be applied directly. For example, it may be rational to use antimicrobials that reach high concentrations in the urine, despite susceptibility reports indicating resistance correlated to achievable plasma concentrations. The reader is referred to chapters 5 and 6 for discussion of pharmacokinetics and the principles of antimicrobial selection.

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3

Antimicrobial Resistance and Its Epidemiology

Patrick Boerlin and David G. White

Introduction

Since the discovery of penicillin in the late 1920s, hundreds of antimicrobial agents have been developed for anti-infective therapy. Antimicrobials have become indispensable in decreasing morbidity and mortality associated with a host of infectious diseases and, since their introduction into veterinary medicine, animal health and productivity have improved significantly (National Research Council, Institute of Medicine, 1998). The emergence of antimicrobial resistance was not an unexpected phenomenon and was predicted by Alexander Fleming, who warned in his Nobel Prize lecture in 1945 against the misuse of penicillin. However, loss of efficacy through the emergence, dissemination, and persistence of bacterial antimicrobial resistance in many bacterial pathogens (defined as the ability of a microorganism to withstand the effect of a normally active concentration of an antimicrobial agent) has become a general problem and a serious threat to the treatment of infectious diseases in both human and veterinary medicine (Salyers and Amiable-Cuevas, 1997; Witte, 1998; Marshall and Levy, 2011).

Infections caused by resistant bacteria are more frequently associated with higher morbidity and mortality than those caused by susceptible pathogens (Helms et al., 2002; Travers and Barza, 2002; Varma et al., 2005). In areas of concentrated use, such as hospitals, this has led to lengthened hospital stays, increased health care costs, and, in extreme cases, to untreatable infections (Maragakis et al., 2008; Shorr, 2009). Contributing to this growing dilemma is the observation that the introduction of new classes or modifications of older classes of antimicrobials over the past 7 decades has been matched, slowly but surely, by the systematic emergence of new bacterial resistance mechanisms. Antimicrobial resistance mechanisms have been reported for all known antibiotics currently available for clinical use in human and veterinary medicine. Therefore, successful sustainable management of current antimicrobials (Prescott, 2008; Doron and Davidson, 2011; Ewers et al., 2011) and the continued development of new ones and of alternatives to antimicrobial drugs are vital to protecting animal and human health against infectious microbial pathogens.

Resistance Mechanisms

A large variety of antimicrobial resistance mechanisms have been identified in bacteria, and several different mechanisms can frequently be responsible for resistance to a single antimicrobial agent in a given bacterial species. The manually curated Antibiotic Resistance Genes Database (ARDB) lists the existence of more than 23,000 potential resistance genes from available bacterial genome sequences (Liu and Pop, 2009). Antimicrobial resistance mechanisms can be classified into four major categories (Figure 3.1): (1) the antimicrobial agent can be prevented from reaching its target by reducing its penetration into the bacterial cell; (2) the antimicrobial agent can be expelled out of the cell by general or specific efflux pumps; (3) the antimicrobial agent can be inactivated by modification or

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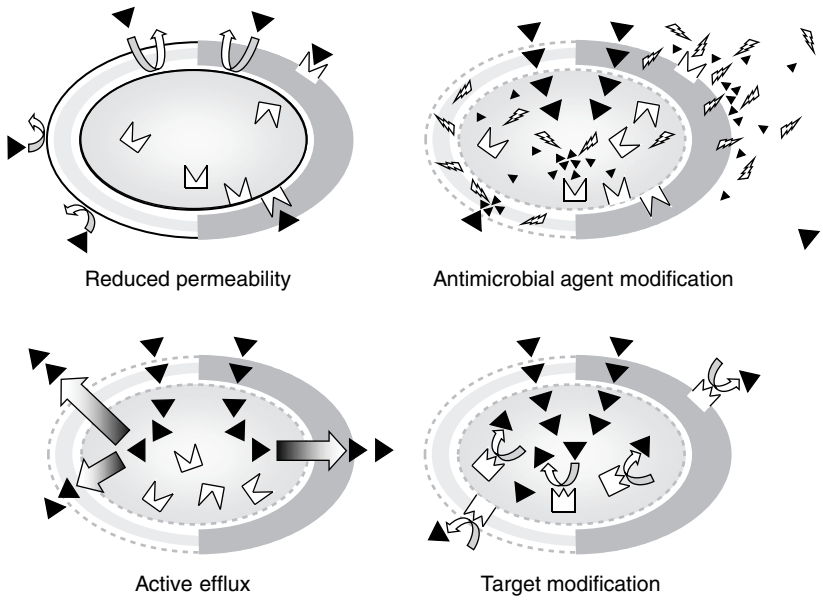

Figure 3.1. The four major mechanisms of antimicrobial resistance. Reduced permeability can be due to either lack of permeability of the outer membrane (e.g., down-regulation of porins in Gram-negatives) or of the cell membrane (e.g., lack of aminoglycoside active transport under anaerobic conditions). Active efflux can pump antimicrobial agents back into the periplasmic space (as with the TetA tetracyclines efflux pump in Enterobacteriaceae) or directly in the outer milieu (as for the RND multidrug efflux transporters). Antimicrobial agent modification by bacterial enzymes can take place either after the agent has penetrated into the cell (e.g., acetylation of chloramphenicol by CAT enzymes), in the periplasmic space (e.g., splitting of the beta-lactam ring by beta-lactamases in *Enterobacteriaceae*), or even outside of the bacterial cell (e.g., beta-lactamase produced by *Staphylococcus aureus*), before the agent has reached its target on the surface of the bacterium. Target modification has been described for both surface-exposed (e.g., peptidoglycan modification in vancomycin-resistant enterococci) and intracellular targets (e.g., macrolide resistance due to ribosomal methylation in Gram-positive bacteria).

degradation, either before or after penetrating the cell; and (4) the antimicrobial target can be modified or protected by another molecule preventing access of the antibiotic to its target, so that the antimicrobial cannot act on it anymore. Alternatively, the antimicrobial agent target can be rendered dispensable by the acquisition or activation of an alternate pathway by the microorganism. A few examples of each one of these resistance mechanisms are listed in Table 3.1 and more systematic information can be found in the following chapters of this book.

Types of Antimicrobial Resistance

In the context of antimicrobial resistance, bacteria display three fundamental phenotypes: susceptibility, intrinsic resistance, or acquired resistance.

Intrinsic resistance is natural to all the members of a specific bacterial taxonomic group, such as a bacterial genus, species, or subspecies. This type of resistance is most often through structural or biochemical characteristics inherent to the native microorganism. For example, many Gram-negative bacteria are naturally resistant to the activity of macrolides since these chemicals are too large to traverse the cell wall and to gain access to their cytoplasmic target. Other examples of innate resistance include the general reduced activity of aminoglycosides against anaerobes, because of the lack of aminoglycoside penetration into the cells under anaerobic conditions, and polymyxin resistance among Gram-positive bacteria because of the lack of phosphatidylethanolamine in their cytoplasmic membrane. A few examples of intrinsic resistance phenotypes for major bacterial taxa are presented in Table 3.2. These intrinsic

Table 3.1. Examples of resistance mechanisms (note that this is by far not a comprehensive list of all the resistance mechanisms known for each category of antimicrobials listed).

Antimicrobial Agent	Resistance Mechanism	Examples of Genetic Determinant
Tetracycline	2. Inducible efflux of tetracycline in *E. coli* and other *Enterobacteriaceae*	*tet*(A), *tet*(B), *tet*(C)
	4. Ribosomal protection in Gram-positive bacteria	*Tet*(O), *tet*(M)
Chloramphenicol	2. Efflux in *Enterobacteriaceae*	*cmlA*, *floR*
	3. Acetylation in *Enterobacteriaceae*	*catA*
Beta-lactams	3. Beta-lactamases in *Enterobacteriaceae*, and *Staphylococcus aureus*	bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{CMY}, bla_{NDM}, bla_{Z}
Oxacillin, methicillin	4. Alternate penicillin-binding proteins in *Staphylococcus aureus*	*mecA*
Imipenem	1. Decreased porin formation in *Enterobacter aerogenes* and *Klebsiella* spp.	Mutations
Aminoglycosides	3. Phosphorylation, adenylation, and acetylation of aminoglycosides in Gram-negative and –positive bacteria	Numerous genes with a broad variety of specificities
Streptomycin	4. Modification of ribosomal proteins or of 16S rRNA in *Mycobacterium* spp.	Mutations
Macrolides, lincosamides, streptogramins	4. Methylation of ribosomal RNA in Gram-positive organisms	*ermA*, *ermB*, *ermC*
Macrolides, streptogramins	2. *Staphylococcus* spp.	*vga(A), msr(A)*
Fluoroquinolones	2. Active efflux	*qepA*
	4. DNA topoisomeases with low affinity to quinolones	Mutations in *gyrA*, *gyrB*, *parC*, *parE*
	4. Target protection	Diverse *qnr* genes
Sulfonamides	4. Bypass of blocked pathway through additional resistant dihydropteroate synthase in Gram-negative bacteria	*sul1*, *sul2*, *sul3*
Trimethoprim	4. Bypass of blocked pathway through additional resistant dihydrofolate reductase	Diverse *dfr* genes

Table 3.2. Examples of intrinsic resistance phenotypes.

Organism	Intrinsic Resistance(s)
Most Gram-negative bacteria (*Enterobacteriaceae Pseudomonas* spp., or *Campylobacter* spp.)	Penicillin G, oxacillin, macrolides, lincosamides, streptogramins, glycopeptides, bacitracin
Klebsiella spp.	Ampicillin
Proteus vulgaris	Ampicillin, cephalosporins I, polymyxins
Proteus mirabilis	Tetracycline, polymyxins
Serratia marcescens	Ampicillin, amoxicillin-clavulanate, cephalosporins I, polymyxins
Enterobacter spp.	Ampicillin, amoxicillin-clavulanate, cephalosporins I, cefoxitin
Pseudomonas aeruginosa	Ampicillin, cephalosporins I and II, ceftriaxone, kanamycine, tetracycline, chloramphenicol, trimethoprim, quinolones
Haemophilus spp.	(Streptomycin, kanamycin), macrolides
Campylobacter jejuni and *Campylobacter coli*	Cephalosporins I, trimethoprim
Most Gram-positive bacteria	Polymyxins, quinolones
Streptococcus spp.	Aminoglycosides (low level)
Enterococcus spp.	Oxacillin, cephalosporins, aminoglycosides (low level), sulfonamides (*in vivo*), trimethoprim (*in vivo*)
Listeria monocytogenes	Oxacillin, cephalosporins, lincosamides
Bacillus anthracis	Cephalosporins, sulfonamides, trimethoprim
Anaerobes (including *Clostridium* spp.)	Aminoglycosides

Adapted from the Communiqué 2005 of the Comité de l'Antibiogramme de la Société Française de Microbiologie.

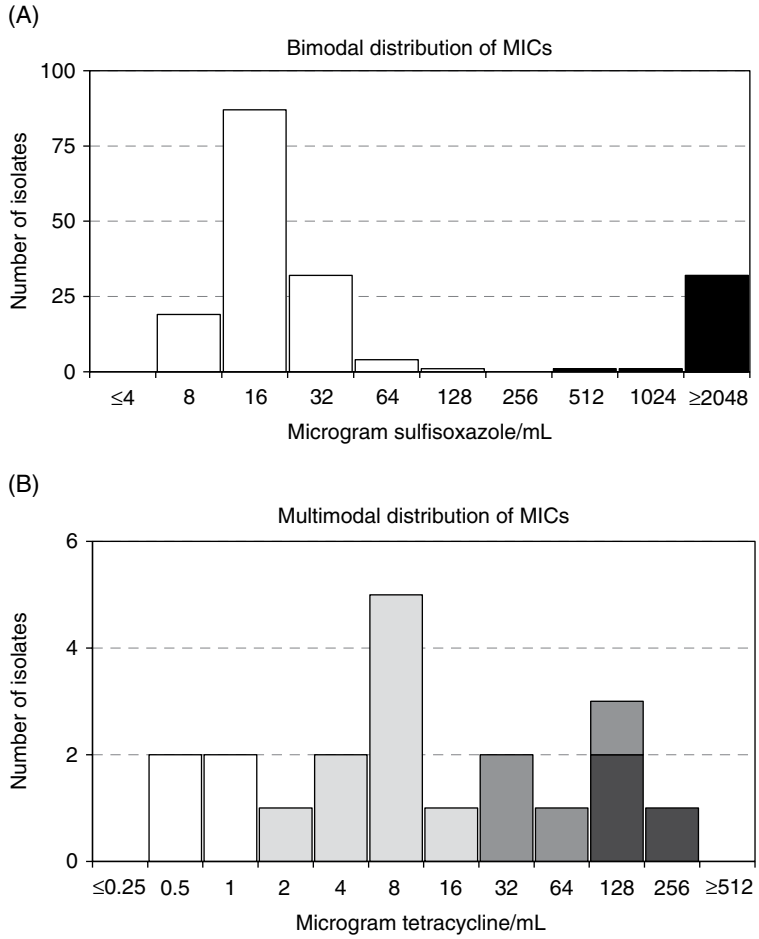

Figure 3.2. Examples of bimodal and multimodal distribution of minimal inhibitory concentrations. (A) Bimodal distribution of MICs for sulfonamides in a sample of commensal *Escherichia coli* isolates from swine and cattle. Susceptible isolates are in white and isolates with a resistance determinant are in black. Note the clear separation between the two groups. (B) Multimodal distribution of MICs for tetracycline in a sample of *E. coli* from a variety of origins. Fully susceptible isolates without any resistance determinant are in white. Isolates with a *tet*(C), *tet*(A), and *tet*(B) are in increasingly dark shades of gray. Note that depending on the respective frequency of each tetracycline resistance determinant, modes may or may not be clearly visible.

resistances should generally be known by clinicians and other users of antimicrobial agents so as to avoid inappropriate and ineffective therapeutic treatments. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) provides a very useful interactive list of antimicrobial susceptibility tables for a variety of organism/antimicrobial combinations on its website (http://mic.eucast.org/Eucast2/).

Antimicrobial resistance can also be acquired, such as when a normally susceptible organism develops resistance through some type of genetic modification. Acquisition of resistance usually leads to discrete jumps in the MIC of an organism and hence to clear bi- or polymodal distributions of MICs (Figure 3.2). However, in some instances such as for fluoroquinolone antimicrobials, acquisition of resistance (elevated MICs) may be a progressive phenomenon, through successive accumulation of multiple genetic modifications blurring the minimal changes in MIC provided by each modification into a smooth continuous MIC distribution curve, since mutations occur in particular topoisomerase genes in a step-wise manner (Hopkins et al., 2005; Table 3.3).

Table 3.3. Characterization of quinolone-resistant avian pathogenic *E. coli* ($n = 56$).[a]

No. of isolates	Mutation in[b]			MIC range (μg/ml)[c]			
	GyrA	GyrB	ParC	Nal	Orb	Enr	Cip
40	Ser83-Leu	None	None	64–>256	0.5–8	0.25–2	0.12–1
7	Asp87-Tyr	None	None	128	0.5–1	0.25–0.5	0.12–0.25
1	Asp87-Tyr	None	Ser80-Ile	>256	>16	16	8
1	Ser83-Leu; Asp87-Gly	None	None	128	1	0.5	0.25
1	Ser83-Leu; Asp87-Ala	None	None	>256	2	1	0.5
1	Ser83-Leu; Asp87-Gly	None	Ser80-Arg	>256	8	4	2
2	Ser83-Leu	Asp426-Thr	None	256	2	0.5	0.25–0.5
1	Ser83-Leu	Glu466-Asp	None	>256	8	2	1
1	Ser83-Leu	Glu466-Asp	Ser80-Ile	>256	>16	8	4
1	Ser83-Leu	Glu466-Asp	Ser80-Ile	>256	>16	8	4

[a]Adapted from Zhao S, et al. 2005. Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. Vet Microbiol 107:215.
[b]Substituted amino acids, and the position number; e.g., Ser83-Leu indicates substitution of a leucine for a serine at position 83. Amino acids: Ser, serine; Asp, aspartic acid; Leu, leucine; Tyr, tyrosine; Glu, glutamic acid; Gly, glycine; I, isoleucine; Arg, arginine; Ala, alanine; Thr, threonine; None, wild-type. No mutations were identified in *parE* sequences.
[c]Nal, nalidixic acid; Orb, orbifloxacin; Enr, enrofloxacin; Cip, ciprofloxacin.

Acquired resistance can be manifested as resistance to a single agent, to some but not all agents within a class of antimicrobial agents, to an entire class of antimicrobial agents, or even to agents of several different classes. In the great majority of cases, a single resistance determinant encodes resistance to one or several antimicrobial agents of a single class of antimicrobials (such as aminoglycosides, beta-lactams, fluoroquinolones) or of a group of related classes of antimicrobials such as the macrolide-lincosamide-streptogramin group. However, some determinants encode resistance to multiple classes. This is, for example, the case for determinants identified in recent years such as the Cfr rRNA methyltransferase (Long et al., 2006) or the aminoglycoside acetyltransferase variant Aac(6′)-Ib-cr (Robiczek et al., 2006), or when multidrug efflux systems are upregulated, as is the case for the AcrAB-TolC efflux pump system (Randall and Woodward, 2002). The simultaneous acquisition of several unrelated genetic resistance determinants located on the same mobile genetic element is, however, more common as an explanation of multidrug resistance.

As should be clear from the discussion above, the acquisition of genetic determinants of resistance is associated with a variety of MICs and does not always lead to clinically relevant resistance levels. Therefore, the use of MIC data rather than categorical classification of bacteria into resistant and susceptible is encouraged. This would avoid many apparent contradictions and compromises between clinicians, microbiologists, and epidemiologists in setting appropriate susceptibility and resistance breakpoints. A clear distinction should be made between epidemiological cut-off values and clinical breakpoints, based on presence of acquired mechanisms causing decreased susceptibility to an antimicrobial or clinical responsiveness, respectively (Kahlmeter et al., 2003; Bywater et al., 2006).

Acquisition of Antimicrobial Resistance

Bacterial antibiotic resistance can result from the mutation of genes involved in normal physiological processes and cellular structures, from the acquisition of foreign resistance genes, or from a combination of these mechanisms. Mutations occur continuously but at relatively low frequency in bacteria, thus leading to the occasional random emergence of resistant mutants. However, under conditions of stress (including those encountered

by pathogens when facing host defenses or in the presence of antimicrobials), bacterial populations with increased mutation frequencies can be encountered (Couce and Blázquez, 2009). This so-called mutator state has been suggested to be involved in the rapid development of resistance *in vivo* during treatment with certain antimicrobials such as fluoroquinolones (Komp Lindgren et al., 2003). However, for the majority of clinical isolates, antimicrobial resistance results from acquisition of extrachromosomal resistance genes.

Foreign DNA can be acquired by bacteria in three different ways (Figure 3.3): (1) uptake of naked DNA present in the environment by naturally competent bacteria (called transformation); (2) transfer of DNA from one bacterium to another by bacteriophages (transduction); and (3) transfer of plasmids between bacteria through a mating-like process called conjugation. Recently, the term *mobilome* was introduced to describe all mobile genetic elements that can move around within or between genomes in a cell. These have been divided into four classes: (1) plasmids; (2) transposons; (3) bacteriophage; and (4) self-splicing molecular parasites (Siefert, 2009). Although there are some examples of bacteriophage-mediated antimicrobial resistance transfer (Colomer-Lluch et al., 2011), the plethora of examples of transferable resistance plasmids found across a broad variety of bacterial hosts suggest that plasmids and conjugation are the major players in the global spread of antimicrobial resistance genes in bacterial populations.

Plasmids are extrachromosomal self-replicating genetic elements that are not essential to survival but that typically carry genes that impart some selective advantage(s) to their host bacterium, such as antimicrobial resistance genes. Despite the apparent efficiency of these transfer mechanisms, bacteria possess a large variety of strategies to avoid being subverted by foreign

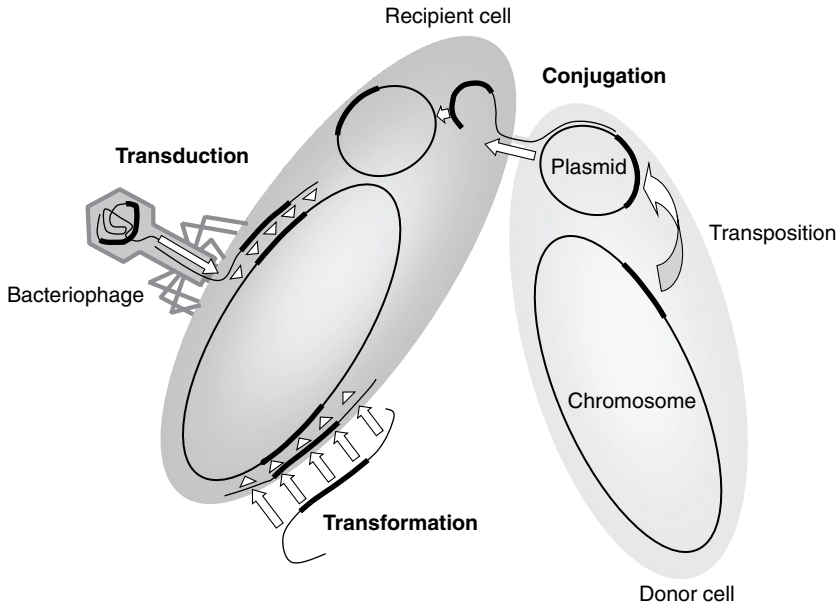

Figure 3.3. The three mechanisms of horizontal transfer of genetic material between bacteria. White arrows indicate the movement of genetic material and recombination events. The bold black line represents an antimicrobial resistance gene (or a cluster of resistance genes). In the case of transduction, a bacteriophage injects its DNA into a bacterial cell, and in the occurrence of a lysogenic phase, this DNA is integrated into the chromosome of the recipient cell. In the case of transformation, "naked" DNA is taken up by a competent cell and may recombine with homologous sequences in the recipient's genome. In the case of conjugation, a plasmid is transferred from a donor bacterium (transfer is coupled with replication and a copy of the plasmid remains in the donor) to recipient cell in which it can replicate. During its stay in various host bacteria, the plasmid may have acquired a transposon carrying antimicrobial resistance genes.

DNA, so that numerous obstacles have to be overcome to allow the stabilization and expression of genes in a new host (Thomas and Nielsen, 2005). In addition, plasmids compete for the replication and partition machinery within cells and plasmids that make use of similar systems and cannot survive for long together in the same cell. This "incompatibility" has led to the classification of plasmids into so-called incompatibility groups, a system widely used to categorize resistance plasmids into similarity groups and to study their epidemiology (Carattoli, 2011). Many studies have shown that antimicrobial resistance plasmids can be transferred between bacteria under a wide variety of conditions. This includes, for example, the relatively high temperature of the intestine of birds as well as other conditions and at the lower temperatures encountered in the environment. Some plasmids can be transferred easily between a variety of bacterial species, for instance between harmless commensal and pathogenic bacteria, thus leading in some cases to the emergence and massive establishment of newly resistant pathogen populations in individual animals within days (Poppe et al., 2005).

In addition to moving between bacteria, resistance genes can also move within the genome of a single bacterial cell and hop from the chromosome to a plasmid or between different plasmids or back to the chromosome, thus allowing development of a variety of resistance gene combinations and clusters over time. Transposons and integrons play a major role in this mobility within a genome. Transposons ("jumping genes") are genetic elements that can move from one location on the chromosome to another; the transposase genes required for such movement are located within the transposon itself. The simplest form of a transposon is an insertion sequence (IS) containing only those genes required for transposition. An advancement on the IS model is seen in the formation of composite transposons. These consist of a central region containing genes (passenger sequences) other than those required for transposition (e.g., antibiotic resistance) flanked on both sides by IS that are identical or very similar in sequence. A large number of resistance genes in many different bacterial species are known to occur as part of composite transposons (Salyers and Amiable-Cuevas, 1997).

Homologous recombination between similar transposons within a genome also play an important role in clustering passenger sequences such as antimicrobial resistance genes together on a single mobile element. Another group of mobile elements called IS*CR* that also help mobilize adjacent genetic material by mechanisms different from classical insertion sequences has been detected increasingly in relation with integrons (see below) and antimicrobial resistance genes (Toleman et al., 2006). Some bacteria (mainly anaerobes and Gram-positive bacteria) can also carry so-called conjugative transposons, which are usually integrated in the bacterial chromosome but can be excised, subsequently behaving like a transferable plasmid, and finally reintegrate in the chromosome of their next host. The magnitude of resistance development is also explained by the widespread presence of integrons, particularly class 1 integrons (Hall et al., 1999; Cambray et al., 2010). These DNA elements consist of two conserved segments flanking a central region in which antimicrobial resistance "gene cassettes" can be inserted. Multiple gene cassettes can be arranged in tandem, and more than 140 distinct cassettes have been identified to date conferring resistance to numerous classes of antimicrobial drugs as well as to quaternary ammonium compounds (Partridge et al., 2009). In addition, integrons are usually part of composite transposons, thus further increasing the mobility of resistance determinants.

The Origin of Resistance Genes and Their Movement across Bacterial Populations

Resistance genes and DNA transfer mechanisms have likely existed long before the introduction of therapeutic antimicrobials into medicine. For example, antimicrobial-resistant bacteria and resistance determinants have been found in Arctic ice beds estimated to be several thousand years old (D'Costa et al., 2011). More recently, molecular characterization of the culturable microbiome of Lechuguilla Cave, New Mexico (from a region of the cave estimated to be over 4 million years old) revealed the presence of bacteria displaying resistance to a wide range of structurally different antibiotics (Bhullar et al., 2012). Resistant microorganisms have also been found among historic culture collections compiled before the advent of antibiotic drugs as well as from humans or wild animals living in remote geographical settings (Smith, 1967; Bartoloni et al., 2004).

It is widely believed that antibiotic resistance mechanisms arose within antibiotic-producing microorganisms as a way of protecting themselves from the action

of their own antibiotic, and some resistance genes are thought to have originated from these organisms. This has been substantiated by the finding of aminoglycoside-modifying enzymes in aminoglycoside-producing organisms that display marked homology to modifying enzymes found in aminoglycoside-resistant bacteria. A number of antibiotic preparations employed for human and animal use have been shown to be contaminated with chromosomal DNA of the antibiotic-producing organism, including identifiable antimicrobial resistance gene sequences (Webb and Davies, 1993). However, as in the case of synthetic antimicrobials such as trimethoprim and sulfonamides, preexisting genes with other resistance-unrelated roles might have evolved through adaptive mutations and recombinations to function as resistance genes. Indeed, some have suggested that in their original host, antimicrobial resistance genes play a role in detoxification of components other than antimicrobials, and in a variety of unrelated metabolic functions (Martinez, 2008). A vast reservoir of such genes, now dubbed the *resistome*, is present in the microbiome of various natural environments (D'Costa et al., 2007; Bhullar et al., 2012), which can be transferred to medically relevant bacteria through genetic exchange (Wright, 2010).

Since resistance genes are frequently located on mobile genetic elements, they can move between pathogens, as well as between non-pathogenic commensal bacteria and pathogens. Thus, the issue of resistance has to be considered beyond the veterinary profession and specific pathogens. Indeed, there is growing evidence that resistance genes identified in human bacterial pathogens were originally acquired from environmental, non-pathogenic bacteria via horizontal gene exchange (Martinez et al., 2011; Davies and Davies, 2010). Resistance genes can spread quickly among bacteria, sometimes to unrelated genera. Even if an ingested bacterium resides in the intestine for only a short time, it has the ability to transfer its resistance genes to the resident microflora, which in turn may serve as reservoirs of resistance genes for pathogenic bacteria. The inclination to exchange genes raises the concern for the possible spread of antimicrobial resistance determinants from commensal organisms in animals and humans to human pathogens (Witte, 1998; Van den Bogaard and Stobberingh, 2000). Thus, the epidemiology of antimicrobial resistance goes beyond the boundaries of veterinary and human medicine. The complexity of movement of microorganisms and of horizontal gene transfer (HGT) involved in the epidemiology of global resistance is difficult to comprehend. The graphical depiction of this complex interaction in Figure 3.4 is the best attempt to date to capture this complexity.

On a long-term evolutionary scale, the epidemiology of antimicrobial resistance should be regarded as dominated by the stochastic or chaotic movement of resistance genes within a gigantic bacterial genetic pool. However, in the shorter term and on a local scale, this unrestricted approach may be too simple and of less practical relevance than considering only resistant pathogens. Because of the complexity of the resistance issue, numerous strategies to control the rise of antimicrobial resistance at every level have emerged in the scientific and medical communities. As with other complex issues that global society faces, no single intervention will be decisive alone, but numerous interventions are needed that cumulatively may preserve acceptable levels of efficacy for current and future antimicrobial drugs (Prescott et al., 2012).

The Effects of Antimicrobial Use on the Spread and Persistence of Resistance

The increased prevalence and dissemination of resistance is an outcome of natural selection, the Darwinian principal of "survival of the fittest." In any large population of bacteria, a few cells that possess traits that enable them to survive in the presence of a toxic substance will be present. Susceptible organisms (i.e., those lacking the advantageous trait) will be eliminated, leaving the remaining resistant populations behind. With long-term antimicrobial use in a given environment, the microbial ecology will change dramatically, with less susceptible organisms becoming the predominant population (Salyers and Amabile-Cuevas, 1997; Levy, 1998). When this occurs, resistant commensal and opportunistic bacteria can quickly become established as dominant components of the normal flora of various host species, displacing susceptible populations. Changes in antimicrobial resistance frequency when new antimicrobials appear on the market or when restrictions are implemented in the use of existing antimicrobials testify for the validity of these evolutionary rules. Several examples

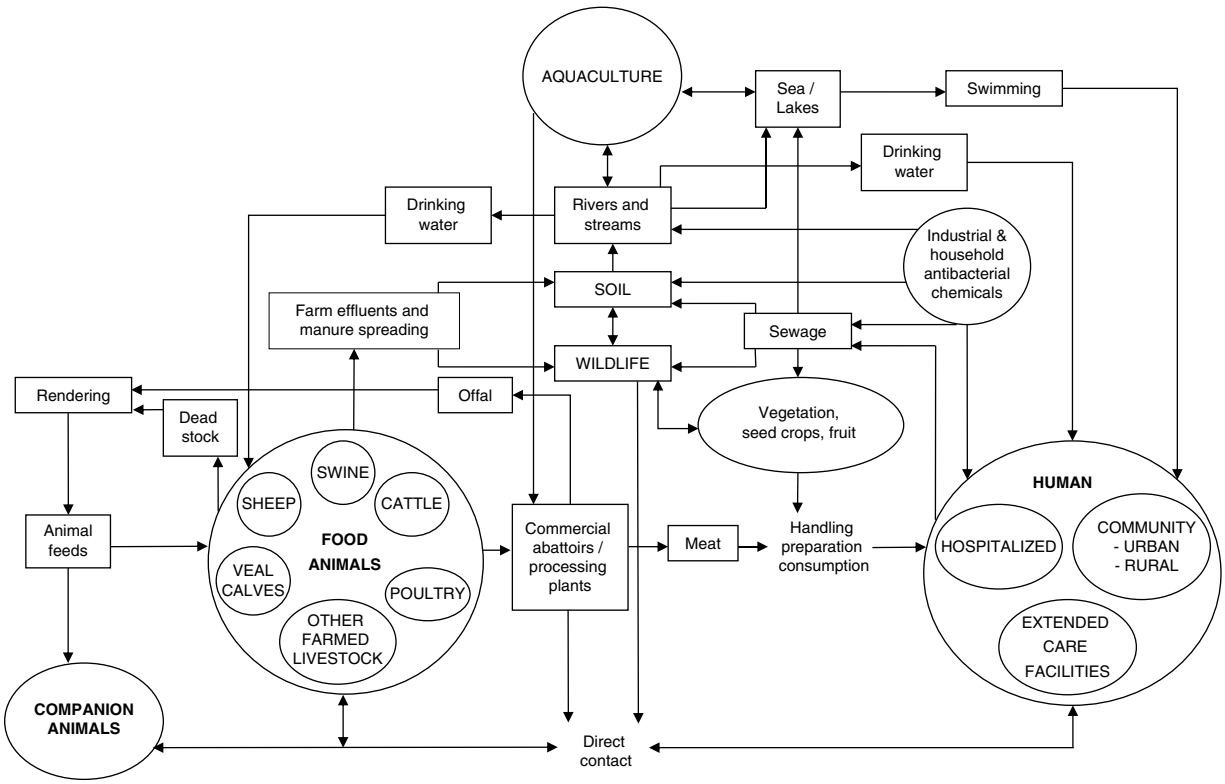

Figure 3.4. The ecology of the spread of antimicrobial resistance and of resistance genes. A schematic representation of resistant bacteria and antimicrobial resistance genes transmission routes across the multiple ecological compartments. This figure is a further development (Irwin et al., 2008) of an original one by Linton, 1977. Reproduced with permission.

of the rise and fall of antimicrobial resistance as selection pressures change are described later in this chapter.

The clustering of multiple resistance genes on plasmids, transposons, and integrons makes the problem of antimicrobial resistance challenging. Exposure to one antimicrobial may co-select for bacteria that are also resistant to several unrelated agents (Cantón and Ruiz-Garbajosa, 2011). There may also be non-antibiotic selection pressure for bacterial antibiotic resistance genes. Although much is only speculative on this subject (Meyer and Cookson, 2010), there is growing evidence showing that disinfectants and biocide may co-select for antimicrobial resistance (Yazdankhah et al., 2006; Hegstad et al., 2010). Not only can resistance determinants for antibiotics of a different class aggregate, but they may also form clusters with resistance genes for non-antibiotic substances such as heavy metals and disinfectants (Baker-Austin et al., 2006; Salyers and Amabile-Cuevas, 1997; Hall et al., 1999) or even with virulence genes (Boerlin et al., 2005; Da Silva and Mendonça, 2012; Johnson et al., 2010).

Carrying genetic material associated with resistance genes when they are not needed represents a burden for bacteria. Therefore, when a bacterial population is not under the selective pressure of antimicrobials, susceptible bacteria not carrying resistance genes may be at an advantage and the population as a whole is expected to slowly revert to a mainly susceptible state. A few examples of such a reversion have been described in the past (Aarestrup et al., 2001; Dutil et al., 2010). However, other studies have also shown that bacteria may exhibit resistance to antimicrobials despite a lack of specific selective pressures, as has been the case, for example, for chloramphenicol, glycopeptides, or streptothricin (Werner et al.,

2001; Bischoff et al., 2005; Johnsen et al., 2005). The mechanisms behind this persistence are unclear but likely to be multifactorial. They may include compensation for the metabolic load imposed by resistance genes by as yet not clearly understood mechanisms (Zhang et al., 2006), regulation of gene expression by the presence/absence of antimicrobials, and plasmid addiction systems. However, the real significance of each one of these mechanisms remains unclear. For instance, compensation for fitness loss has been shown to play a role in the case of resistance mechanisms associated with chromosomal mutations, but its role in the persistence of resistance associated with mobile genetic elements is much less evident. Although plasmid addiction systems may avoid reversion of plasmid carriers to a susceptible state, it is not clear if this is a real advantage for the affected bacteria (Mochizuki et al., 2006). When resistance genes are physically linked together or to other selectively advantageous genes, co-selection will lead to the persistence of all the resistance genes as part of the cluster. Several examples of co-selection are known, such as the maintenance of glycopeptide resistance in porcine enterococci by the use of macrolides, or the persistence and higher frequency of antimicrobial resistance in some pathogen populations due to linkage between virulence and resistance genes (Martinez and Baquero, 2002).

Finally, the effects of diverse drug administration protocols (administration route, timing, dosage) on the dynamics and persistence of susceptible and resistant bacteria and on the spread of resistance genes among bacterial populations at the global and individual level are complex and poorly understood (MacLean et al., 2010). Every effort should be made to define treatment protocols that avoid or minimize the windows for selection of resistant bacteria. This is of particular direct concern when low-level resistance mechanisms elevate the mutant selection window high enough to allow *in vivo* selection of fully resistant mutants, as can be the case for fluoroquinolones (Drlica and Zhao, 2007; Cantón and Morosini, 2011).

Antimicrobial Resistance and Public Health

Although most of the bacterial antimicrobial resistance observed in human medicine may be ascribed to use in human patients, it is being resolutely argued that antimicrobial use in veterinary medicine and food animal agriculture contributes to antimicrobial-resistant food-borne bacterial pathogens. These concerns are not new and in the 1960s led to the release in the United Kingdom of the Swann Report (Anonymous, 1969), which resulted in changes in antimicrobial use in agriculture. Despite the best efforts to date, there is no agreement regarding the scale of the impact of antimicrobial use in animals on human health. The fundamental and obvious concern over the agricultural use of antibiotics arises from the potential that antimicrobials used on the farm select for resistant bacterial strains that are transferred to humans via direct contact and ingestion of contaminated food and/or water (Figure 3.4). Numerous cases of transmission of resistant bacteria between animals and humans at risk, such as farmers, abattoir workers, and veterinarians, support these concerns (Hunter et al., 1994; van den Bogaard et al., 2002; Garcia-Graells et al., 2012). The parallel rise and decrease of resistance to glycopeptides in animal and human enterococci in some European countries after the introduction and subsequent ban of avoparcin (see below) and other antimicrobial growth promoters substantiate these fears. The identification of fluoroquinolone-resistant *Campylobacter* and quinupristin/dalfopristin-resistant enterococci from animal sources or their immediate environment has intensified this debate (Piddock, 1996; Witte, 1998). Food of animal origin has recently even been suggested to represent a potential reservoir of resistant extraintestinal pathogenic *E. coli* for humans, and uropathogenic *E. coli* in particular (Manges and Johnson, 2012). Methicillin-resistant *Staphylococcus aureus* (MRSA) seems to represent another resistant zoonotic agent (see below). This suggests that, because of their intimate contact with humans, pets and not just farm animals may represent another source of resistant bacteria and resistance genes of public health relevance (Ewers et al., 2010; Platell et al., 2011). A historical perspective on the issue of agricultural use of antimicrobial drugs and its impact on human health is available (Prescott, 2006).

Overall, there are clear and compelling data demonstrating that the use of antimicrobials in animals can have negative effects on antimicrobial resistance in bacteria and pathogens from humans. Although more research is needed to quantify the risk associated with this use in animals and the fraction of resistance in human pathogens attributable to it, this situation clearly warrants some caution and preventive measures.

Examples of Antimicrobial Resistance in Veterinary Medicine of Public Health Significance

Resistance in *Salmonella*

Although a large body of science is available on the prevalence of antimicrobial resistance and associated mechanisms in *Salmonella*, many aspects related to the emergence, persistence, and dissemination of antimicrobial resistance in these pathogens remain unclear.

Salmonella can colonize and cause disease in a variety of food-producing and non-food-producing animals. Although all serotypes may be regarded as potential human pathogens, the great majority of infections are caused by only a limited number. Resistance in non-typhoidal *Salmonella* spp. has become an international problem (Threlfall, 2000; Poppe et al., 2001; Williams, 2001). The levels and extent of resistance vary and are influenced by antimicrobial use practices in humans and animals, as well as by geographical differences in the epidemiology of *Salmonella*. Drug resistance phenotypes have been associated with the use of antimicrobials in food-producing animals (Piddock, 1996; Wiuff et al., 2000; Molbak, 2004; Alcaine et al., 2005), in which resistance profiles generally reflect how long an agent has been in use. Thus, irrespective of source (food animals, food, humans), the most frequent resistances are usually to older antimicrobials such as ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (Anderson, 1968; Chiappini et al., 2002; Molbak, 2004; Sun et al., 2005). However, there are increasing reports of *Salmonella* isolates worldwide displaying reduced susceptibility or resistance to extended-spectrum cephalosporins or fluoroquinolones (Threlfall et al., 2000; Zhao et al., 2001; Gupta et al., 2003; Alcaine et al., 2005; Johnson et al., 2005; Su et al., 2008; chapters 9 and 18). This is particularly troublesome since these antimicrobial classes are frequently used to treat *Salmonella* infections in children and adults, respectively (Angulo et al., 2004; Alcaine et al., 2005). Treatment will be more difficult with the recent emergence of carbapenemases in *Salmonella* (Savard et al., 2011).

Salmonella Typhimurium continues to be one of the serovars most frequently recovered from food animals worldwide (Zhao et al., 2005). In the United States, it is among the top four serovars most frequent in cattle, swine, chickens, and turkeys. Because of its broad host range, *S*. Typhimurium is also one of the most common serotypes isolated from human salmonellosis. Historically this serovar has often been associated with multiresistance, particularly in relation with phage type DT104, but this type may be decreasing in frequency, and a new multiresistant monophasic *S*. Typhimurium variant is now spreading globally (Butaye et al., 2006; Hauser et al., 2010).

An increase in *S*. Newport infections was reported by the CDC in 2000. Many of these strains exhibited a multidrug-resistant phenotype (commonly referred to as *S*. Newport MDR-AmpC) characterized by resistance to nine antimicrobials, including amoxicillin-clavulanic acid and ceftiofur. In addition to the characteristic resistance to nine specific antimicrobials, these strains also exhibited decreased susceptibility to ceftriaxone (MIC 16–32 μg/ml; Zhao et al., 2003). These strains are of particular clinical concern, as they possess plasmid- or chromosomally encoded AmpC beta-lactamases (e.g., bla_{CMY}) that confer decreased susceptibility to a wide range of beta-lactams, including ceftriaxone, the drug of choice for treating complicated salmonellosis in children (Gupta et al., 2003). Slightly later, a similar increase in third-generation cephalosporin resistance related to bla_{CMY} plasmids was observed in *S*. Heidelberg in Canada, which was attributed to the use of this class of antimicrobials in poultry (Dutil et al., 2010; chapter 9). In both cases, MDR-AmpC strains found their way into the food chain and were linked to human foodborne infection (Gupta et al., 2003; Zhao et al., 2003; Dutil et al., 2010). Multidrug-resistant *Salmonella* have also been associated with illness in animals and humans in equine and companion animal veterinary facilities (Wright et al., 2005). These latter reports frequently describe poor hand-washing practices by employees, eating in work areas, and previous antimicrobial drug therapy in affected humans or animals.

Methicillin-Resistant *Staphylococcus aureus*

MRSA has emerged as a major nosocomial pathogen in human hospitals. This problem had remained limited to hospital settings, but MRSA is now present in the human community too. However, MRSA has been emerging rapidly in animals in recent years, for reasons that are not clear (chapter 8), and represents an important example of both the spread of resistance and the links between resistance in human and animal medicine.

There are an increasing number of reports on MRSA colonization and infections in animals (Weese, 2010), demonstrating spread into animal populations (chapter 8). Most early reports of MRSA in animals were from horses and from dogs and cats; MRSA have remained a rarity in cattle despite extensive use of cloxacillin in mastitis treatment. A recent report from Belgium (Vanderhaegen et al., 2010) suggests that this situation may be changing. MRSA isolates were originally recovered more frequently from horses in relation with nosocomial surgical wound infections possibly originating from humans (Seguin et al., 1999). Equine MRSA usually belong to a specific clone that seems to be maintained within equine populations (Weese et al., 2005a,b). This clone is also occasionally found in humans, particularly in horse personnel, but is not one of the most prevalent human MRSA clones. Investigations suggest that transmission of MRSA goes in both directions between humans and horses and may be associated with clinical disease in both groups.

The epidemiology of MRSA in dogs and cats may be different since the clones found in dogs and cats, and occasionally transmitted between animals, are the same as those frequently found in nosocomial and community infections in humans. In addition, many reports show that the same MRSA strain from clinical infections or from healthy carriage can be found in pets and humans with close contact (van Duijkeren et al., 2004a,b; Rankin et al., 2005). In recent years, the MRSA ST398 clone has emerged massively in livestock (Smith and Pearson, 2011). This clone seems to be particularly frequent in pigs and veal calves (Voss et al., 2005) but has also been described in poultry, dairy cattle, and other species, as well as in meat products. The reasons for the emergence of this clone in livestock are not completely understood. Although people working with livestock (farm workers, veterinarians) are at higher risk of carrying MRSA ST398, its transmission between humans seems not to be as active as for other MRSA.

Antimicrobials in Animal Feeds and Association with Resistance in Bacteria of Human Health Significance

It has been known for decades that continuous oral administration of low concentrations of antimicrobials increases feed conversion and weight gain and reduces shipping stress-associated diseases in food animals (Butaye et al., 2003; Dibner and Richards, 2005). Past studies have shown that this practice is also a potentially significant driving force in accelerating the emergence of resistant bacteria that could infect humans (Wegener, 2003; Kelly et al., 2004; Dibner and Richards, 2005). The use of antimicrobial agents for growth promotion is discussed in chapter 22.

Most classes of antimicrobials used in animals have analogues used in humans and are therefore capable of selecting for resistance to human medical antibiotics. The important exceptions are the ionophores (e.g., lasalocid, monensin, narasin, salinomycin), the quinoxalines (e.g., olaquindox), bambermycins (flavophospholipol), and avilamycin (Turnidge, 2004). Among the former group, two classes of antimicrobials that have received particular attention in the scientific community are the streptogramins (quinupristin/dalfopristin, virginiamycin) and glycopeptides (avoparcin, vancomycin).

Virginiamycin in feed has been approved since 1975 for food-producing animals for growth promotion and prevention or control of certain diseases in turkeys, swine, cattle, and chickens (Kelly et al., 2004). The human analogue, Synercid, a mixture of the two streptogramin antibiotics quinupristin and dalfopristin (QD), was approved in September 1999 by the U.S. FDA for treatment of bacteremias in humans, particularly against vancomycin-resistant *Enterococcus faecium* (VREF) and for the treatment of skin and soft tissue infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. Synercid was considered then to be a last resort of therapy for potentially life-threatening bloodstream infections caused by VREF. The approval of Synercid focused increased attention on the use of virginiamycin in animal husbandry; specifically, whether farm use of virginiamycin resulted in streptogramin resistance in bacteria that could result in impaired Synercid therapy in humans (Wegener 2003; Kelly et al., 2004). Synercid-resistant *E. faecium* (SREF) are common in the poultry production environment, including samples from litter and transport containers (McDermott et al., 2005). SREF is also common on poultry meat products at retail, suggesting that such meats serve as a continual source of resistant strains and/or their resistance genes (McDermott et al., 2005). Foodborne strains might transfer plasmidborne resistance determinants to human native enterococci *in vivo* (Jacobsen et al., 1999), which in turn might donate

these genes to other strains causing human infections. The food safety implications prompted the FDA (http://www.fda.gov/downloads/AnimalVeterinary/NewsEvents/CVMUpdates/UCM054722.pdf) and others (Cox and Popken, 2004; Kelly et al., 2004) to propose risk assessment models examining the potential public health consequences of virginiamycin use. The potential for streptogramin resistance genes to transfer from foodborne enterococcal isolates to those causing disease in humans remains difficult to assess, because of complex interplays between bacterial specificity for hosts and gene transfer (Hammerum et al., 2010). In addition, while new resistance genes and new variants thereof keep emerging and spreading in Gram-positive organisms (Witte and Cuny, 2011), a significant proportion of the streptogramin-resistance determinants from enterococci remain unknown in many recent studies. Therefore, estimations of the potential health risks to humans resulting from virginiamycin use in animal husbandry require further study.

Early studies in the 1990s provided evidence in favor of a causal association between the use of avoparcin and the occurrence of VREF on farms in Europe (Bager, 1999; Aarestrup et al., 2000). This suggested that food animals constitute a potential reservoir of infection for VREF in humans (Wegener, 2003). In response to continued pressure from the "major harm" position, the European Union took the "precautionary principle" and followed the earlier move of Scandinavian countries by suspending the use of the "growth promoter" in feed antibiotics: avoparcin, bacitracin, virginiamycin, spiramycin, and tylosin because of their ability to select for resistance to antimicrobials of human importance (Turnidge, 2004; chapter 26). The frequency of resistance to vancomycin and to growth promoters in enterococci from animal origin generally declined after the ban of antimicrobial growth promoters (Aarestrup et al., 2001; Sorum et al., 2004). Interestingly, because of the plasmid-based linkage of glycopeptide and macrolide resistance genes in swine VREF, the decrease of VREF frequency in swine isolates after the ban on avoparcin was slow until tylosin was also banned as a growth promoter (Aarestrup et al., 2001). Some studies have also demonstrated a parallel declining trend in VREF isolated from food and humans after the ban, thus supporting the effectiveness of the ban (Klare et al., 1999; Pantosti et al., 1999). However, VREF are still persisting in animals (Heuer et al., 2002) and isolates similar to those from animals could be recovered from humans several years after the ban of avoparcin (Hammerum et al., 2004; Hammerum, 2012). Thus, antimicrobial resistance associated with the use of antimicrobial growth promoters will not vanish as quickly as early studies had led us to hope (Johnsen et al., 2011). In addition, the global ban of antimicrobial growth promoters might have undesirable consequences on animal health, consequences that remain to be assessed precisely (Casewell et al., 2003). It also increases, at least initially, the use of therapeutic antimicrobials (Grave et al., 2006). As part of the federal strategy for controlling antimicrobial resistance in the United States, the Food and Drug Administration (FDA) in 2012 released Guidance for Industry #209 "The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals," which focuses on two primary principles: (1) limiting medically important antimicrobial drugs to uses in food-producing animals that are considered necessary for assuring animal health; and (2) limiting such drugs to uses in food-producing animals that include veterinary oversight or consultation (http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM216936.pdf). This guidance, which represents FDA's current thinking on this topic, is a very important development in the field (chapter 26).

Surveillance Programs and the Role of Diagnostic Laboratories

The seriousness of the antimicrobial resistance threat has prompted many governments to initiate surveillance programs, which include bacteria of animal origin. These programs provide a tool to globally assess the extent of the problem, to follow its evolution over time, and to evaluate the effectiveness of control measures. Such systems include, among others, the National Antimicrobial Resistance Monitoring System (NARMS) in the United States, the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) in Canada, and the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) in Denmark. On the veterinary side, most of the national surveillance programs only include bacteria considered as indicators of the general resistance situation (i.e., *Escherichia coli* and *Enterococcus* spp.)

and zoonotic bacterial agents (*Salmonella enterica* and *Campylobacter* spp.). Only a few surveillance programs obtain antimicrobial susceptibility data from bacterial pathogens of animals, the most visible being the BfT-GermVet Monitoring Program in Germany (Schwarz et al., 2007). Surveillance programs are of particular interest when, like DANMAP, they include the collection of data on antimicrobial use and try to link the latter with the evolution of resistance. Because of the past problems in lack of standardization of antimicrobial susceptibility testing, it is encouraging that these national surveillance programs use similar (if not identical) methodologies and provide increasingly comparable data.

There is a wealth of information on the prevalence of antimicrobial resistance in animal pathogens (Aarestrup, 2006). However, because of the geographically local and temporarily limited nature of these studies and their different sampling and susceptibility testing methodologies, it is difficult to draw reliable conclusions on the global antimicrobial resistance situation in veterinary medicine. Constant efforts are made by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) to develop agreed veterinary standards for susceptibility testing methodologies (chapter 2). However, investigation shows that many veterinary laboratories do not strictly follow these standards. There is a great need for diagnostic laboratories to adhere to standards so as to provide reliable and reproducible susceptibility data for clinicians and other users. It should be recognized, however, that most studies of antimicrobial resistance in veterinary pathogens are not based on a representative sample of pathogen populations but rather on diagnostic laboratory submissions, so that these reports may overestimate the prevalence of resistance in target pathogen populations. Consequently, better-designed studies are needed for the assessment of the real antimicrobial resistance situation in veterinary pathogens at every level, starting from the farm and all the way up to the global national and international level.

Susceptibility testing of clinical isolates is a cornerstone for prudent use of antimicrobials and for an adequate management of single clinical cases (chapters 2 and 7). Unfortunately, microbiological analysis and susceptibility testing are still frequently performed only when a problem has not been resolved by empirical antimicrobial therapy.

Nosocomial Infection and Antimicrobial Resistance in Veterinary Hospitals

Because of the high selection pressure exerted by the heavy use of antimicrobial agents in human hospitals, resistance first emerged as a significant problem in bacteria associated with nosocomial infections. Veterinary hospitals and practices, and their intensive care units, keep increasing in size. In parallel, companion animal medicine is increasingly more sophisticated and intensive. Consequently, antimicrobial resistance problems similar to those from human hospitals have appeared in companion animal practice. Compared, however, to human medicine, few publications are available on nosocomial infections with multiresistant pathogens in animals. Nevertheless, what there is shows that the similarities between veterinary and human hospitals are striking. The heavy use of antimicrobial agents in intensive care units is associated with increased antimicrobial resistance (Ogeer-Gyles et al., 2006a), multidrug resistant organisms are widespread in veterinary clinics and hospital environments (Murphy et al., 2010), and indwelling devices as well as surgical procedures are "hot spots" for nosocomial infections (Ogeer et al., 2006b; Bubenik et al., 2007; Marsh-Ng et al., 2007; Jones et al., 2009).

Besides the problem with MRSA in horses (Anderson et al., 2009) and companion animals (Wieler et al., 2011) mentioned above, and increasingly frequent outbreaks in veterinary clinics (van Duijkeren et al., 2010), methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is now emerging as a major problem organism in the veterinary world, including in hospital settings (van Duijkeren et al., 2011; chapter 8). These organisms seem to be resistant to a large number of other antimicrobials of a variety of classes, making treatment of MRSP infections even more challenging than treatment of MRSA (Steen, 2011). Interestingly, the emergence of MRSP is related to the spread of a very few major clonal lineages (Perreten et al., 2010), suggesting the importance of infection control as one approach to improving antimicrobial stewardship (chapter 7).

Other multiresistant nosocomial pathogens have been reported in veterinary hospital and intensive care units, including *Salmonella enterica*, *E. coli*, *Acinetobacter baumannii*, and enterococci, but other resistant pathogens common in human hospitals are also reported sporadically.

Multiresistant *Salmonella* is one of the most regularly encountered causes of nosocomial infections in veterinary hospitals. Equine clinics seem to be particularly prone to such problems (Dargatz and Traub-Dargatz, 2004), and resistance profiles are increasingly problematic (Dallap Schaer et al., 2010). However, multiresistant *Salmonella* outbreaks also happen in companion animal clinics (Wright et al., 2005). As in human hospitals, multidrug-resistant *Enterobacteriaceae* resistant to extended-spectrum cephalosporins are increasingly being reported in veterinary nosocomial infections. Both AmpC- and ESBL-type beta-lactamases have been described in *Salmonella*, *E. coli* (Sanchez et al., 2002), and *Klebsiella* (Haenni et al., 2011). This may also be a precursor trend toward the emergence of carbapenemases in these organisms (chapter 10).

Acinetobacter baumannii is another often multiresistant Gram-negative organism of environmental origin causing major nosocomial human hospital infection problems. Recent reports suggest that this may also occur in veterinary clinics (Endimiani et al., 2011; Zordan et al., 2011). Multiresistant *A. baumannii* strains seem to persist better in hospitals under antimicrobial pressure than susceptible organisms. This was the case in a series of *A. baumannii* infections in a veterinary hospital, in which persistent strains were multiresistant, whereas sporadic ones all presented only few resistances. After eradication of a first multiresistant strain through hygienic measures, another persistent multiresistant strain readily replaced the first (Boerlin et al., 2001).

Antimicrobial stewardship and clinical use guidelines are discussed in chapter 7.

Accumulation and Persistence of Antimicrobial Resistance in Pathogens

Resistance gene linkage and co-selection are one of the reasons for the accumulation and persistence of resistance in bacterial populations (Bischoff et al., 2005; Johnsen et al., 2005). However, this does not in itself explain why pathogens are more frequently resistant to antimicrobials than the normal flora. The most frequently cited explanation for this difference is the higher selection pressure exerted on pathogens by repeated treatments. Linkage of resistance and virulence genes on plasmids is likely to be an additional factor explaining the higher prevalence of resistance among many pathogens. Such linkages have already been described sporadically in the past (Martinez and Baquero, 2002), but evidence gathered in molecular epidemiology studies is accumulating to show that it may be a relatively widespread phenomenon, at least in organisms such as *E. coli*. For instance, tetracycline resistance genes are frequently linked to enterotoxin genes in enterotoxigenic *E. coli*, which may explain why tetracycline resistance is more frequent in ETEC than in commensal *E. coli* populations (Boerlin et al., 2005). Similarly, the linkage of chloramphenicol resistance genes to enterotoxins genes may partially explain why, despite the ban of chloramphenicol approximately 2 decades ago, chloramphenicol resistance is still widespread in porcine ETEC but less frequent in commensal *E. coli*.

Recent research aimed at characterizing broad host range plasmids recovered from numerous bacterial species has shed additional light on potential gene linkage associations. For example, DNA sequencing of multidrug resistant plasmids from *Salmonella* Kentucky revealed highly conserved backbones shared with avian pathogenic *E. coli* (APEC) virulence plasmids (Fricke et al., 2009). Specifically, the largest plasmid identified carried resistance determinants for streptomycin and tetracycline as well as important virulence genes found in APEC strains. Given the shared intestinal habitat, it is likely that *S.* Kentucky acquired APEC-like plasmids from commensal and/or pathogenic *E. coli* strains in the chicken intestine. These results show that antimicrobial resistance determinants and APEC virulence factors important in avian and possibly human *E. coli* pathogenesis can be encoded by the same plasmid. Under antimicrobial selection, the propagation of these virulence factors within bacterial communities could potentially lead to the emergence of new virulent strains from the commensal microflora of both animals and humans.

Do virulence genes accumulate in bacterial populations because of their genetic linkage with resistance genes and because of the selection exerted by antimicrobial use? The extent of genetic linkage and the degree to which co-resistance and virulence are related is an important consideration in assessing risks associated with antimicrobial use.

The Control of Antimicrobial Resistance

It is doubtful whether new classes of antimicrobial agents will be available for veterinary use in the coming years. Novel antimicrobials are likely to be restricted to

human medicine and economic considerations will limit development of new antimicrobials only for animal use. Thus, the antimicrobials available to veterinary medicine will probably remain the same as today. Therefore, continued efforts should be made to preserve their efficacy. Many professional associations, governmental agencies worldwide, and international committees are developing or have provided guidelines for responsible and prudent use of antimicrobial agents in veterinary medicine and agriculture (chapter 7). Additionally, economic incentives and the development of new market segments, such as the production of food from organic farms and "antibiotic-free" animals may reduce the use of antimicrobial agents in animals. The role of alternatives to antimicrobials such as vaccines, as well as pre- and probiotics, also remains to be thoroughly assessed and defined. Finally, maintenance and improvement of good management practices in companion animal medicine as well as in food animal husbandry represent cornerstones in the reduction of antimicrobial use and in the control of antimicrobial resistance.

In conclusion, the optimism of the early antimicrobial discovery era has been tempered by the emergence of bacterial strains displaying resistance to almost every antimicrobial therapeutic in use. Today, many clinically important bacteria are characterized by multiple antibiotic resistance phenotypes, the legacy of past decades of antimicrobial use and misuse. This modern predicament of widespread antimicrobial resistance has led recognition internationally that the benefits of these agents may be lost, unless there is comprehensive and concerted action to combat the present problem and to reverse anticipated developments. Resistance is an inevitable biological phenomenon: the challenge is to prevent it from continuing to be a persistent and serious obstacle to modern medicine.

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4

Principles of Antimicrobial Drug Bioavailability and Disposition

J Desmond Baggot and Steeve Giguère

In treating microbial infections it is important that an effective concentration of antimicrobial drug be rapidly attained at the focus of infection and that it be maintained for an adequate duration. The concentration achieved depends on the systemic availability of the drug, which varies with the dosage form (drug preparation) and route of administration, the dosing rate, and ability of the drug to gain access to the infection site. The chemical nature and physicochemical properties (in particular lipid solubility and degree of ionization) of the drug influence the extent of absorption (systemic availability), pattern of distribution, and rate of elimination (pharmacokinetic characteristics). The location of the infection can have a major influence on the drug concentration achieved where its action is required, as some sites (e.g., central nervous system) are protected by cellular barriers to drug penetration, while others (e.g., mammary glands) have a local pH that may favor drug accumulation (systemically administered lipid-soluble organic bases) but could alter antimicrobial activity. The urinary tract is unique in that very high concentrations may be attained in urine, particularly of antimicrobial agents that are mainly eliminated by renal excretion. Microbial susceptibility to the drug concentration achieved at the site of infection is critical in determining the clinical response to therapy (chapter 2). Thus effective antimicrobial therapy depends on a triad of bacterial susceptibility, pharmacokinetic characteristics of the drug, and the dosage regimen. In addition, the competence of host defense mechanisms influences the outcome of therapy.

Routes of Administration

Drugs are administered as prepared dosage forms, such as parenteral preparations for injection, and tablets, capsules, suspensions, or pastes for oral administration. It is highly important that drug preparations be administered only by the route(s) and to the animal species for which their formulation was developed; this information is provided on the label of authorized products. When veterinary preparations of an antimicrobial agent are not available, preparations intended for use in humans could be administered to companion animals. Knowledge of the pharmacokinetics of the drug is important, since dosage must be appropriate for the animal species.

Parenteral therapy should always be used in the treatment of severe infections, and in horses and ruminant species, it is generally preferable to oral therapy. Long-acting parenteral preparations should always be administered by IM or SC injection. In mild-to-moderate infections, oral therapy is preferred in dogs and cats, particularly for antimicrobial agents that are reliably absorbed from the gastrointestinal tract and those for which parenteral preparations cause tissue irritation at

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the IM site of injection. In the treatment of systemic infections caused by susceptible Gram-negative aerobic bacteria, aminoglycosides (such as gentamicin, amikacin) must be administered parenterally (generally IM or SC). Parenteral cephalosporins, with the notable exceptions of ceftiofur and cefquinome, which are given by IM injection, should be administered by slow IV injection. Certain antimicrobials are approved for administration in the feed or drinking water to pigs or poultry, providing convenience of administration.

Intravenous Administration

The IV injection of a parenteral drug solution ensures that the total dose enters the systemic circulation. The high concentration initially produced in the blood declines rapidly as the drug distributes to other tissues of the body including the organs of elimination (liver and kidneys). Since passive diffusion is the process by which most drug molecules enter cells and penetrate cellular barriers, the chemical nature of a drug, the lipid solubility and degree of ionization of those that are weak organic acids or bases, and the concentration gradient are the factors that determine the concentrations attained in cells, transcellular fluids (e.g., cerebrospinal, synovial, and ocular), and glandular secretions (e.g., milk, saliva, prostatic fluid). After the attainment of pseudodistribution equilibrium, the plasma concentrations decline at a slower rate that is associated entirely with elimination (i.e., metabolism and excretion) of the drug. It is on the elimination phase of drug disposition that the half-life of the drug is based (Figure 4.1).

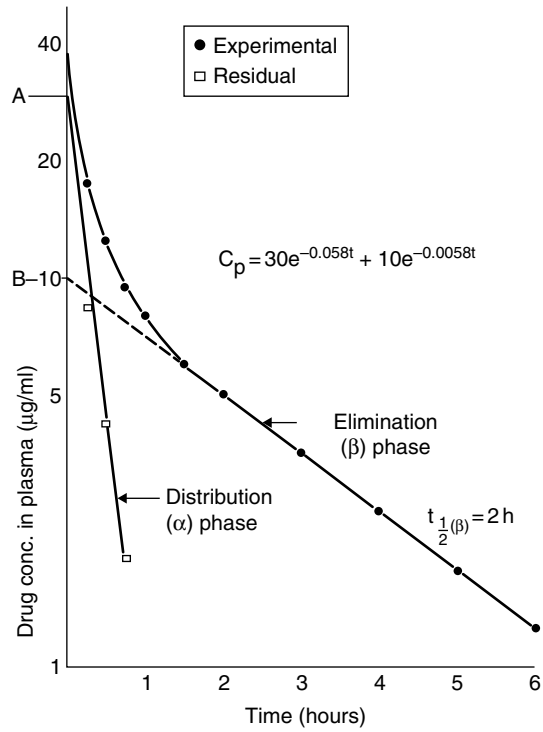

Figure 4.1. Plasma concentration-time curve for a drug after intravenous injection of a bolus dose. The disposition curve is described by a biexponential equation (inset) and separated into its component phases (distribution and elimination). The half-life of the drug is obtained from the exponent of the elimination phase ($\beta = 0.0058$ min^{-1}; $t_{1/2} = 0.693/0.0058 = 120$ minutes). Reproduced with permission from Baggot, 1997.

The IV administration of a parenteral solution assures complete systemic availability of the drug. Intravenous injection provides higher plasma concentrations that may enhance tissue distribution, but effective plasma concentrations generally persist for a shorter duration than following extravascular drug administration. A shorter dosage interval is required to maintain effective concentrations, and the concentrations achieved will fluctuate to a greater degree. Parenteral solutions contain a drug in salt form dissolved in a vehicle, and the pH reaction of some solutions is far outside the physiologic range. To avoid excessively high initial drug concentrations in the systemic circulation and adverse effects that could be produced by the drug per se or by constituents of the formulation, IV injections should be given slowly. Parenteral solutions (conventional formulation only) that would produce tissue irritation at IM injection sites may be administered IV, but care must be taken to avoid perivascular damage. Pharmacokinetic parameters describing the disposition of a drug are based on the plasma concentration-time data following the IV injection of a single dose.

IV infusion of a parenteral solution containing a fixed concentration of drug is the only method of administration that allows precise control over the rate of drug entry into the systemic circulation and the plasma concentration that will ultimately be attained. Assuming knowledge of the systemic clearance of the

drug, this method can be used to achieve and maintain a desired steady-state concentration and avoid fluctuation in concentrations, which is a feature of multiple dosing. While the rate of infusion determines the steady-state concentration attained, the time taken to reach steady-state is determined solely by the rate of elimination (half-life) of the drug. For practical purposes it can be predicted that a plasma concentration within 90% of the desired steady-state concentration will be achieved after infusing the drug at a constant rate for a period corresponding to 4 times the half-life. It follows that the use of continuous infusion is most suitable for drugs with short half-lives (< 2 hours). Should a change from one steady-state concentration to another be contemplated, infusion at a different rate for a similar length of time (i.e., 4 half-lives) will be required to effect the change in steady-state concentration.

Intramuscular and Subcutaneous Injections

Parenteral dosage forms (solutions and suspensions) of most antimicrobial agents can, in general, be administered by IM or SC injection to animals. The composition of the formulation, the concentration of the drug in the preparation, and the total dose to be administered will determine suitability of the dosage form for administration to a particular species. With regard to species, particular attention must be given to the concentration of drug in the preparation since drug concentration, together with the total dose required, determine the volume to be administered. A volume exceeding 20 mL should not be administered at any one IM injection site. The lateral neck is the preferred site for IM injection in large animals. While non-irritating parenteral solutions are frequently administered by SC injection in cats, this route of administration is seldom used in horses. Most antimicrobial agents are rapidly and completely absorbed from non-irritating solutions; peak plasma concentrations are reached within 1 hour of giving the injection. Although drug absorption from IM injection sites is generally assumed to be a first-order process, the validity of this assumption is often questionable. Oil-based formulations and unbuffered aqueous solutions or suspensions may cause irritation and produce tissue damage at IM injection sites. Slow and erratic absorption occur and systemic availability of the drug is often incomplete.

Absorption from IM and SC injection sites is determined by the formulation of the parenteral preparation, the vascularity of the injection site, and, to a lesser extent, the chemical nature and physicochemical properties of the drug substance. When single doses (10 mg/kg) of amikacin were administered SC to dogs at three different concentrations (50, 100, and 250 mg/mL), the concentration of the solution did not influence the absorption and elimination kinetics of the drug. The bioavailability of gentamicin (50 mg/mL) was not affected by the location of the injection site (Gilman et al., 1987; Wilson et al., 1989). Comparison of the plasma concentration-time curves after IM injection in calves of five different parenteral preparations of ampicillin at similar dose levels (7.7 ± 1.0 mg/kg) shows the marked influence of formulation on the pattern of ampicillin absorption (Nouws et al., 1982; Figure 4.2). Only drug preparations that are bioequivalent in the target animal species would be expected to have similar clinical efficacy.

The concentration of drug in a parenteral suspension can influence the plasma concentration profile. For example, when an aqueous suspension of amoxicillin trihydrate was administered IM to horses at the same dose level (10 mg/kg) but at different concentrations (100 and 200 mg/mL), the lower concentration (10%) was better absorbed and produced a more consistent plasma concentration profile. While the commercially available aqueous suspension of amoxicillin trihydrate may be administered by IM injection to cattle, it is unsuitable for clinical use in horses because of tissue irritation caused at the injection site. Location of the injection site may also affect the systemic availability and peak plasma concentration of drugs administered as prolonged-release parenteral preparations. This was shown in a study of the influence of injection site location on the plasma concentration-time curves for penicillin G administered as procaine penicillin G to horses (Firth et al., 1986; Figure 4.3).

The systemic availability and peak plasma concentration of penicillin G were highest following IM injection of the drug product in the neck region (*M. serratus ventralis cervicis*). This site was followed, in descending order, by *M. biceps* > *M. pectoralis* > *M. gluteus* or subcutaneously in the cranial part of the pectoral region. It appears that tissue irritation caused by some parenteral preparations is more severe after subcutaneous than

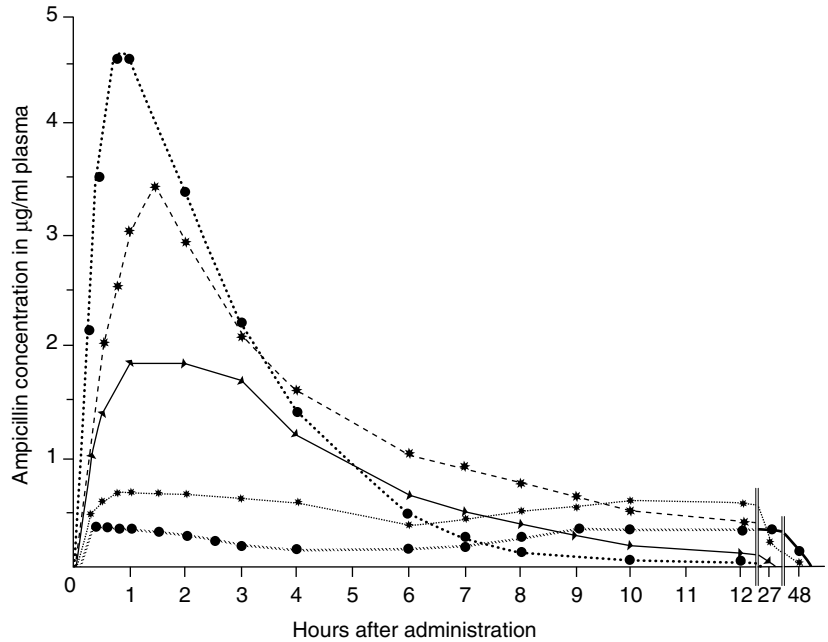

Figure 4.2. Mean plasma ampicillin concentrations after IM injection of 5 different parenteral ampicillin formulations at similar dose levels (7.7 ± 1.0 mg/kg) to 5 calves. Reproduced with permission from Nouws et al., 1982.

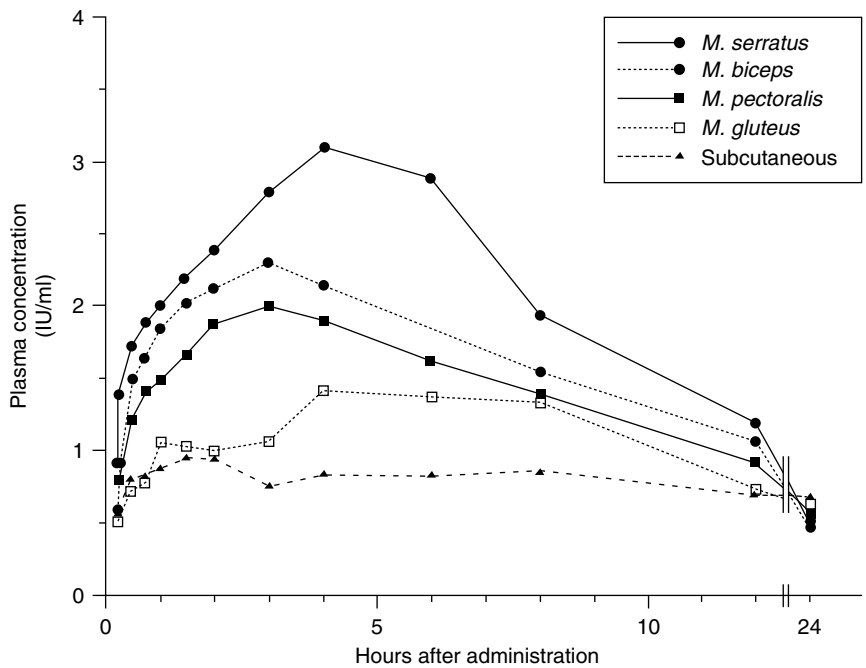

Figure 4.3. Mean plasma penicillin concentration-time curves after 20,000 IU of procaine penicillin G/kg was administered to 5 horses at 5 different sites. Reproduced with permission of Firth et al., 1986.

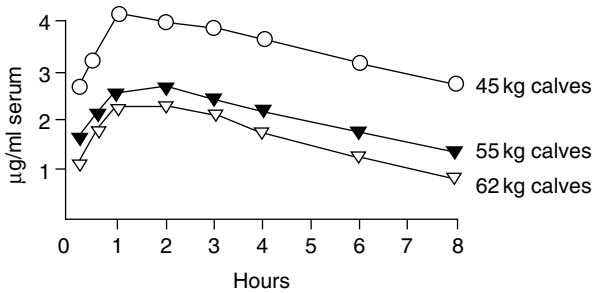

Figure 4.4. Effect of age and weight on systemic availability of amoxicillin in calves after IM injection of amoxicillin trihydrate aqueous suspension (100 mg/ml) at a dosage of 7 mg/kg body weight. Reproduced with permission from Marshall and Palmer, 1980.

intramuscular injection (Nouws and Vree, 1983; Korsrud et al., 1993). The systemic availability of amoxicillin, administered as amoxicillin trihydrate 20% aqueous suspension, was shown in dairy cows to vary as widely with IM injection site as between IM and SC sites (Rutgers et al., 1980). Based on this study and others in which the conventional formulation of oxytetracycline was administered IM at different sites, it can be concluded that the shoulder and neck regions for IM injection are superior to the buttock and to subcutaneous injection in cattle (Nouws and Vree, 1983). Better antimicrobial absorption from the former sites could be attributed to greater access of drug to a larger absorptive surface area with perhaps greater blood flow. Age or body weight of calves influenced the relative systemic availability, based on comparison of area under the curve, of amoxicillin (7 mg/kg) administered IM as amoxicillin trihydrate 10% aqueous suspension (Marshall and Palmer, 1980; Figure 4.4). When the same preparation was administered IM to different animal species, the trend was for smaller animals (piglets, dogs, cats) to show an early high peak concentration followed by a rapid decline, while larger animals (calves, horses) showed a lower and relatively constant plasma concentration of amoxicillin over at least an 8-hour period.

Useful methods of evaluating the extent of tissue irritation and rate of resolution at the IM injection site include the use of ultrasonography (Banting and Tranquart, 1991) and determination of the kinetics of plasma creatinine kinase (CK) activity (Aktas et al., 1995; Toutain et al., 1995). The use of a tissue-damaging drug preparation in food-producing animals must entail a correspondingly long withdrawal period. The withdrawal period for a drug varies with formulation of the dosage form (preparation) and may differ between animal species. Parenteral preparations should be formulated in a manner such that their IM injection does not cause tissue damage with persistence of drug residues at the injection site. Irritating preparations and drugs in oil-based vehicles should *never* be administered to horses. With the notable exceptions of procaine penicillin G (aqueous suspension) and, when specifically indicated, oxytetracycline formulated in polyethylene glycol, long-acting parenteral preparations currently available are unsuitable for use in the horse.

Since avian and reptilian species appear to have a well-developed renal portal system, first-pass renal excretion may decrease the systemic availability of drugs, especially those that undergo proximal tubular secretion such as beta-lactam antibiotics, injected IM in the legs (thighs) of birds or the posterior half of the body of reptiles.

Long-Acting Preparations

Long-acting formulations of antimicrobial agents provide sustained concentrations at the site of infection. Such preparations have been particularly popular in cattle and swine because of the convenience of a single injection, although long-acting antimicrobial agents are now also labeled for use in horses, dogs, and cats. Long-acting preparations may be drugs with a particularly long half-life, drugs formulated to delay absorption (Figure 4.5A), or drugs that concentrate in and are slowly released by phagocytic cells (Figure 4.5B.)

Drug with a Long Elimination Half-Life. Very few antimicrobial agents have an elimination half-life long enough to provide sustained therapeutic plasma concentrations. One example is cefovecin, a cephalosporin labeled for use in dogs and cats. The elimination half-life of cefovecin sodium in dogs is approximately 133 hours after intravenous or subcutaneous administration (Stegemann et al., 2006). As a result, a single

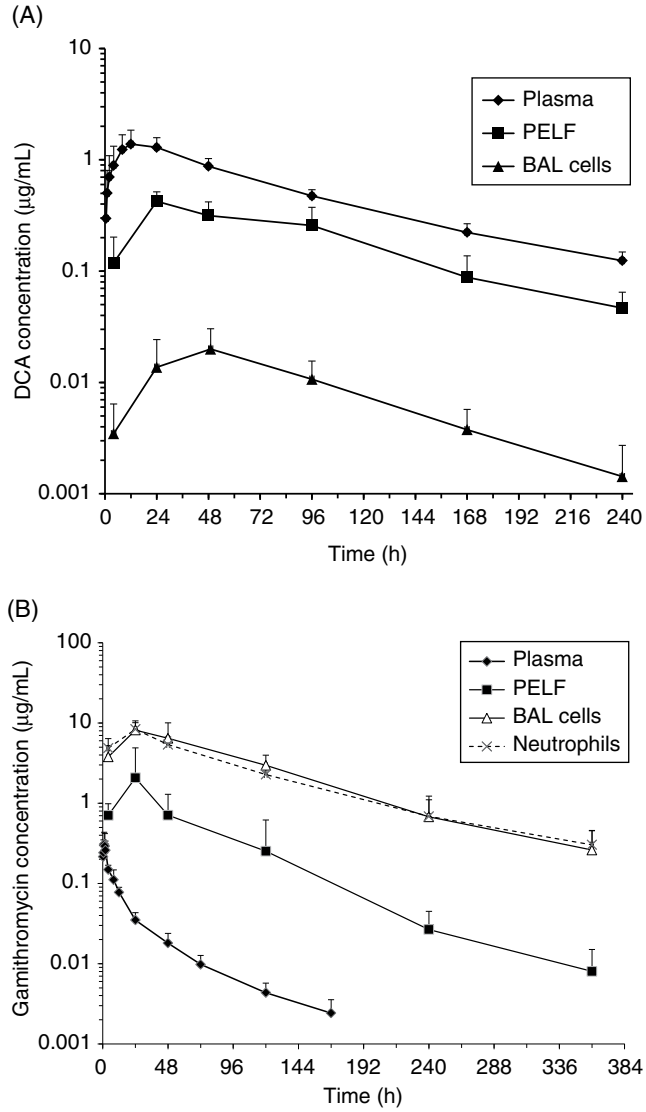

Figure 4.5. Mean (± SD) concentrations of desfuroylceftiofur and related metabolites (A) or gamithromycin (B) in plasma, bronchoalveolar (BAL) cells, neutrophils (gamithromycin only), and pulmonary epithelial lining fluid (PELF) of healthy foals after a single IM dose of ceftiofur crystalline free acid (6.6 mg/kg of body weight; n = 6) or gamithromycin (6.0 mg/kg of body weight; n = 6). (A) Ceftiofur crystalline free acid is slowly absorbed from the injection site, resulting in sustained plasma concentrations. Concentrations in PELF and BAL cells are lower than concurrent plasma concentrations but the drug follows a similar pattern of distribution and elimination at all sites. (B) Gamithromycin concentrations in neutrophils, BAL cells, and PELF are considerably higher than concurrent plasma concentrations. Drug concentrations in PELF are lower than intracellular concentrations but follow the same pattern of elimination, suggesting that cells may act as a delivery system for release of the drug in PELF. Adapted from Credille et al. and Berghaus et al., 2012.

subcutaneous injection is sufficient to provide 7–14 days of therapeutic coverage.

Prolonged-Release Preparations. Drugs with short elimination half-lives when administered intravenously may be formulated in a prolonged-release formulation. Prolonged-release (long-acting) preparations are designed to delay absorption and thereby maintain effective drug concentrations for an extended period, which infers several times the elimination half-life of the drug. The aqueous suspension of procaine penicillin G (300,000 IU/mL) provides an example that decreasing the rate of absorption can be usefully applied to lengthen the dosage interval for penicillin G. A single dose (25,000 IU/kg) of this preparation will maintain effective concentrations against susceptible bacteria for at least 12 hours, and generally for 24 hours. An essential feature of prolonged-release preparations is that the rate of drug release be adequate to maintain effective plasma concentrations for the duration of the dosage interval.

A single IM dose (20 mg/kg) of a long-acting preparation of oxytetracycline base in 2-pyrrolidone provided plasma oxytetracycline concentrations greater than 0.5 μg/mL for 48 hours in ruminant calves, cattle, goats, red deer and fallow deer. Pronounced tissue damage at the injection site was found on examination of excised muscle tissue of pigs slaughtered 1 and 2 weeks after IM administration of the long-acting preparation, whereas the conventional preparation, administered at the same dose level (20 mg/kg), produced little tissue irritation (Xia et al., 1983). Comparison of the pharmacokinetics of three injectable oxytetracycline preparations administered IM in the lateral neck of pigs (20 mg/kg) indicates that 48 hours would be an appropriate dosage interval for either of the long-acting preparations and 24 hours for the conventional preparation (Banting and Baggot, 1996; Table 4.1). Oxytetracycline formulated in polyethylene glycol has been used for IM administration in horses (Dowling and Russell, 2000).

More recently, a prolonged-release formulation of ceftiofur has gained in popularity because of the convenience of only 1 or 2 injections and potential resulting improvement in owner compliance. Ceftiofur crystalline free acid is labeled for use in cattle, swine and horses. Formulation in a caprylic/capric triglyceride and cottonseed oil–based suspension allows slow release of the drug from the site of injection (Figure 4.5A).

Table 4.1. Pharmacokinetic parameters describing the absorption and disposition of three oxytetracycline formulations administered intramuscularly (lateral neck) to pigs.

Pharmacokinetic term	Product A	Product B	Product C
C_{max} (μg/ml)	6.27 ± 1.47	5.77 ± 1.0	4.68 ± 0.61
t_{max}(h)	3.0 (2.0–4.0)	0.5 (0.083–2.0)	0.5 (0.083–2.0)
AUC (μg ¥ h/ml)	79.22 ± 25.02	91.53 ± 20.84	86.64 ± 14.21
MRT (h)	11.48 ± 2.01	25.27 ± 9.22	37.66 ± 15.62
$C_{p(24h)}$ (μg/ml)	0.81 ± 0.34	1.01 ± 0.26	0.97 ± 0.29
$C_{p(48h)}$ (μg/ml)	<LOQ	0.40 ± 0.17	0.50 ± 0.09

Note: n = 8; dose = 20 mg/kg body weight. Results are expressed as mean ± standard deviation. LOQ = limit of quantification (0.1 μg/ml). Product A: engemycine 10% in polyvinylpyrrolidone; product B: Oxyter LA 20% in dimethylacetamide; product C: terramycin LA 20% in 2-pyrrolidone and polyvinylpyrrolidone.
Source: Banting and Baggot (1996) with permission.

Drugs That Concentrate in and Are Slowly Released by Phagocytic Cells. Other long acting formulations are absorbed and eliminated from the plasma fairly rapidly. However, phagocytic cells act as a delivery system for slow release of the drug at the site of infection (Figure 4.5B). Most of these drugs are macrolides and azalides (chapter 13) that are potent weak bases that become ion-trapped within acidic intracellular compartments, such as lysosomes and phagosomes. In veterinary medicine, these drugs are most commonly used for the treatment and control of bovine respiratory disease. Examples of such drugs include tulathromycin, gamithromycin, and tildipirosin (Cox et al., 2010; Giguère et al., 2011; Menge et al., 2012). Plasma concentrations of these drugs are considerably lower than their respective minimum inhibitory concentrations (MICs) against the pathogens causing bovine respiratory diisease. Nonetheless, multiple studies have demonstrated the efficacy of these drugs in the treatment of bovine respiratory disease indicating that drug concentrations at the site of infection provide more clinically relevant

information than simple reliance on plasma concentrations. High intracellular concentrations combined with slow and sustained release of the drugs in pulmonary epithelial lining fluid (PELF) likely contribute to their efficacy despite administration as a single injection and rapid disappearance of the drug from the plasma. This evidenced by the fact that calves are at least partially protected from experimental infection with *Mannheimia haemolytica* 5 and 10 days after administration of a single dose of gamithromycin, when plasma concentrations are considerably below therapeutic concentrations but PELF and bronchoalveolar cell concentrations are still within the therapeutic range (Forbes et al., 2011).

Oral Administration

There are a wide variety of oral dosage forms available for use in animals. They include oral solutions, suspensions, pastes, capsules, tablets of various types and powders. The rate of drug absorption varies with the type of dosage form; oral solutions provide rapid absorption. Dissolution must precede absorption from a solid dosage form and frequently controls the rate of drug absorption. Oral suspensions and pastes generally provide drug for absorption at a rate that is intermediate between solutions and solid dosage forms. Reticular groove closure may enable drug solutions to bypass the rumen, while drug suspensions are largely deposited in the rumen. This distinction may be of significance with regard to the clinical efficacy of some anthelmintics. Although the rumen has good absorptive capacity, drug absorption takes place slowly from ruminal fluid (pH 5.5–6.5) because of its large volume and slow onward passage to the abomasum. In monogastric species, gastric emptying is the principal physiologic factor governing the rate of drug absorption. Medication of feed or of drinking water provides a convenient means of antimicrobial administration, to pigs and poultry. By contrast, the addition of an antimicrobial agent to the feed is an unreliable method of dosing horses and should not be considered.

The systemic availability, which is the fraction of an oral dose that reaches the systemic circulation unchanged, is of greater clinical importance than the rate of absorption of an antimicrobial agent. Systemic availability is influenced by the stability of an antimicrobial agent in the highly acidic gastric contents (pH 3–4) or its susceptibility to inactivation (by hydrolytic or reductive reaction) by ruminal microorganisms, and by the chemical nature and physicochemical properties of the drug. Since absorption takes place by passive diffusion across the mucosal epithelial barrier, high solubility in lipid is an important property. Having passed through the mucosal barrier, drug molecules are conveyed in hepatic portal venous blood to the liver, the major organ of drug metabolism, prior to reaching the systemic (general) circulation. Presystemic metabolism, referred to as the first-pass effect, can occur in the gut lumen or mucosal epithelium or, most importantly, in the liver. The first-pass effect decreases the systemic availability of drugs that undergo extensive hepatic metabolism. Presystemic metabolism activates prodrugs of ampicillin, such as pivampicillin and bacampicillin, by hydrolysis of the ester in the intestinal mucosa. Metabolic conversion (N-dealkylation) of enrofloxacin to ciprofloxacin and of difloxacin to sarafloxacin is likely to occur to some extent but the products formed possess high antimicrobial activity, being drugs in their own right.

The systemic availability of aminoglycoside antibiotics, which are polar organic bases, is very low following oral administration, whereas they are rapidly absorbed and completely available systemically when administered by IM or SC injection. It is the absorption process that differs between the gastrointestinal tract and parenteral sites. Passage across the mucosal barrier requires that the drug be at least moderately lipid-soluble while absorption from parenteral sites is mainly controlled by capillary blood flow at the absorptive surface.

The presence of food in the stomach or binding to feed constituents decreases the systemic availability of most penicillins, apart from amoxicillin and ampicillin prodrugs, oral cephalosporins, and tetracyclines (except doxycycline). The systemic availability of some drugs (e.g., doxycycline, erythromycin estolate, ketoconazole) is increased when administered to dogs after feeding. Some drugs might interfere with oral absorption of other drugs. For example antacids are known to decrease absorption of many fluoroquinolones and drugs that increase gastric pH such as omeprazole are known to decrease absorption of itraconazole. Concurrent administration of rifampin considerably reduces absorption

of clarithromycin and potentially other macrolides in foals possibly by inhibition of an unknown intestinal uptake transporter (Venner et al., 2010; Peters et al., 2011, 2012).

It may be feasible to administer certain antimicrobial agents orally to young foals, calves and kids, even though these drugs are not suitable for oral use in older and adult herbivorous animals. This is not only due to better absorption, but to the fact that neither the microflora indigenous to the specialized fermentation regions of the gastrointestinal tract nor the hepatic microsomal oxidative reactions have developed.

Applied Clinical Pharmacokinetics

The chemical nature and related physicochemical properties largely govern the absorption, distribution and elimination, which refers to biotransformation (metabolism) and excretion, of antimicrobial agents. The majority of antimicrobial agents are weak organic electrolytes, either weak acids or weak bases, while fluoroquinolones, tetracyclines and rifampin are amphoteric compounds. Lipid solubility and the degree of ionization, which is determined by the pK_a of the drug and the pH of the biologic fluid in question (pH of blood is 7.4), influence the extent of absorption, the pattern of distribution, and the elimination process(es) for antimicrobial agents. Lipid solubility is a requirement for passive diffusion of drugs across cell membranes and it is the non-ionized form of weak organic acids and bases that is lipid-soluble.

Since antimicrobial agents, like other drugs, are available as prepared dosage forms, the type and formulation of the dosage form (drug preparation) determine the route of administration, the bioavailability and overall rate of elimination of the drug. Because it affects pharmacokinetic processes, the drug preparation influences the dosage regimen for each animal species and the withdrawal period(s) in food-producing animals.

Distribution and Elimination

Following the entry of an antimicrobial agent into the systemic circulation, the free (unbound) fraction is available for distribution to extravascular tissues and for removal from the body by the organs of elimination (liver and kidneys). The extent and pattern of distribution vary between antimicrobial agents of different classes due to differences in their chemical nature. Distribution is determined by blood flow to tissues and the ability of a drug to penetrate (mainly by passive diffusion) cellular barriers. The rate of distribution is largely influenced either by perfusion (lipophilic drugs) or by diffusion (ionized and polar compounds). Extensive (> 80%) binding to plasma proteins limits the immediate availability of a drug for extravascular distribution. Accumulation in tissues (pH partition effect) influences the extent of distribution. Selective binding to a tissue component (e.g., aminoglycosides to phospholipid-rich tissues of the inner ear and kidney cortex) may account for only a small fraction of the amount of drug in the body but could produce an adverse, even toxic, effect or the residue could limit the use of the drug in food-producing animals. Definitive information on the distribution pattern of a drug can only be obtained by measuring levels of the drug in the various organs and tissues of the body, such as kidneys, liver, skeletal muscle, adipose tissue, and skin. Selective binding can reasonably be suspected and should be further investigated when a specific lesion is produced in a tissue or terminal elimination is prolonged.

While some antimicrobial agents are almost entirely eliminated by renal excretion (aminoglycoside, most beta-lactam antibiotics), others are eliminated by hepatic metabolism and, to a lesser extent, by renal or biliary excretion. The extent to which liver damage decreases the rate of elimination of drugs is difficult to assess. However, certain antimicrobial agents (chloramphenicol, erythromycin, tiamulin, ketoconazole) inhibit hepatic microsomal enzyme activity, while rifampin and griseofulvin induce hepatic microsomal enzymes by increasing their synthesis. The rate of elimination of several therapeutic agents used concurrently with one of these antimicrobials can be affected by the altered microsomal-mediated oxidative reactions. Metronidazole inhibits aldehyde dehydrogenase and thereby produces a disulfiram-like effect in people. Decreased renal function requires adjustment of aminoglycoside dosage (see below). Renal impairment may lead to the accumulation of drug metabolites although formed in the liver or at other sites of biotransformation.

Table 4.2. Half-life and urinary excretion of trimethoprim.

Species	Half-life (h)	Fraction of Dose Excreted Unchanged (%)
Goat	0.7	2
Cow	1.25	3
Pig	2.0	16
Horse	3.2	10
Dog	4.6	20
Human	10.6	69 ± 17

Lipophilic antimicrobial agents readily penetrate cellular barriers, with the exception of the blood-brain barrier. Consequently, these drugs are well absorbed from the gastrointestinal tract, become widely distributed in body fluids and tissues, and can generally attain effective concentrations at sites of infection. Examples of lipophilic antimicrobial agents include fluoroquinolones, macrolides and lincosamides, minocycline and doxycycline, trimethoprim, rifampin, metronidazole and chloramphenicol. Some of these drugs (erythromycin, clindamycin, doxycycline) bind extensively to plasma proteins, which limits their availability for extravascular distribution. Clindamycin, however, may attain effective concentrations in bone. Of the lipophilic antimicrobials, only certain individual drugs penetrate the blood-brain and blood-CSF barriers and attain effective concentrations in cerebrospinal fluid (e.g., trimethoprim, metronidazole, chloramphenicol). In the presence of meningitis, most intravenously administered third-generation cephalosporins (except cefoperazone) penetrate the blood-CSF barrier. Fluconazole may be the only azole antifungal drug that penetrates the blood-brain barrier. Individual tetracyclines differ in lipid solubility, which influences the tissue concentrations attained and their clinical efficiency. Lipophilic antimicrobial agents are eliminated mainly by the liver (metabolism and biliary excretion), while a fraction of most of these drugs (with the notable exception of doxycycline) is excreted unchanged (and as metabolites) in the urine. The more rapidly a drug is metabolized, the smaller the fraction of dose that is excreted unchanged, for example, trimethoprim (Table 4.2). The metabolic pathways, various hepatic microsomal oxidative reactions and glucuronide conjugation, are determined by the functional groups present in the drug molecule. Apart from some fluoroquinolones, rifampin and metronidazole the metabolites of lipophilic antimicrobials are inactive. Enrofloxacin is converted to ciprofloxacin, difloxacin to sarafloxacin, and pefloxacin to norfloxacin by N-dealkylation (oxidative reaction). The half-lives of individual lipophilic antimicrobials may differ within a species and between animal species. For example, the half-lives of various fluoroquinolones in the dog are: ciprofloxacin (2.2 hours), enrofloxacin (3.4 hours), norfloxacin (3.6 hours), difloxacin (8.2 hours), and marbofloxacin (12.4 hours). The half-lives of metronidazole in various species are: cattle (2.8 hours), horse (3.9 hours), dog (4.5 hours), chicken (4.2 hours) and of chloramphenicol are: horse (0.9 hours), dog (4.2 hours), cat (5.1 hours), chicken (5.2 hours).

The pharmacokinetic properties of different antibacterial drug classes and their members, and factors affecting these properties, are discussed extensively under the description of each drug.

Pharmacokinetic Parameters

Drug disposition is the term used to describe the simultaneous effects of distribution and elimination, that is the processes that occur subsequent to the absorption of a drug into the systemic circulation. The major pharmacokinetic parameters that describe the disposition of a drug are the systemic (body) clearance (Cl_B), which measures the ability of the body to eliminate the drug, and the volume of distribution (V_d), which denotes the apparent space in the body available to contain the drug. The half-life ($t_{½}$) expresses the overall rate of drug elimination; it is only when the dose is administered intravenously that the "true" (elimination) half-life of a drug can be determined. When a drug preparation is administered orally or by a non-vascular parenteral route (e.g., IM or SC), the systemic availability (F), that fraction of the dose that reaches the systemic circulation unchanged, is an important parameter. Since the absorption process influences the rate of drug elimination, the value obtained for half-life is "apparent"; it will vary with route of administration and formulation of the dosage form (drug preparation). Bioavailability, which refers to both the rate and extent of drug absorption, provides a more complete description of the absorption process. The rate and pattern of absorption assume importance when a drug is administered as a prolonged-release (long-acting) preparation.

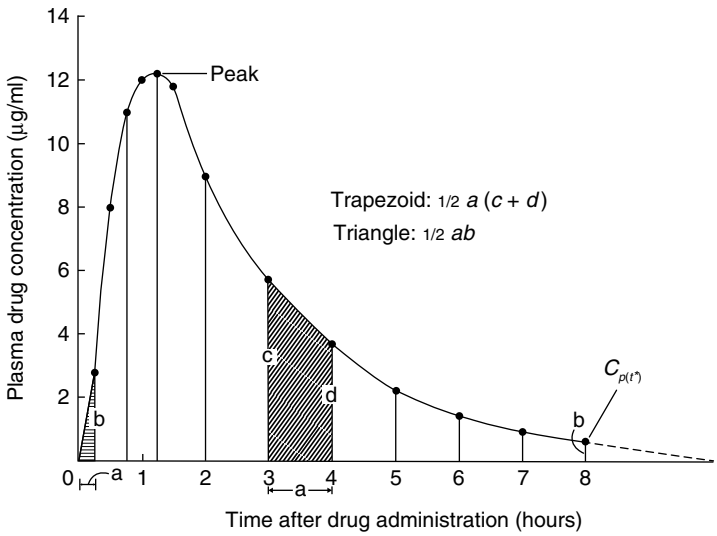

Figure 4.6. Typical plasma drug concentration profile following oral administration or non-vascular (IM, SC) injection of a conventional form of a drug. AUC may be calculated by the trapezoidal rule. From Baggot, 1977, with permission.

Bioavailability

Bioavailability is defined as the rate and extent to which a drug enters the systemic circulation unchanged. It is influenced not only by the factors that determine drug absorption but also by formulation of the dosage form and the route of administration. Complete systemic availability (extent of absorption) can be assumed only when a drug is administered intravenously.

An estimation of the rate of drug absorption can be obtained from the peak (maximum) plasma concentration (C_{max}) and the time at which the peak concentration is attained (t_{max}), based on the measured (observed) plasma concentration-time data. However, the blood-sampling times determine how well the peak is defined; t_{max} often lies between measured plasma concentrations. Both C_{max} and t_{max} may be influenced by the rate of drug elimination, while C_{max} is also affected by the extent of absorption. The parameter C_{max}/AUC, which can be calculated and is expressed in units of reciprocal time (h^{-1}), is an additional term that may be used to indicate the rate of drug absorption. Even though an absorption rate constant (and half-life) can be calculated, the generally small number of data points on which it is based makes it an inaccurate measurement of the rate of drug absorption. The usual technique for estimating systemic availability (F), extent of absorption, employs the method of corresponding areas:

$$F = \frac{AUC_{PO}}{AUC_{IV}} \times \frac{Dose_{IV}}{Dose_{PO}}$$

where AUC is the total area under the plasma concentration-time curve relating to the route of drug administration (IV and PO, IM, or SC). The application of this technique involves the assumption that clearance of the drug is not changed by the route of administration. Following the administration of a single dose by any route, the total area under the curve can be estimated by the linear trapezoidal rule, from time zero to the last measured plasma drug concentration, with extrapolation to infinite time, assuming log-linear decline (Figure 4.6). The accuracy of this method for estimating the total area under the curve (AUC) depends on the number of plasma concentration-time data points from the time of drug administration (time zero) to the last measured plasma concentration and on the relative area under the extrapolated portion of the curve, which should be less than 10% of the total area. When comparison is made of the AUC for an oral dosage form with that for an intravenous preparation of the drug, the absolute bioavailability (systemic availability) is obtained, whereas comparison of the AUCs for two oral dosage forms (test and reference) estimates the relative bioavailability. The latter comparison is used in

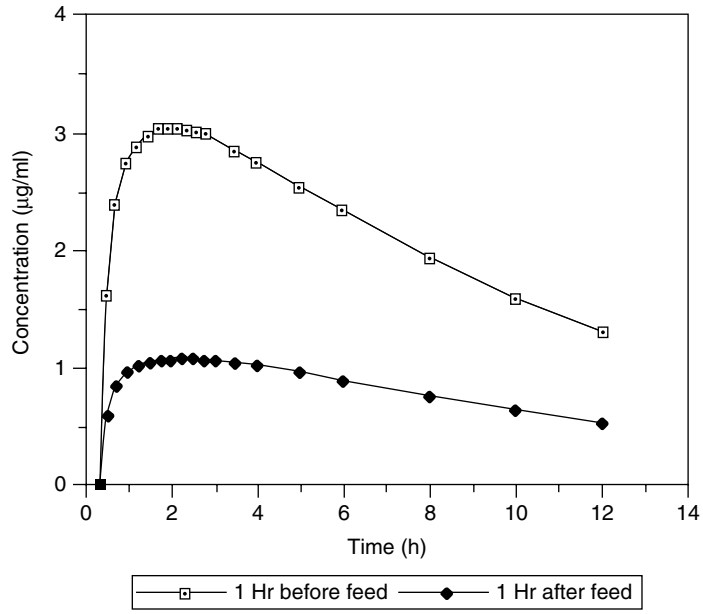

Figure 4.7. Mean plasma rifampin concentration curves in horses (n=5) after oral administration of the drug (5 mg/kg) 1 hour before or 1 hour after feeding.

bioequivalence assessment. In bioavailability studies a crossover design, with an appropriate washout period between the phases of the study, should be used whenever feasible.

The systemic availability of orally administered antimicrobial drugs is often incomplete (< 100%). This may be due to poor absorption, degradation in the stomach or rumen, or presystemic metabolism (first-pass effect). Incomplete systemic availability can often be compensated for by administering a higher oral dose. The time of feeding relative to oral dosing may affect the systemic availability (oral bioavailability) of an antimicrobial agent, as discussed earlier. For example, the oral bioavailability of enrofloxacin, trimethoprim and sulfadiazine is high (> 80%) in pigs and is not influenced by the intake of feed. In contrast, the presence of feed in the gastrointestinal tract markedly decreases the oral bioavailability of spiramycin (60–24%) and lincomycin (73–41%; Nielsen, 1997). The systemic availability of rifampin (5 mg/kg) was 26% when the drug was administered to horses 1 hour after feeding, compared with 68% when given 1 hour before feeding (Figure 4.7). Because of species differences in digestive physiology and in anatomical arrangement of the gastrointestinal tract, the systemic availability and rate of absorption of drugs administered orally differ widely between ruminant and monogastric species.

Parenteral preparations administered by IM injection often vary in systemic availability, while the rate of absorption differs between conventional (immediate-release) and long-acting (prolonged-release) dosage forms. Incomplete systemic availability of parenteral preparations could be attributed either to partial precipitation of the drug at the injection site or to tissue irritation caused by the drug per se, the vehicle or the pH of the preparation. By decreasing the rate of drug absorption, long-acting preparations provide a prolonged duration of effective plasma concentrations and allow the use of a longer dosage interval. For example, the dosage interval for procaine penicillin G is 12 hours in horses, and 24 hours in pigs and cattle; the dosage interval for the long-acting parenteral formulation of oxytetracycline is 48 hours in pigs, cattle and goats. Repeated dosage with prolonged-release preparations produces less fluctuation in plasma concentrations than the degree of fluctuation produced by conventional preparations. It is usual to determine the relative bioavailability of a prolonged-release preparation by comparing area under the curve with AUC for a conventional preparation administered by the same route to the same animals (crossover design). The mean residence times should be compared. The plasma concentration-time

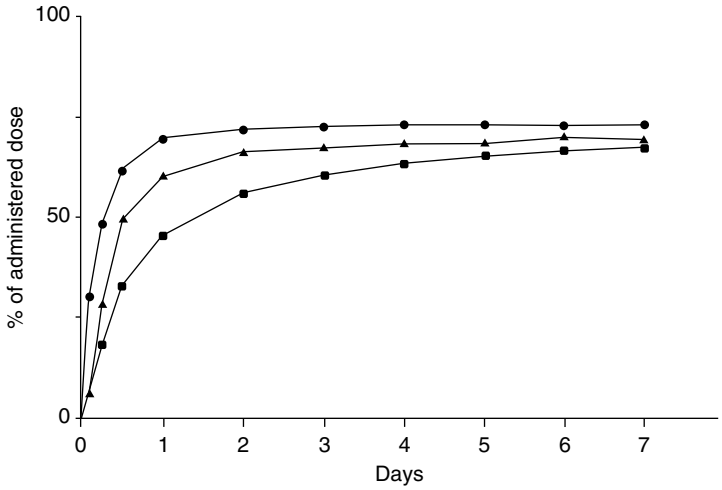

Figure 4.8. Cumulative urinary excretion of oxytetracycline in pigs after IV injection of conventional OTC preparations (circle, n = 3) and IM injection of conventional (triangle, n = 4) and a long-acting OTC preparation (square, n = 6). From Xia et al., 1983, with permission.

Table 4.3. Systemic availability of three oral dosage forms of sulfamethazine in cattle.

Dosage Form	Systemic Availability (%)
Solution	80.8
Rapid-release bolus	63.2
Sustained-release bolus	32.0

curve, plotted on arithmetic coordinates, shows the pattern of drug absorption and the duration of effective plasma concentrations. It is on the latter, rather than the apparent half-life, that the dosage interval is based.

The systemic availability of a drug can be estimated by comparing the cumulative urinary excretion of the unchanged (parent) drug after extravascular administration with the amount excreted unchanged after IV administration. Using this approach, the systemic availability of oxytetracycline was determined in pigs following the IM injection (biceps femoris) of single doses (20 mg/kg) of a conventional (OTC-C) and a long-acting (OTC-LA) preparation (Figure 4.8). Both preparations provided over 95% systemic availability of the antibiotic (Xia et al., 1983). Cumulative urinary excretion was used to compare the systemic availability of sulfamethazine administered as three oral dosage forms to yearling cattle (Bevill et al., 1977). The results obtained (Table 4.3) indicate that the oral solution (107 mg/kg) and oral rapid-release bolus (27.8 g of sulfamethazine; similar dose level as for oral solution) provide relatively effective availability of the drug for absorption from the rumen, whereas the sustained-release bolus (67.5 g of sulfamethazine) is a less satisfactory dosage form. This method is an alternative to comparing area under the plasma concentration-time curves, but it is cumbersome to apply since the total volume of urine voided during the excretion period for the drug (at least 4 half-lives) must be measured. In addition, the stability of the drug in urine during the collection period and storage of the samples must be assured. Use of cumulative urinary excretion data to compare the systemic availability of different dosage forms of a drug administered by the same extravascular route (PO or IM), ie, relative bioavailability, assumes that the ratio of the total amount excreted unchanged to the amount absorbed remains constant. It is always preferable to base estimation of the rate of drug absorption on plasma concentration data rather than on urinary excretion data.

Clearance

Clearance indicates the volume of blood or plasma from which a drug (or marker substance for an elimination process) would have to be cleared per unit of time to account for its elimination. For comparative purposes, clearance is expressed in units of mL/min × kg.

When based on plasma drug concentrations clearance can assume values that are not "physiologic"; conversion from plasma to blood clearance can be accomplished.

The systemic (body) clearance of a drug represents the sum of the clearances by the various organs (liver, kidneys, "other" organs or tissues) that contribute to elimination of the drug. It can be calculated by dividing the systemically available dose by the total area under the plasma concentration-time curve (from time zero to infinity):

$$Cl_B = \frac{F \times Dose}{AUC}$$

where F is the fraction of the dose that enters the systemic circulation unchanged and AUC is the total area under the curve. By definition, the systemic clearance of a drug is the product of the volume of distribution, calculated by the area method, and the overall elimination rate constant:

$$Cl_B = V_{d(area)} \times \beta$$

When an intravenous dosage form of the drug is not available, F cannot be determined; in this situation the term Cl_B/F should be used.

The concept of clearance is extremely useful in clinical pharmacokinetics, since the systemic clearance of most therapeutic (including antimicrobial) agents is constant over the clinically useful range of plasma concentrations. This is because the overall elimination of most drugs obeys first-order kinetics whereby a constant fraction is eliminated per unit of time (e.g., 50% is eliminated each half-life). Systemic clearance is probably the most important pharmacokinetic parameter to consider in defining a drug dosage regimen and is required for calculating dosing rate adjustment that may be necessitated by functional impairment of an organ of elimination. When multiple doses are administered at a constant dosage interval, systemic clearance relates the average steady-state plasma concentration to the dosing rate of the drug. Systemic or individual organ clearance, depending on the elimination processes for a drug, may be the pharmacokinetic parameter of choice in applying the allometric technique to interspecies scaling of drug elimination. As an example of one species, values of pharmacokinetic parameters describing the disposition of some antimicrobial agents are presented for dogs (Table 4.4).

Table 4.4. Disposition kinetics of antimicrobial agents in dogs.

Drug	Half-life (h)	V_d(area) (ml/kg)	Cl_B (ml/min × kg)
Penicillin G	0.50	156	3.60
Ampicillin	0.80	270	3.90
Ticarcillin	0.95	340	4.30
Cephalexin	1.71	402	2.70
Cefazolin	0.80	700	10.40
Cefotaxime	0.73	480	7.50
Ceftizoxime	1.07	300	3.25
Ceftazidime	0.82	220	3.15
Ceftriaxone	0.85	240	3.26
Gentamicin	1.25	335	3.10
Amikacin	1.10	245	2.61
Kanamycin	0.97	255	3.05
Norfloxacin	3.56	1,770	5.53
Enrofloxacin	3.35	2,454	8.56
Marbofloxacin	12.40	1,900	1.66
Difloxacin	8.20	3,640	5.10
Trimethoprim	4.63	1,849	4.77
Sulfadiazine	5.63	422	0.92
Sulfadimethoxine	13.20	410	0.36
Sulfisoxazole	4.50	300	0.77
Chloramphenicol	4.20	1,770	4.87
Thiamphenicol	1.75	765	5.20
Metronidazole	4.50	948	2.50
Erythromycin	1.72	2,700	18.2
Clindamycin	3.25	1,400	5.25
Oxytetracycline	6.02	2,096	4.03
Doxycycline	6.99	1,010	1.72
Minocycline	6.93	1,952	3.55

Volume of Distribution

The volume of distribution, which relates the amount of drug in the body to the concentration in the plasma, provides an estimation of the extent of distribution of a drug. It quantifies the apparent space, in both the systemic circulation and the tissues of distribution, available to contain the drug, but does not reveal the pattern of distribution. The distribution pattern of a drug can only be described by measuring the level (amount) of drug in the various organs and tissues of the body.

The volume of distribution can be calculated (area method) from the equation:

$$V_{d(area)} = \frac{Dose}{AUC \times \beta}$$

where AUC is the total area under the plasma concentration-time curve and β is the overall elimination rate constant of the drug, obtained from the linear terminal (elimination) phase of the semilogarithmic disposition curve (Figure 4.1). This implies that the drug was administered as an IV bolus dose. When the drug is administered orally (PO) or by a non-vascular parenteral route (IM, SC), correction must be made for systemic availability (F) and the apparent first-order elimination rate constant (k_d) be substituted for β.

Drugs that are predominantly ionized in plasma or are relatively polar (penicillins, cephalosporins, aminoglycosides) have volumes of distribution in the range 150–300 mL/kg; this infers no more than their distribution is limited in extent. Lipophilic antimicrobial agents (macrolides, lincosamides, chloramphenicol, trimethoprim, fluoroquinolones) have volumes of distribution that are generally between 1 and 3 L/kg. The volumes of distribution of moderately lipid-soluble antimicrobials (e.g., metronidazole, rifampin, sulfonamides) are intermediate (400–800 mL/kg). The tetracyclines differ in lipid solubility and their volumes of distribution vary accordingly.

Species variations in the volume of distribution of a drug can be largely attributed to differences in body composition (Table 4.5), in particular anatomical features of the gastro-intestinal tract while differences in plasma protein binding may contribute. The greatest variation is found between ruminant and monogastric species, mainly for lipophilic organic bases.

Since volume of distribution, serving as a proportional factor, relates the plasma concentration to the amount of drug in the body, knowledge of this parameter is required for calculating the dose (mg/kg) that would provide a desired plasma drug concentration:

$$Dose_{iv} = C_{p(ther)} \times V_{d(area)}$$

Drug administration by the oral or a non-vascular parenteral route may require upward adjustment of the dose to compensate for incomplete systemic availability of the drug. No provision can be made for variation in the rate of drug absorption.

Volume of distribution has useful applications, but it is a parameter (volume term) that must be properly interpreted. Although $V_{d(area)}$ may be determined following drug administration by any route, it varies with change in the elimination rate constant for a drug, even when the distribution space has remained unchanged. The volume of distribution at steady-state $V_{d(ss)}$ is not subject to this disadvantage, but can only be determined when the drug is administered as an IV bolus dose. The volume of distribution at steady-state can be calculated by the use of areas (Benet and Galeazzi, 1979):

$$V_{d(ss)} = \frac{Dose_{iv} \times AUMC}{(AUC)^2}$$

where AUC is the total area under the curve (zero moment) and AUMC is the area under the first moment of the plasma concentration-time curve, that is, the area under the curve of the product of time and plasma concentration ($t \times C_p$) over the time span zero to infinity. This non-compartmental method of calculating V_d does not require the application of a compartmental pharmacokinetic model or mathematical description of the disposition curve. The volume of distribution at steady-state represents the volume in which a drug would appear to be distributed during steady-state if the drug existed throughout that volume at the same concentration as in the plasma.

The volume of distribution at steady-state is somewhat smaller than that calculated by the area method. Volumes of distribution of trimethoprim in dogs are $V_{d(ss)}$ 1675 mL/kg, $V_{d(area)}$ 1849 mL/kg, and of sulfadiazine are $V_{d(ss)}$ 392 mL/kg, $V_{d(area)}$ 422 mL/kg. When interpreting the influence of disease or physiologic state on the disposition kinetics of a drug, the systemic clearance (Cl_B) and $V_{d(ss)}$, rather than $V_{d(area)}$, are the pharmacokinetic parameters that should be used. Neither volume of distribution term allows one to predict drug concentrations that are attained in tissues or at infection sites.

Half-Life

The half-life of a drug expresses the time required for the plasma concentration, as well as the amount in the body, to decrease by 50% through the process of elimination.

Table 4.5. Body composition of various species (% live weight).

Organ/Tissue	Horse	Dog	Goat	Cow	Human
Blood	8.6	7.2	8.0	7.7	7.9
Brain	0.21	0.51	0.29	0.06	2.0
Heart	0.66	0.82	0.48	0.37	0.47
Lung	0.89	0.89	0.88	0.71	1.4
Liver	1.3	2.32	1.95	1.22	2.6
Spleen	1.11	0.26	0.25	0.16	0.26
Kidney	0.36	0.61	0.35	0.24	0.44
Gastrointestinal tract	5.8	3.9	6.4	3.8	1.7
Gastrointestinal contents	12.7	0.72	13.9	18.4	1.4
Skin	7.4	9.3	9.2	8.3	3.7
Muscle	40.1	54.5	45.5	38.5	40.0
Bone	14.6	8.7	6.3	12.7	14.0
Tendon	1.7	–	–	–	2.0
Adipose	5.1	–	–	18.9	18.1
Body weight (kg)	308	16	39	620	70
Source	a	b	b	c	d

Sources: a, Webb and Weaver (1979); b, Neff-Davis et al. (1975); c, Matthews et al. (1975); d, International Commission on Radiological Protection (1975).

Half-life ($t_{1/2}$) measures the rate of decline in plasma drug concentrations during the elimination phase of the disposition curve, and is calculated from the expression:

$$t_{1/2} = \frac{0.693}{\beta}$$

where β is the overall elimination rate constant of the drug; 0.693 is ln 2. The half-lives of antimicrobial agents are independent of the dose administered (at least within the recommended dose range), since their overall elimination obeys first-order kinetics. The characteristic of first-order elimination is that the time required for a given concentration to decrease by a certain fraction (e.g., 50% each half-life) is usually independent of the concentration.

Half-life is the pharmacokinetic parameter that is used to compare the rate of elimination of drugs in different species (Table 4.6). Even though the relative contribution of hepatic metabolism or renal excretion to antimicrobial elimination may differ between species, this approach is useful for comparative purposes. The half-lives of antimicrobials (and pharmacologic agents) that are mainly eliminated by hepatic metabolism can vary widely among species. Apart from oxytetracycline, which undergoes enterohepatic circulation, variation between mammalian species in the half-lives of antimicrobials that are eliminated by renal excretion is not of clinical significance. For comparative purposes the half-life of gentamicin, which is eliminated by glomerular filtration, is about 1 hour in guinea pigs and rabbits, 1.1–1.4 hours in dogs and cats, 1.4–1.8 hours in cattle, sheep, and goats, 1.9 hours in pigs, 2–3 hours in horses and people, approximately 3 hours in llamas and camels, 2.5–3.5 hours in chickens and turkeys, 12 hours in channel catfish (*Ictalurus punctatus*) at 22 ± 2°C, and an average of 51 hours in reptiles.

Compared with mammalian (and avian) species, the half-lives of antimicrobials in poikilothermic species (fish and reptiles) are prolonged, which is consistent with their much lower metabolic turnover rate (Calder, 1984; chapters 37 and 39). The half-life of an antimicrobial agent in fish is influenced by the temperature of the water in which the fish are acclimatized (Table 4.7). The overall rate of antimicrobial elimination increases (i.e., half-life decreases) with increase in water temperatures. The average half-life of trimethoprim, administered IV as trimethoprim-sulfadiazine combination, in carp (*Cyprinus carpio L.*) is 40.7 hours at 10°C and 20 hours at 24°C (Nouws et al., 1993) compared with cattle (1.25

Table 4.6. Average half-lives of antimicrobial agents in various species.

Drug	Process(es) of Elimination	Half-life (h)			
		Cattle	Horses	Dogs	Humans
Trimethoprim	M + E(r)	1.25	3.2	4.6	10.6
Sulfadiazine	M + E(r)	2.5	3.6	5.6	9.9
Sulfamethoxazole	M + E(r)	2.3	4.8	–	10.1
Sulfamethazine	M + E(r)	8.2	9.8	16.8	–
Sulfadimethoxine	M + E(r)	12.5	11.3	13.2	40
Sulfadoxine	M + E(r)	10.8	14.2	–	150
Norfloxacin	M + E(r)	2.4	6.4	3.6	5.0
Enrofloxacin	M + E(r)	1.7	5.0	3.4	–
Chloramphenicol	M + E(r)	3.6	0.9	4.2	4.6
Metronidazole	M + E(r)	2.8	3.9	4.5	8.5
Tinidazole	M + E(r)	2.4	5.2	4.4	14.0
Erythromycin	E(h) + M	3.2	1.0	1.7	1.6
Oxytetracycline	E(r ± h)	4.0	9.6	6.0	9.2
Penicillin G	E(r)	0.7	0.9	0.5	1.0
Ampicillin	E(r)	0.95	1.2	0.8	1.3
Cefazolin	E(r)	–	0.65	0.8	1.8
Ceftriaxone	E(r)	–	1.62	0.85	7.3[a]
Gentamicin	E(r)	1.8	2.2–2.8	1.25	2.75
Amikacin	E(r)	–	1.7	1.1	2.3

[a]Eliminated by the liver (biliary excretion) in human beings.
Note: M, metabolism; E, excretion; r, renal; h, hepatic.

hours), horse (3.2 hours), dog (4.6 hours), and human beings (10.6 hours). Sulfadiazine half-life similarly differs widely: carp (47 hours at 10°C; 33 hours at 24°C), cattle (2.5 hr), horse (3.6 hours), dog (5.6 hours), and human being (9.9 hours). The prolonged half-lives of lipid-soluble antimicrobials in fish could be attributed to a greater contribution made by enterohepatic circulation. The half-life of oxytetracycline in African catfish (*Clarias gariepinus*) is 80.3 hours at 25°C and in rainbow trout (*Salmo gairdneri*) is 89.5 hours at 12°C (Grondel et al., 1989), compared with half-lives in the range 3.4–9.6 hours in domestic animals. When developing antimicrobial products for use in farmed fish, studies of the relationship between pharmacokinetics of the drugs and ambient (water) temperature should be performed (chapter 39). Furthermore the quantitative susceptibility (MIC) of bacterial pathogens isolated from poikilothermic animals may be temperature-dependent.

Half-life is the parameter on which selection of the dosage interval for a drug is based. The rate at which a drug administered by constant infusion or as multiple doses at a fixed interval (e.g., approximately equal to the half-life) approaches a steady-state concentration is determined solely by the half-life of the drug; a duration of 4 times the half-life is required to attain an average plasma concentration during the dosage interval within 90% of the eventual steady-state concentration. A drug that selectively binds to tissues or is sequestered in a body compartment may have more than one half-life in any species. The relevance of the half-life chosen depends on the proposed application. The half-life based on the decline in plasma concentrations of clinical interest is relevant to dosage interval selection. That based on the gradual decline in subinhibitory plasma concentrations in the case of an antimicrobial agent may find application in predicting the withdrawal period for the drug in a food-producing species. The half-life of gentamicin (10 mg/kg, IV) in sheep based on the clinically relevant (β) elimination phase is 1.75 hours, while that based on the prolonged terminal (γ) phase is 88.9 hours (Brown et al., 1986). For drugs that show linear pharmacokinetic behavior (antimicrobial agents),

Table 4.7. The half-lives of various antimicrobial agents in fish.

Antimicrobial Agent	Species	Acclimatization Temperature (°C)	$t_{1/2}$ (h)
Trimethoprim	Carp	10	40.7
	(Cyprinus carpio L.)	24	20.0
Sulfadiazine	Carp	10	47.0
	(Cyprinus carpio L.)	24	33.0
Oxytetracycline	Rainbow trout *(Salmo gairdneri)*	12	89.5
	African catfish *(Clarias gariepinus)*	25	80.3
Florfenicol	Atlantic salmon *(Salmo salar)*	10.8 ± 1.5 (seawater)	12.2
Enrofloxacin	Fingerling rainbow trout *(Oncorhynchus mykiss)*	15	27.4
Enrofloxacin (5 mg/kg, IM)	Red pacu *(Colossoma brachypomum)*	25	28.9
Gentamicin	Channel catfish *(Ictalurus punctatus)*	22	12.0
Sulfadimidine	Carp	10	50.3
	(Cyprinus carpio L.)	20	25.6
	Rainbow trout	10	20.6
	(Salmo gairdneri)	20	14.7

dissimilar values of clearance based on single dose and average steady-state plasma concentrations (multiple dosing) provides definitive evidence of the presence of a "deep" peripheral compartment (Browne et al., 1990). Requirements of the study design are that the duration of blood sampling be prolonged and the sensitivity of the analytical method be sufficiently high to detect the presence of a deep peripheral compartment; the plasma concentration-time data is analyzed according to a three-compartment open model.

Mean Residence Time

The mean residence time (MRT) represents the average time the molecules of a drug reside in the body after the administration of a single dose. This parameter is the statistical moment analogy to half-life and may vary with the route of administration. The calculation of MRT is based on total areas under the plasma concentration curves, which are estimated by numerical integration using the trapezoidal rule (from time zero to the last measured plasma concentration) with extrapolation to infinite time:

$$\mathrm{MRT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}}$$

where AUC is area under the curve (zero moment) and AUMC is area under the (first) moment curve obtained from the product of plasma concentration and time *versus* time from time zero to infinity. The areas under the extrapolated portion of the curves are estimated by:

$$\frac{C_{p(last)}}{\beta} \text{ for AUC, and } \frac{t^{*} \cdot C_{p(last)}}{\beta} + \frac{C_{p(last)}}{\beta} \text{ for AUMC}$$

where β is the overall elimination rate constant of the drug and t^{*} is the time of the last measured plasma drug concentration ($C_{p(last)}$). The elimination rate constant β is obtained by least squares regression analysis of the terminal 4–6 data points. It is desirable that the areas under the extrapolated portion of the curves be less than 10% of the total AUC and less than 20% of total AUMC.

Values of mean residence time and other pharmacokinetic parameters obtained for metronidazole in horses are presented (Table 4.8).

The advantage of using non-compartmental methods for calculating pharmacokinetic parameters, such as mean residence time (MRT), systemic clearance (Cl_B), volume of distribution ($V_{d(area)}$) and systemic availability (F), are that they can be applied to any route of administration and do not require the selection of a compartmental model. The only assumption made is that the absorption and disposition of the drug obey first-order (linear) pharmacokinetics. After intravenous administration of a bolus dose of drug, the volume of distribution at steady-state is given by:

$$V_{d(ss)} = Cl_B \times MRT_{IV}$$

Changes in Drug Disposition

Certain physiologic conditions (neonatal period, pregnancy), prolonged fasting (48 hours or longer), disease states (fever, dehydration, chronic liver disease, renal function impairment), or pharmacokinetic-based drug

Table 4.8. Bioavailability, absorption, and disposition kinetics of metronidazole after administration of single IV and oral doses to quarter horse mares.

Pharmacokinetic Terms and Units		Mean ± s.d.
Intravenous		
$V_{d(area)}$	(ml/kg)	661 ± 44
$V_{d(ss)}$	(ml/kg)	651 ± 45
Cl_B	(ml/kg ? h)	115 ± 10.8
$t_{1/2}$	(h)	4.04 ± 0.45
MRT_{IV}	(h)	6.02 ± 0.91
Oral		
Lag time	(h)	0.3 (0–0.88)[a]
t_{max}	(h)	1.5 (0.75–4.0)[a]
C_{max}	(μg/ml)	21.2 ± 3.1
$t_{1/2(d)}$	(h)	6.0 ± 2.94
MRTPO	(h)	9.4 ± 4.32
F	(%)	74.5 ± 13.0
		72.7 (58.4–91.5)[a]

[a]Median (range) of F; note wide individual variation.
Note: n = 6; IV dose = 10 mg/kg; oral dose = 20 mg/kg.
Source: Baggot et al. (1988a) with permission.

interactions may alter the disposition of drugs. Assessment of changes in the disposition of a drug should include a comparison of the plasma concentration-time curves in healthy and affected animals and of the following pharmacokinetic parameters: systemic clearance, volume of distribution at steady-state as well as that calculated by the area method, and the half-life of the drug.

The time course of a drug in the body depends upon both the volume of distribution and the systemic clearance, while half-life reflects the relationship between these two parameters:

$$t_{1/2} = \frac{0.693 \times V_{d(area)}}{Cl_B}$$

It follows that an alteration in either or both of the basic parameters, V_d and Cl_B, may result in a change in the half-life, which is a derived parameter. Because of the variables on which the half-life depends, it cannot be used as the sole pharmacokinetic parameter to interpret the underlying changes associated with altered disposition of a drug.

Changes in volume of distribution may occur in disease or physiologic states where membrane permeability is altered (fever), extracellular fluid volume is changed (dehydration, neonatal period), or drug binding to plasma proteins is decreased (hypoproteinemia, uremia, competitive drug displacement). In studies of the effect of *E. coli* endotoxin-induced fever in dogs and etiocholanolone-stimulated fever in people on the serum concentrations of gentamicin, it was shown that serum gentamicin concentrations were lower during the febrile state, while the renal clearance (gentamicin is eliminated entirely by glomerular filtration) and the half-life of the drug were not significantly changed (Pennington et al., 1975). The lower serum concentrations could be attributed to increased extravascular distribution, although not of sufficient extent to significantly increase the half-life, of the aminoglycoside. Penicillin G distributes more widely in febrile than in normal animals (Figure 4.9). Even though infectious diseases have in common the presence of fever, the alterations produced in drug disposition will vary with the pathophysiology of the disease. When corresponding changes occur in volume of distribution and clearance of a drug, the half-life remains unchanged (Abdullah and Baggot, 1984, 1986). Corresponding significant increases in both the volume of distribution and systemic clearance of trimethoprim administered in combination with sulfadimethoxine or sulfamethoxazole occurred in febrile pneumonic pigs compared with healthy pigs; the half-life of trimethoprim remained unchanged. The disposition kinetics of neither sulfonamide was altered in the disease state (Mengelers et al., 1995). In the presence of an experimentally induced *E. coli* infection in pigs, the systemic clearance of enrofloxacin was significantly decreased while the volume of distribution remained unchanged. This resulted in an approximately 2.5-fold increase in the half-life of enrofloxacin (Zeng and Fung, 1997).

Changes in systemic clearance may occur when glomerular filtration is decreased (renal function impairment) or hepatic microsomal metabolic activity is altered. Alteration of blood flow to the organ of elimination may affect the clearance of antimicrobials. Halothane anaesthesia, for example, significantly decreased the clearance of gentamicin resulting in significantly higher plasma concentrations at 8 hours after IV administration of the drug (Smith et al., 1988).

Chloramphenicol, metronidazole and erythromycin

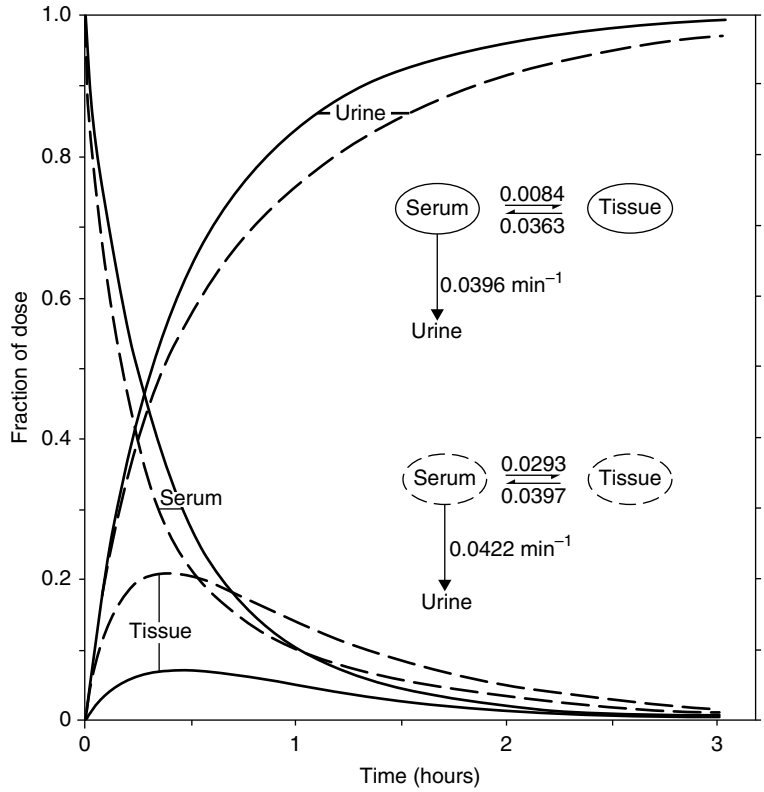

Figure 4.9. Analog computer–generated curves showing penicillin G concentrations (as fraction of the IV dose) in the central and peripheral compartments of the two-compartment open model and the cumulative amount excreted in the urine as a function of time. The curves are based on the first-order rate constants associated with the model that was used to describe the disposition kinetics of the drug in normal (solid line) and febrile (dashed line) dogs. From Baggot, 1980, with permission.

inhibit hepatic microsomal enzymes, while rifampin and various lipid-soluble drugs (e.g., phenobarbital) and xenobiotics induce hepatic microsomal enzymes. Prolonged fasting (> 48 hours), which is accompanied by hyperbilirubinemia, appears to decrease hepatic microsomal metabolic activity and thereby the rate of oxidative reactions and glucuronide synthesis. Although chronic liver disease or altered hepatic function can change the disposition of drugs that undergo extensive hepatic metabolism, indicator tests that would quantify the affected elimination process are not available for clinical application.

There is limited information regarding the influence of disease states, including gastrointestinal disease, on drug absorption. Decreased cardiac output (a feature of congestive heart failure) and, as a consequence, altered blood flow to the intestinal tract may influence the rate but probably not the extent of absorption (systemic availability). Combined IV and oral dose studies (i.e., determination of absolute bioavailability) are required to differentiate between altered absorption and disposition processes.

Dosage Regimen

Factors that affect drug dosage regimens are discussed in chapter 5. A dosage regimen entails the administration of a series of maintenance doses at a constant dosage interval. Additional features relating to clinical efficacy are the dosage form, which determines the route of administration, of the drug selected and the duration of therapy. The dosing rate and duration of therapy should be appropriate to treat the infection. It is because

bacterial susceptibility can be determined *in vitro* and values of the pharmacokinetic parameters describing bioavailability and disposition are known that dosing rates for antimicrobial agents can be calculated. The minimum effective plasma/serum concentration used in calculating the usual dosing rate for an antimicrobial is based on the MIC for the majority of pathogenic microorganisms that are susceptible to the drug. Variation in the degree of infection and in drug concentrations attained at various sites of infection is partly catered for by the range of doses that is recommended for use in an animal. With the notable exception of penicillins, the maximum dose that can safely be administered does not generally exceed 5 times the dose that would provide minimum effective plasma concentrations. It is usual to estimate the dose that would provide safe and effective plasma concentrations for an 8- or 12-hour dosage interval, depending on the (apparent) half-life of the antimicrobial agent.

Even though antimicrobial agents do not have a defined range of clinically effective plasma concentrations, a dosing rate based on maintaining a desired average steady-state plasma concentration throughout the dosage interval is a useful approach to therapy, especially for drugs that produce a bacteriostatic effect. They include tetracylclines, macrolides and lincosamides, sulfonamides when used alone, chloramphenicol and its derivatives. The dosing rate of a drug can be defined as the systemically available dose (F × Dose) divided by the dosage interval (τ):

$$\text{Dosing rate} = \frac{F \times \text{Dose}}{\text{Dosage interval}} = C_{p(avg)} \times Cl_B$$

where $C_{p(avg)}$ is the average plasma concentration of the drug at steady-state associated with multiple dosing at a fixed (selected) dosage interval and Cl_B is the systemic clearance of the drug. The relationship can be applied only to drugs that show linear pharmacokinetic behavior, that is, absorption and elimination are first-order processes. The desired average steady-state plasma concentration is a multiple of the MIC_{90}; the multiple represents the area under the inhibitory curve (AUIC) associated with the dosage interval. This approach may be applied to the calculation of dosing rates for fluoroquinolones, which produce a concentration-dependent effect on Gram-negative aerobic bacteria, using a number within the range 3–5 as the multiple of MIC_{90}. Because only the free (unbound to plasma proteins) drug is microbiologically active, the plasma drug concentration (measured as total drug) may be corrected for the extent of protein binding by calculating the fraction unbound (f_u) and expressed as free drug concentration in the plasma. This refinement is clinically worthwhile but infrequently applied.

Assuming knowledge of the systemic availability and the clearance of a drug, the average plasma concentration at steady-state that would be achieved by applying a fixed dosing rate can be predicted:

$$C_{p(avg)} = \frac{F \times \text{Dose}}{Cl_B \times \tau}$$

The longer the dosage interval (τ) relative to the half-life of the drug, the greater will be the degree of fluctuation in plasma concentrations at steady-state. By selecting a dosage interval similar to the (apparent) half-life of the drug, fluctuation in plasma concentrations will be minimized; fluctuation would be non-existent when the drug is administered by IV infusion.

When a drug is administered by IV infusion, the clearance of the drug determines the rate of infusion (R_o) that would be required to produce a desired steady-state (plateau) concentration:

$$R_o = C_{ss} \times Cl_B$$

The steady-state plasma concentration achieved by continuous infusion or the average plasma concentration at steady-state produced by multiple dosing at a constant dosage interval depends on the clearance of the drug. The time required to attain steady-state depends solely upon the half-life of the drug. After infusing the drug solution for a period corresponding to 4 times the half-life, the plasma concentration will be within 90% of the eventual steady-state concentration. Some third-generation cephalosporins (e.g., cefotaxime, ceftazidime) and ampicillin are among the relatively few antimicrobial agents for which continuous intravenous infusion is a feasible method of administration to animals. Steady-state can be attained either gradually by continuous infusion or

multiple dosing or promptly by administering a loading dose. The size of the loading dose that would provide a desired plasma concentration can be calculated:

$$\text{Loading dose} = C_{p(avg)} \times V_{d(area)}$$

Alternatively, the loading dose can be based on the fraction of drug eliminated during the dosage interval and be related to the maintenance dose of the dosage regimen. This approach is generally applied to antimicrobial agents that produce a bacteriostatic effect and have half-lives between 8 and 24 hours (e.g., conventional dosage forms of sulfonamides and tetracyclines).

Duration of Therapy

The success of antimicrobial therapy depends upon the administration of multiple doses, at an appropriate dosage interval, of a drug to which the causative pathogenic microorganisms are susceptible at the concentrations attained at the site of the infection and also on the duration of treatment. While both the microbiological and pharmacokinetic properties of the antimicrobial agent selected are taken into account in the dosage regimen, the duration of treatment is largely empirical. It is imperative that antimicrobial therapy be maintained for an adequate duration, which should be based upon monitoring the response both by clinical assessment of the animal patient (resolution of fever, leukocytosis and other signs of acute inflammation) and bacterial culture of properly collected specimens. Definitive diagnosis at an early stage of infection and the application of specific therapy, based on knowledge of the causative pathogenic microorganism and its susceptibility, will decrease the overall duration of treatment and minimize residual sequelae. An extended course of treatment is generally required in immunocompromised animals. Because of their potential to produce toxicity, due to preferential accumulation associated with selective binding to phospholipid (phosphatidylinositol)-rich tissues of the inner ear and kidney cortex, and ability (with the possible exception of amikacin) to induce plasmid-mediated bacterial resistance, therapy with an aminoglycoside antibiotic should not be extended beyond the duration required to treat the infection.

There are certain infections that, due to the relative inaccessibility of the causative pathogenic microorganisms to antimicrobial agents, invariably require a prolonged duration (3–5 weeks, rather than 6–10 days) of therapy. They include prostatitis, osteomyelitis and skin infections in dogs, and *Rhodococcus equi* pneumonia in foals.

Development of Antimicrobial Preparations

Blood concentration profiles generated at the low and high ends of the approved dose range, coupled with MIC data for commonly isolated bacterial pathogens, provide a basis for selection of the appropriate dose to use for the particular disease, organ system affected, and causative pathogenic microorganism. The dose range may be defined by a clinically confirmed dose at the lower end and target species safety (including consideration of human food safety for food-producing animals) at the upper end of the dose range (Martinez and Berson, 1998).

Pharmacokinetic/pharmacodynamic (PK/PD) relationships have been well described for many antimicrobials and provide the basis for development of veterinary drug product labels that bear a range of doses. This topic is discussed extensively in chapter 5.

Penetration into Cerebrospinal Fluid

The distribution of drugs from the blood into the central nervous system is unique because functional barriers, the blood-brain and blood-CSF barriers, are present that restrict entry of drugs into the CNS. Because brain capillary endothelial cells and choroidal epithelial cells have continuous tight junctions between adjacent cells, drug entry into the brain interstitial fluid and cerebrospinal fluid depends entirely on transcellular transport for which lipid solubility is a prerequisite. Drug penetration of the blood-CSF barrier is influenced by the concentration and rate of decline of the drug in blood plasma, the extent of binding to plasma proteins, and for drugs that are weak organic electrolytes, their degree of ionization in plasma (which is determined by pKa) and lipid solubility of the non-ionized moiety. Lipid-soluble, non-ionized drug molecules that are free (not bound to plasma proteins) in the blood plasma may enter brain interstitial and cerebrospinal fluids by passive diffusion.

Antimicrobial agents that penetrate the blood-CSF barrier include cefuroxime, cefotaxime, ceftazidime, ciprofloxacin, trimethoprim, sulfamethoxazole, sulfadiazine, metronidazole, chloramphenicol and fluconazole

(triazole antifungal agent). In the presence of meningeal inflammation and fever, the penetrative capacity of these antimicrobial agents is increased and penicillin G, which poorly penetrates the uninflamed meninges, may attain concentrations in CSF adequate to treat infection caused by susceptible microorganisms.

Drugs may leave the CSF by bulk flow into the venous sinuses, by passive diffusion of the non-ionized (lipid-soluble) form into the blood and, in addition, there are efflux carriers present in the choroid plexus that actively secrete the ionized form of organic acids from CSF into the blood. When the meninges are inflamed, carrier-mediated active transport of penicillins from the CSF to the blood is impaired (Spector and Lorenzo, 1974).

Passage into Milk

The bovine udder is richly supplied with blood mainly through the external pudendal arteries and supplemented by a subsidiary supply, cranially through the subcutaneous abdominal artery and caudally via the perineal artery. The ratio of the volume of blood circulating through the mammary gland to volume of milk produced has been estimated to be 670:1, at a moderate level of milk production. This provides ample opportunity for the unbound fraction of lipid-soluble drugs to passively diffuse from the systemic circulation into milk. The passage of antimicrobial agents into milk shows the influence of chemical nature, degree of ionization and lipid solubility, and extent of plasma protein binding on the equilibrium concentration attained across a cellular barrier. The validity of using the milk-to-plasma equilibrium concentration ratio for predictive purposes is highly dependent on the experimental design applied in obtaining the results. Steady-state can be achieved either by infusing the drug intravenously for a period exceeding 4 times the half-life or by administering a loading dose followed by maintenance doses, each one-half the loading dose, at intervals equal to the half-life of the drug. After attaining equilibrium, blood and milk samples should be collected at regular (30-minute) intervals and drug concentration be determined in ultrafiltrates of plasma and milk.

The majority of antimicrobial agents cross the blood-milk barrier, which is a somewhat restrictive functional rather than an anatomical barrier, by passive diffusion. Both non-polar lipid-soluble compounds and polar substances that possess sufficient lipid solubility passively diffuse through the predominantly lipoidal barrier. The rate of transfer is directly proportional to the concentration gradient across the barrier and the lipid solubility of the drug. The equilibrium concentration ratio of total (non-ionized plus ionized) drug is determined by the degree of ionization in blood and milk, the charge on the ionized moiety, and the extent of binding to plasma proteins and milk macromolecules. It has been shown that only the lipid-soluble, non-ionized moiety of a weak organic acid or base that is free (not bound to protein) in the plasma can penetrate cell membranes, enter the milk and diffuse into transcellular fluids. The milk-to-plasma equilibrium concentration ratio ($R_{m/p}$) can often be predicted (Rasmussen, 1966):

For an acid,

$$R_{milk/plasma} = \frac{1+10(pHm - pK_a)}{1+10(pHp - pK_a)}$$

or, for a base,

$$R_{milk/plasma} = \frac{1+10(pKa - pHm)}{1+10(pKa - pHp)}$$

where pH_m and pH_p are the pH reactions of milk and plasma, respectively, and pK_a is the negative logarithm of the acidic dissociation constant of an organic acid or base. In normal lactating cows (milk pH range 6.5–6.8), weak organic acids attain milk ultrafiltrate-to-plasma ultrafiltrate concentration ratios less than or equal to 1; organic bases, excluding aminoglycosides and spectinomycin (which are polar), attain equilibrium concentration ratios greater than 1 (Table 4.9). Some lipophilic bases concentrate (ion-trapping effect) in milk, these drugs have an advantage over other antimicrobial agents in the systemic treatment of mastitis. The significance of this favored distribution decreases with increasing pH of milk, particularly for macrolides (Table 4.10). The higher pH of mastitic milk (6.9–7.2) does not interfere with antibacterial activity of macrolides and aminoglycosides, whereas their activity would be decreased in a more acidic environment. An undesirable feature of the distribution of macrolides is diffusion from the systemic circulation into ruminal fluid (pH 5.5–6.5) where the

Table 4.9. Comparison of calculated and experimentally obtained milk:plasma concentration ratios for antimicrobial agents under equilibrium conditions.

Drug	Lipid Solubility	pK_a	Milk pH	Concentration Ratio (milk ultrafiltrate: plasma ultrafiltrate)	
				Theoretical	Experimental
Acids					
Penicillin G	Low	2.7	6.8	0.25	0.13–0.26
Cloxacillin	Low	2.7	6.8	0.25	0.25–0.30
Ampicillin	Low	2.7, 7.2	6.8		0.24–0.30
Cephaloridine	Low	3.4	6.8	0.25	0.24–0.28
Cephaloglycin	Low	4.9	6.8	0.25	0.33
Sulfadimethoxine	Moderate	6.0	6.6	0.20	0.23
Sulfadiazine	Moderate	6.4	6.6	0.23	0.21
Sulfamethazine	Moderate	7.4	6.6	0.58	0.59
Bases					
Tylosin	High	7.1	6.8	2.00	3.5
Lincomycin	High	7.6	6.8	2.83	3.1
Spiramycin	High	8.2	6.8	3.57	4.6
Erythromycin	Very high	8.8	6.8	3.57	4.6
Trimethoprim	High	7.3	6.8	2.32	2.9
Aminoglycosides	Low	1.8[a]	6.8	3.13	0.5
Spectinomycin	Low	8.8	6.8	3.87	0.6
Polymyxin B	Very low	10.0	6.8	3.97	0.3
Amphoteric					
Oxytetracycline	Moderate	–	6.5–6.8	–	0.75
Doxycycline	Moderate/high	–	6.5–6.8	–	1.53
Rifampin[b]	Moderate/high	7.9	6.8	0.82	0.90–1.28

[a]The pKa value given for aminoglycosides is unconfirmed.
[b]Theoretical concentration ratio for rifampin is based on its behavior as an organic acid (pK_a 7.9).

ion-trapping effect also applies. Because spiramycin avidly binds to tissue components, the persistence of drug residues is a major disadvantage associated with its use. Lipid solubility appears to be the principal factor that governs the passage of tetracyclines (amphoteric compounds) into milk and the equilibrium concentration ratios attained. Even though doxycycline is 85–90% bound to plasma proteins and oxytetracycline is only 20% bound, the equilibrium concentration ratio of doxycycline is 1.53 while that of oxytetracycline is 0.75 at milk pH within the range 6.5–6.8. Tetracyclines exert their greatest activity at an acidic pH close to their isoelectric point (5.5 for all tetracyclines apart from minocycline, 6.0). This implies that their antimicrobial activity would be less in mastitic milk (pH 6.9–7.2). Enrofloxacin and its active metabolite ciprofloxacin, formed by N-deethylation (a microsomal-mediated oxidative reaction) in the liver, would be expected to attain concentrations in milk that would be effective against Gram-negative aerobic bacteria, in particular *Escherichia coli* (Kaartinen et al., 1995).

The principal differences in mammary gland physiology are in the relative volume of milk produced by various species and in the composition of the milk, particularly the fat (triglycerides) and protein (casein) content.

Considerations in Pregnant Animals

Physiological adaptations that occur during pregnancy and could influence the oral bioavailability and disposition of drugs include an increase in gastric pH, an increase in the circulating blood (plasma) volume and in renal blood flow, an alteration in body fluid compartments, and hormonal-induced change in hepatic microsomal enzyme activity. A major concern in the use of

Table 4.10. Comparison of the fraction of dose recovered in normal and mastitic milk for antibiotics administered intramuscularly to cows.

i		Percentage Non-ionized in Plasma	Percentage of Dose Recovered in Milk	
Drug	pK_a		Normal	Mastitic
Acids				
Penicillin G	2.7	0.002	0.001	0.001
Cloxacillin	2.7	0.002	0.001	0.001
Ampicillin	27, 7.2		0.08	0.10
Amoxicillin	27, 7.2		0.06	0.15
Bases				
Tylosin	7.2	66.67	2.60	1.40
Spiramycin	8.2	13.68	6.80	2.40
Erythromycin	8.8	3.85	3.80	2.20
Spectinomycin[a]	8.8	3.85	0.04	0.08
Gentamicin[a]	7.8	28.47	0.006	0.01
Polymyxin	10.0	0.25	0.001	0.001
Amphoteric				
Oxytetracycline	–	–	0.07	0.08
Doxycycline	–	–	0.15	0.15

[a]Polar drug with low solubility in lipid.

drugs during pregnancy is the potential for adverse effects on the fetus, since all drugs administered to the mother cross the placental barrier, although at different rates, and the fetus is ill equipped to eliminate drugs. To what extent enzymes located in the placental membranes (e.g., microsomal drug-metabolizing system, which mediates various oxidative reactions, and cholinesterase) contribute to the conversion of drugs to inactive, more active or potentially toxic metabolites does not appear to have been established in domestic animal species.

Placental drug transfer by passive diffusion is similar to passage across any epithelial barrier, and in many respects resembles passage from the systemic circulation into milk of lactating animals. Since the pH of arterial blood in the fetus (7.27) is only slightly lower than in the mother (7.37), the ion-trapping effect whereby lipophilic organic bases attain higher concentrations in the milk does not apply to the fetal circulation. Drug diffusion across the placenta from mother to fetus is favored by lipid solubility, a large concentration gradient of unbound drug between the maternal and fetal circulations, and the presence of drug in the non-ionized form in the maternal circulation. Blood flow to the placenta limits the rate of delivery of drug to the fetal circulation. Conversely, molecules that are ionized (penicillins, cephalosporins), hydrophilic (aminoglycosides), and present in low free drug concentrations (doxycycline, macrolides, lincosamides) have restricted access to the fetus. Differences in the extent of plasma protein binding by the mother and fetus (in which it is lower) affects the total plasma drug concentrations in the maternal and fetal circulations. Regardless of the physicochemical properties of a drug, the duration of maternal therapy with the drug influences the concentrations that will be attained in the fetus. Some drugs known to diffuse well in other body fluids such a synovial and abdominal fluid do not necessarily reach therapeutic concentrations in fetal fluids. For example, concentrations of ceftiofur and related metabolites in placenta, fetal fluids, and fetal tissues are well below therapeutic concentrations after IM administration of ceftiofur to pregnant mares (Macpherson et al., 2012). In contrast, penicillin G and gentamicin undergo effective placental transfer in pregnant mares (Murchie et al., 2006).

Because toxic effects could be produced in the fetus caution should be exercised with the use in pregnant animals of a wide variety of antimicrobial agents (Table 4.11), while some others (fluoroquinolones, tetracyclines, griseofulvin) are contraindicated. When selecting an antimicrobial for administration to a pregnant animal, due consideration must be given to the potential of some of these drugs to produce adverse effects on the fetus.

Renal Excretion

Polar drugs and drug metabolites have restricted extravascular distribution, which may be largely confined to extracellular fluid, and undergo elimination by renal excretion. This is because of their limited capacity to passively diffuse through lipid membranes. Even though lipid-soluble drugs are mainly eliminated by hepatic metabolism, a fraction of the systemically available dose is usually eliminated by renal excretion. Because herbivorous species metabolize most lipid-soluble drugs more rapidly than carnivorous species, a smaller fraction of the dose is eliminated by renal excretion in herbivorous species, for example, trimethoprim (Table 4.2).

The renal excretion of drugs and drug metabolites involves glomerular filtration and, for some drugs and most metabolites, carrier-mediated proximal tubular

Table 4.11. Suggested cautions or contraindications of potentially toxic antimicrobial drugs in pregnant animals.

Drug	Toxicity	Recommended Use	
		Caution[a]	Contraindicated
Antibacterial			
Aminoglycosides	Auditory nerve toxicity?	+	
Chloramphenicol	Gray syndrome in newborn	+	
Fluoroquinolones	Arthropathy in immature animals	+	+
Metronidazole	Carcinogenic in rodents?	+	At term
Nitrofurantoin	Hemolytic anemia newborn	+	
Sulfonamides	Increased risk neonatal jaundice, teratogenic in some studies	+	
Tetracyclines	Tooth discoloration; inhibited bone growth in fetus; hepatic toxicity in pregnant animals with impaired renal function		+
Trimethoprim	Folate antagonist may cause congenital anomalies	+	
Antifungal			
Imidazoles, triazoles	Teratogenic	+	
Griseofulvin	Teratogenic		+

[a]Caution: Do not use if suitable alternative is available.

secretion. Extensive binding to plasma proteins limits the availability of drug molecules for glomerular filtration but might not hinder their secretion by proximal renal tubules because of rapid dissociation of the drug-protein complex. The glomerular filtration rate (GFR) varies among animal species. Average values of GFR, expressed in units of mL/min × kg body weight are: horse, 1.65; sheep, 2.20; cattle and goats, 2.25; pig, 2.80; cat, 2.94; dog, 3.96. At least in companion animal species (horses, dogs, and cats), endogenous creatinine clearance provides a clinically useful index of renal function (GFR).

Aminoglycoside antibiotics are eliminated almost entirely by glomerular filtration. Their half-lives reflect the relative rates of glomerular filtration in domestic animal species; the higher the glomerular filtration rate, the slower the half-life of an aminoglycoside. The half-life of gentamicin, for example, is 1.25 hours in dogs, 1.8 hours in cattle, and 2.6 hours in horses. The primary route of elimination for most tetracyclines including oxytetracycline is renal excretion. Doxycycline and minocycline, which are more lipid-soluble than other drugs in this class, are exceptions in that doxycycline is excreted in the feces as an inactive conjugate or chelate and minocycline may be eliminated mainly by metabolism. Enterohepatic circulation largely accounts for the relatively slow elimination of oxytetracycline that takes place by glomerular filtration. Fluconazole, unlike other azole antifungal drugs, is eliminated by renal excretion.

The majority of beta-lactam antibiotics (penicillins and cephalosporins) are eliminated both by glomerular filtration and proximal tubular secretion. Nafcillin (a staphylococcal penicillinase-resistant penicillin) and ceftriaxone and cefoperazone (third-generation cephalosporins) are exceptions in that they are mainly excreted by the liver in bile. Ceftiofur, following absorption into the systemic circulation, is converted by ester hydrolysis to desfuroylceftiofur, which has antibacterial activity similar to that of the parent drug.

Ciprofloxacin, a drug in its own right and the active metabolite of enrofloxacin, is mainly eliminated by renal excretion (glomerular filtration and proximal tubular secretion). Probenecid, by inhibiting renal tubular secretion, decreases the renal clearance of penicillins and ciprofloxacin. The beta-lactamase inhibitor clavulanic acid does not alter the disposition (i.e., distribution and elimination) of the penicillins (amoxicillin and ticarcillin) with which it is combined.

While a drug may enter tubular fluid both by glomerular filtration and proximal tubular secretion, its renal clearance may also be influenced by reabsorption from the distal nephron. As tubular reabsorption takes place by passive diffusion, it is influenced by lipid solubility and concentration of the drug in distal tubular fluid, and by the pKa/pH determined degree of ionization of weak organic acids and bases. The reabsorption of weak organic acids and bases is confined to the lipid-soluble

non-ionized form of these drugs. Alkalinization of the urine, by favoring ionization of weak organic acids (e.g., sulphamethoxazole, pKa 6.0; sulphadiazine, pKa 6.4) in distal tubular fluid, may increase their elimination while it may decrease the elimination, by promoting reabsorption, of weak organic bases (e.g., trimethoprim, pKa 7.3). At urine pH reactions of 6.0 and 8.0, the percentages of sulfadiazine that exist in the lipid-soluble non-ionized form are 71 and 2.4, respectively, and of trimethoprim are 5 and 83.4, respectively. The urine pH-excretion rate dependency is significant only when the fraction of the dose excreted in urine exceeds about 20% and the non-ionized moiety in distal tubular fluid is lipid-soluble.

Renal Impairment

Renal disease decreases the rate of elimination of drugs that are cleared predominantly by the kidneys. Reduced glomerular filtration rate decreases the elimination of penicillins (except nafcillin), cephalosporins (except ceftriaxone and cefoperazone), ciprofloxacin, fluconazole, and especially aminoglycosides. While reduced GFR decreases the elimination of tetracyclines, with the notable exception of doxycycline, changes in extravascular (tissue) distribution may exert an influence. Doxycycline, unlike other tetracyclines, is entirely eliminated by non-renal mechanisms and does not accumulate significantly in the presence of renal failure. These features make doxycycline the tetracycline of choice for use in dogs (half-life 7.0 hours) and cats (half-life 4.6 hours) with renal function impairment without a need to adjust the dosing rate. Renal blood flow can influence all of the processes involved in the excretion of drugs, but changes in renal blood flow are likely to have a more pronounced effect on the tubular processes than on glomerular filtration. Because of its narrow therapeutic index and high potential to cause nephrotoxicity associated with a dose-related decrease in renal blood flow, dosage with amphotericin B requires particular attention and renal function must be monitored during the course of treatment of systemic mycoses with this drug.

In the presence of impaired renal function modification of the usual dosing rate of an aminoglycoside antibiotic may be required to prevent accumulation of the drug with an increased risk of producing either ototoxicity or nephrotoxicity, or both. The dosing rate of an aminoglycoside should be adjusted in accordance with the decrease in renal function. An indication of the magnitude of the decrease in GFR may be obtained by measuring endogenous creatinine clearance in the animal. Dosage adjustment may be made either by reducing the dose and maintaining the usual dosage interval or by administering the usual dose at a longer dosage interval; the latter adjustment is preferable. Whatever dosing rate is used, trough plasma concentrations of gentamicin should not be allowed to exceed 2 μg/mL. Because dehydration enhances the toxicity of aminoglycosides, the concurrent use of an aminoglycoside and a diuretic agent, especially furosemide, which also has ototoxic potential, should be avoided. The nephrotoxic potential of aminoglycosides is influenced both by the dosing rate and the duration of therapy, which should not be extended beyond that required to cure the infection.

The dosage interval for fluconazole should be increased in the presence of impaired renal function. The adjustment could be based on the decrease in creatinine clearance. Depending upon the degree of renal impairment, consideration should be given to dosage adjustment of enrofloxacin that would allow for the decreased rate of excretion of ciprofloxacin. Since a significant fraction of the systemically available dose of marbofloxacin is eliminated by renal excretion, adjustment of dosage should be considered in the presence of renal impairment. Even though beta-lactam antibiotics, especially penicillins, have a wide margin of safety, the size of the dose should be decreased, depending on the decrease in creatinine clearance, in animals with renal failure. Since nafcillin, ceftriaxone, and cefoperazone are excreted by the liver in bile, dosage adjustment is not required in renal insufficiency.

In the presence of uremia associated with chronic renal failure, the binding of acidic drugs to plasma albumin is reduced and the rate of certain biotransformation pathways (e.g., reductive and hydrolytic reactions) is decreased. The significance of these alterations on the activity and dosage of antimicrobial agents that could be affected remains to be determined. It is likely that the activation of prodrugs (such as pivampicillin) would be decreased. The hydrolytic conversion of ceftiofur to desfuroylceftiofur could be decreased.

Modification of Dosage Regimens

The primary pathophysiologic sequelae relevant to antimicrobial dosing in renal dysfunction is a decreased GFR, which results in a decreased clearance of drugs eliminated by the kidney. Because of the large renal

Table 4.12. Antimicrobial drug dosage adjustments in the presence of renal failure.

Drug Class	Examples	Route of Elimination	Dosage Adjustment
Aminoglycosides	Amikacin, gentamicin, tobramycin	Renal	Contraindicated; interval extension if used[a]
Cephalosporins	Cefazolin, cephalexin,	Renal	Interval extension
	Cefaclor	Hepatic	No change
	Cephalothin	Renal, hepatic	2 × interval with severe renal failure
Lincosamides, macrolides	Clindamycin	Hepatic	No change
	Erythromycin, tylosin	Hepatic, renal	No change
	Lincomycin	Hepatic, renal	3 × interval in severe renal failure
Fluoroquinolones	Ciprofloxacin, enrofloxacin	Renal	Dosage reduction
Penicillins	Cloxacillin, oxacillin	Hepatic	No change
	Ampicillin, amoxicillin, carbenicillin, penicillin G, ticarcillin, clavulanic acid, imipenem-cilastatin	Renal, hepatic	Half-dose or 2 × interval in severe renal failure
Phenicols	Chloramphenicol	Hepatic	No change, but avoid in renal failure
Polymyxins	Polymyxin B		Contraindicated
Sulfonamides	Sulfisoxazole	Renal, hepatic	2–3 × interval in severe renal failure
	Trimethoprim-sulfamethoxazole	Renal, hepatic	No change, but do not use in severe renal failure
Tetracyclines	Tetracyclines	Renal, hepatic	Contraindicated, except for doxycycline
	Doxycycline	GI mucosa	No change
Miscellaneous	Amphotericin	Hepatic	Half-dose in severe renal failure
	Metronidazole	Hepatic, renal	Unknown

[a]Individual therapeutic drug monitoring should be used when available because of variability in disposition.

functional reserve, 75% of GFR must generally be lost before signs of clinical disease are readily evident. Adjustments to dosage regimens generally account only for decreased GFR, and unless therapeutic drug monitoring (TDM) is employed, other changes seen in severe renal dysfunction will not be accounted for.

The construction of modified dosage regimens in renal failure assumes that renal drug clearance directly correlates with clinical estimates of GFR (e.g., creatinine clearance or 1/serum creatinine), that the intact nephron hypothesis holds true, and that relative glomerular-tubular balance is present. In these cases, an antimicrobial's renal clearance is a linear function of GFR independent of whether the drug is filtered, secreted, and/or absorbed in the kidney. In addition, the volume of distribution of the drug is assumed to be unchanged.

When TDM is available, both a drug's clearance and volume of distribution may be directly determined in an individual pharmacokinetic study. The resulting individualized dosing regimen thus accounts for the renal insufficiency present. However, even in this scenario, as is true for other approaches, the shape of the serum concentration-time profile in an animal with renal failure cannot be made to precisely duplicate that in a healthy animal since the drug's clearance is reduced and half-life prolonged (Frazier and Riviere, 1987). In general, TDM is only employed for toxic antimicrobials whose accumulation would adversely affect the animal's health. A great deal of effort, both in veterinary and human medicine, has therefore been spent on the nephrotoxic aminoglycoside antibiotics. The effort is further necessitated by the great variability often seen in aminoglycoside pharmacokinetics in diseased animals where both creatinine clearance (Cl) and fluid status (Vd) are often changed (Frazier et al., 1988), necessitating close monitoring to avoid drug-induced nephrotoxicity.

The initial loading dose of the drug should be the same as in the normal animal. Dose-reduction schemes attempt to decrease the subsequent maintenance doses or increase the dosing interval, both in proportion to decreased Cl. Table 4.12 lists the antimicrobial agents commonly used in veterinary medicine for which modified dosage regimens can be formulated on the basis of existing data. Extrapolation from human studies is often necessary because of a lack of such work in animals. For drugs eliminated primarily by hepatic mechanisms (e.g.,

chloramphenicol) or drugs with wide safety indices (e.g., penicillins), dosage modification is often unnecessary. In cases in which such antimicrobials would be efficacious, the drugs that are cleared by hepatic mechanisms are preferred. When modification based on Cl is indicated, the following two methods are suggested.

Interval extension—administer normal maintenance dose:

$$\text{Interval} = \text{Normal Interval} \times \frac{(\text{Normal Cl}_{\text{Cr}})}{(\text{Patient Cl}_{\text{Cr}})}$$

Dose reduction—administer at the normal dose interval:

$$\text{Dose} = \text{Normal dose} \times \frac{(\text{Patient Cl}_{\text{Cr}})}{(\text{Normal Cl}_{\text{Cr}})}$$

In severe renal failure, use of the interval extension method may result in excessively prolonged periods of subinhibitory drug concentrations. In this case, half or one-third of the dose should be given at half or one-third, respectively, of the calculated intervals.

If Cl_{cr} is not available, some researchers have suggested that 1/SCR (serum creatinine) or 1/BUN (blood urea nitrogen) be substituted. In severe renal failure, this may not be accurate.

Considerable effort has been expended to define how a dosage regimen in a diseased individual can be constructed to maintain efficacy and avoid toxicity. This may be an impossible goal suggesting that trade-offs must be made. Close clinical monitoring is required to ensure antimicrobial efficacy and no drug-induced toxicity. The latter is especially difficult when detecting aminoglycoside-induced nephrotoxicity is confounded by the underlying renal dysfunction. For aminoglycosides, interval extension has been shown to produce less toxicity than dose reduction. The data are not as clear for other drugs. Serial monitoring of renal function tests (SCR, BUN), urinary enzymes, or TDM are the only approaches that may be used.

Finally, it must be stressed that if renal disease is present in food-producing animals, the decreased GFR would be expected to result in a prolonged elimination half-life, possibly necessitating a prolonged withdrawal time. Guidelines have not been established in veterinary medicine to address this problem, other than using drugs not eliminated by the kidney or using drugs with very short half-lives so that prolonged withdrawal times would suffice. Again, aminoglycosides, characterized by prolonged tissue half-lives, would be contraindicated. On-site urine monitoring could also be employed to reduce the chance of residues.

Urinary Drug Concentration

The concentration of a drug in the urine depends on the dose administered, the dosage form and route of administration, the extent of absorption (systemic availability) of the drug, the fraction of the systemically available drug excreted unchanged (as parent drug and/or active metabolite) in the urine, and the volume of urine produced, which is related to the hydration status of the animal. The urine pH reaction (usual range is pH 5.5–7.0 in dogs and cats; pH 7.2–8.4 in horses) may influence antimicrobial activity. Fluroquinolones, with the probable exception of difloxacin, are more active against Enterobacteriaceae and other Gram-negative aerobic bacteria in an alkaline environment.

The success of treatment of urinary tract infections depends on the maintenance for most of the dosage interval of high urinary concentrations (at least four-fold the MIC) of an antimicrobial agent to which the causative pathogenic microorganisms are at least moderately susceptible (chapter 23).

Elimination by the Liver

Most lipid-soluble drugs are eliminated by hepatic metabolism, principally by hepatic microsomal oxidation and glucuronide formation, and some are excreted unchanged (as parent drug) in bile. Antimicrobial agents that are mainly eliminated by hepatic metabolism include some fluoroquinolones (enrofloxacin, difloxacin, marbofloxacin), trimethoprim, sulfonamides, minocycline, chloramphenicol and its derivatives, clindamycin, metronidazole, rifampin, and azole antifungal drugs with the notable exception of fluconazole. Macrolides and lincosamides, nafcillin, cefoperazone and ceftriaxone are eliminated by biliary excretion, while marbofloxacin is partly eliminated by excretion in bile as well as in urine. Even though tetracyclines (except minocycline and doxycycline) are excreted in bile they are reabsorbed from the intestine and returned to the liver (enterohepatic cycle) for re-entry into the systemic circulation.

Liver blood flow and capacity of the liver to eliminate lipid-soluble drugs by metabolism or excretion in bile are the principal factors that determine hepatic clearance. Extensive binding to plasma proteins may limit access of a drug to metabolizing enzymes within hepatocytes. Differences in the rates of hepatic microsomal oxidative reactions or in conjugate (especially glucuronide) formation often account for species variations in the hepatic clearance of a lipid-soluble drug. The extent to which differences in clearance affect half-life is influenced by the apparent volume of distribution of the drug, since half-life is a hybrid pharmacokinetic parameter.

Moderate or severe liver damage reduces the capacity of the liver to eliminate antipyrine (a marker substance for microsomal oxidative activity) and indocyanine green (marker substance for biliary secretion that may be influenced by liver blood flow). The uncertainty associated with quantification of the degree of hepatic dysfunction and its influence on the clearance of lipid-soluble drugs makes it difficult to predict dosage adjustment that might be required. In general, the dosage interval for a drug that is mainly eliminated by hepatic metabolism should be increased in the presence of impaired liver function and preference should be given to the use of a drug that has a bactericidal action. Likewise, the dosage interval of an antimicrobial agent that is extensively metabolized by the liver should be increased when it is used concomitantly with a drug that inhibits microsomal oxidative reactions (such as ketoconazole, omeprazole, or cimetidine). A somewhat contrasting situation applies when griseofulvin and phenobarbital are used concomitantly in epileptic dogs in that the dose level (mg/kg) of phenobarbital may have to be increased in order to prevent convulsive seizures from occurring. Both griseofulvin and phenobarbital induce hepatic microsomal oxidative activity. The rate of oxidative metabolism of metronidazole is increased by phenobarbital and rifampin.

Absorption and Disposition in Neonatal Animals

The neonatal period, which is generally considered to be the first month of postnatal life, varies among species. It appears to be 1–2 weeks in foals; about 8 weeks in calves, kids, lambs, and piglets; and 10–12 weeks in puppies. However, the most profound adaptive changes in physiological variables occur during the first 24 hours after birth in all species. This coincides with the time that the pharmacokinetic behavior of drugs is most "unusual" (Baggot and Short, 1984). Some characteristics of the neonatal period include better absorption from the gastrointestinal tract, lower binding to plasma proteins, lower ratio of body fat-to-fluids, increased volume of distribution of drugs that distribute in extracellular fluid or total body water, increased permeability of the "blood-brain" barrier, and slower elimination (longer half-life) of most drugs.

Antimicrobial agents, such as penicillins, that are poorly absorbed and cause digestive disturbances in older foals (over 4 months of age) and adult horses can be administered orally to neonatal and young (up to 4 months of age) foals for the treatment of systemic bacterial infections caused by susceptible microorganisms. Oral administration of amoxicillin trihydrate (30 mg/kg), as a 5% oral suspension, to 5- to 10-day-old foals produced serum amoxicillin concentrations above 1 μg/ml for 6 hours (Love et al., 1981). Systemic availability of amoxicillin was 30–50% in the foals compared with 5–15% in adult horses (Baggot et al., 1988). Pivampicillin, a prodrug of ampicillin, has systemic availability (ampicillin) of 40–53% in foals between 11 days and 4 months of age (Ensink et al., 1994). In adult horses, the systemic availability of ampicillin administered as pivampicillin is within the range 31–36%. The half-life of aminobenzyl penicillins is approximately two-fold longer following oral than intravenous administration. It may be because of their moderate extent of absorption that the detrimental effect of oral penicillins, which is due to severe disturbance of the balance between the commensal bacterial flora in the colon of adult horses, is avoided in neonatal and young foals. There is no need to adjust the dosage interval in neonatal foals since penicillins in the systemic circulation have a wide margin of safety. Penicillin V, the phenoxymethyl analog of penicillin G, does not have a place (due to low systemic availability and the production of digestive disturbances) in the treatment of bacterial infections in foals or adult horses (Baggot et al., 1990).

The systemic availability of cefadroxil (5% oral suspension) decreases progressively from 68% in 1-month-old foals to 14.5% in foals 5 months of age (Duffee et al., 1997). The half-life of the drug remains unchanged over this age range. Cephradine, another first-generation oral cephalosporin, administered in sucrose syrup to 10- to 14-day-old foals has an average systemic availability of 64% and half-life of 1.1 hours (Henry et al., 1992).

Table 4.13. Age-related changes in the disposition kinetics of chlorampenicol in calves (50 mg/kg, IV) and foals (25 mg/kg, IV).

Age	$V_{d(ss)}$ (mL/kg)	Cl_B (mL/min/kg)	$t_{1/2}$ (h)
Calves (n = 5)			
1 day	1130 ± 50	1.1 ± 0.24	11.7 ± 1.7
7 days	1180 ± 70	1.9 ± 0.03	7.5 ± 0.9
10–12 weeks	1230 ± 60	3.1 ± 0.63	4.9 ± 0.7
Foals (n = 6)			
1 day	992 ± 269	2.25 ± 0.67	6.19 ± 2.43
3 days	543 ± 173	6.24 ± 2.22	1.48 ± 0.51
7 days	310 ± 67	8.86 ± 1.90	0.64 ± 0.14

Table 4.14. Disposition kinetics of enrofloxacin and formation of ciprofloxacin in newborn and 1-week-old Finnish Ayrshire calves.

Pharmacokinetic Parameter	Age of Calves		Statistical Significance
	1 day	1 week	
Enrofloxacin			
$V_{d(ss)}$ (l/kg)	1.81 ± 0.10 (1.54–2.01)	2.28 ± 0.14 (1.88–2.52)	P = 0.035
Cl_B (l/h × kg)	0.19 ± 0.03 (0.14–0.28)	0.39 ± 0.06 (0.31–0.56)	P = 0.021
$t_{½}$ (h)	6.61 ± 1.12 (4.28–9.36)	4.87 ± 0.68 (3.13–6.43)	Not significant
Ciprofloxacin			
t_{max} (h)	15.0 ± 3.0 (12–24)	2.8 ± 0.8 (1–4)	P = 0.007
C_{max} (mg/L)	0.087 ± 0.017 (0.07–0.14)	0.142 ± 0.005 (0.13–0.15)	P = 0.023

Note: A single dose (2.5 mg/kg) of enrofloxacin was administered by intravenous injection to the calves (n = 4 in each age group). Results are expressed as mean ± SEM (and range).

Since the rumen takes 4–8 weeks to develop and become functional, the bioavailability (rate and extent of absorption) of drugs administered orally to preruminant calves resembles that in monogastric species rather than in cattle. Although chloramphenicol is not approved for use in food-producing animals, a comparison between preruminant calves and neonatal foals is informative. Chloramphenicol, administered as an oral solution, is well absorbed in preruminant calves and oral dosage (25 mg/kg at 12-hour dosage intervals) will maintain therapeutically effective plasma concentrations (> 5 μg/ml) of the antibiotic (Huffman et al., 1981). In ruminant calves and adult cattle, orally administered chloramphenicol fails to provide effective plasma concentrations since the antibiotic is inactivated (reductive reaction) in the rumen. A single oral dose (50 mg/kg) of chloramphenicol solution administered to foals between 3 and 8 weeks of age produced an average peak plasma/serum concentration of 6 μg/ml, which was lower than the peak concentration produced in adult horses (18 μg/ml) given the drug at the same dose level (Buonpane et al., 1988). Changes in the disposition kinetics of chloramphenicol (administered as a single IV dose) are age-related and the pattern of the change differs between species; a marked increase in the rate of chloramphenicol elimination (hepatic metabolism) during the first week after birth is a consistent finding (Table 4.13). Assuming that chloramphenicol is mainly eliminated by glucuronide conjugation, it would appear that this microsomal-associated metabolic pathway develops far more rapidly in foals (within 1 week; Adamson et al., 1991) than in calves (8–12 weeks; Reiche et al., 1980). This finding is consistent with the shorter neonatal period in foals than in calves.

Antimicrobial agents that undergo extensive first-pass metabolism by hepatic microsomal oxidative reactions would be expected to have higher systemic availability in neonatal animals. This applies to trimethoprim, which has far higher systemic availability in newborn kids than in older kids and adult goats. In addition to lower hepatic microsomal oxidative activity, the ruminal microflora have not developed in neonatal ruminant species.

Since disposition refers to the simultaneous effects of distribution and elimination, it is necessary to consider both components of the process when interpreting changes that occur during the neonatal period or in the presence of a disease state. Enrofloxacin is converted by N-dealkylation, a hepatic microsomal oxidative reaction, to ciprofloxacin. Both enrofloxacin and ciprofloxacin are active antimicrobially. Comparison of the disposition kinetics of enrofloxacin (2.5 mg/kg administered IV) in 1-day-old and 1-week-old calves shows that the volume of distribution at steady-state is smaller and the systemic clearance of the drug is lower in the 1-day-old calves, while the half-life does not differ significantly between the 1-day-old and 1-week-old calves (Table 4.14). The

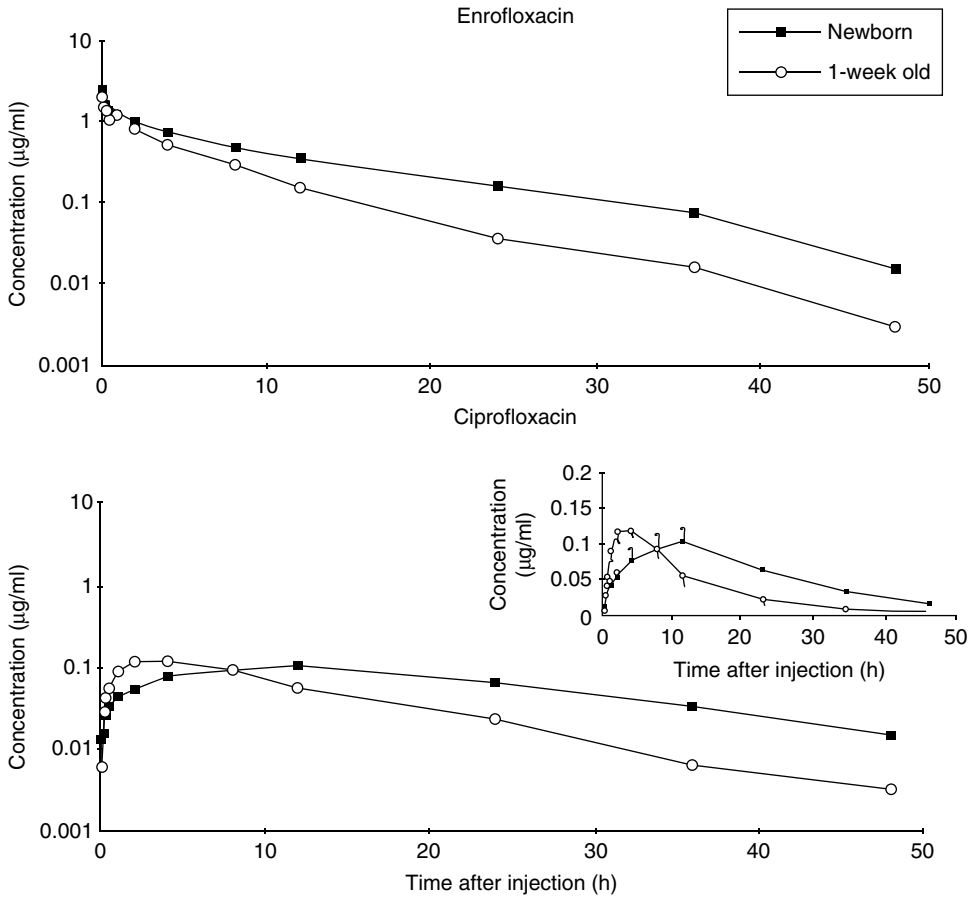

Figure 4.10. Mean concentration-time curves of enrofloxacin and its metabolite ciprofloxacin in newborn and 1-week-old calves (4 calves per group). Enrofloxacin was administered IV at a dosage of 2.5 mg/kg. Drug concentrations were analyzed using an HPLC method. The insert in the lower panel shows the mean ciprofloxacin concentrations and standard errors of mean on a non-logarithmic scale.

changes in the disposition kinetics of enrofloxacin that occur during the first week of postnatal life in calves could be attributed to differences in plasma protein binding of enrofloxacin and in the body fat-to-fluids ratio since fluoroquinolones are lipid-soluble drugs. Newborn calves metabolize enrofloxacin to ciprofloxacin but the rate of formation of the active metabolite is slower and the peak serum concentration (C_{max}) is lower than in the 1-week-old calves; mean t_{max} is about 5 times longer in newborn calves (Figure 4.10; Kaartinen et al., 1997). Since the content of cytochrome P-450 has been shown to double during the first week of postnatal life in calves (Shoaf et al., 1987), it can be concluded that the rate of conversion of enrofloxacin to ciprofloxacin is age-related. Following IV administration of a single dose (2.5 mg/kg) of enrofloxacin, the sum of enrofloxacin and ciprofloxacin concentrations in plasma/serum was above 0.1 µg/ml at 30 hours and 24 hours in 1-day-old and 1-week-old calves, respectively. The minimum inhibitory concentration for the majority of susceptible *E. coli* strains (MIC_{90}) isolated from calves is 0.25 µg/ml.

Although there are species differences in the degree to which some drug metabolic pathways are deficient in neonatal animals, a relative lack of development of hepatic smooth-surfaced endoplasmic reticulum and its associated drug metabolizing enzyme systems (mediate

oxidative reactions and glucuronide conjugation) appears to be a characteristic of the neonatal period in all mammalian species. Because of the low activity of most metabolic pathways, the half-lives of drugs that undergo extensive hepatic metabolism are prolonged in neonatal animals, particularly during the first 24 hours after birth. The maturation of the various metabolic pathways could be related to hormonal influence on postnatal enzyme induction. In the majority of species (ruminant animals, pigs, dogs, and presumably cats), the hepatic microsomal-associated metabolic pathways develop rapidly during the first 3–4 weeks after birth, and at 8–12 weeks of age have developed activity approaching that of adult animals (Nielsen and Rasmussen, 1976; Reiche, 1983). The foal appears to be an exception in at least the rate of development of glucuronide synthesis, which develops very rapidly during the first week after birth (Adamson et al., 1991). While a long dosage interval should be applied during the first 3 days after birth, it can gradually be decreased, depending on the animal species, as the neonate matures.

Conversion of ceftiofur to desfuroylceftiofur, a third-generation cephalosporin, is catalyzed by an esterase, which is most active in the kidneys followed by the liver (Olson et al., 1998). Desfuroylceftiofur has antibacterial activity similar to that of the parent drug and the active metabolite rapidly becomes reversibly bound to proteins in plasma and tissues and forms conjugates with glutathione and cysteine. The high-performance liquid chromatographic (HPLC) assay method measures the combined plasma concentration of ceftiofur and desfuroylceftiofur conjugates as a single derivative, desfuroylceftiofur acetamide, which is expressed as micrograms of ceftiofur free acid equivalents per ml (Jaglan et al., 1990). In a study of the influence of age on the disposition kinetics of ceftiofur, administered IV as ceftiofur sodium at a dose of 2.2 mg ceftiofur free acid equivalents per kg body weight, in Holstein bull calves, the volume of distribution at steady-state ($V_{d(ss)}$) decreased and systemic clearance (Cl_B) increased during the first 3 months after birth (Brown et al., 1996). The progressive decrease in volume of distribution of ceftiofur and desfuroylceftiofur conjugates could be attributed to the age-related decrease in extracellular fluid volume. The lower clearance in the 7-day-old and 1-month-old calves than in the older calves is probably due to maturation of the processes of elimination for ceftiofur and desfuroylceftiofur metabolites.

Table 4.15. Comparison of pharmacokinetic values derived from plasma concentrations of ceftiofur and metabolites after IV injection of ceftiofur sodium in Holstein bull calves of various ages.

Age	$V_{d(ss)}$ (ml/kg)	Cl_B (ml/h/kg)	$t_{½}$ (h)
1 week	345 ± 62	17.8 ± 3.2	16.1 ± 1.5
1 month	335 ± 92	16.7 ± 3.1	17.2 ± 3.1
3 months	284 ± 49	30.3 ± 4.6	8.2 ± 2.8
6 months	258 ± 72	39.8 ± 14.9	5.95 ± 1.2

Note: Plasma concentrations of ceftiofur and metabolites were measured as desfuroylceftiofur acetamide by HPLC. Dosage of ceftiofur sodium was 2.2 ceftiofur free acid equivalents per kilogram.

Because the decreases in volume of distribution were proportionally less than the increases in clearance in calves 1 month of age and older, the half-life decreased more or less in accordance with the increased clearance (Table 4.15). Plasma concentrations of ceftiofur and its metabolites (measured as a single derivative) remained above the limit of quantification (LOQ, 0.15 μg/ml) of the assay method for the entire 72-hour blood-sampling period in 7-day-old and 1-month-old calves, but decreased to below the LOQ within 48 hours of drug administration to 6- and 9-month-old calves. In foals, half-life, clearance and $V_{d(ss)}$ of desfuroylceftiofur acetamide after intravenous administration of ceftiofur sodium to neonatal (< 1 week of age) and 4- to 5-week-old foals are not significantly different (Meyer et al., 2009).

The renal excretion mechanisms (glomerular filtration and active, carrier-mediated tubular secretion) are incompletely developed at birth in all mammalian species. During the neonatal period renal excretion mechanisms mature independently at rates that are species-related. GFR, based on inulin clearance, attains adult values at 2 days in calves; 2–4 days in lambs, kids, and piglets; and may take at least 14 days in puppies. Proximal tubular secretion, based on clearance of para-aminohippurate, matures within 2 weeks after birth in the ruminant species and pigs, but may take up to 6 weeks in dogs. Indirect evidence, provided by pharmacokinetic studies of some antimicrobial agents, suggests that renal function develops rapidly in foals at a rate similar to that in ruminant species. In a recently published study of the maturation of renal function in

full-term pony foals during the first 10 days postpartum, it was shown (using the single injection technique) that the glomerular filtration rate and effective renal plasma flow remain relatively constant throughout the postnatal period (Holdstock et al., 1998). This implies that the neonatal foal, like the calf, has relatively mature renal function compared with neonates of most other species. The hydration state of newborn animals would affect renal function (GFR). Even though renal function is immature in neonatal, particularly newborn, animals it has the capacity adequate to meet physiological requirements. However, when lipid-soluble drugs are administered to neonatal animals, the combined effect of slow hepatic microsomal associated metabolic reactions (oxidation and glucuronide conjugation) and relatively inefficient renal excretion mechanisms considerably decreases the rate of elimination of the parent drugs and their polar metabolites. Urinary pH is acidic in neonates of all species; this would favor renal tubular reabsorption and extend the half-life of drugs that are weak organic acids and of sufficient lipid solubility to be reabsorbed by passive diffusion (e.g., most sulfonamides).

The pharmacokinetic parameters describing the disposition of gentamicin (4 mg/kg, IV) were determined in foals of various ages (12–24 hours, 5, 10, 15, and 30 days) and in mares (Cummings et al., 1990). The apparent volume of distribution of the aminoglycoside did not change significantly with age of the foals, but was approximately two-fold larger than in mares. In another study, administration of gentamicin at a dose of 12 mg/kg to foals of various ages (1–3 days, 2, 4, 8, and 12 weeks of age) resulted in a significantly higher volume of distribution in 1- to 3-day-old foals than in 8- or 12-week-old foal (Burton et al., 2012). Since the distribution of gentamicin is virtually restricted to the extracellular fluid (ECF), it could be concluded that ECF volume is larger in young foals than in adult horses. Gentamicin is eliminated solely by glomerular filtration. The systemic clearance of gentamicin in newborn foals is similar to that in adult horses; this indicates that glomerular filtration is well developed in newborn foals. Because of the larger volume of distribution and unchanged systemic clearance, the half-life of gentamicin in newborn foals is twice as long as in adult horses, while in foals between 5 and 15 days of age, it is approximately 1.5 times the half-life in adult horses. The pattern of age-related changes in the disposition of gentamicin in calves (Clarke et al., 1985) is similar to that in foals (Table 4.16).

Table 4.16. Age-related changes in the disposition of gentamicin in foals and calves.

Age (days)	$V_{d(ss)}$ (mL/kg)	Cl_B (mL/min/kg)	$t_{1/2}$ (min)
Foals			
1	307 ± 30	1.75 ± 0.47	127 ± 23
5	350 ± 66	2.98 ± 1.48	90 ± 32
10	344 ± 95	2.60 ± 0.96	101 ± 33
15	325 ± 48	2.40 ± 0.87	106 ± 33
Mares	156 ± 22	1.69 ± 0.65	65 ± 55
Calves			
1	376 ± 41	1.92 ± 0.43	149 ± 38
5	385 ± 44	2.44 ± 0.34	119 ± 20
10	323 ± 20	2.02 ± 0.27	118 ± 13
15	311 ± 29	2.10 ± 0.32	111 ± 8.5
Cows	129 ± 17	1.29 ± 0.26	76 ± 11

The disposition of gentamicin differs significantly between newborn (4–12 hours of age at the time of dosing) and 42-day-old piglets (Giroux et al., 1995). The age-related pattern of changes in piglets is consistent with that in foals and calves. As the neonate matures, the apparent volume of distribution decreases, systemic clearance increases and the half-life of gentamicin becomes shorter. The average half-life of gentamicin is 5.2 hours in newborn piglets, and 3.8 hours, 3.5 hours, and 2.7 hours in 4-, 6-, and 10-week-old piglets, respectively. In a study of the pharmacokinetics of amikacin in critically ill full-term foals ranging in age from 2 to 12 days, the systemic clearance of the aminoglycoside was lower and the half-life was considerably prolonged in uremic compared with non-uremic foals (Adland-Davenport et al., 1990). Renal excretion mechanisms appear to mature within the first 2 weeks after birth in ruminant species, horses and pigs, whereas their maturation in dogs may take 4–6 weeks.

The half-life of ceftriaxone, a third-generation cephalosporin that distributes widely in body fluids, penetrates the blood-brain barrier and is eliminated by biliary rather than renal excretion, is two-fold longer in 2- to 12-day-old foals (Ringger et al., 1998) than in adult horses (Ringger et al., 1996). The longer half-life of the drug could be attributed to the larger volume of extracellular fluid in the neonatal foals. The average half-life of erythromycin, administered IV as erythromycin

gluceptate, in Shetland-cross foals of various ages (from 1 to 12 weeks old) is the same (1 hour) as in mares. It is likely that biliary and renal excretion mechanisms mature at the same rate in neonatal animals of any species, while hepatic formation of conjugates controls the rate of their excretion in bile and/or urine.

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5

The Pharmacodynamics of Antimicrobial Agents

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Introduction

For any infectious disease process, the efficacy of antimicrobial therapy is dependent upon the ability of the pathogen to respond to the antimicrobial therapy, the drug exposure characteristics necessary to elicit the targeted microbiological response, and the ability to achieve the necessary active drug concentrations at the site of the infection. The relationship between systemic drug exposure and its corresponding clinical and microbiological effects is termed *pharmacokinetics/pharmacodynamics* (PK/PD). This PK/PD relationship, in turn, dictates the dose, dosing frequency, and duration of drug administration necessary to achieve the desired clinical and microbiological outcome.

The PK component describes the handling of the drug by the host (absorption, distribution, metabolism, and elimination). Since basic PK principles have already been covered in chapter 4 of this text, we will not reiterate this information except as necessary to expand concepts to a potential patient population. The PD component describes the effect of the drug over time on the bacteria at the site of infection. Thus, the interplay between PK and PD reflects the relationship between the fluctuating concentrations of biologically active drug at the site of infection, as reflected by its serum or plasma drug concentrations, versus its effects on the targeted microbial pathogen (Drusano, 1998; Levison, 2004).

It is the goal of this chapter to enumerate the many factors that influence this overall paradigm. In addition, we discuss how these relationships influence the multidimensional uses of antimicrobial agents as encountered across veterinary species.

Interpreting the MIC from a Clinical Perspective

When assessing the selection of drug, dose, and dosing regimen, a fundamental question is the pathogen's ability to respond to that antimicrobial agent. In this regard, tests describing the minimum inhibitory concentration (MIC) are invaluable. Although the *in vitro* growth conditions associated with these tests differ in many ways from those occurring *in vivo*, the strength of these methods is their ability to be standardized. In so doing, the MIC derived with one set of isolates from a particular clinical laboratory should not differ from that which would be derived from any other clinical laboratory utilizing the same standardized procedure.

The MIC is measured using a range of antimicrobial concentrations in a suitable growth medium into which the strain is inoculated, incubated for a period relevant to the growth rate of the strain (usually ~18–24 hours), and then examined for complete or near complete inhibition of growth (usually prevention of turbidity). The MIC is the lowest concentration in the range tested that inhibits growth.

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The features of MIC measurement differ significantly from what might be observed *in vivo*:

- Concentrations of antimicrobial are fixed over the time of incubation (assume the agent is stable), rather than the fluctuating concentrations occurring *in vivo* with intermittent dosing.
- The growth medium may differ physiologically in many ways (pH, osmolarity, redox potential, cation and protein concentration, etc.) from the fluid environment at the site of infection.
- The test does not include host factors such as phagocytic cells, antibodies, complement, or other immunologically active molecules.
- The end point is growth inhibition, rather than killing, the latter being the therapeutic goal for many antimicrobial agents.
- It provides no information on the persistent effects of antimicrobials, which have been well documented for most antimicrobial classes (Levison, 1995).
- Traditional *in vitro* susceptibility test methods also cannot describe the impact of a drug on pathogen virulence factors (Clatworthy et al., 2007: Barczak and Hung, 2009; Cegelski et al., 2008; Alksne and Projan, 2000). These factors are responsible for anchoring to and invasion into host cells, quorum sensing (the bacterial production of autoinducers that regulate gene expression within the bacterial colony), and the production of toxins and factors that influence host immune functions.

For these reasons, the MIC is frequently criticized for being a poor indication of *in vivo* antimicrobial activity and the clinical relevance of the MIC values has been called into question (Müller et al 2004; Firsov et al., 1998, 1999). Nevertheless, the MIC is a value that investigators frequently compare to antimicrobial exposure. From this perspective, criticisms against the MIC and its use for comparison with *in vivo* drug concentrations are misdirected. The MIC is simply a standardized measure of antimicrobial activity whose true value resides in its property as a unifying factor in the PD indices (Craig, 2002). In that sense, the actual values do not matter: what does matter is how it is measured. To be an effective measure, it must be performed in a robust way that is reliable and reproducible wherever it is performed); hence the value of an internationally standardized approach. The importance of such standardization has only recently been recognized (International Organization for Standards, 2006).

Ultimately, factors impacting the *in vivo* activity of an antimicrobial need to be appreciated in order to understand how antimicrobials act over time at the site of infection. In this regard, the full range of properties include bactericidal activity, as measured for instance by the minimum bactericidal concentration (MBC; NCCLS, 1999), shape of the concentration-effect profile, sub-MIC effects, post-antibiotic effects (PAE), post-antibiotic sub-MIC effects, and post-antibiotic leucocyte enhancement (Levison, 1995; Craig, 2002). Furthermore, different strains of pathogen may have similar MIC values but require different levels of drug exposure to achieve the desired clinical response (Andes and Craig, 2002).

In vivo generation time (O'Reilley et al., 1996) can also influence the *in vivo* exposure-response relationship (Erlendsdottir et al., 2001). For example:

- Ceftriaxone (an antibiotic closely related to the veterinary compound, ceftiofur), is rapidly bactericidal against fast growing bacteria but even a modest decrease in bacterial growth rate (engendered by nutrient limitation) renders it bacteriostatic. This loss of beta-lactam cidal activity is drug specific and certain compounds, including amoxicillin and benzylpenicillin, maintain their bactericidal activity in the presence of an increased generation time (Cozens et al., 1986). The selective loss of cidal activity against slow or non-growing bacteria has been explained by an alteration in outer membrane composition, the impact of which is related to the permeability of the specific antibiotic.
- When bacterial growth rate is reduced by limiting nutrient supply, the bactericidal activity of the quinolones against *E. coli* is minimally affected. Loss of cidal activity against *S. aureus* is slightly more pronounced as compared to that seen with *E. coli*. However, the killing activity of several fluoroquinolones (including ciprofloxacin, floxacin, norfloxacin, and ofloxacin) is markedly enhanced (up to 176%) when the growth rate of *P. aeruginosa* is reduced (Dalhoff et al., 1995). These changes have been explained as a function of an adaptive response in the *Pseudomonas* outer membrane in the presence of nutrient limitation. This change, while enhancing

penetration of a limited nutrient supply simultaneously sensitizes the bacterium to the killing activity of these compounds.

- Biofilms include slow-growing or stationary phase cells, and only those bacteria in non-growing zones of a biofilm survive an antimicrobial challenge. However, factors other than simply a slow growth rate may contribute to antibiotic resistance in biofilms (Stewart, 2002). For example, in the case of *P. aeruginosa*, biofilm antimicrobial resistance appears, at least in part, to relate to the gene ndvB, which is involved in the formation of periplastic glucans. These periplastic glucans are thought to sequester drug molecules, thereby preventing drug interaction with their bacterial drug targets. Simultaneously, expression of the ndvB gene is believed to enhance the expression of multiple genes that have also been linked with biofilm-associated antimicrobial resistance (Mah et al., 2003; Beaudoin et al., 2012).

Because of its power as a unifying factor in PD indices, an understanding of the MIC as a measurement is important. MICs are always measured on an interval scale, with the 2-fold dilution series being the most often recommended, although any other scale could be used, such as the arithmetic scale (Legett and Craig, 1989). The 2-fold dilution series is a logarithmic scale, based on logarithms to the base 2. The most popular 2-fold dilution series is that based on the integer powers of 2, (e.g., . . . 2^{-2}, 2^{-1}, 2^{0}, 2^{1}, 2^{2}, . . . = . . . 0.25, 0.5, 1, 2, 4, . . .). The original choice of the logarithmic scale for MIC testing was serendipitous, for when a large number of strains of a microbial species have their MICs measured on this scale, the wild-type stains (i.e., those lacking an acquired resistance mechanism) show a lognormal distribution of MICs (Turnidge et al., 2006).

Like any assay, MIC measurements have an intrinsic variance. This is easily appreciated by examination of any study that attempts to establish quality control ranges for MIC testing (Brown and Traczewski, 2009). The variance of the assay is frequently stated to be "± one 2-fold dilution" (CLSI, M23-A3), but this is a gross oversimplification, as any inspection of raw data from a quality control range-setting study will reveal. An MIC distribution for a particular antimicrobial-species combination, such as one of those found on the EUCAST website (http://mic.eucast.org/Eucast2/), is therefore a composite of biological variation between strains, and assay variance. This composite variation is accounted for when undertaking Monte Carlo simulation of antimicrobial dosing regimens to construct target attainment graphs at different MICs, the latter being used to establish PD cutoff values that are incorporated into the development of clinical breakpoints for susceptibility testing (Turnidge and Paterson, 2007).

Understanding the Drug Response

The first step in understanding PK/PD relationships is to identify the mechanisms through which the drug-pathogen interactions occur (Table 5.1). In general, these mechanisms of action dictate the PD characteristics of the drug, including whether it will result in static or cidal activity, its rate of kill, the ability to suppress growth after local drug concentrations have dropped below the microbial MIC, and its ability, if any, to act on bacteria that are in a stationary growth phase.

Table 5.1. Actions of the various classes of antimicrobial.

1. Agents that inhibit cell wall synthesis:
   a. Penicillins
   b. Cephalosporins
   c. Carbapenems
   d. Monobactams
   e. Vancomycin
2. Agents affecting the function of 30s and 50s ribosomal subunits, resulting in a reversible inhibition of protein synthesis, and are generally considered to exert primarily bacteriostatic effects:
   a. Chloramphenicol and florfenicol
   b. Tetracyclines
   c. Macrolides
   d. Ketolides
   e. Azolides
   f. Lincosamides
3. Agents binding to the 30s ribosomal subunit, inhibiting bacterial protein synthesis or leading to aberrant proteins and eventually leading to cell death:
   a. Aminoglycosides
   b. Aminocyclitols
4. Agents affecting nucleic acid metabolism:
   a. Rifamycins (inhibit RNA polymerase activity)
   b. Quinolones (inhibit topoisomerases)
5. Agents acting as antimetabolites (e.g., trimethoprim and sulphonamides that block folate metabolism)
6. Membrane depolarizing agents (lipopeptide, e.g., daptomycin)

Another critical determinant of the success or failure of therapy is the interaction between the pathogen and the host-immune system. In the presence of an immune-competent host (as is generally the case for prophylaxis and metaphylaxis), lower total drug exposures are needed to achieve therapeutic success as compared to that needed when the immune system is compromised. Thus, in addition to its influence on the pathogen load, the host immune response determines the dosing regimen necessary to achieve the targeted clinical outcome.

While drugs are often listed as being bactericidal or bacteriostatic, there are instances where compounds can exhibit both kinds of effects. In addition to the previously mentioned impact of bacterial growth rate:

- At concentrations equal to the MIC of the pathogen, fluoroquinolones act as bacteriostatic rather than as bactericidal compounds.
- At clinically relevant concentrations, chloramphenicol, which is bacteriostatic against most Gram-negative bacteria, is cidal against *Haemophilus influenzae* and *Streptococcus pneumoniae* (Feder, 1986).
- Linezolid, which binds to a site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex, is bacteriostatic against enterococci and staphylococci but is bactericidal for the majority (not all) of streptococcal strains (Physician's Desk Reference).
- A drug's bactericidal activities can vary with the intracellular pH, oxygen content, and intracellular enzymatic activity (Butts, 1994). Therefore, it is necessary to consider each drug-microbe combination independently to accurately address the characteristics of the rate and nature of its effect.

The question sometimes raised is, "When is it preferable to administer a bacteriostatic versus a bactericidal agent?" The answer to this question depends upon the host immune response, the bioburden in the host, and the pathology of the disease process. For example, there appears to be an increased risk of shigatoxin release secondary to antimicrobial-induced bacteriolysis when bactericidal agents are used to treat foodborne *E. coli* 0157:H7 infections in humans. In contrast, bacteriostatic agents, such as macrolides and newer-generation carbapenens, do not appear to increase the risk of toxin release from these vetotoxogenic *E. coli* (VTEC; Keir et al., 2012). In fact, a relationship between VTEC strain and the nature of the antimicrobial used in the treatment of *E. coli* 0157:H7 enteritis may be one of the reasons for the intense debate (e.g., Safdar et al., 2002) regarding the role of antibiotics in increasing the risk of developing hemolytic uremic syndrome, especially in children.

In terms of PK/PD, the distinction between cidal and static activity is generally of minor relevance in an immune competent host unless the bioburden is sufficiently high that it will either induce granulocyte saturation (Drusano et al., 2010, 2011) or will increase the risk of selecting for resistant strains. When a primary cause of host pathology is the release of toxins synthesized by the bacteria, antimicrobials capable of inhibiting protein synthesis may be far more effective than those that simply kill the bacteria (Bottcher et al., 2004). In this regard, when it comes to a risk of septicemia, bacteriostatic compounds may result in a lower release of endotoxins as compared to many cidal drugs such as the beta-lactams and, to some extent, the fluoroquinolones (Prins et al., 1994), although some argue that there is little evidence to support the clinical relevance of this perspective (Hurley, 1992).

Understanding the Exposure-Response Relationship

The relationship between drug exposure and antimicrobial response is a function of the drug's mechanism of action. The resulting PK/PD characteristics can be categorized as: (1) the percentage of the dosing interval for which the plasma concentration exceeds the MIC (%T > MIC); (2) the maximum plasma concentration (C_{max}) divided by MIC (C_{max}/MIC); and (3) the area under the concentration versus time curve (AUC) divided by the MIC (AUC/MIC). While classical dose fractionation studies conducted in rodent experimental infection models have frequently been used to define the nature of these PK/PD relationships (e.g., Craig, 1998), to some degree, all of these relationships contain both time and exposure elements.

As discussed by Toutain (2002), the concentration-effect relationship is empirically described by the sigmoidal E_{max} model (also known as the Hill equation):

$$E(t) = E_0 + \frac{E_{max} \times C^h(t)}{EC_{50}^h + C^h(t)}$$

where:

- *E(t)* is the effect observed for a given concentration (C) at time *t*.
- E_{max} is the maximal effect attributable to the drug.
- EC_{50} is the plasma concentration producing 50% of E_{max}.
- *h* is the so-called Hill coefficient, which describes the steepness of the sigmoidal relationship between the concentration and effect.
- E_0 is the rate of background response in the absence of drug (such as that achieved by the host immune response.

When $h = 1$ and C is expressed in logarithms of the concentration, the E_{max} model reduces to a logistic function.

For PD purposes, the killing properties of antimicrobials are often classified as concentration-dependent and concentration-independent (or time-dependent). This classification stems from observations made on standard time-kill curves at various fixed drug concentrations. Concentration-dependent antimicrobials (e.g., aminoglycosides and fluoroquinolones) show more rapid and profound killing over a very wide range of concentrations, while concentration-independent agents show increasing killing rates over a very narrow range of concentrations (e.g., beta-lactams). It is generally stated that concentration-dependent antimicrobials will have AUC/MIC or C_{max}/MIC as their PD parameter, while concentration-independent agents with have T > MIC. However, all antibiotics obey the E_{max} (Hill) model to a greater or lesser extent. Therefore, as discussed by Mattie (2000), this segregation into two categories of action is a simplification for convenience. A further caveat relates to the PD parameter C_{max}/MIC: if a concentration-dependent drug had a high peak but an ultra-rapid half-life (i.e., the AUC would be miniscule), then the very large C_{max}/MIC values would not adequately suppress the selection of resistant strains. Therefore, the duration of exposure to these peak drug concentrations need to consistent with the duration of time necessary to precipitate a killing effect.

What does differ between antimicrobials is the domain of the curve, which is a function of the shape describing the concentration-effect relationship. For an antimicrobial where this relationship is steep, there is little difference in the prevailing concentration needed to achieve maximum killing effect. Drugs, such as the beta-lactams, also exhibit relative small post-antimicrobial effects, and only small changes in concentrations can lead to maximum or submaximal killing activity. Accordingly, the only way to further increase the response is to increase the duration of the maximal possible effect (i.e., higher doses will remain above the MIC for a longer duration, or more frequent dosing will insure adequate coverage throughout a 24-hour period). Conversely, the more shallow the curve, the greater the relationship between the rates of bacterial kill versus the antimicrobial drug concentration. This type of E_{max} relationship has been coined "concentration-dependent" killing because the degree of bactericidal activity increases as concentrations increase, up to the point where maximum killing effects (E_{max}) are achieved.

For drugs exhibiting time-dependent killing, the duration of exposure needed to achieve a targeted log-reduction in colony forming units (CFUs) is a function of the magnitude of the PAE (Nicolau, 2001). The PAE itself may differ when estimated *in vivo* versus *in vitro*. Mouton et al. (2005) defined the *in vitro* PAE as the period of suppression of bacterial growth after the drug has been removed following a short duration of exposure to that antimicrobial compound (unit = time). Owens and Ambrose (2007) argued that although these predictions have often proven useful, the sudden on-off modality of these *in vitro* tests may not adequately reflect *in vivo* conditions where concentrations are always changing with time. Therefore, they defined an *in vivo* PAE as the difference in the time needed for the number of bacteria in a tissue of treated versus control animals to increase by ten-fold once the drug concentrations in serum or at the infection site have decreased below the MIC (unit = time). Accordingly, the *in vivo* PAE includes any effect associated with drug concentrations that are less than the MIC (sub-MIC effects). Furthermore, the PAE obtained in immunocompetent versus neutropenic animals often differ (Fantin et al., 1991), underscoring the importance of the host defense system in when describing the PK/PD relationship.

As has been the case with so many of the other variables, the duration of the PAE must be evaluated from the perspective of the drug class, the pathogen, the conditions of measurement, the site of infection, etc. For those bacteria-drug combinations that exhibit a PAE, *in vivo*

PAEs have been shown to be longer than *in vitro* PAEs for most organisms. β-hemolytic streptococci are notable exceptions. Thus, optimizing the exposure to MIC ratio will delay the regrowth of the pathogen, sometimes by several hours.

For many compounds, the duration of the *in vivo* and *in vitro* PAE is substantially greater for Gram-positive than for Gram-negative pathogens. Because the duration of the *in vitro* and *in vivo* PAE of beta-lactams tends to be negligible for Gram-negative species and streptococci, the T > MIC for Gram-negative bacteria tend to be substantially greater than that for Gram-positive pathogens (except streptococci). This difference in the duration of the *in vitro* and *in vivo* PAE appears to also be one of the reasons why the *in vivo* AUC/MIC for fluoroquinolones tends to be less for Gram-positive as compared to Gram-negative organisms. Typically, penicillins and cephalosporins have moderate *in vitro* PAEs against staphylococci, but not against streptococci or Gram-negative bacilli. In contrast, carbapenems tend to have a moderate PAE against all susceptible species (Craig et al., 1990).

As has been demonstrated *in vitro* for a carbapenem and across a variety of fluoroquinolones, the duration of the PAE can vary as a function of the magnitude and duration of drug exposure (Munckhof et al., 1997; Carbone et al., 2001). In fact, PAE is related to AUC, even for agents with time-dependent killing (Munckhof et al., 1997). This explains why AUC/MIC is the PD parameter for time-dependent drugs with long PAE (e.g., some macrolides). AUC/MIC also serves as the pivotal PK/PD parameter when the infection is caused by relatively slow-growing bacteria.

Another concept frequently discussed is the mutation selection window (MSW). The impact of varying the magnitude of drug exposure on the selection of resistant strains were first seen in early *in vitro* studies by Gerber and Craig (1982), Blaser et al. (1987), and Dudley et al. (1987). For example, Blaser et al. (1987) showed that while profound killing effects of a fluoroquinolone (enoxacin) and an aminoglycoside (netilmicin) on *P. aeruginosa*, *E. coli*, *Klebsiella pneumoniae* and *S. aureus* occurred after the first dose of these compounds, the prevention of bacterial regrowth and selection of a resistant subpopulation occurred only when C_{max}/MIC exceeded some threshold value. For *P. aeruginosa*, that value was an enoxacin C_{max}/MIC that was at least a factor of 8. However, these same authors questioned the magnitude of therapeutic impact of this selection window in animals with a functional immune system.

Drlica and Zhao (2007) defined the MSW on the basis of three discrete concentrations:

- The MIC of the wild-type bacteria.
- Concentrations above the MIC of the wild-type bacteria, where there is a plateau in killing due to the survival of the least susceptible microbial subpopulation of the first-step resistant variant.
- Concentrations at which even the least susceptible organisms are killed. The latter has been termed the *mutant prevention concentration* (MPC).

In vitro, the MPC is defined as the drug concentration that blocks growth when 10^{10} cells are applied to agar; that is, in a rich inoculum yet containing a subpopulation resulting from spontaneous mutation. In contrast, the classical MIC is typically obtained from a 10^5 culture size (it is unlikely to have a mutated subpopulation in 10^5 organisms since the mutation rate is about one in 10^9).

Unless the PK/PD target factors in the likelihood for resistance selection, the estimated dosage regimens may risk the generation of concentration-time profiles that oscillate within a region that encourages the selection and amplification of resistant microbial strains. As resistant bacteria proliferate and disseminate to a fresh host, bacterial population expansion occurs and a new round of antimicrobial pressure can further enrich the mutant population, leading to a loss of antimicrobial effectiveness over time (Epstein et al., 2004). Nevertheless, it is important that oversimplification of MSW concepts be avoided: the region defined as the MSW does not consist of a homogeneous risk of mutant selection but rather represents gradations within which little risk occurs at the upper margins of this window.

It is now evident that it is not necessarily the presence of drug concentrations within the MSW that is important but rather where within that window the majority of the drug exposure occurs. Firsov et al. (2008) confirmed that even if the time within the MSW is identical between dosing regimens, it is the location of the oscillation that determines whether or not there will be resistance amplification. In other words, what is important is that drug concentrations

exceed the concentrations needed to prevent resistance amplification. These investigators concluded that for this reason, it is the AUC/MIC rather than the time above the MSW (TMSW) that is important. Similar findings were reported by Tam et al. (2007). Moreover, there is little correlation between MIC and MPC (Drlica et al., 2006) and the MIC/MPC ratio is both drug and pathogen specific (Weitzstein, 2005).

Ultimately, clinical experience shows that only some bacteria (e.g., *P. aeruginosa*) have a significant risk of resistance selection during treatment. Therefore, the therapeutic relevance of the MSW concept has yet to be clearly determined.

Mouton et al. (2005) attempted to standardize the interpretation of the PK/PD parameters. Some of the basic definitions included:

- **AUC:** should be expressed in terms of *unbound* drug. If multiple dosing regimens are applied, AUC should be measured over a *24-hour dosing interval at steady state*. In this regard, it should be noted that for compounds exhibiting linear PK, the AUC over a single dosing interval at steady state ($AUC_{0-\tau}$) is equal to AUC extrapolated to time infinity (AUC_{0-inf}) following a single administration.
- **AUC/MIC:** although sometimes given the dimension of time, because it is measured over a set period of incubation (generally 18–24 hours), this ratio is frequently expressed as a dimensionless value. Please note that for veterinary medicine where a single extended-release injection may be the totality of the therapy, the appropriate portion the AUC used to estimate the ratio must be specified, for example, AUC_{0-24} or $AUC_{0-\infty}$.
- **C_{max}/MIC:** the peak concentration relative to the MIC of the targeted pathogen.
- **T > MIC:** the cumulative percentage of a 24-hour period that the free drug concentration exceeds the MIC at steady-state pharmacokinetic conditions. Once again, please note that for veterinary medicine where a single extended-release injection may be the totality of the therapy, the portion of the profile defining the T > MIC may extend well beyond 24 hours.
- ***In vitro* PAE:** the period of suppression of bacterial growth after short exposure of an organism to an antimicrobial compound (unit = time). In this case, drug has been removed.
- ***In vivo* PAE:** the difference in time for the number of bacteria in a tissue of treated versus control animals to increase 1 log10 over values when drug concentrations in serum or at the infection site fall below the MIC (unit = time). The *in vivo* PAE includes any effect associated with sub-MIC concentrations.
- **Sub-MIC effect:** any effect of an antimicrobial on a microorganism at concentrations below the MIC.
- **Post-antibiotic sub-MIC effect:** the effect of sub-MIC drug concentrations on bacterial growth following serial exposure to drug concentrations exceeding the MIC.

PK/PD Targets

PK/PD targets influence dose selection and the estimation of susceptibility breakpoints (discussed later in this chapter). The level of kill needed to treat any infection is a subjective question for which patient response, potential risk of antimicrobial drug resistance, cost and safety (both to the target animal species and human food safety) must be weighed. This is a judgment call, which cannot be definitively solved by any mathematical technique.

Examples of factors that can influence the PK/PD target include:

- The therapeutic target: The PK/PD target will differ depending upon whether one wishes to achieve stasis, a 1-log kill, 2-log kill, etc (Andes and Craig, 1998, 2002). When the immune system is fully functional, the antimicrobial "assists" the body's own ability to combat the infection. Accordingly, substantially lower drug exposures may be needed to achieve the same effect as would be necessary in the presence of compromised immune functions (e.g., Craig, 1993). Andes and Craig (2002) observed that to achieve stasis, 1-log kill or 2-log kill for *S. aureus* infection in the thighs of neutropenic mice, AUC/MIC ratios of 69.7, 129 and 235 were needed, respectively. Similarly, ratios to achieve the same goal for this thigh infection model in non-neutropenic mice were 32.2, 62.2 and 165 respectively.
- In addition to host immune response, other variables that influence the PK/PD target include:
  - Virulence of organism
  - Pathogen growth rate

  - Pathogen load
  - The likelihood of emerging resistant strains.
- The site of the infection: Site can influence drug responsiveness, even at identical drug concentrations (Erlendsdottier et al., 2001; O'Reilly et al., 1996). One potential reason for this observation is a relationship between site of infection and microbial generation time.

Although PK/PD targets have "MIC" in the denominator, it is incorrect to assume that two different species with similar MIC will have the same MBC or require the same AUC/MIC, C_{max}/MIC, or T > MIC to achieve a given magnitude of kill (Andes and Craig, 2002). Nevertheless, there are generalizations that can be made with regard to bacterial species and organism group patterns in terms of their PK/PD targets. For instance, it has been demonstrated both in rodent models and in human clinical studies that the free (unbound) AUC/MIC targets of fluoroquinolones are around 70–80 hours for Gram-negative bacilli and 30 hours for *S. pneumoniae* (Ambrose et al., 2007).

Ultimately, antimicrobial PK/PDs are exposure-response relationships that reflect the conditions under which these estimates are derived. Evaluation of the PK/PD target should be based upon the desired therapeutic outcome. In this regard, the clinical endpoint associated with the use of antimicrobial compounds in companion animal species versus food animal species may not be the same. With food-producing animals, the treatment is often focused on the health of the group (e.g., herd) and the therapeutic objective may include prophylaxis, metaphylaxis, and/or curative strategies. Conversely, in companion animals, treatment is aimed at the individual. Furthermore, with companion animal medicine, we are confronted with multiple sources of population PK variability, such as a wide range of ages, concomitant diseases or use of concomitant medications, which may not be as problematic with animals intended for human consumption.

Optimally, clinically relevant PK/PD relationships would be derived from prospective clinical studies. However, such a goal is no small task, often necessitating information derived on hundreds of patients (Ambrose et al., 2004; Preston et al; 1998). For this reason, there are few examples of large datasets being generated in animal species.

In an effort to remedy this void, alternative sources of information are frequently employed that, while falling short of defining the population variability in drug response, do provide a characterization of pathogen-drug interaction within a limited set of conditions. Potential methods for describing these relationships include *in vitro* systems, and animal model experiments involving a range of dosage regimens. However, inherent limitations of these methods need to be considered:

- Many animal models involve the use of neutropenic rodents or estimate exposure-response relationships at infection sites that differ from the intended site of action. The interaction between antimicrobial activity and the site of infection was found to be particularly important in the evaluation of daptomycin for the treatment of *S. pneumonia* (Silverman et al., 2005).
- *In vitro* kill curves provide biased information on kill kinetics because it subjects the bacteria to constant drug exposure. This is in contrast to the typical fluctuations associated with *in vivo* drug exposure. To reduce this source of error, *in vitro* kinetic models have been developed (e.g., Blaser et al., 1985).
- *Ex vivo* models have also been used where tissue cages are implanted to collect the exudate (inflammatory fluid obtained with carrageenan) and transudate, and the resulting antimicrobial activity of the drug in that fluid is estimated *in vitro* (Brentnall et al., 2012). In both *in vitro* kinetic models and *ex vivo* effectiveness studies, the *in vivo* conditions influencing the exposure-response relationship are ignored.

For drugs exhibiting concentration-dependent killing, C_{max}/MIC ratios may be particularly important when the pathogen has a high MIC value or is rapidly proliferating (Craig and Dalhoff, 1998). Rapidly proliferating bacteria have a greater likelihood of undergoing a mutational event that could lead to the genesis of a less susceptible population. Similarly, in the presence of a high bacterial burden (inoculum effect), the risk of a mutational event is increased due simply to the laws of probability (Craig and Dalhoff, 1998; Drusano et al., 1993).

For the fluoroquinolones, the targeted C_{max}/MIC ratios are approximately 10–12 to ensure increased killing of susceptible organisms and to kill or inhibit organisms with higher MICs. However, exceptions to this rule have

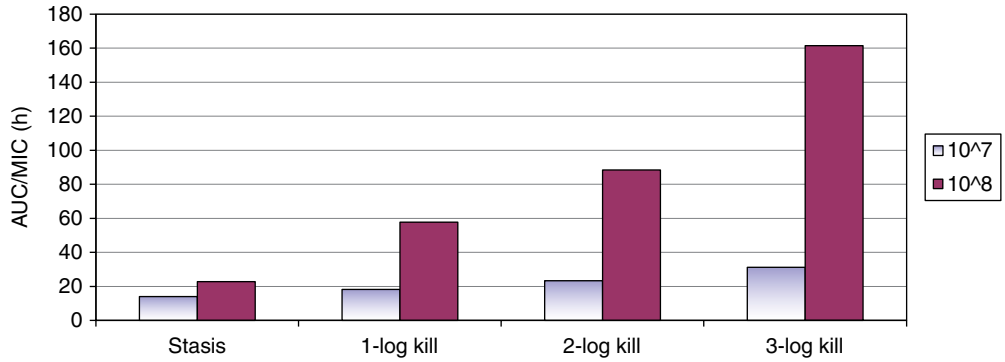

Figure 5.1. *P. aeruginosa* dose response. Normal mice were inoculated with about 10^7 (a) or 10^8 (b) bacteria per thigh. The levofloxacin MIC and MBC were 0.8 µg/mL and 1.6 µg/mL, respectively. The x axis displays the exposures in mg/kg doses. The model allowed calculation of the dose necessary to achieve stasis (i.e., to return the colony counts at sacrifice to that used for the challenge), as well as 1, 2, and 3 $\log_{10}$ (CFUs/g) reductions in bacterial counts from the stasis point. Based upon information published by Jumbe et al., 2003.

been observed. For example, in the case of *Bacillus anthracis*, hollow fiber studies suggest that AUC/MIC was more predictive of success as compared to C_{max}/MIC (Deziel et al., 2005). This result relates to the findings described by MacGowen et al. (2001; 2002), where time to kill 99% of the inoculum depends on C_{max}/MIC, but the ability to maintain this decrease in microbial counts is related to AUC/MIC (*in vitro* test conditions), thereby including time as a consideration in the exposure-response relationship. If the duration of time between doses is extended beyond 24 hours, effectiveness may also depend upon T > MIC (MacGowan and Bowker, 2002).

The Inoculum Effect

Numerous studies have examined the influence of inoculum size on the killing activity of antimicrobial compounds with the claim that the size of the inoculum influences the MIC value and the amount of drug needed to obtain a 3-log kill (a bactericidal effect). In some cases, this observation is artificial, reflecting *in vitro* test conditions and the effect of confined volume on the relationship between bacterial concentration and the concentration of bacterial-generated hydrolyzing enzymes (Craig et al., 2005). On the other hand, *in vivo* inoculum effects have been demonstrated to affect the bactericidal AUC/MIC ratios, a finding postulated to be the result of a microbial population burden that exceeds the mutation frequency.

The following are examples of an inoculum effect:

- Figure 5.1 provides a comparison of microbiological outcome endpoints following levofloxacin treatment of *P. aeruginosa* infections in mice. The data clearly show an inoculum-dependent killing effect. Isolation of drug-resistant *P. aeruginosa* mutants was common and occurred with a frequency of 0.1×10^{-6} to 2×10^{-6}. At the higher infection inoculum, the microbial population burden significantly exceeded the mutational frequency. At exposures that killed the sensitive population, the resistant population was able to survive. This allowed resistant subpopulation to be selected and amplified by the drug pressure. Subsequently, a subpopulation of mutant organisms emerged. Only with sufficient exposure to inhibit and kill the resistant subpopulation is a larger overall reduction of bacterial load attained (Jumbe et al., 2003).
- After exposure to marbofloxacin, *in vitro* and *in vivo* studies in mice confirmed that when inoculum sizes increased, the selection of resistant bacteria likewise increased (Ferran et al., 2007, 2009). Similarly, a much higher dose of marbofloxacin was needed to ensure the survival of mice infected with a high versus low pulmonary bacterial burden of *P. multocida* (Ferran et al., 2011).
- Although an increase in bacterial inoculum had no significant impact on MIC values when raised from

Table 5.2. Examples of PK and PD targets reported for various antimicrobial classes. Adapted from Craig WA. 2002. Pharmacodynamics of antimicrobials: general concepts and applications. In: Nightingale CH et al (eds). Antimicrobial Pharmacodynamics in Therapy and Practice. New York: Marcel Dekker.

General Class	Target Site	Action	Drug Class	Subclass (closely related to class)	Examples	Exposure-Effect Relationship	PAE	PK/PD Parameter	PK/PD Target		
									Gram-	*Strep*	*Staph*
Protein Synthesis Inhibitors	30S ribosome	Inhibitor	Aminoglycoside	*Streptomyces*-derived *Micromonosora*-derived	Amikacin, neomycin Gentamicin	*In vitro* time kill response dependence on drug concentration is large (maximum at >4×MIC and usually much greater) = often described as concentration-dependent killing	Moderate to large	AUC_{24}/MIC or C_{max}/MIC	C_{max}/MIC ≥ 8		
	t-RNA	Binding	Tetracyclines Glycylcylines		Doxycycline, Chortetracycline, Oxytetracycline Tigecycline	*In vitro* time kill response dependence on drug concentration is small (maximum at 1–4× MIC) = often described as time-dependent killing	Large	AUC_{24}/MIC	For a discussion, refer to Agwuh and MacGowan, 2006		
	50S	Initiation inhibitor	Oxazolidinone		Linezolid	*In vitro* time kill response dependence on drug concentration is small (maximum at 1–4×MIC only) = often described as time-dependent killing	Minimal to moderate	T>MIC	85 (Ambrose et al., 2007)		
		Inhibits peptidyl transferase	Amphenicols		Chloramphenical, Florfenicol	Time-dependent		T>MIC*	50% (Burgess et al., 2007)		
	Transpeptidation/ ribosomal translocation	Inhibition	Pleuromutilins Macrolides Lincosamide Streptogramins	Erythromycin-like Azalide Ketolide	Tiamulin, Valnemulin Erythromycin Azithromycin Telithromycin Clindamycin Virginiamycin	*In vitro* time kill response dependence on drug concentration is small (maximum at 1–4×MIC only) = often described as time-dependent killing	Minimal Moderate	AUC_{24}/MIC* T>MIC AUC_{24}/MIC	Novac, 2011 Approximately 50% 5 3.75 16–30	4	12
Cell Wall Synthesis Inhibitors			Beta-lactams	Penicillins		*In vitro* time kill response dependence on drug concentration is small (maximum at 1–4×MIC only) = often described as time-dependent killing	Minimal to moderate	T>MIC	30	50	30

10^5 to 10^8 cfu/mL, a significant markedly higher doses of several carbapenems and fluoroquinolones (expressed as ED_{50}) were needed for the treatment of mice infected with > 10^8 cfu/mL *S. aureus* and *P. aeruginosa* (Mizunag et al., 2005).

- Vancomycin appears to be subject to the inoculum effect *in vitro* and *in vivo* (Craig and Andres, 2006), and may in part explain poorer outcomes in patients with high bacterial burdens at the beginning of treatment that cannot be managed surgically (Kim et al., 2003).
- Consistent with the role of bacterial burden on clinical outcome, retrospective work on doxycycline in swine has indicated that although PK/PD point to the need for a daily dose of ≥ 20 mg/kg is necessary to insure clinical success in the treatment of swine respiratory disease, a dosage of 11 mg/kg/day in feed is effective in the control of swine pneumonia due to *Pasteurella multocida* (Toutain, 2005; Bousquet et al., 1998). Toutain suggests that this disparity may, at least in part, be related to an inoculum effect, supporting the importance of metaphylactic therapeutic strategies (i.e., administering the antimicrobial agent prior to the establishment of disease).
- A density-dependent decline in the rate and extent of antibiotic-mediated killing has been reported with a variety of antimicrobial compounds including daptomycin, linezolid, gentamicin, oxacillin, vancomycin, and ciprofloxacin (Udekwu et al., 2009).

The relationship between bacterial numbers may be particularly meaningful in situations where there is purulent fluids (pus), which has been correlated with cfu/mL averaging 2×10^8 (samples from human patients soft tissue and intra-abdominal infections). In fact, some patients were found to present with a cfu/mL in pus that was as high as 10^9 (König et al., 1998).

Putting It All Together

Examples of PK/PD targets (for infection sites freely accessible to the antimicrobial agent) are provided in Table 5.2. These relationships are expressed in terms of the free (unbound) concentration of the active drug and (its metabolites) in the free (unbound) plasma or serum as a surrogate for infection site exposure.

Several potential shortcomings of these metrics need to be considered when addressing unique situations encountered within veterinary medicine. For example, the MIC determined in milk can be markedly different than that determined in broth or agar. This point is an important consideration when treating bovine mastitis. While the MIC of *E. coli* and *S. aureus* for penicillin G in milk is the same as that obtained in Mueller-Hinton (MH) broth, the MIC of tetracycline in milk is 4–32 times higher than that observed in MH broth (Kuang et al., 2009). For oxytetracycline, the MIC can be 20–30 times higher in serum, exudate, and transudate as compared to that seen in MH broth. Since the plasma protein binding of oxytetracycline is only about 50%, alternative explanations are necessary (Brentnall et al., 2012).

Finally, basing the AUC/MIC or T > MIC upon a 24-hour dosing interval does not address the kinds of alternative dosing strategies sometimes used with food-producing animal (Toutain et al., 2007). Therefore, when dealing with the types of dosing regimens and formulations encountered for veterinary use, there are numerous situations where we need alternative metrics that are not restricted to the traditional daily dosing regimens typically encountered in human medicine.

Achieving Targeted Drug Exposure

For any antimicrobial agent, the primary target is the invading pathogen. Thus, effectiveness will depend upon the ability to achieve the necessary magnitude and duration of drug exposure at the infection site. The site of a bacterial infection is seldom the vascular bed (septicemia). Instead, bacterial infections nearly always occur in tissues, necessitating that the drug diffuse out of the systemic circulation. Once at the site of infection, the drug needs to interact with the bacterial cell to exert a cidal or static effect.

It is only the unbound (free, F) drug that gains access to the extracellular fluids through porous capillaries. For this reason, PK/PD relationships should be based upon free plasma or serum drug concentrations (Liu et al., 2002; Liu et al., 2005; Mueller et al., 2004; Drusano, 2004). During a Clinical and Laboratory Standards Institute (CLSI) workshop, Dr. William Craig (2011) provided an excellent overview of the fluoroquinolones and cephalosporins, demonstrating that when expressed in terms of free drug concentrations, the PK/PD targets across a variety of compounds within each class were effectively identical, despite marked differences in total drug concentrations.

There are also examples of infection sites and drug classes for which infection site concentrations differ markedly from those observed in the blood. The pulmonary epithelial lining fluid (ELF) concentrations of macrolides, ketolides, some fluoroquinolones and oxazolidinones tend to be higher than the concentrations observed in plasma (Rodvold et al., 2011; Honeybourne et al., 1994; Shryock et al., 1998). For that reason, ketolides such as telithromycin require a plasma AUC/MIC ratio of only 3.375 to achieve 90% bacterial eradication of respiratory pathogens in human patients with intact immune function (Drusano and Preston, 2002). Conversely, ELF concentrations of the aminoglycosides and glycopeptides tend to be less than that in plasma (Rodvold et al., 2011). For example, the ELF concentration of the aminoglycoside tobramycin is substantially lower than that observed in the plasma (Carcas et al., 1999).

Lung homogenate concentrations should not be used to estimate infection site exposure. These concentrations largely reflect drug that is bound/constrained by intracellular and extracellular elements and therefore misrepresent free drug concentrations at the site of infection. Rather, measurements such as drug concentrations in the ELF is frequently used to estimate bacterial drug exposure in the lung, That said, the accuracy of ELF drug concentrations has recently been called into question. Although the very high local concentrations have frequently been attributed to partitioning of drug into leukocytes (Maglio et al., 2003; Scorneaux and Shryock, 1999), Kiem and Schentag (2008) concluded that the apparent concentrating of antibiotics such as azithromycin, clarithromycin, ketolides, fluoroquinolones, itraconazole, tigecycline, and rifampin in the ELF may be exaggerated if captured through the use of broncoalveolar lavage (BAL). These investigators demonstrated that BAL may lead to sample contamination by drug that is released upon lysis of surrounding cells (including alveolar macrophages). If their conclusions are correct, then measurements of drug concentrations in the ELF (when captured via BAL) will be compromised by the same sources of bias encountered with lung tissue homogenization. Alternatively, Kiem and Schentag suggested that lung microdialysis may offer an overall better correlation with microbiological outcomes and that until the BAL problem is remedied, it is preferable to simply continue expressing PK/PD parameters using serum drug concentration as these values are better correlated with patient microbiological outcomes. Similarly, Muller et al. (2004) concluded that in general, acute inflammatory events have little influence on tissue penetration and that reports on the increase in the target site availability of antibiotics by macrophage drug uptake and preferential release at the site of infections is unfounded.

Some tissues have permeability limitations at the capillary level and/or possess an efflux pump. In these situations drug accumulation at the site of action (e.g., blood-brain barrier) is impaired, and only lipophilic drugs can cross such barriers (e.g., the fluoroquinolones). Blood perfusion can also be a limiting factor (clot, abscess, or sepsis), impairing tissue perfusion and therefore drug partitioning.

When bacteria are located within cells (facultative or obligatory intracellular pathogens), intracellular drug concentrations (e.g., polymorphonuclear neutrophils) can vary across organelles (e.g., cytosol, phagosome, and phagolysosome). Macrolides, for example, are trapped in phagolysosomes that have a low pH (about 4–5), leading to a "high" total cell concentration. However, as the antibacterial potency of macrolides is pH dependent (low or no activity at acidic pH), these high concentrations reflect ionized (trapped) drug that have significantly reduced antimicrobial effects (Toutain et al., 2002).

Occasionally, penetration in healthy tissue does not reflect the penetration that occurs in diseased tissues. Because the volume of the infection site is small as compared to that of the rest of the body, changes in infection site drug concentrations are rarely discernable from concentrations in the blood. Reasons for this difference in drug distribution into healthy versus infected tissue can be multi-fold. For example, by increasing the rate of blood flow to the tissues, such as the increase in circulation that may occur during an acute inflammatory response, we can anticipate an increase in the rate of drug exchange between the blood and the inflamed tissue (Ryan, 1993). Using microdialysis to measure unbound ciprofloxacin concentration in subcutaneous adipose tissue and microcirculatory blood flow by laser Doppler flowmetry, it was shown that the warming of a lower extremity was able to increase the microcirculatory blood flow by approximately three- to four-fold over baseline blood flow, and that the corresponding ratio of ciprofloxacin C_{max} for the warmed thigh versus

the C_{max} of the non-warmed thigh was 2.1 ± 0.90 (Joukhadar et al., 2005).

However, inflamed tissue may respond differently than would be predicted solely on the basis of purely a heat response. Blood vessel dilation during inflammation may not be synonymous with an increase in local blood flow. In fact, acute inflammation can be associated with a decrease of blood flow, as was reported for lung inflammation (Henson et al., 1991). The lungs of horses affected by recurrent airway obstruction (RAO) secreted inflammatory mediators that resulted in vasospasm and poor local circulation. Such circulatory changes are likely to impede local access to drugs. For mastitis, a far more complicated situation may exist where an initial phase (0–12 hours) of blood flow increase is followed by a subsequent decrease during the next 12 hours (Potapow et al., 2010).

For urinary tract infections (UTIs), it is frequently the concentrations in the urine that are thought to be biologically relevant. In this regard, high concentrations of active drug in the bladder have been found to work effectively against bacteria in the urine and have been correlated with bacteriological cure for uncomplicated UTI. However, these luminal drug concentrations appear to be virtually ineffective against bacterial growth in the bladder wall (Frimodt-Møller, 2002). In the latter situation, the drug needs to reach the infected tissue via the blood (Frimodt-Møller et al., 1981). In addition, at least in human patients, chronic bladder infections may be attributable to the intracellular invasion of uropathogenic *E. coli* (UPEC). There is evidence suggesting that the UPEC invade bladder epithelial cells where they replicate and form large bacterial inclusions. This may trigger host exfoliation of these infected cells, as well as cytokine production. Prior to completion of cellular sloughing, the UPEC emerge from the infected cell, forming new contacts with the exposed transitional epithelium. This intracellular phase provides a significant challenge to the effective use of antimicrobials for the treatment of chronic UTIs (Schilling and Hultgren, 2002), underscoring the importance of identifying the location of the pathogen when determining the targeted destination for the antimicrobial therapy.

Finally, as described in the next section, pH changes associated with infection and inflammation can markedly influence the concentration and activity of drug at the site of action.

Importance of pH Considerations

Ionization facilitates compound solubilization in an aqueous environment, and this solubilization is an initial step in drug absorption. This ionization, at least in part, explains the intracellular accumulation (particularly in neutrophils and macrophages) seen with many macrolides (Carbon, 1998). However, it is the un-ionized drug that crosses into the systemic circulation or into cells and organelles (Martinez and Amidon, 2002). This difference in the behavior of the ionized and ionized drug molecules is critical because while ionization can lead to elevated drug concentrations via ion trapping, ionized drug cannot readily penetrate into the bacteria. Accordingly, this pH effect can influence concentration-effect relationships of ionizable compounds such as weak acids (beta-lactams), weak bases (e.g., macrolides), and zwitterions (e.g., fluoroquinolones; Siebert et al., 2004).

As an example, lowering the pH of MH broth from 8 to 5.8 caused 8- to 31-fold increases in the ciprofloxacin and sparfloxacin MICs for *E. coli* (Tsutsumi et al., 1999). Similarly, Table 5.3 provides an example of this pH-related change in the MIC of a macrolide (tulathromycin) as a function of pH. For this weak base, as pH decreases, a greater proportion of the drug exists in its ionized form, leading to a decrease in its potency (Microbial Risk Assessment). This pH sensitivity can also be problematic under *in vitro* test conditions as incubation with CO_2 can lower the pH of the growth medium.

Potential changes in pH at the site of the infection can affect the accuracy of *in vitro* PK/PD predictions. For example, bovine mastitis generally results in an increase in the pH of milk (the pH of milk is normally 6.6–6.8 but can go as high as 7), although rare instances of a

Table 5.3. The effect of pH on tulathromycin activity.

	Mean MIC (μg/mL) at pH:†					
Microorganism*	6.5	7.0	7.2	7.4	7.6	8.0
E. coli ATCC 25922	>128	18.4	4.59	2.0	2.0	2.0
E. faecalis ATCC 29212	>128	36.8	12.1	3.48	2.0	2.30
S. Aureus ATCC 29213	>128	24.3	8.0	3.03	1.74	2.0

*Quality control isolates obtained from the American Type Culture Collection (ATCC).

†Standard testing conditions consistent with NCCLS methods were used, except that pH of the culture medium was varied as indicated [31,3].

decrease in milk pH have been reported. These changes in pH affect the ratio of ionized to un-ionized drug. Therefore, drug concentrations in normal healthy quarters may not reflect active drug concentrations in inflamed and infected udders (Gips and Soback, 1999).

UTIs are another example of where pH may be a concern. Ionization can be an issue in uncomplicated UTI. The pH of horse urine exhibits a bimodal distribution with pH values ranging between 5 and 9 (Stanley et al., 1995). Depending upon diet, the urinary pH of sows can between 4.7 and 7.7 (DeRouchey et al., 2003; Figure 5.2). Therefore, drug entering sow urine will be in an environment with a markedly different pH as compared to the blood and may fail to exhibit the expected exposure-response relationship if ionization is not considered.

Tolerance versus Resistance

Unlike acute infections, chronic infections are often associated with biofilm formation and slow cell division rates (Owens et al., 1997). The propensity to form biofilms is considered one of the major virulence factors involved in coagulase negative staphylococcal infections (Otto, 2004). Drugs that act on any of these virulence factors may have effectiveness that extend well beyond predictions that are based solely upon the static effects on planktonic organisms. Currently such effects can be incorporated into our targeted serum concentrations only when the PK/PD model is based upon a retrospective analysis of clinical outcome (Sánchez-Navarro et al., 2001; Drusano et al., 2004).

In these situations, *in vitro* MIC values alone cannot predict whether or not a drug will work. Traditional *in vitro* susceptibility data reflect the impact of therapeutic agents on bacteria that are in the active growth phase and are free floating (planktonic) cells. These tests do not describe the differential activity across the various life phases of the bacteria (Cerca et al., 2005). As bacteria form biofilms, they become sessile (attached) and constitute an organized community of bacteria able to survive in an adverse environment. When in a biofilm, bacteria enter into a non-growth phase where many antibacterial compounds lose their effectiveness. This is particularly evident with those compounds that are dependent upon internal synthetic mechanisms, such as the beta-lactams (Tanaka et al., 1999). For example, the reduced susceptibility to beta-lactams is related to a diminished expression of penicillin-binding proteins and a decrease in the drug-induced inhibition of transpeptidases (Gilbert and Brown, 1998). Such findings have lead to the term "drug indifference" (Jayaraman, 2008). However, biofilm-associated bacteria are not "resistant" to antimicrobials in the traditional sense, ultimately reverting back to a sensitive state once they return to the planktonic form. Furthermore, even non-growing biofilm cells remain sensitive to the fluoroquinolones or to drugs that interact with and disrupt cell membranes (Tanaka et al., 1999; Jayaraman, 2008; Smith et al., 2009).

While clonal bacterial populations were previously considered to be both genotypically and phenotypically identical, newer techniques have caused this view to be challenged. It is now well established that phenotypic heterogeneity can exist within a clonal population due to "noise" in gene expression. This "noise" can arise as a result of stochastic variations in gene expression or in

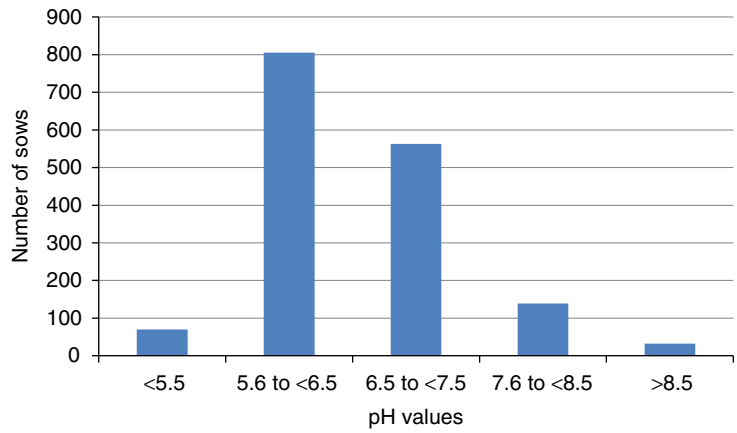

Figure 5.2. Frequency of observed urinary pH values in sows (based upon data from Madee, 1984).

response to environmental perturbations (Jayaraman, 2008). Ultimately, these variations cause drugs to lose, either partially or totally, their ability to fight infection (drug tolerance).

The issue of biofilms is very challenging (Costerton et al., 1999). Nearly all bacteria are capable of forming biofilms, and biofilms have been postulated to involve up to 65% of all human infections (Potera, 1999). It may likewise be of substantial clinical consequence in veterinary medicine (for a discussion on biofilms and their relevance to veterinary medicine, see Clutterbuck et al., 2007). Examples include chronic wound infection of the distal limb in horses, chronic bovine mastitis, indwelling catheter infection in horses, pyoderma and periodontal conditions in dogs. It was recently shown that dogs leaving an intensive care unit may carry a large multidrug-resistant enterococcal population with a capacity for biofilm formation and presenting a risk of horizontal gene transfer to humans (Ghosh et al., 2011).

In addition to biofilm issues described in the beginning of this chapter (*P. aeruginosa*), there are several factors that may contribute to antimicrobial failure in the treatment of biofilms, and mathematical models have been developed to describe these multifactorial interactions. (Keren et al., 2004a,b; Cera et al., 2005; Cogan et al., 2005). Variables include:

- Components of the biofilm may interact with and neutralize the antimicrobial compound, imposing a penetration barrier.
- Some investigators conclude that biofilms tend to retain a population of cells that remain unaffected by an antibacterial challenge. These persister cells remain dormant and therefore, unaffected by the cidal effects of antimicrobial agents, even those that are active against slow-growing cells.
- Quorum-sensing has been proposed as a mechanism by which bacteria can up-regulate resistance mechanisms. Recent studies suggest that interference with the quorum-sensing communication system may increase bacterial susceptibility.
- Some investigators suggest the emergence of biofilm-specific phenotypes. Much of the work done on bovine mastitis has supported this postulate.

Importantly, it is now recognized that there are chemical communication signals between cells of eukaryotic organisms, within and across species of prokaryotic organisms and between the microorganisms and their hosts (Hughes and Sperandio, (2008). Each of these aspects of biofilm physiology exerts a drug-specific effect on antimicrobial activity (König et al., 2001).

Some people (and publications) are now promoting the use of antibiotics (e.g., cephalosporins) by pulse dosing when presented with a biofilm infection (e.g., dog dermatitis). For example, they may recommend treating dogs systematically for 3 days per week for the entire life of the animal. Considering what is now known about biofilms, such pulse dosing will only periodically control the release of planktonic pathogens, leading to symptomatic relief without biofilm eradication. In dairy cattle epidemiological studies have shown that treatment failure of old cows that have a chronic *S. aureus* infection is due to the presence of biofilm (Clutterbuck et al., 2007).

PK/PD and Dose Predictions: Putting It All Together

When considering the relationship between dose-exposure effect for antimicrobial compounds, it is important to recognize the potential pitfalls associated with such generalizations and the numerous interacting variables that can influence these relationships. These interacting variables are diagrammed in Figure 5.3.

Drug and dosage regimen can be considered a function of nested and interacting variables that ultimately dictate the necessary dosage regimen (dose, dosing frequency, and duration of administration) for achieving the desired clinical outcome. In this regard, the starting point in any course of therapy is diagnosis of the disease and a determination of the therapeutic objective. For example, if treatment is being administered for the prevention of disease (such as the prophylactic use of a drug on a farm where there is an effort to minimize the risk of a disease outbreak), then the therapeutic objective may be to simply maintain a sufficiently low bioburden at the herd level such that the host immune system of healthy animals within the herd can successfully constrain an outbreak. Alternatively, there may be an active and life-threatening infection necessitating rapid eradication of the infectious agent. However, with the latter example, a simultaneous consideration is the fundamental

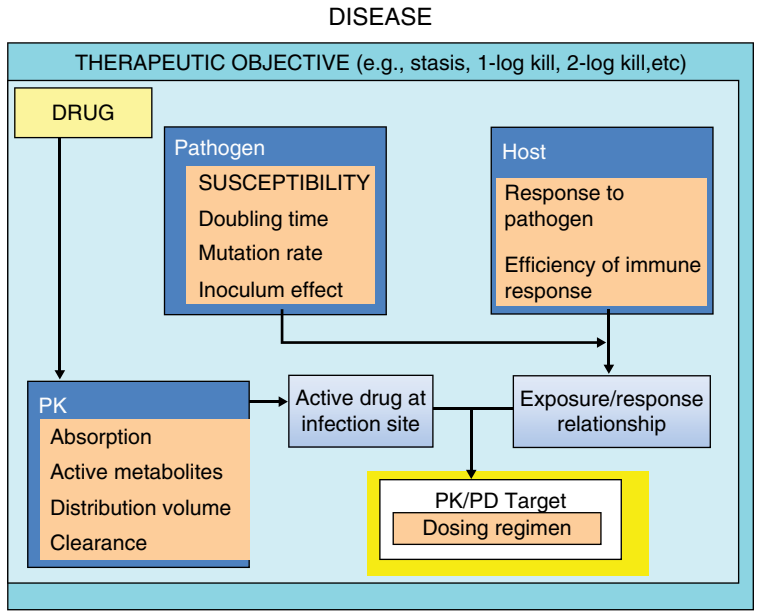

Figure 5.3. A schematic of the factors influencing the dose-response relationship of antimicrobial agents.

pathology of the disease itself. Understanding the host immune status and response will dictate if there is a need for rapid versus slow kill or stasis, if there are other mechanisms of the disease process (e.g., host-induced tissue damage due to host immune response), or the mechanism of bacterial toxin production requiring some specific decisions (treatments?).

Drug Use in a Population

The use of "mean" PK estimates (e.g., average AUC_{0-24} value or average T > MIC) does not take into account the uncertainties that influence the range of responses to a compound when used in the actual patient population. As stated by Ambrose and Quintiliani (2000), "It is important to remember that population pharmacokinetic and microbiological data are stochastic in nature and analytically need to be treated as such." For this reason, Monte Carlo methods provide an excellent mechanism for examining the probabilistic outcomes within a range of MIC values (Drusano et al., 2001; Drusano, 2003).

While individual data obtained under laboratory conditions provide important information on drug PK, these data may not adequately reflect the kinetics of the drug under field conditions. Changes in drug clearance due to compromised hepatic or renal function can result in higher than anticipated drug concentrations and a positive clinical outcome if the drug is safe. On the other hand, the availability of active drug concentrations at the site of infection may be compromised. This is particularly problematic when, due to either sepsis or swelling at the infection site, there is a decrease in the delivery of drug from the systemic circulation. Additional reasons for differences between tissue concentrations in normal versus healthy individuals include such factors as changes in drug diffusivity through the infected tissues and changes in concentration of un-ionized drug due to the relationship between drug pKa and the pH at the site of the infection.

We also know that there can be significant changes in PK as a function of breed, age, gender and species. Using population methods, Preston et al. (1998) noted that substantially higher levofloxacin AUC_{0-24} values were needed to achieve therapeutic success in older as compared to younger human patients. Their contention for this finding related to the physiological status of the patient. For this reason, when assessing the likelihood for success within a population of potential recipients, population variability in PK, host response, and organism susceptibility needs to be considered. Differences in drug metabolism can also occur depending upon whether or not the animal is castrated (Skálová et al., 2003).

When we consider the dose needed to achieve the PK/PD target in 90% of the population, the estimates

will be biased if only normal healthy subjects are used rather than including PK data generated in the patient population. An example of this was published by Peyrou et al., (2004) where he showed that clearance had a lower mean and a higher variance for diseased horses (various pathogens) than healthy horses, with respectively a mean of 0.209 and 0.284 L/h/kg and a coefficient of variation of 52 and 15%. Consequently, although the average AUC tended to be greater in diseased than in healthy horses, the magnitude of variability was greater in diseased than in healthy horses and the corresponding ability to hit specific PK/PD targets in healthy animals did not reflect the target attainment rate (TAR) in the presence of infection. Rubino et al. (2009) showed that ovitavancin (a glycopeptides) clearance in human patients with complicated skin infections or bacteriemia was substantially higher in patients (phase 2 and 3 studies) as compared to that observed in normal healthy subjects (phase 1). Similarly, mean AUC and C_{max} values in normal human subjects were higher (252 μg·hr/mL and 35.7 μg/mL, respectively) than the corresponding values in patients (146 μg·hr/mL and 28.5 μg/mL, respectively). A plot of the mean ± 1 standard deviation of the observed values in these patients following oritavacin via IV infusion is provided in Figure 5.4.

Within veterinary medicine, PK information is frequently derived from normal healthy animals. Such study designs may fail to accurately describe the shift in drug exposure that can occur during disease. Furthermore, these PK studies are generally conducted on a single breed using animals that are maintained under carefully controlled conditions. As a result, the estimated means and variances may have limited population inferential value, underestimating the range of exposures encountered across the infected patient population. In this regard, we note that within the context of veterinary medicine, antibiotics are extensively used at the herd level for prophylaxis and metaphylaxis (control), two conditions for which PK data obtained in healthy animals are likely relevant. In these situations, variability is likely to be more influenced by husbandry factors (modalities of drug administration, interindividual competition, etc.) than by the health status of the animal.

Monte Carlo simulation procedures are often used for generating population predictions. Experimentally generated estimates of parameter means, variances and relevant covariate information (e.g., age, gender, breed, creatinine clearance) are used to generate PK parameter distributions that conform to their respective

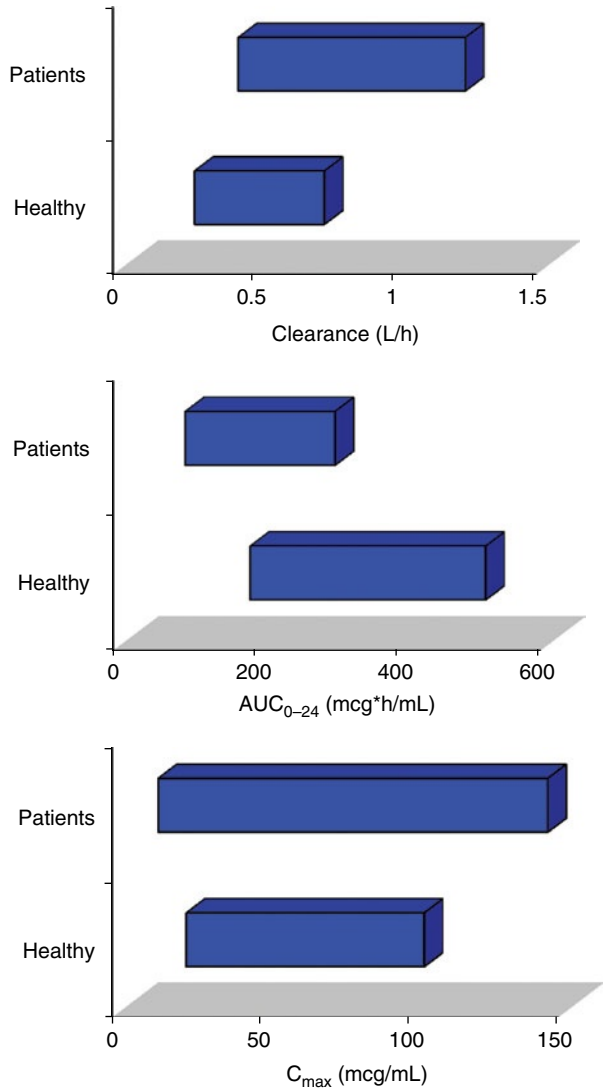

Figure 5.4. Oritavancin clearance, AUC_{0-24} and C_{max} values observed in healthy versus infected human subjects. Infected patients presented with complicated skin and skin structure infections caused by susceptible strains of Gram positive pathogens. Floating bar graphs reflect reported mean ± 1 standard deviation of observed values. Graphs are based upon data presented in the study report by Rubino et al., 2009.

probabilities. From these randomly generated values, the simulation procedure generates a large number (generally thousands) of PK/PD values that can be used to assess the probability of achieving specific PK/PD values for any specified MIC. This simulation outcome is not weighted by the probability of achieving a specific MIC value but rather examines the likelihood of achieving some PK/PD target for any MIC value in question. Figure 5.5 provides an example of the use of MICs to compare the likelihood of achieving an AUC/MIC value of 30 with the approved doses of gatifloxacin and levofloxacin (Ambrose and Grasela, 2000), where the value of 30 was determined on the basis of human survival data.

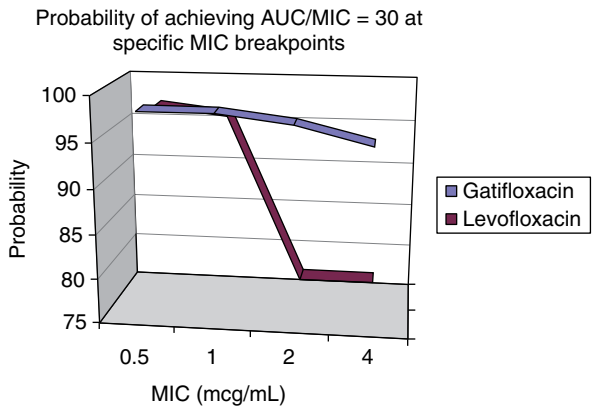

Figure 5.5. Probability of achieving an AUC/MIC ratio of 30 across a range of MIC values (based upon figure by Ambrose and Grasela, 2000).

There are several ways that these assessments can be generated (Dudley and Ambrose, 2000):

- Simulate the pivotal PK metric based upon an estimate of population means and variances for a specific parameter (e.g., AUC). For the microbial susceptibility, examine the proportion of the patient population expected to reach the targeted PK/PD relationship (e.g., AUC/MIC = 30). Examine the probability of obtaining that targeted PK/PD value for various doses using a fixed MIC value, such as the MIC_{90} (e.g., Figure 5.4). When the targeted PK/PD parameter is T > MIC or C_{max}/MIC, a more scientifically robust approach would be to simulate the PK profiles based upon mean vectors and variance/covariance matrices of the various pharmacokinetic parameters (e.g., volume of distribution, clearance, percent absorbed). In this situation, the investigator uses the population PK parameter values from these simulated profiles and then estimates the target attainment based upon the use of fixed MIC values. This method can be used in dose estimation (e.g., altering the dose to obtain the desired TAR). Alternatively, as discussed later in this chapter, by repeating this with a specific dose but with varying MIC values, this method can be valuable in the evaluation of susceptibility breakpoints.
- As seen in Figure 5.6, due to the population distribution of drug PK, it is incorrect to assume that a doubling of the dose will necessarily result in a doubling of the TAR. When nearing a plateau, a doubling

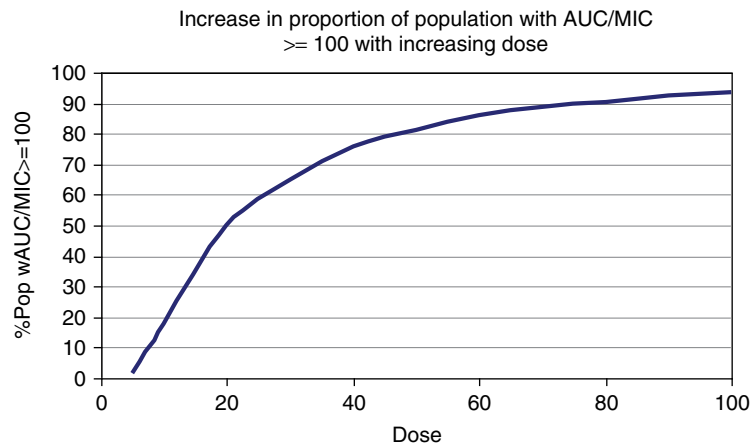

Figure 5.6. Impact of increasing the dose on the percent of the simulated population achieving the PK/PD target of AUC/MIC = 100.

of the dose will have minimal additional therapeutic benefits but will likely result in substantial deleterious effects associated with target animal safety, human food safety, and cost. On the other hand, when the dose is on the linear portion of the profile, substantial benefit may be achieved by increasing the dose or the frequency of administration.

- This kind of analysis could be extremely beneficial during attempts at dose optimization. Simulate the PK/PD parameter distributions based upon simulations that factor both the distribution of the PK parameters (using either of the two approaches described above) and the MIC population distribution. With using this approach, each simulated individual is randomly assigned an MIC value based upon probability distribution derived from the epidemiological MIC data. The PK/PD population distribution obtained by MICs is weighted by the population distribution of MIC values associated with the pathogen. In this case, the probability of achieving the PK/PD target for a given MIC value is multiplied by the percentage of the microbial population associated with that MIC value. When the weighted probabilities are summed across all of the MIC values, we obtain an overall weighted (weighted TAR) for that dose. This method may be of value when selecting a course of therapy where the intervention associated with the highest weighted TAR (dosing regimen and/or drug) may be selected (Tam et al., 2006). An example of the determination of the weighted target attainment, similar to the one published by Drusano et al. (2001a) is provided in Table 5.4.

Table 5.4. Estimation of a weighted target attainment rate.

MIC µg/ml	%AUC/MIC = 100	% Bacteria w/MIC	Product of Fraction
0.125	0.99	0	0
0.25	0.94	0.3	0.282
0.5	0.57	0.35	0.1995
1	0.09	0.2	0.018
2	0.03	0.1	0.003
4	0	0.05	0
			Weighted TAR = 0.5025

Clinical Susceptibility Breakpoints

The use of susceptibility tests that rely upon validated clinical breakpoints can be of tremendous therapeutic benefit. However, its strengths and limitations need to be appreciated.

Interpretive criteria are intended to help the human or veterinary practitioner *avoid* choosing an antimicrobial that is likely to result in therapeutic failure. In other words, the clinical breakpoint is used by the diagnostic laboratory as a mechanism for discouraging the clinician from prescribing antimicrobials that are likely to be ineffective under a specific set of disease conditions.

There are three values of interest—Susceptible (S), Intermediate (I), and Resistant (R):

- Susceptible (S): a category that implies that an infection due to the isolate *may* be successfully treated with the normal dosage regimen of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise indicated.
- Intermediate (I): a category that implies that an infection due to the isolate may be successfully treated in body sites where the drugs are physiologically concentrated or when a higher approved dosage of drug can be used; also indicates a "buffer zone" that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations.
- Resistant (R): isolates not inhibited by the usually achievable concentrations of the agent with normal dosage regimens and/or fall in the range where specific microbial resistance mechanisms are likely (e.g., beta-lactamases), and clinical efficacy has not been reliable in treatment studies.

Antibacterial susceptibility testing is used to determine if the bacteria that are isolated from a patient with an infection are likely to be killed or inhibited by a particular antibacterial drug at the concentrations of the drug that are attainable at the site of infection using the dosing regimen(s) indicated on the drug product's label (CDER/CDRH Draft Guidance: Updating Labeling for Susceptibility Test Information in Systemic Antibacterial Drug Products and Antimicrobial Susceptibility Testing Devices, June 2008). However, a classification of "susceptible " does not insure that the use of a particular compound will result in therapeutic success. In fact, as diseases become more severe, the likelihood of efficacy will drop, even with susceptible isolates.

The distinction between clinical resistance (which is related to pathogen susceptibility, PK, and the approved dosage regimen) versus epidemiological cut-off values (which is purely a function of pathogen susceptibility) is fundamental to how we consider and interpret clinical breakpoints (Bywater et al., 2007; Simjee et al., 2008; Turnidge and Paterson, 2007). In human medicine, it is reported that a clinical breakpoint classification of "susceptible" is associated with a favorable therapeutic response in 90–95% of patients. Although two-thirds of patients will also respond when the infecting bacterium has been determined to be "resistant" (depending on the type of infection), it is evident that a test result of "susceptible" succeeds in predicting the likelihood of a positive clinical outcome (Rex et al., 2002).

Three components are considered when establishing susceptibility breakpoints:

- The CO_{WT} is the microbiological (wild-type) MIC cutoff value derived from geographically diverse diagnostic laboratory collections.
- The CO_{CL} is clinical MIC cutoff value derived from the clinical field trial.
- The CO_{PD} is the PK/PD MIC cutoff value, which is based upon the relationship between achievable drug concentrations at the site of infection and the dynamics of the drug's antimicrobial activities.

Given the theme of this chapter, we will close with how PK/PD is used in the breakpoint assessment.

Deriving a CO_{PD}

The information contained in this section reflects the method for deriving a clinical breakpoint and the CO_{PD} as described in the CLSI Veterinary Antimicrobial Susceptibility Testing Subcommittee (VAST) M37-A3 document.

The first step is to understand the exposure-response relationship necessary to achieve the desired therapeutic outcome (e.g., stasis? 1-log kill? 2-log kill?). This implies knowledge of how the drug works (e.g., time-dependent effects vs concentration-dependent effects). The magnitude of the PAE, and the relationship between host factors and the magnitude of the PK/PD ratio that is necessary to achieve the desired therapeutic outcome. Generally, for the sake of obtaining of dose selection, the MIC_{90} is used (i.e., the MIC associated with 90% of the tested bacterial population for a given microbial species).

Table 5.5. MCS of the percentage of pigs for time spent above the different MICs (from 0.0625 to 4 μg/mL) following a single IM administration of amoxicillin at a dosing rate of 30 mg/kg.

	MIC value (μg/mL)						
	0.0625	0.125	0.25	0.5	1	2	4
0%	100	100	100	100	100	100	100
10%	100	100	100	100	99.6	88.9	34.45
20%	100	99.95	99.45	96.05	79.95	34.2	0.3
30%	99.85	98.85	91.5	73.4	49.05	5.85	0
40%	99.45	95.3	78.9	57.25	33.8	0.25	0
50%	98.75	90.4	68.8	49.9	16.45	0	0
60%	97.95	86.8	63	45.15	4.55	0	0
70%	97.5	84.3	59.9	36.1	0.9	0	0
80%	96.45	81.25	56.5	25.6	0.2	0	0
90%	95.25	79.3	51.95	16.7	0	0	0
100%	94.25	77.1	45.9	9.2	0	0	0

The flip side of this is the CO_{PD} where the question is the MIC at which the PK/PD target is achieved in 90% of the patient population. In other words, we are looking for the MIC_{90} at which 90% of the patients achieve the PK/PD target. Fundamental to this evaluation are the specified dose and dosing regimen, as well as all of the other factors previously discussed in this chapter.

Recently such an approach was followed to establish a CO_{PD} for amoxicillin in pigs. Based upon a meta-analysis of amoxicillin PK in 191 pigs, a population model of amoxicillin disposition was developed and was subsequently used to explore various dosing regimen scenarios by the oral and IM route using MCS. Of particular interest with respect to the issue of the CO_{PD} are the results obtained for a single IM injection of 30 mg/kg. As shown in Table 5.5, based upon these simulations, it was shown that to guarantee a 90% TAR of T > MIC of 50% over a 24-hour dosing period with the single IM dose, the MIC could not exceed 0.125 μg/ml (Rey et al., 2010) It is interesting to note that the clinical breakpoint for amoxicillin in swine was recently set as 0.5 μg/mL by the CLSI VAST.

Conclusion

What is the role for traditional PK/PD metrics in antimicrobial therapy? The answer to this question is clear: PK/PD provides the basis for selecting a starting point for dose prediction and facilitates the estimation of

clinical breakpoints. If concentrations are suboptimal, the use of the antimicrobial may increase the risk of driving an infection into chronic form or the risk of selecting for resistant pathogenic strains. In this regard, animal model studies and *in vitro* investigations provide valuable insights into the exposure-response relationship for the planktonic forms of the pathogen. We need to keep in mind that an antimicrobial agent can produce a short-term therapeutic success but may contribute to long-term therapeutic failure. Bovine mastitis and urinary tract infections are two excellent examples of where this phenomenon occurs. Particularly for those classes of compounds where concentration-response relationships have been well defined, PK/PD can help avoid the selection of doses that can lead to therapeutic failures, both in the long term and short term.

There are numerous examples where antimicrobial agents were expected to be highly effective but failed to produce the desired clinical outcome. Reasons for this may include the growth phase of the bacteria, release of toxins and *in vivo* drug inactivation. There are also examples where clinical cure have been seen at doses that were not expected to be effective. Reasons for this outcome may include drug effects on toxin production, the enhancement of host immune responses, and drug anti-inflammatory properties. These observations all point to the fact that antimicrobial agents do far more than simply inhibit or kill the bacterial pathogens. These are complex compounds that precipitate an array of events, any of which may produce a therapeutic or adverse response. Attempts to summarize such complexities as a simple two-dimensional AUC/MIC, C_{max}/MIC or T > MIC metric has the inherent assumption that it is only the killing (inhibition) of the planktonic cell that is of therapeutic relevance. This clearly is an incorrect assumption. Optimally, concentration-effect controlled clinical trials help to establish whether that predicted dose is appropriate. When such data are available, the appropriate PK/PD relationship (ratios) for dose optimization can be defined.

We need to consider not only short-term effectiveness but also long-term maintenance of an effective therapeutic arsenal. In that regard, we should not ignore the potential long-term impact of suboptimal antimicrobial drug use. We must ask ourselves if we are willing to select a dose that provides a positive response for some short-term acute clinical outcome and ignore the risk that we may be creating a long-term problem (both in terms of chronic infections in that treated individual and in terms of the risk of selecting for resistant bacterial strains). In this regard, we should not discount the importance of these two-dimensional PK/MIC estimates for helping to avoid this potential problem.

The scientific community needs to strive to understand the mechanisms of action for each new molecular entity because it is only through this understanding that we can truly define the PK/PD relationships for these compounds and the host factors that affect the response to therapy. Ultimately, because of the numerous complex interactions that can influence a drug's effect, it is only after years of actual field experience that we can have a higher level of certainty that a drug will be safe, effective, and produce minimal risk of long-term therapeutic failures when administered to the targeted patient population.

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6

Principles of Antimicrobial Drug Selection and Use

Steeve Giguère

The aim of antimicrobial therapy is to assist the host's defense mechanisms in containing and eliminating the invading microorganism(s). The ability to do this is enhanced when therapeutic drug concentrations are rapidly produced at the site of infection and maintained for sufficient length of time. In doing so the pathogen's ability to replicate is reduced or eliminated, thus also decreasing the production of toxic substances, both from the host and the pathogen. The overall result is elimination of the infection with a decrease in the disruption of function of adjacent tissues and acceleration of the host's return to health.

Antimicrobial therapy involves a calculated risk that selective toxicity of the drug for the microorganism will occur before any toxic effect of the drug on the host. A requirement of all drug therapy is also that it be rational. With the increasing choice of a wide array of highly effective antimicrobial drugs, with dosages based on pharmacokinetic analysis of drug disposition in the species of interest and with selection of the appropriate drug based on clinical microbiological data and pharmacodynamic indices, rational antimicrobial therapy is more applicable today than in the history of antimicrobial therapy.

The considerations affecting the choice of an antimicrobial drug are illustrated in Figure 6.1

Risks Associated with Antimicrobial Treatment

Antimicrobial agents can have a wide variety of damaging effects, including (l) direct host toxicity, (2) adverse interactions with other drugs, (3) interference with the protective effect of normal host microflora or disturbance of the metabolic function of microbial flora in the digestive tract of herbivores, (4) selection or promotion of antimicrobial resistance, (5) tissue necrosis at injection sites, (6) drug residues in animal products that are intended for human consumption, (7) impairment of the host's immune or defense mechanisms, and (8) damage to fetal or neonatal tissues.

Direct Host Toxicity

Direct host toxicity is the most important factor limiting drug dosage. The selective toxicity of antimicrobials is variable. Some agents, such as beta-lactams, are generally considered to be safe, whereas others, such as the aminoglycosides, are potentially toxic. Antimicrobial drugs can damage the function of many organs or tissues, particularly the kidneys (e.g., aminoglycosides, amphotericin B), nervous system (e.g., aminoglycosides, polymyxins), liver (e.g., tetracyclines, chloramphenicol), heart (e.g., aminoglycosides, monensin, tilmicosin, and tetracyclines), immune system (e.g., penicillin G), hematopoietic system (e.g., sulfa drugs, chloramphenicol), retina (e.g., fluoroquinolones), and joint cartilage (e.g., fluoroquinolones). The toxicity of antibiotics with a narrow margin of safety can be minimized by using the lowest effective doses and the shortest duration of treatment, by substituting equally effective but less toxic agents, or by using a combination of antimicrobial agents that work synergistically against the pathogen without increased toxicity to the host.

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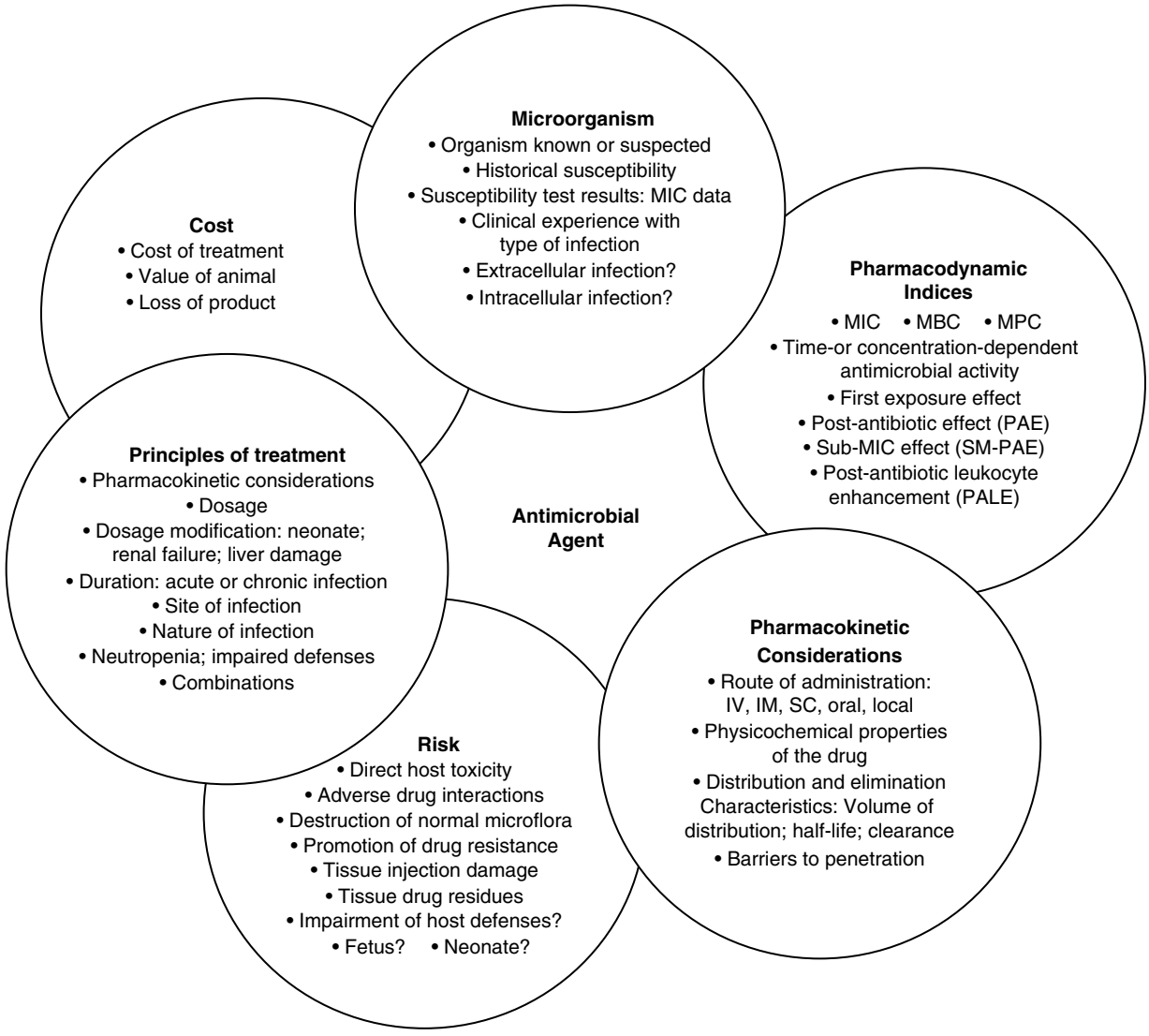

Figure 6.1. Some considerations in selection and use of antimicrobial drugs.

Drug Interactions Involving Antimicrobial Agents

Adverse drug interactions can occur in many ways, both *in vitro* and *in vivo*, and should be anticipated. These interactions can affect intestinal absorption, enhance or slow liver metabolism, interfere with kidney excretion, or result in competition for receptors or plasma proteins. Examples are shown in Table 6.1.

Absorption from the intestine may be affected nonspecifically by food, or through pH, fat, or ionic chelating effects (i.e., divalent or trivalent cations). The influence of food on oral absorption of some antibiotics is summarized in Table 6.2. Antibiotics may also affect liver microsomal enzymes. Notable examples are described in sections on individual drugs. In the kidney, the pH of the urine may, depending on the pka of the drug, affect the excretion and absorption of weak acids and weak bases. Many acidic drugs such as penicillins and sulfonamides are secreted actively in the proximal tubules and may interact with

Table 6.1. Examples of adverse *in vivo* effects of drug interactions between antibiotics and other agents.

Antimicrobial Drug	Interacting Drug	Adverse Effect
Aminoglycoside	Cephaloridine, cephalothin, polymyxins, furosemide	Nephrotoxicity
	Polymyxins, curare-like drugs, anesthetics	Neuromuscular blockade
Amphotericin B	Aminoglycosides	Nephrotoxicity
Azoles (except fluconazole)	Acid suppressant	Decreased absorption
Chloramphenicol	Dicoumarol, barbiturates	Prolonged anesthesia, anticoagulation
Griseofulvin	Dicoumarol, barbiturates	Reduced anticoagulant effect
Lincomycin	Kaolin-pectate	Decreased lincomycin absorption
Monensin	Tiamulin	Neurotoxicity
Polymyxins	Aminoglycosides	Nephrotoxicity, neuromuscular blockade
Rifampin	Macrolides, many others	Decreased plasma concentrations
Sulfonamides	Oral anticoagulants	Prolonged anticoagulant effect
Tetracyclines	Barbiturates Oral iron, calcium, magnesium	Anesthetic potentiation Decreased tetracycline absorption

Table 6.2. Suggested oral administration in relation to feeding.

Better when Fasting[a]	Better with Food	Indifferent to Feeding
Azithromycin	Cefadroxil[b]	Cephalexin[b]
Cephradine	Chloramphenicol palmitate[d]	Chloramphenicol capsules, tablets[b,d]
Most erythromycin preparations[b]	Doxycycline[e]	Chloramphenicol palmitate[b]
Fluoroquinolones[c]	Griseofulvin	Clarithromycin[b]
Isoniazid	Itraconazole	Ethambutol
Lincomycin	Ketoconazole	Fluconazole
Most penicillins[b]	Metronidazole[e]	Hefacillin
Rifampin	Nitrofurantoin[e]	Spiramycin[f]
Most sulfonamides		
Most tetracyclines		

[a]Absorption of these drugs may be reduced or delayed in ingesta. Fasting means no food for 1–2 hours before and 1–2 hours after dosing.
[b]Canine data.
[c]Enrofloxacin availability is reduced in ingesta in dogs. Effects of ingesta on fluoroquinolones are considered generally mild, but milk and yogurt should be avoided.
[d]Feline data.
[e]Food may reduce gut irritation without hindering absorption significantly.
[f]Human data. Porcine data indicate better when fasting.
Source: Data are from studies in humans, except as indicated.

other drugs that are similarly excreted. For example, probenecid has been used for many years to block the active tubular secretion of ampicillin. When probenecid is administered simultaneously with ampicillin, the serum concentration of ampicillin is increased by a factor of two.

Drug Incompatibilities

Antimicrobials may be physicochemically incompatible with other agents *in vitro*. For example, tetracyclines are incompatible with any solution containing calcium or magnesium. Although it is appropriate and common practice to use a combination of a cephalosporin and an aminoglycoside *in vivo*, many cephalosoprins are not compatible with aminoglycosides in suspension. Thus, it is not a good practice to mix antimicrobial agents in the same vessel. The lack of an obvious interaction, for example, precipitate, does not mean a chemical inactivation has not occurred.

Antibiotics and the Immune System

Antimicrobial drugs may enhance or suppress host defenses (see chapter 5 for more detailed description). These effects may be associated with alterations in cytokine production or the production of other inflammatory mediators. Microorganisms that have been damaged by antimicrobial drugs are more susceptible to killing by phagocytes. The ability of some antibiotics to penetrate and to concentrate within cells, particularly phagocytic cells, while not guaranteeing efficacy, is an important consideration in the treatment of intracellular bacterial infections. For example, phagocyte alteration of pathogen metabolism or structure may render the pathogen more susceptible to the effect of the antimicrobial agent and drug concentrations too low to have a cidal effect may render the microbe more susceptible to leukocyte action, an event associated with post-antibiotic leukocyte enhancement.

Factors Determining Choice of Antibiotic

Appropriate antimicrobial chemotherapy requires the attending clinician to have a reasonable idea as to the most likely pathogen(s) involved in the infectious disease process and the ability of the chosen antimicrobial agents to reach therapeutic concentrations at the site of infection. While clinical experience may aid the clinician in suspecting a given etiological agent, it is optimal to obtain samples for culture and susceptibility testing in order to select the most appropriate drug and dose to use. Samples for bacteriologic culture should be collected from the actual site of infection, preferably before administering an antimicrobial drug. A Gram stain, of an appropriately collected sample, may provide insight as to the etiological agent but in many cases it is necessary to isolate and identify the pathogen and determine its susceptibility profile.

In certain situations, antibacterial therapy is begun before a specific bacterial pathogen has been identified. The choice of agent is guided by the results of studies identifying the most common pathogens at a given site or in that clinical setting, by pharmacodynamic considerations, and by the resistance profile of the expected pathogen in a particular hospital or geographic area. Situations in which empirical antimicrobial therapy is appropriate include:

1. Life-threatening infections: suspected bacterial infections in an animal with a life-threatening illness should be treated empirically while awaiting culture and susceptibility results. Unless the clinical disease is characteristic for a specific microorganism, it is common practice to initiate therapy with one or more antimicrobial agent(s) to provide broad-spectrum coverage. Therapy is later tailored to address the specific susceptibilities of the pathogen cultured.
2. Treatment of mild infections in unhospitalized patients: in many situations it is appropriate to treat individual animals with non-life-threatening infections without obtaining culture. However, if the infection recurs or fails to respond to initial therapy, efforts should be made to obtain a proper sample for culture to guide retreatment. In situations where many animals are affected by the same disease it is preferable to obtain samples for culture and susceptibility from at least a few affected animals.

The selection of an antimicrobial agent depends on: (1) the likely identity of the infecting microorganism(s) at a given site of infection; (2) knowledge of the usual susceptibility profile of the suspected pathogen(s); (3) knowledge of factors that affect drug concentration at the site of infection; (4) knowledge of drug toxicity and factors that enhance it; (5) the cost of treatment; and (6) considerations of regulations about drug use including drug withdrawal times where applicable.

Bacterial Susceptibility

Antimicrobial susceptibility of some bacterial pathogens, notably beta-hemolytic streptococci and *Arcanobacterium pyogenes*, is predictable to certain antimicrobial agents, for example, benzyl penicillins (chapter 2). This is not the case for most Gram-negative bacteria that readily acquire resistance genes (chapter 3). Every veterinary practice should have access to a laboratory that has the capabilities to determine the antimicrobial susceptibility of bacterial pathogens. For properly collected and transported samples, a laboratory should be able to provide information regarding the pathogens identification and susceptibility within 48 hours of receipt. As critical as the identification of the pathogen is the assurance that the susceptibility testing has been done appropriately. Improperly conducted susceptibility tests can result in a resistant organism being reported as susceptible and vice versa (chapter 2).

Laboratory results may be misleading for several reasons. Some of these may include (1) failure to isolate the causative agent, which may be due to poor sample collection and poor sample transport, for example, anaerobes were involved in the infection but died out due to aerobic transport; (2) misinterpretation of the significance of normal flora, which could be due to inexperience of the laboratory personnel, poor sample collection and transport, or an error in interpreting the laboratory results by the submitting clinician; and (3) inappropriate antimicrobial susceptibility testing. It is not uncommon for a laboratory to overlook the importance of running appropriate quality control microorganisms when performing antimicrobial susceptibility tests. Failure to do so could result in erroneous results. Detailed discussion on *in vitro* antimicrobial susceptibility testing is provided in chapter 2.

Choice of Antimicrobial Drugs

The ideal drug is one to which the pathogen is susceptible, reaches effective concentration at the site of infection, is non-toxic to the host, requires minimal stress to the animal, and is inexpensive. To assist the veterinarian in choosing the appropriate antimicrobial agent a laboratory should provide as much information as possible. Along these lines the most basic information the laboratory can provide is qualitative susceptibity (susceptible, intermediate, or resistant [SIR]) results. Quantitative (MIC) results may be more useful than the traditional SIR data because MIC data define more precisely the degree of susceptibility of the pathogen. Armed with this information the clinician can more precisely define the dosing regimen that fits the criteria listed above. In making the decision as to which drug to use, the clinician should also keep in mind that bactericidal drugs are preferable for (1) serious life-threatening infections; (2) when host defenses are seriously impaired; (3) in infections of vital tissues such as central nervous system, cardiovascular, and bones where host defenses may not be fully functional; and (4) in immunodeficient or immunosuppressed animals. For infection of a less severe nature, bacteriostatic agents may be as or more useful than bactericidal drugs.

Where appropriate, a narrow-spectrum drug may be more appropriate than a broad-spectrum antibacterial because the narrow spectrum is less likely to interfere with the normal microbial flora. In this regard, pharmacokinetic considerations are also relevant. For example, drugs excreted via the bile may disturb the intestinal flora more than those excreted via the kidneys. Drug combinations should be considered in seriously ill patients with severe infections when results of bacteriologic tests are not available. The availability of a dosage form of the antimicrobial drug of choice that is suitable for administration to the particular species of animal is another factor influencing the final choice of antimicrobial drug.

Principles of Antimicrobial Treatment

To some extent drug dosage can be tailored to the susceptibility of the organism, the site of infection and the pharmacokinetic and pharmacodynamic properties of the selected antimicrobial agent. However, it should be recognized that *in vitro* susceptibility data are laboratory derived and the standardized conditions under which the susceptibility data was generated does not exist at the site of infection. It is also important to recognize that pharmacokinetic data represents mean data obtained from different healthy animals and that the immune status of the host, as well as its physiological and psychological status can influence the therapeutic outcome.

Factors involved in tailoring a dosing regimen include, among other things, the susceptibility of the pathogen in terms of MICs, the concentration of the antimicrobial agent at the site of infection in active form (pharmacokinetic properties of the drug), and the pharmacodynamic properties of the antimicrobial agent. The principles of pharmacodynamics are discussed in detail in chapter 5. Briefly, antimicrobial agents may be categorized as those that exhibit concentration-dependent killing, time-dependent killing, a combination of time-dependent and concentration-dependent killing, and those that are primarily bacteriostatic. Examples of concentrations and time-dependent killing are illustrated in Figure 6.2. For an aminoglycoside, such as tobramycin, as the concentration of the antimicrobial agent increases above the MIC of the pathogen, *Pseudomonas aeruginosa*, the number of viable organisms decreases dramatically. Thus, optimal dosing of concentration-dependent antimicrobial agents involves administration of high doses with long dosing intervals. On the other hand, for a beta-lactam drug such as ticarcillin, the number of viable organisms decreases as concentration of ticarcillin increases from 0.25 of the MIC to 1 time the MIC to 4 times the MIC. However, there is very little decrease in viable organisms as the concentration of ticarcillin continues to increase to 16 and 64 times the MIC. Optimal dosing of such antimicrobial agents involves frequent administration. Bacteriostatic agents typically exert time-dependent activity.

Although the factors listed above can contribute to determining the optimal dosage, the factor that most frequently limits dosage is toxicity to the host. In most instances the upper level of the recommended dosage should not be exceeded, because this is often determined by toxicity. Sometimes, however, a drug's antibacterial effects may be limiting and may determine the upper level of dosage. For example, as discussed above, the killing rate of penicillin G (and other beta-lactam drugs) has an optimal concentration, whereas that of the aminoglycosides or fluoroquinolones is proportional to

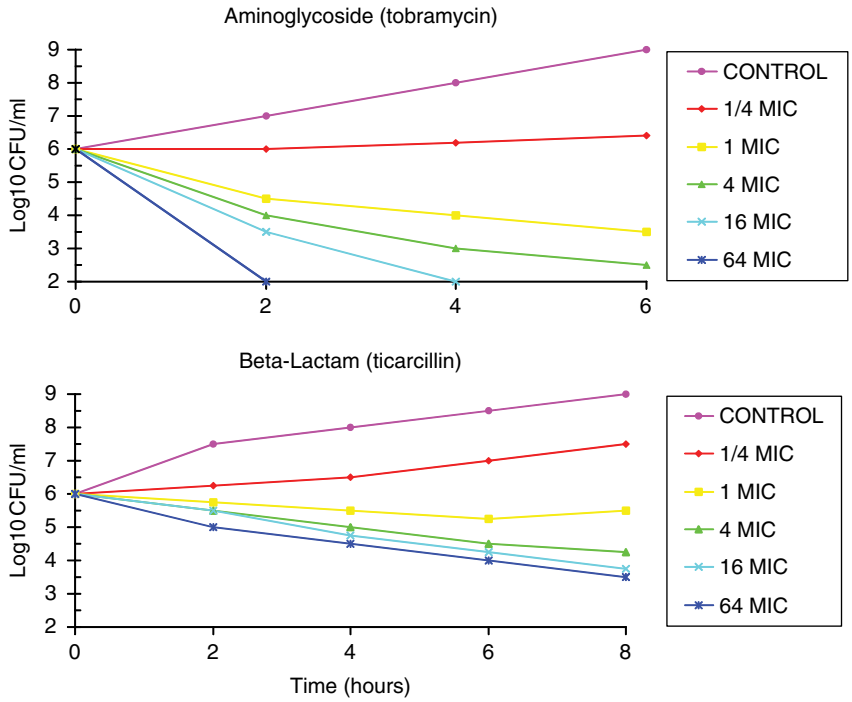

Figure 6.2. Example of concentration-dependent killing by an aminoglycoside (tobramycin). This effect contrasts to the killing by beta-lactams, which depends on the presence of drug concentration above the MIC (time-dependent killing) but is otherwise independent of drug concentration. Reproduced with permission from Craig and Ebert, 1990.

drug concentration. Penicillin G is virtually non-toxic in non-allergenic patients, but its dosage is limited by its antibacterial action, whereas the dosage of aminoglycoside is limited not by antibacterial effects but rather by its toxicity to the host.

Recommended dosing intervals should be followed. With the exception of the penicillins, fluoroquinolones, and aminoglycosides, the interval for IV-administered drugs required to maintain therapeutic plasma concentrations should not usually exceed twice their elimination half-life. Because elimination half-life is based on IV dosing, however, administering appropriate formulations by other routes can be an effective way to lengthen the interval between doses, since absorption may be delayed. For example, a single dose of procaine penicillin G administered IM can maintain effective drug levels for 12–24 hours because of slow absorption from the site of administration, even though in all species the elimination half-life of penicillin G is less than 1 hour. There are detrimental effects of not following appropriate dosing recommendations as the concentration on active drug at the site of infection may not be sufficient to inhibit the pathogen.

Dosages may have to be modified in neonates and in animals with impaired liver or kidney function (chapter 4).

Duration of Treatment

Although it is universally recognized that a drug must be present at a sufficient concentration for an adequate length of time at the site of infection, the variables affecting length of treatment have not been fully defined. Responses of different types of infections to antimicrobial drugs vary, and clinical experience with many infections is important in assessing response to treatment. For acute infections, it will be clear within 2–3 days whether or not therapy is clinically effective. If no response is seen by that time, both the diagnosis and treatment should be reconsidered. Treatment of acute infections should be continued for at least 2 days after clinical and microbiologic resolution of infection. For serious acute infections, treatment should last at least 7–10 days. For chronic

infections, particularly intracellular infections, treatment will be considerably longer and may involve months. Some uncomplicated infections, such as cystitis in human females, have been treated successfully with single doses of antibiotics, and some antimicrobial agents are now being marketed for single administration in cattle for the treatment of acute respiratory disease. However, in animals, the efficacy of such treatment must be well established before this approach is recommended. Such approaches may, inappropriately, be driven by market competition and cost-efficacy considerations rather than by optimum therapeutic benefit.

Adjunctive Treatment

Adjunctive treatments to antimicrobial therapy are essential in promoting healing. They include debriding necrotic tissues, removing purulent exudate, removing foreign bodies, correcting acid base and fluid balance successfully, and identifying and removing predisposing causes and providing rest and nursing, when appropriate. It is virtually impossible to treat infection associated with a foreign body without removing the foreign body.

Other Considerations

Other considerations in antimicrobial therapy include the cost of the drug and convenience of administration. In food-producing animals, one must know the likelihood of drug residues remaining in tissues or in milk, and therefore the required withdrawal times of agents. For example, aminoglycosides residues are known to be present in the kidney and liver of cattle for months after administration. Label directions for drug products for food-animal use must be followed or intelligently interpreted. The danger of selection of resistant bacteria by antibiotic use is another important consideration.

Extra-Label Drug Use

Antimicrobial drugs are licensed in many countries only for use for particular purposes at specific dosage, as shown on the manufacturer's product label. Because of the high costs of obtaining approval, many drugs are not approved or may only be approved for narrowly specified purposes at dosages that, in food animals at least, may be more concerned with the potential for drug residues or economics of treatment than with optimal clinical efficacy. In the United States, the Food and Drug Administration's Center for Veterinary Medicine has (with notable exceptions, chapter 26) a discretionary "extra-label" policy. This means that veterinarians who use drugs in ways not in accord with label directions, with certain other specifications, will not be prosecuted so long as no illegal tissue residues occur in edible products. The specifications include the need for a careful diagnosis within the context of a valid veterinarian-client-patient relationship, a determination that there are no alternative drugs or that dosage is inappropriate, treated animals are identified, and extended drug withdrawal ensures no tissue residues. The increasing availability of simple in-house tests for drug residues (chapter 25) has aided extra-label drug use. For non-food-producing animals, the position is generally that veterinarians may use any legally obtainable antimicrobial drug to treat disease, subject only to subsequent scientific justification before the law courts or veterinary licensing body should this use need to be defended.

Corticosteroid Use

The benefits of using corticosteroids with antimicrobial drugs in the treatment of acute bacterial infections are both controversial and poorly investigated. Clear guidelines are not available. Corticosteroids have many effects on non-specific and specific host defenses, for example, suppressing inflammation, impairing phagocytosis, delaying healing, reducing fever, and impairing the immune response. Use of corticosteroids in the treatment of infections would therefore generally be expected to have deleterious effects and should be avoided. However, in the virtual absence of experimental or clinical data supporting their concurrent use, certain circumstances may justify their short-term use: (1) in infections with concurrent life-threatening autoimmune or immune-mediated disorders; (2) in selected extensive, acute local infections to prevent lysosomal enzyme release from neutrophils and resulting tissue destruction; and (3) in the early treatment of meningitis to control inflammation caused by beta-lactam antibiotic-induced release of inflammatory mediators and to control cerebral edema (chapter 23). Corticosteroids have been considered for decades for the treatment of severe sepsis and septic shock, based on their pivotal role in the stress response and their hemodynamic and anti-inflammatory effects. Whereas short-term therapy with high doses of corticosteroids has been ineffective or even harmful in humans with septic shock, prolonged therapy with low doses of hydrocortisone

(200–300 mg for 5–7 days or longer) has been shown to have beneficial effects in recently conducted randomized, controlled trials (Dellinger et al., 2008). The role of this modality in the treatment of bacterial sepsis in domestic animals is unknown at this point.

Rapid Attainment of High Tissue Concentrations of Drugs

In acute bacterial infections, especially when using bacteriostatic drugs, it may be useful to administer a priming (loading) dose, usually by giving a high dose by IV injection, to rapidly establish therapeutic drug levels.

Local Administration of Antimicrobial Drugs

Antimicrobial drugs are administered locally in the treatment of a wide variety of infections including endometritis; skin, outer ear, and wound infections; corneal infections; mastitis; osteomyelitis, septic arthritis and tenosynovitis; and occasionally in bronchopneumonia by endotracheal or aerosol administration. Local administration has the potential to achieve higher and more persistent drug concentrations than systemic administration. Because of this local treatments may be administered less frequently than systemic treatments but this is very site and drug dependent. The principles of drug selection and use are those described for systemic antimicrobial drugs with the caution that the drug vehicle and the drug must not provoke tissue inflammation. For endometritis, local treatment may not penetrate important sites, such as the oviducts or cervix, in comparison to systemic treatment. In the cow and the mare, intrauterine treatment of the involuted uterus is often with 1 gram of antibiotic dissolved in 100–250 ml sterile saline administered daily for 3–5 days, depending on the severity and chronicity of infection. Acute, severe metritis requires systemically administered antibiotics, which may be supplemented by local treatments.

Endotracheal administration of antibiotics, particularly aminoglycosides, results in high, persistent drug concentrations in the tracheobronchial tree but may be limited in distribution. Because of this, endotracheal administration of antimicrobial agents is generally not recommended except for those tracheobronchial infections that have responded poorly to systemic treatment. Aerosol administration of antimicrobial drugs results in better diffusion throughout the bronchial tree and may have a place in severe infections of the bronchial tract that are unresponsive to other treatments. In some experimental models of pneumonia in mechanically ventilated animals, even poorly ventilated and consolidated areas of the lungs contained higher antimicrobial drug concentrations after aerosol administration than after IV administration (Michalopoulos et al., 2011). Nevertheless, the administration of antimicrobial agents by inhalation alone may not be sufficient in patients with severe parenchymal involvement or substantial consolidation. In these cases, aerosol therapy may be more appropriate as an adjunct to oral or systemic administration.

Local delivery of antimicrobial agents is an important adjunct to joint lavage, systemic antimicrobial therapy and, when necessary, surgical therapy in animals with severe infections of the musculoskeletal system (chapter 23). Intra-articular administration of antimicrobial agents is a common local delivery method in cases of septic arthritis. Regional intravenous or intraosseous infusions are useful alternatives in animals with osteomyelitis of the distal limb, when multiple joints are involved, or when the drug of choice is too irritant for intra-articular use. Antimicrobial-impregnated polymethyl methacrylate for the treatment of osteomyelitis may maintain effective local concentrations of drug for several weeks. Gentamicin-impregnated collagen sponges have also been used successfully in the local treatment of septic arthritis in animals (chapter 23).

Preventing Selection of Resistant Bacteria

Development of resistance is common collateral effect of antimicrobial drug use. However, emergence of resistance can be minimized with appropriate dosing regimens. As explained in chapter 2, current measurement of antimicrobial drug activity against bacterial pathogens relies on measurement of the MIC and, by comparison with established breakpoints, bacteria are classified as susceptible, intermediate, or resistant. At drug concentrations higher than the MIC, susceptible bacteria should in principle be inhibited, whereas a very small proportion of mutants harboring resistance mechanisms will not be inhibited. Nevertheless, these resistant variants will be inhibited at higher drug concentrations (i.e., the MIC of the resistant mutants). The mutant prevention concentration (MPC) is defined as the drug concentration that prevents generation of first-step resistant mutants within a susceptible

population. The range of concentrations between the MIC and the MPC is the mutant selection window (MSW). The MSW represents the danger zone for emergence of resistant mutants. Minimizing the length of time that the drug concentrations remain in the MSW may reduce the likelihood for development of resistance during therapy. In the future, integration of the MPC with pharmacodynamic and pharmacodynamic concepts may lead to development of dosage regimen that will not only maximize efficacy but also minimize development of resistance (see Figure 6.3).

Antimicrobial Drug Combinations

From the earliest days of antibiotic use it was known that combinations of drugs sometimes had synergistic effects where individual agents had failed (Pillai et al., 2005). On the other hand, early studies of the use of a combination of penicillin and chlortetracycline to treat certain types of bacterial meningitis showed that antagonism between drugs might have fatal results. The importance of antagonism is greatest in patients with suppressed immune defenses or severe infections, such as in meningitis, endocarditis, or chronic osteomyelitis. Mechanisms of synergism and antagonism were discussed in chapter 1. There are four indications for the use of antimicrobial combinations:

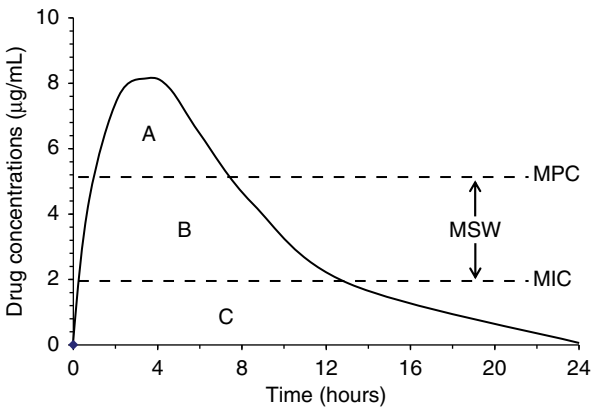

Figure 6.3. Mutant prevention concentration (MPC) and mutant selection window (MSW). The curve represents the concentration versus time profile of an antimicrobial agent. MIC and MPC are represented by dashed horizontal lines. The range of concentrations between the MIC and the MPC is the MSW. (A) Drug concentrations are above the MPC; both susceptible and first-step resistant mutants are inhibited and there is no selective amplification of resistant subpopulations. (B) The susceptible cells are inhibited but the first-step resistant cells are not; there is selective amplification of resistant subpopulations. (C) Neither susceptible nor first-step resistant mutants are inhibited; there is no selective amplification of resistant subpopulations.

1. Antimicrobial synergism: there is a considerable body of literature investigating the role of antimicrobial synergism in the treatment of various bacterial infections in humans and laboratory animals. However, there are surprisingly few examples where *in vitro* documentation of antimicrobial synergism has been predictive of superior clinical activity. In addition to the well documented merit of fixed combinations such as trimethoprim/sulfonamide, combinations of bactericidal agents such as penicillin (or ampicillin or vancomycin) with an aminoglycoside (streptomycin or gentamicin) has proved superior to monotherapy for the treatment of enterococcal endocarditis in humans. Potential advantages of synergistic bactericidal combinations have been observed primarily in patients with impaired host defenses.
2. Polymicrobial infections: two or more agents may be administered to treat documented or suspected polymicrobial infections (e.g., peritonitis, aspiration pneumonia, female genital tract infections). A classic example is the rat peritonitis model of intestinal perforation in which treatment against both Enterobacteriaceae (e.g., aminoglycoside) and anaerobes (e.g., clindamycin) is necessary to clear infection. The increasing availability of highly active, broad-spectrum, bactericidal drugs has reduced the need for combination therapy in humans. However, the high cost of many of these newer broad-spectrum drugs is prohibitive especially in large animal species. As a result, drug combinations are commonly used in veterinary medicine for the treatment of polymicrobial infections.
3. Decrease the emergence of resistant isolates: the simultaneous use of two or more agents to treat infections caused by bacteria that may develop resistance by different mechanisms reduces the possibility that resistance to the agents will develop. This is best illustrated in the treatment of tuberculosis in people, where concurrent therapy with multiple drugs clearly decreases the risk of resistance. This rationale is often

discussed for other combinations but it is particularly relevant for rifampin, an agent to which many bacteria develop resistance when used alone.

4. Decrease dose-related toxicity: several antimicrobials have dose-related toxicity that may limit their use. There are theoretical grounds on which combined therapy may allow dosage reduction of a toxic drug, while ensuring successful therapy. A clinically relevant example is the combination of flucytosine and amphotericin B in the treatment of cryptococcal meningitis, which allowed a reduction in the dose of amphotericin B, therefore limiting its toxicity.

A few examples of clinically effective combinations used in veterinary medicine are shown in Table 6.3. Combinations should only be used where their efficacy is established.

Combining antimicrobial agents may also have disadvantages. For example, a bacteriostatic drug may neutralize bactericidal effects where these effects are required. Combinations may have additive or synergistic toxicity. They may produce super-infection after destroying normal microbial flora and may have possible adverse pharmacokinetic interactions. When combination therapy is used, it should be done so in such a way as to maximize the synergistic effect. For example, in using an aminoglycoside/beta-lactam combination, the aminoglycoside should be administered once a day for its concentration-dependent killing effect whereas the beta-lactam should be administered so as to maintain continues serum concentrations above the MIC of the organism for the majority of the dosing interval.

Failure of Antimicrobial Therapy

Treatment failure has many causes. The antibiotic selected may be inappropriate because of misdiagnosis, poor drug diffusion at the site if infection, inactivity of a given drug at the site of infection (e.g., aminoglycosides in purulent material), failure to identify the etiological agent including inaccurate results of laboratory tests (chapter 2), resistance of pathogens, intracellular location of bacteria, metabolic state of the pathogen, or errors in sampling. These factors are more likely to cause failure than inadequate dosage or the use of drugs of low bioavailability, although these may also be important.

When failure occurs, diagnosis must be reassessed and proper samples collected for laboratory analysis. Patient factors such as the persistence of foreign bodies, neoplasia, and impairment of host defenses are important to consider. It is important also to ensure that persons medicating their own animals comply with dosing instructions.

Drug Withdrawal

Most countries require that antimicrobial drugs not be present in foods for human consumption and specify the time during which animals cannot be slaughtered and milk cannot be sold after antibiotic treatment. These withdrawal periods are specified for different agents (chapter 25) and extra-label drug use.

Table 6.3. Examples of clinically useful antimicrobial drug combinations in veterinary medicine.

Indication	Drug Combination	Comment
Bovine *Staphylococcus aureus* mastitis	Penicillin-streptomycin; Ampicillin-clavulanic acid Penicillin-novobiocin	Synergistic combination Also approved for bovine mastitis caused by streptococcal species
Rhodococcus equi pneumonia of foals	Macrolide*-rifampin	Synergistic; prevent emergence of resistance
Brucella canis in dogs	Minocycline-streptomycin	Synergistic combination
Peritonitis after intestinal spillage	Gentamicin-clindamycin; Cefuroxime-metronidazole	Broad-spectrum antibacterial activity
Coliform meningitis	Trimethoprim-sulfamethoxazole	Synergistic, good CSF penetration
Cryptococcal meningitis	Amphotericin-flucytosine	Synergistic decreased toxicity
Severe undiagnosed infection	Beta-lactam-gentamicin; Cefoxitin-clindamycin	Broad-spectrum, often synergistic combination

*Azithromycin, clarithromycin, or erythromycin.

FARAD (Food Animal Drug Avoidance Databank) is an organization that has been developed to assist veterinarians in estimating residue depletion times for antimicrobial agents that are administered at doses in excess of label recommendations. More information may be found on FARAD in chapter 25.

Targeted Drug Delivery

Therapeutic efficacy of antimicrobial drugs *in vivo* may be reduced by their inability to reach the site of infection in adequate amounts. Considerable effort has been devoted to finding ways to target drugs to the appropriate site. One approach has been to encapsulate drugs in liposomes—microscopic, closed lipid vesicles. After IV injection, liposomes are taken up by macrophages in the liver and spleen. Experimentally, liposome-entrapped antimicrobial drugs have enhanced activity when compared to conventionally delivered drugs against facultative intracellular pathogens with the advantage, for example with amphotericin B. of reduced toxicity. Liposomally entrapped drugs have been used for many years in human medicine but the use in veterinary medicine is still under investigation.

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7

Antimicrobial Stewardship in Animals

J. Scott Weese, Stephen W. Page, and John F. Prescott

Introduction

Antimicrobials play a critical role in the health and welfare of humans and animals. The advent and subsequent widespread availability of antimicrobial agents was one of the most transformative milestones in modern medicine. Without effective antimicrobials for treatment and prophylaxis, major medical and surgical advances would have been impossible, and infectious diseases would account for a much greater health and economic burden. Modern medicine is built on antimicrobials. The rapidly rising problem of resistance requires vigorous multipronged counter-action across a broad field (World Health Organization, 2012).

The optimism that accompanied entry into the "antibiotic era" was quickly tempered by recognition that microorganisms would not simply surrender to these new therapeutic agents. In addition to discovering penicillin, Sir Alexander Fleming may also be the forefather of antimicrobial stewardship, when he warned of the dangers of antimicrobial misuse, the selection and dissemination of resistant pathogens and the consequences of untreatable infections in his Nobel Prize acceptance speech (Fleming, 1945). Clearly, the reality of Fleming's concerns have been repeatedly validated through successive waves of the pandemic of antimicrobial resistance that now threatens human and animal health.

While the concept of antimicrobial resistance and prudent use originated early in the antibiotic era, concern about antimicrobial resistance was largely tempered by continued development of new drugs. Whereas development of new antimicrobials and antimicrobial classes outpaced development of resistance in the 1950s to early 1970s, the trend has now reversed, and emergence and dissemination of resistant bacteria is a problem worldwide. Antimicrobial resistance is a major threat in medicine, associated with increased morbidity, mortality, and treatment costs (Roberts et al., 2009). Achieving good stewardship of antimicrobials is now one of the major challenges facing veterinary and human medicine.

Based on the increasing prevalence of antimicrobial resistance and the negative effect on patient outcomes, it is not surprising that factors promoting the development and spread of resistance have come under increasing scrutiny. Antimicrobial misuse and overuse are common in human medicine, where as much as 50% of antimicrobial use may be inappropriate (Gonzales et al., 2001; Lemmen et al., 2001; Paskovaty et al., 2005). Emergence and dissemination of multidrug-resistant pathogens has gathered attention well beyond the medical fields, as evidenced by the heightened political and public concern raised by pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and New Delhi metalloproteinase-1 (NDM-1) producing Enterobacteriaceae.

Although there is less information in veterinary medicine, it is clear that excessive and inappropriate use of antimicrobials can be an important problem in food animals and companion animals (World Health Organization, 2000; Weese, 2006; Wayne et al., 2011;

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Knights et al., 2012). Additionally, increased awareness of antimicrobial use in animals and antimicrobial resistance in zoonotic pathogens has increased awareness of veterinary antimicrobial use as a public health concern.

Although any use of antimicrobials can potentially contribute to development of antimicrobial resistance, antimicrobials are a required component of veterinary medicine to prevent and treat disease, improve animal welfare and potentially to increase the safety of food. From these standpoints, veterinarians have an ethical responsibility to use antimicrobials when indicated. However, veterinarians also have a responsibility to ensure the wise use of antimicrobials and to ensure that antimicrobial use is not a replacement for good management or infection control practices. Accordingly, the focus must be on the appropriate or "prudent" use of antimicrobials to provide a balance between concerns regarding antimicrobial resistance and positive effects of antimicrobial use.

What Is "Prudent Use" of Antimicrobials?

Prudent use, also referred to as *judicious use* (Prescott, 2008), is often defined broadly as the optimal selection of drug, dose, and duration of antimicrobial treatment, along with reduction of inappropriate and excessive use, as a means of achieving the best clinical outcome while slowing the emergence of antimicrobial resistance (Shlaes et al., 1997; Society for Healthcare Epidemiology of America, 2012). While a good general concept, this definition provides minimal practical guidance to clinicians and in veterinary medicine is hampered by the widespread lack of evidence regarding treatment practices, particularly duration of therapy. Without a clear understanding of goals and optimal practices, it is difficult to implement effective surveillance and mitigation practices. Accordingly, while this overriding definition is commonly used, specific details defining prudent use are not widely accepted. It may be both more inspiring and effective to subsume the concept of "prudent use" under the broader, more encompassing, concept of antimicrobial stewardship.

What Is Antimicrobial Stewardship?

Antimicrobial stewardship is the term increasingly used in medicine to describe the multifaceted approaches required to sustain the efficacy of antibiotics and minimize the emergence of resistance. The complexity of factors affecting the efficacy of antibiotic use (chapters 2 and 4–6), and of resistance and its epidemiology (chapter 3), mean that effective stewardship requires multiple approaches. In large human hospitals this often encompasses multidisciplinary teams, including clinicians, clinical microbiologists, pharmacists, epidemiologists, and infection control practitioners. The term *stewardship* resonates with a sense of religious obligation, as in its use in the concept of "environmental stewardship." Stewardship in the context of antimicrobial stewardship takes on the original (Middle English) meaning, which refers to the higher order of management of a situation that is necessary when the steward takes personal responsibility for the management of a valuable resource entrusted to his or her care. In human hospital practice a key strategy of stewardship has been drug restriction and preauthorization of use of certain antibiotics, but the concept of stewardship has to go beyond its use as simply a euphemism for restriction. It has been recommended that antimicrobial stewardship programs be mandatory for human healthcare (ACHQHC, 2011; Society for Healthcare Epidemiology of America et al., 2012), and it is difficult to justify any lesser expectation in veterinary medicine.

In human medicine, successful antimicrobial stewardship programs, like broader infection control programs, tend to be multimodal interventions incorporating different components. These may include a combination of educational initiatives, formulary restriction, antimicrobial use audits and similar measures (Toth et al., 2010; Avdic et al., 2012; Society for Healthcare Epidemiology of America et al., 2012; Teo et al., 2012). The complex scourge of antimicrobial resistance will undoubtedly benefit from multiple interventions targeting variations of disease prevention, management improvement and antimicrobial use practices, all elements encompassed within the framework of antimicrobial stewardship.

There is a need for veterinary medicine to embrace antimicrobial stewardship in its multifaceted dimensions (Edwards and Gould, 2012). Good Stewardship Practice (GSP) takes a continuous improvement and dynamic approach to addressing resistance and sustaining the future of antimicrobial therapy. Numerous small actions that can have large cumulative effects are required to address a problem of such multidimensional complexity. Each on their own may seem minor or insignificant; cumulatively they will have effect. Good

stewardship is required to bridge the current gap between the problem of resistance and the eventual development of new antibiotics and other new approaches to control of infection. Everyone associated with antibiotic use, whether government regulators, individual veterinarians or animal owners, needs to be involved in a stewardship approach. At the "front-line" veterinary practitioner level, embracing the stewardship concept will involve moving beyond concepts into generally accepted practice standards. Included in this is the need to find ways to measure outcomes of implementation of new antimicrobial use practices, to promote continuing education and to ensure that guidelines are dynamic and able to change as needed.

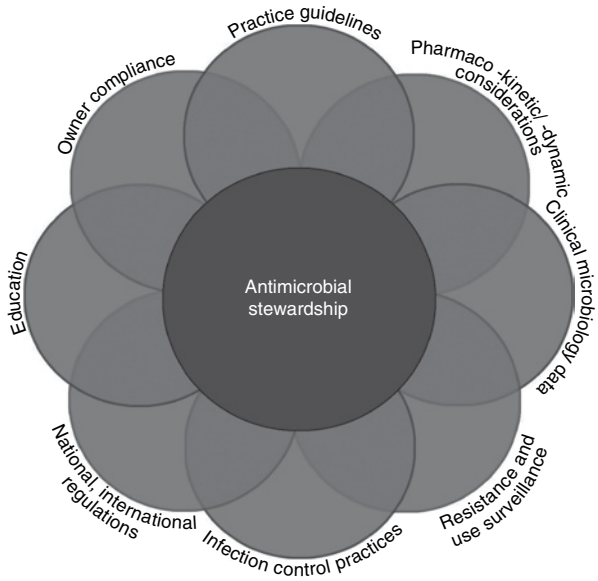

Figure 7.1. *Antimicrobial stewardship* is the term increasingly used in medicine to describe the multifaceted approaches required to sustain the efficacy of antibiotics and minimize the emergence of resistance. Good Stewardship Practice (GSP) takes a continuous improvement and dynamic approach to addressing resistance and sustaining the future of antimicrobial therapy. This figure illustrates some elements of a stewardship approach in veterinary medicine.

Considerations in Facilitating Antimicrobial Stewardship

Antimicrobial stewardship has multidimensional components encompassing the effective use of antibiotics while minimizing the development and spread of resistance. It needs to involve everyone, but particularly those involved in antibiotic use. Only a stewardship mind-set will ensure the long-term sustainability of antimicrobial drugs. Although the veterinary practitioner is on the front line of stewardship, and this chapter is focused on the practitioner, the evolving concept of stewardship involves many other elements and actors (Figure 7.1 and Table 7.1).

Antimicrobial Use Guidelines: Human Medicine

The Society for Healthcare Epidemiology in America (SHEA) has published a comprehensive position paper on guidelines for prevention of antimicrobial resistance in human hospitals (Shlaes et al., 1997), along with guidelines for development of institutional antimicrobial stewardship programs (Society for Healthcare Epidemiology of America et al., 2012). Development and implementation of specific antimicrobial use guidelines improve both patient care and stewardship through provision of clear, clinically oriented information to guide treatment decisions, including both when to use antimicrobials and optimal dosing regimens. A diverse and rapidly increasing range of clinical antimicrobial use guidelines are available in human medicine, providing clear treatment recommendations for selected diseases (American Society of Health-System Pharmacists, 1999; Bisno et al., 2002; Nicolle et al., 2005; Antibiotic Expert Group, 2010; Liu et al., 2011). Guidelines are typically created by expert panels under the auspices of organizations such as the Infectious Diseases Society of America and published after undergoing peer review (Hillier et al., 2011; Institute of Medicine, 2011; Kuehn, 2011; Lee and Vielemeyer, 2011). Evidence of a positive impact of international or local guidelines has been shown repeatedly through measures such as decreased overall antimicrobial use and the use of more appropriate drugs and dosing regimens (Angoulvant et al., 2012; Doco-Lecompte et al., 2012; Slekovec et al., 2012). Conversely, hospitals with the fewest controls on antimicrobial prescribing tend to have the greatest frequency of antimicrobial resistance (Conly, 2002). A statewide program to promote appropriate antimicrobial use in Wisconsin was associated with a decrease of 20% in antimicrobial prescription by primary care physicians (Belongia et al.,

Table 7.1. Antimicrobial stewardship: Professional management to reduce resistance selection and to preserve the efficacy of antimicrobial agents.

Responsibility	The prescribing veterinarian accepts responsibility for the decision to use an antimicrobial agent and recognizes that such use can have adverse consequences beyond the recipient. The prescriber acknowledges that an explicit risk assessment of the particular circumstances has found the benefits of such use together with any risk management measures recommended will minimise the likelihood of any immediate or longer term adverse impacts.
Reduction	Wherever possible means of reducing the use of antimicrobial agents should be implemented. Reduction in use may arise, for example, from enhanced infection control, biosecurity, vaccination, targeted treatment of individual animals, or reduction in the duration of treatment.
Refinement	Each use of an antimicrobial agent should incorporate into the design of the dosage regimen all available information on the patient, the pathogen, the epidemiology, and the antimicrobial agent (especially species-specific pharmacokinetic and pharmacodynamic profiles) to ensure the likelihood of selecting antimicrobial resistance is minimized. For example, responsible use implies right drug, right time, right dose, right duration.
Replacement	The use of antimicrobial agents should be replaced whenever available evidence supports the efficacy and safety of an alternative whose benefit-to-risk balance is assessed by the prescriber as superior to the intended use of an antimicrobial agent.
Review	Antimicrobial stewardship initiatives should be reviewed regularly and a process of continuous improvement adopted to ensure that antimicrobial use practices set or reflect contemporary best practice.

2005). However, it was concluded that increased emphasis on local interventions, such as academic detailing by physician opinion leaders, feedback on antimicrobial performance and economic incentives for careful antimicrobial use, might be more successful at changing individual prescribing behavior.

Evaluation of overall success of guideline implementation can be difficult to measure in part because of the different outcomes that can be considered, including compliance with guidelines, clinical outcome, reduction of overall or class-specific antimicrobial use and reduction of antimicrobial resistance. Evaluation of the impact of guidelines over time on clinical behavior is required to ensure that there is not simply a short initial response with rapid reversion to previous practices. A guideline cannot be realistically considered effective unless there is fulfillment of most or all goals (and explicit goals should be defined in each guideline), and maintained success. Objective evaluation of changes in antimicrobial resistance is, however, difficult, and development of resistance by pathogens other than the target pathogen, such as Enterobacteriaceae, is even more difficult to evaluate but should be considered. A guideline that decreases resistance in the target pathogen but increases patient morbidity or increases overall resistance among other pathogens (referred to as "squeezing the balloon" by Burke, 1998) cannot be considered a success, nor can a guideline that results in poor compliance. Guidelines must be flexible, updated frequently, and locally relevant because of differences in factors such as patient risk and antimicrobial resistance patterns.

Guideline development is an intensive and often exhausting process. Experience suggests that the effort spent introducing guidelines, educating healthcare providers and monitoring the response to guidelines is minimal compared with the effort required to develop the guidelines, but all aspects are critical to success. Compliance with antimicrobial use guidelines may be a challenge because of inadequate communication, differences in opinion regarding recommended treatments, resentment of measures to proscribe individual decisions, and failure to adapt guidelines to reflect cultural differences (Diamond and Kaul, 2008; Chu et al., 2011; Hoomans et al., 2011; Borg et al., 2012). A study evaluating compliance with perioperative guidelines in a human hospital reported good compliance with many aspects of the guidelines, but overall compliance of only 28% (van Kasteren et al., 2003). Reasons for lack of compliance included ineffective distribution of guidelines, lack of awareness of guidelines, and organizational or logistic constraints (van Kasteren et al., 2003).

Antimicrobial Use Guidelines: Veterinary Medicine

General prudent use guidelines have been developed in recent years by most national veterinary organizations, mostly providing statements of principles of prudent antimicrobial use (Table 7.2). As discussed above, although

Table 7.2. General principles of appropriate antimicrobial use.

- Antimicrobials should only be used when there is reasonable likelihood that a bacterial infection is present or at risk of developing.
- Antimicrobial therapy should be based on culture and susceptibility testing whenever possible.
- As narrow a spectrum therapy as possible should be used.
- Antimicrobials should be used for as short a time as possible.
- Antimicrobial, pathogen, infection site, and patient factors should be considered when choosing an appropriate treatment.
- Antimicrobials that are important for treating refractory or serious infections in humans should be used sparingly and only after careful consideration.
- Extra-label use should be avoided when on-label options are reasonable.
- Clients should be educated to improve compliance, particularly with respect to completing the entire treatment course.
- Antimicrobial therapy should never be used as a substitute for good infection control, medical and surgical practices, and animal husbandry.
- Methods to reduce the risk and incidence of infection should be emphasized to decrease the need for antimicrobials.
- Peri-operative prophylaxis should only be used when indicated, and following standard guidelines.
- Antimicrobials should only be used in the context of a valid veterinarian/client/patient relationship.

these are important from a conceptual standpoint, their clinical guidance and impact is likely limited. This lack of effect may be greatest in companion animal medicine because there are fewer clinical trials, fewer standard treatment practices and a wider range of drug regimens that may be used, many with little or no underlying objective published evidence. Antimicrobial use guidance based on the experience of authors and a review of the literature is available in a number of sources including, since 1993, this textbook. Other standard texts have included author recommendations on first choice, second choice and last resort antimicrobial agents (Guardabassi et al., 2008). Most recently, true evidence-based clinical antimicrobial use guidelines have been developed using approaches similar to those for human guidelines. These guidelines have typically involved expert panels reviewing and assessing the quality and strength of published literature to produce recommendations for diagnosis and management of specific conditions (Nielsen, 2010; Noli and Morris, 2011; Weese et al., 2011; Mueller et al., 2012; Roberson, 2012; Teale and Moulin, 2012). Many veterinary specialty organizations have also developed guidelines, ranging from generic prudent use guidelines (Morley et al., 2005) to practice- or disease-specific guidelines (Littman et al., 2006; Sykes et al., 2011). National veterinary organizations have also coordinated practice guideline development allowing for consideration of national differences in agricultural and veterinary practices, antimicrobial availability and disease trends. While there has been a dramatic increase in available guidelines, there has been little assessment of the impact of these guidelines on practices or evaluation of different measures to disseminate information. Objective study is required to better understand the impact of veterinary practice guidelines and ways to improve their use.

Antimicrobial Use Categories

It has been recommended that veterinarians and veterinary practices categorize antimicrobials used at their facilities (Morley et al., 2005). Use categories define when the use of specific drugs is appropriate. Various category names have been employed, such as primary, secondary, and tertiary, or first-line, second-line, and third-line (Table 7.3). The key principle of use categorization is that second- or third-line drugs (discussed further below), which are typically broad-spectrum and often of particular importance in human medicine, should only be used when first-line drugs are not appropriate, when alternative approaches (e.g., local therapy alone) are not appropriate, and when treatment with second- or third-line drugs has a reasonable potential to positively affect the outcome of the case. Culture and susceptibility testing of appropriate diagnostic specimens is essential for determining the use of different classes, particularly for use of second-line or third-line drugs.

The line concept is straightforward, but application is difficult because of a lack of consensus regarding membership of each category. The categorization of antimicrobial drugs for animal use based on their importance in human medicine is an area of intense discussion; criteria used in assessing their importance in animals and in humans are shown in Table 7.4. The World Health Organization divides antimicrobials into three categories: critically important, highly important, and important (World Health Organization, 2005; Table 7.5). It is immediately obvious that many antimicrobials in the critically important class are commonly used in both food and companion animals.

Table 7.3. An example of antimicrobial classification in a small animal veterinary hospital.

Class	Definition	Examples
Primary, front-line	Used as initial treatments with known or suspected bacterial infection, in advance of or in lieu of culture and susceptibility results. These drugs may be commonly used in human medicine but are typically considered less important for treating serious human infections or where development of resistance is of lesser concern.	Penicillin; first- and second-generation cephalosporins; tetracyclines; trimethoprim-sulfonamides
Secondary, second-line	Used when culture and susceptibility testing, plus patient and infection factors, indicate that no first-line drugs are reasonable options. Drugs in this class may be more important for treatment of serious infection in humans, or there may be particular concern about development of resistance.	Fluoroquinolones; third- and later generation cephalosporins
Tertiary, third-line	Used in serious, life-threatening infections, with the support of culture and susceptibility testing, when no first- or second-line drugs are indicated.	Carbapenems
Restricted, voluntarily prohibited	Used only in life-threatening infections where culture and susceptibility testing indicates no other options. Additional requirements may be indicated, or use may be voluntarily prohibited.	Vancomycin

Table 7.4. World Health Organization (2009) and World Organisation for Animal Health (2007) criteria for classification of antimicrobials.

Criterion	Veterinary Critically Important Antimicrobials (OIE, 2007)	Human Critically Important Antimicrobials (WHO, 2009)
1	Response rate: majority of respondents identified the importance of the antimicrobial class	Antimicrobial agent is used as sole therapy or one of few alternatives to treat serious human disease
2	Treatment of serious animal disease and availability of alternative antimicrobials: agents in class considered essential, few alternatives	Antimicrobial agent is used to treat diseases caused by either: (1) organisms that may be transmitted via non-human sources; or (2) diseases caused by organisms that may acquire resistance genes from non-human sources
1 and 2	Veterinary critically important antimicrobials	Critically important antimicrobials
1 or 2	Veterinary highly important antimicrobials	Highly important antimicrobials
Not 1, not 2	Veterinary important antimicrobials	Important antimicrobials

Table 7.5. World Health Organization (2009) categorization of antimicrobials used in human medicine.

Critically Important	Highly Important	Important
Aminoglycosides (excluding kanamycin/neomycin)	Aminocyclitols	Bacitracin
Ansamycins	Kanamycin/neomycin	Fosfomycin
Carbapenems	Amphenicols	Lincosamides
Third- and fourth-generation cephalosporins	First- and second- generation cephalosporins	Nitrofurantoins
Glycopeptides	Cephamycins	Nitroimidazoles
Lipopeptides	Fusidic acid	
Glycylcyclines	Mupirocin	
Macrolides and ketolides	Antistaphylococcal penicillins	
Oxazolidinones	Pleuromutilins	
Penicillins (natural, aminopenicillins, antipseudomonal)	Polymyxins	
Quinolones	Sulfonamides and combinations with dihydrofolate reductase inhibitors	
Streptogramins	Monobactams	
Tetracyclines		

Placement of specific drugs into use categories may vary somewhat with practice type and animal species, but the general principles remain the same. It is important to remember that primary (first-line) drugs (Table 7.3) are not necessarily less useful than second- or third-line drugs, and that seriousness of infection does not indicate the need for second- or third-line drugs. In fact, first-line drugs are useful for the treatment of most bacterial infections, and second- and third-line options should rarely be needed. At one tertiary care small animal teaching hospital, first-line drugs accounted for over 90% of antimicrobial prescriptions, despite a caseload skewed toward critically ill referral cases, many of which had been treated with a variety of antimicrobials prior to presentation and were immunocompromised (Weese, 2006). Because of the potential for overuse and uncommon need for second- and third-line options, consultation with clinical microbiologists, clinical specialists such as internists, microbiologists and infection control personnel should be undertaken when situations seem to indicate that second- or third-line antimicrobials are indicated, and should reasonably be considered a mandatory component of third-line drug selection (Table 7.3).

Voluntary Antimicrobial Restriction Policies

Antimicrobial restriction is a contentious measure, but formal or informal restriction of the availability of antimicrobials is a well-recognized approach in human medicine. Restriction may involve an outright ban of certain drugs, but more commonly requires demonstration of the need for a specific antimicrobial and authorization by designated officials prior to its use. A 1996 survey reported that 81% of surveyed human teaching hospitals had a policy that restricted use of certain antimicrobials (Lesar and Briceland, 1996). Although beneficial results have been demonstrated in human medicine (Kaki et al., 2011; Slain et al., 2011), implementation of voluntary restriction policies remains controversial, under-used and under-investigated in veterinary medicine. Some clinicians may think that antimicrobial restriction causes undue obstruction to their privilege of using off-label therapies, whereas others may believe that restriction of certain drugs or classes is an appropriate response to concerns regarding antimicrobial misuse and veterinary use of drugs that are deemed critically important in human medicine.

Some veterinary teaching institutions voluntarily restrict the use of certain drugs, such as vancomycin. This may consist of an outright prohibition to restriction to only selected circumstances (e.g., life-threatening infection, where local therapy alone is not reasonable, where there is the potential for survival with therapy, when culture and susceptibility testing indicates that vancomycin is the only option, and with the approval of designated infectious diseases or infection control personnel). All veterinary facilities should consider whether specific antimicrobial restriction policies are indicated. Even in situations where the targeted drug has never been used, it is important proactively to develop a policy so that decisions can be made in advance, with consultation of relevant parties, as opposed to a reactionary process when a clinician wishes to use the drug. Education and open dialogue between all involved parties is critical to facilitate development and successful implementation of voluntary restriction policies. Where third-line antimicrobials considered of critical importance to human medicine are used an assessment of the likelihood of adverse impacts to public health should be undertaken and if indicated appropriate risk mitigation measures introduced. Such risk assessments should become a standard part of the prescribing process.

Formularies in Promoting Antimicrobial Stewardship

Drug formularies can take two major forms. One type lists antimicrobial drugs that are available for an institution or region. Another type involves treatment recommendations for common conditions, including antimicrobial agent, dose, route, and duration. In human medicine, formularies are widely used to control antimicrobial costs, but they can have a significant impact on antimicrobial use trends. In some areas, most (66–91%) human hospitals have a formulary (Lawton et al., 2000; Woodford et al., 2004). Changes in formulary composition can have a profound impact on the use of certain antimicrobials. Local formulary decisions can include consideration of institutional antimicrobial resistance trends, and regular reviews can ensure that protocols remain adequate.

Formularies are not widespread in veterinary medicine. As with antimicrobial restriction policies, formularies may be met with resistance based on perceptions of negative impact on the “freedom” of practice and

the "art" of veterinary medicine. Development of standardized antimicrobial use recommendations for common situations has been advocated (Morley et al., 2005), with the understanding that the goal is not to dictate the practice of medicine, but rather to supplement clinical judgment and ensure that the art of medicine remains based in science.

Stop-Orders in Promoting Antimicrobial Stewardship

A potential cause of excessive antimicrobial use is failure to discontinue therapy at the appropriate time. In some cases, antimicrobial treatment may be continued for longer than planned simply because no one wrote orders to stop therapy. Stop orders are automatic orders to stop antimicrobial therapy after a certain time, and these have been components of successful antimicrobial control practices in human medicine (Diamond and Hales, 1997; Singer et al., 1998). Longer treatment is permitted but must be requested; therefore inadvertent continuation of therapy is avoided. A similar concept is ensuring that animals are re-evaluated before renewal of any antimicrobial prescription and not dispensing bulk quantities of antimicrobials to be used at an owner's or producer's discretion.

Antimicrobial Use Monitoring

Monitoring of antimicrobial use practices can provide useful information about baseline antimicrobial use practices, changes in prescription patterns over time and the impact of interventions. Data can also be used for educational programs designed to improve prescribing practices. Obtaining good-quality antimicrobial use data can be facilitated by electronic medical records systems, but recovery and interpretation of data can be difficult. Because of the large variation in size of veterinary patients, overall mass of antimicrobial is a relatively poor indicator of use unless study involves a population of similarly sized animals (e.g., cats, adult horses, feedlot cattle). Methods to alleviate this biasing factor include use of total prescriptions (which can be impacted by variable length of use), defined daily doses, and biomass. Regardless of the method, antimicrobial use monitoring is perhaps best used for evaluation of trends in the same facility over time, on the assumption that any data biases would be consistent between time periods and any identified changes would represent true changes.

Retrospective study of antimicrobial use practices has been reported in small animal veterinary teaching hospitals (Weese, 2006; Escher et al., 2011; Wayne et al., 2011). One study reported a positive effect of increased education, with a reduction in the overall use of antimicrobials and the use of certain targeted antimicrobials (fluoroquinolones, carbapenems; Weese, 2006). Another study reported no documented evidence of infection in 38% of cases where therapeutic antimicrobials were prescribed (Wayne et al., 2011). However, this study did not report any efforts to educate clinicians or modify practices. While unpublished efforts might have been undertaken, monitoring use practices for academic reasons without attempting to modify local behavior limits the impact of any monitoring program. As medical records systems evolve to facilitate timely and easy collection of antimicrobial use data, routine antimicrobial use monitoring should be considered as part of a veterinary practice's routine infection control activities.

A common debate between individuals in human medical, veterinary medical and public health fields is the relative use of antimicrobials in humans and animals. In some countries, especially those where antimicrobials for animals are only available by prescription and dispensed by pharmacies, accurate use data can be obtained (e.g., DANMAP, 2011). In other countries, sales and use data is collected through myriad sources such as voluntary disclosure by pharmaceutical companies and feed mills, sentinel veterinarians, drug importation data and farmer records, yet the accuracy of the data cannot be verified and may be poor. It is remarkable how difficult it can be to accurately determine the amount of antimicrobials that are produced or consumed in many regions and how different groups (often with inherent biases) can produce different estimates of human and animal antimicrobial consumption for the same region. International standards require the creation of effective national systems to monitor antimicrobial use in animals (World Health Organization, 2012).

While there are limitations in antimicrobial consumption data, this information is valuable (Apley et al., 2012; Merle et al., 2012), particularly for evaluation of year-to-year variation, for "benchmarking" between different countries, determination of the impact of use interventions and for comparison of antimicrobial use to antimicrobial resistance data obtained in properly designed surveillance studies.

Laboratory Diagnostic Testing

It important that reasonable measures be taken to achieve a diagnosis so that antimicrobials are not used unnecessarily and so that appropriate antimicrobials are used if indicated. A common limitation is the reluctance of owners to pay for diagnostic testing. Although it is unreasonable to expect that all animal owners will agree to recommended testing, the manner in which diagnostic testing is presented can have an impact on compliance. Use of susceptibility testing and result interpretation is reviewed in chapter 2.

The increasing availability of rapid diagnostic tests, particularly the expansion of nucleic acid amplification tests, may have an impact on antimicrobial use patterns by providing a quicker diagnosis and resistance gene detection. However, if new tests have higher sensitivities, lower detection thresholds or lower specificities compared to existing tests, there may be a fine line between increased diagnosis and overdiagnosis/misdiagnosis, with the latter potentially contributing to unnecessary antimicrobial use. It is imperative that new diagnostic tests are validated prior to introduction.

Monitoring resistance trends in important pathogens can be useful in any practice as a guide to appropriate antimicrobial use in advance of susceptibility testing results, or in cases where testing is not performed. However, as noted in chapter 3, data from diagnostic labs can be misleading, since results indicate the prevalence of resistance in bacteria isolated from clinical specimens submitted to the diagnostic laboratory. This population can be biased toward more severe infections, infections that develop despite prophylactic antimicrobial therapy, and infections that are non-responsive to initial therapy; hence results do not necessarily indicate the overall prevalence of resistance. Thus, such data can provide useful information but the inherent biases must be recognized.

The diagnostic laboratory can also have an impact on antimicrobial use based on reporting of culture and susceptibility testing results (chapter 2). Veterinarians should only use high-quality laboratories, with certified standards. The largely unregulated nature of veterinary diagnostic testing means that diagnostic laboratories are not required to use standard guidelines (e.g., Clinical and Laboratory Standards Institute [CLSI], European Committee on Antimicrobial Susceptibility Testing [EUCAST]) for testing and reporting of antimicrobial susceptibility. These guidelines provide information on testing and quality control practices, what antimicrobials should be tested for certain bacteria, how results should be reported, and which organisms should be considered resistant to certain antimicrobials regardless of test results (e.g., cephalosporin resistance in enterococci). Reporting of antimicrobial susceptibility results for a large number of drugs, particularly second- and third-line drugs, can result in increased unnecessary use of these agents. Another important component is not performing susceptibility testing on bacterial isolates that are considered to be part of the normal microflora, and not considered clinically relevant given the site of sampling and clinical process. Reporting of susceptibility results for commensal microflora undoubtedly results in inappropriate antimicrobial use.

Good diagnostic laboratories serve as important conduits for isolates for molecular epidemiology studies that can pinpoint the spread of resistant clones of pathogens in animals.

Education in Promoting Antimicrobial Stewardship

Veterinarians

Improvement in education about antimicrobial resistance and prudent use has been advocated for medical students and physicians (Society for Healthcare Epidemiology of America et al., 2012), and parallel education of veterinarians, from students to experienced practitioners, is required to ensure appropriate use of antimicrobials. Better education will facilitate making optimal antimicrobial treatment decisions and foster better communication to counter pressure from owners or producers to dispense antimicrobials in situations where antimicrobial therapy is not indicated. Practices such as routine perioperative antibiotic prophylaxis for "clean surgery" need to become regarded as obsolete (Knights et al., 2011).

Education pertaining to antimicrobial stewardship requires acceptance that antimicrobial stewardship is important. Despite overwhelming evidence, some prominent groups still display resistance to the notion that veterinary antimicrobial use is of concern. While the American Veterinary Medical Association has stated that "There is little to no evidence that restricting or eliminating the use of antimicrobials in food-producing

animals would improve human health or reduce the risk of antimicrobial resistance to humans" (http://www.avma.org/public_health/antimicrobial_use.asp), it is hoped that this statement does not infer that veterinarians should not be stewards of antimicrobial agents. Clearly, broader acceptance of the role of veterinary medicine in antimicrobial resistance is needed to facilitate development of GSP. Fortunately, many national and international veterinary organizations have taken a proactive approach in the support of antimicrobial stewardship.

Although general awareness of antimicrobial resistance and concerns regarding overuse of antimicrobials is increasing in the lay population, veterinarians may still face knowledge gaps that lead to inappropriate antimicrobial use. Increased education, both on an individual veterinarian-client level, and from broader initiatives, could reduce overall and inappropriate antimicrobial use.

Farmers

In many countries, farmers play an important and direct role in antimicrobial use decisions, sometimes through the ability to purchase and administer antimicrobials without veterinary involvement. While clearly an illogical practice from animal health and welfare as well as international stewardship standpoints (World Health Organization, 2012), in areas where this practice still exists, education of producers may be critical first step for reduction and improvement in antimicrobial use. All antimicrobials used for disease control in animals should require prescription (World Health Organization, 2012) and be under veterinary oversight. Knowledge regarding antimicrobial use and resistance among producers is variable and may be poor (Eltayb et al., 2012). Therefore, education regarding indications for antimicrobial therapy, when veterinary involvement is critical, and proper dosing regimens might help improve antimicrobial use. Antimicrobial stewardship requires veterinary oversight.

Even where there is good veterinary oversight of antimicrobial use, education of producers is important to ensure completion of recommended antimicrobial regimens and to help producers understand stewardship considerations that might be taken by their veterinarian when developing preventive or treatment regimens. Indeed, under GSP, farmers should question the use of antimicrobials and take active steps to ensure infection control, biosecurity, and other measures are in place to reduce the likelihood of infectious disease.

Companion Animal Owners

It is well recognized in humans that antimicrobials are sometimes prescribed to satisfy patients in some situations where treatment is not indicated (Murphy et al., 2012). Although not investigated in veterinary medicine, it is likely that the same phenomenon occurs. Accordingly, education of clients is crucial because client pressure to "do something" or prescribe antimicrobials can lead to inappropriate antimicrobial use. Various approaches have been tried in human medicine to address this, including the use of "non-prescription pads," a "prescription" that says that antimicrobial treatment is not needed. While not satisfying all individuals, it conveys the concept that the clinician has considered antimicrobials and has determined that they are not needed. Novel approaches such as this could also fulfill an important function in veterinary medicine.

An additional important role of owners in stewardship is proper antimicrobial administration. Failure to administer the antimicrobial as directed or to complete the prescribed treatment regimen may have an impact on both clinical outcome and antimicrobial resistance. Good communication can overcome the pressure to prescribe inappropriately and can emphasize the need to complete the entire treatment course. Additionally, veterinarians must take into consideration the abilities of the owner and the behavior of the animal when prescribing and decrease the likelihood of poor compliance because of difficulty in administering the drug (route or frequency of administration), which can lead to premature cessation of therapy. Even the timing of dosing may be a particular challenge for some owners (Adams et al., 2005), and should be considered when selecting a treatment regimen.

Access to Antimicrobials and Implications for Stewardship

In most countries, the veterinarian is directly responsible for overseeing and directing antimicrobial use in animals. This is obviously a logical approach because of veterinarians' training in animal diseases, animal husbandry and antimicrobial use, and consistent with modern stewardship approaches (World Health Organization, 2012). However, in many countries, as outlined above,

some antimicrobial agents remain available for purchase over-the-counter by lay untrained personnel, with no direct veterinary involvement. As such, it has been recommended that all antimicrobials be available only through a veterinarian with an established veterinarian-patient-client relationship (Morley et al., 2005). Not only should this reduce resistance, it potentially facilitates accurate monitoring of total antimicrobial drug use, optimizes animal care, reduces the likelihood of antimicrobial residue problems in food animals and facilitates dialogue regarding other treatment and prevention measures.

An additional concern pertaining to antimicrobial access is bulk dispensing of drugs by veterinarians to producers, something that is allowed in many countries. Although under the auspices of a veterinary/client/patient relationship, this approach may often be inappropriate for many reasons, including that it can lead to imprudent use by allowing lay personnel to guide decisions about which animals to treat.

Internal versus External Regulation in Promoting Stewardship

Currently, veterinarians in many countries have almost complete freedom in prescribing and dispensing any antimicrobial, with the most significant regulatory issues involving the absence of maximum residue limits or tolerances and the appropriate withdrawal period. In contrast, in other countries veterinary dispensing of antimicrobials is tightly controlled. (Regulatory aspects are discussed in chapter 26.) Increased awareness of antimicrobial use, misuse (real or perceived) and resistance has led to recurring efforts in some countries to restrict veterinary access to antimicrobials that are of critical importance in human medicine. This has raised concern because of the potential for excess restriction, particularly since the majority of therapeutic antimicrobials used in animals are classified by the World Health Organization as "critically important" or "highly important" (Table 7.5).

One approach that can be taken to decrease the likelihood of restrictions being placed on antimicrobial use is demonstration of appropriate use and self-regulation. Internal "policing" by self-audit, including guideline development, education, monitoring, and emphasizing an evidence-based approach to antimicrobial use, demonstrate that care and attention are being paid to antimicrobial use and resistance issues and may help allay concerns by non-veterinary groups. To decrease the perceived need for external control, it is critical to demonstrate to regulatory bodies that the veterinary profession is taking a proactive, stewardship approach to antimicrobial use. Concerns, frequently unjustified, regarding inappropriate antimicrobial use practices in animals that have been raised, sometimes quite vocally, should serve as a stimulus for the veterinary profession to take measures to demonstrate GSP, rather than the frequent defensive posturing that ensues in an attempt to deflect criticism. Although antimicrobial misuse is rampant in human medicine and human antimicrobial use plays a major role in antimicrobial resistance, the veterinary profession must acknowledge, accept and address the many areas of weakness over which it has control. Failure to do so could increase the likelihood of severe restrictions being placed on veterinary access to antimicrobials.

Addressing Potential Conflicts of Interest in Prescribing Practices

A contentious area in many regions is the financial benefit that veterinarians receive from prescribing antimicrobials. Unlike most human medicine, veterinarians in most regions both prescribe and sell antimicrobials, and therefore benefit financially from antimicrobial use. This is of particular concern in food animal medicine, where antimicrobial sales may generate a substantial percentage of practice revenue. Although that does not mean that treatment decisions will be made with profit rather than prudent use in mind, this conflict of interest needs to be removed (Grave and Wegener, 2006).

Importance of Regulatory Monitoring for Evaluation of Antimicrobial Stewardship Measures

Regulatory bodies play critical roles in evaluating potential risks with new antimicrobials, licensing of veterinary antimicrobials and post-licensing surveillance for resistance (chapter 26). The strength of these efforts is variable and these bodies may be under considerable pressure from competing interest groups. Monitoring programs are important to determine the impact of new antimicrobials on resistance in both target organisms and in indicator bacteria (organisms that are not the target of therapy but in which antimicrobial resistance is of

relevance). In some cases resistance in indicator bacteria may be more important because they can be important zoonotic pathogens (chapter 3). Regulatory bodies may withdraw access to the antimicrobial if alarming trends develop in either group; however, this is most likely to occur if resistance in human pathogens develops. A rare example of post-marketing regulatory intervention was the U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine's withdrawal of approval of the use of enrofloxacin in poultry because of emergence of fluoroquinolone resistance in *Campylobacter jejuni*.

Linking surveillance of food animals, food and zoonotic pathogens from humans can be useful to clarify the role of veterinary antimicrobial use on resistance patterns in human disease isolates (Dutil et al., 2010; World Health Organization, 2012) but integrated programs are limited in number globally. Few countries have embarked on programs to actively reduce the use of antimicrobials in animals and monitor the outcome. One of the most comprehensive is the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP), which reports antimicrobial use data from animals and humans, as well as the occurrence of antimicrobial resistance in bacteria from animals, humans, and food products (DANMAP, 2011). Other programs, such as CIPARS and NARMS, are described in chapter 3.

A weakness of most monitoring programs is the focus on food animals, with little or no attention paid to companion animals. The more frequent use of critically important drugs (e.g., fluoroquinolones and third-generation cephalosporins), occasional administration of drugs used for the treatment of multidrug-resistant infections in humans (e.g., carbapenems) and the close nature of contact between humans and companion animals indicates the potential importance of companion animals (including horses) in the emergence of antimicrobial resistance (Heuer et al., 2005; chapter 3).

Introduction of New Antimicrobials

Veterinary medicine is a progressive medical field, and practitioners typically strive to remain on the cutting edge of medicine. As a consequence, there may be inclinations to try new antimicrobials, or drugs that are used in humans but rarely used in animals, as a perceived way to advance the "quality of medicine" practiced. The use of newer antimicrobials when traditional options are reasonable does not constitute progress in the practice of medicine but rather may indicate inappropriate use. Indeed, the need to use new antimicrobials may reflect failure of infection control and preventive medicine practices, rather than a normal progression of veterinary medicine. However, as antimicrobial resistance develops, there will be increased pressure, and in many cases need, to use newer antimicrobials, if any are developed or allowed for use in animals. The key for GSP is avoiding these changes until absolutely required, and ensuring that use of these drugs will have as minimal impact on antimicrobial resistance in human and animal pathogens as possible.

One conundrum is at what stage one starts using new antimicrobials. Objective guidelines have not been developed to assist with this process. Consideration should be given to defining situations where first-line empirical use of a drug for a particular pathogen is inappropriate. This is difficult to do without supporting evidence, and is confounded by regional variations in susceptibility, other factors that might influence clinical outcome (e.g., toxicity, adverse reactions, drug interactions) and the bias of clinical laboratory surveillance results.

With increased awareness and scrutiny of extra-label antimicrobial use, educational and regulatory campaigns have targeted extra-label use of antimicrobials. However, unintended consequences of increased regulation of antimicrobial use must be considered. Since newer drug approvals tend to be for broad-spectrum drugs such as fluoroquinolones and later-generation cephalosporins, there may be increasing conflict between the use of newer on-label drugs versus more narrow-spectrum, "older" options that do not have specific label claims. Thus, while label indications must be a consideration when choosing an antimicrobial, particularly in food animals, this cannot be done at the exclusion of broader aspects of prudent use.

Use of Non-antimicrobial Treatment Options

The best way to control resistance is often not to use antimicrobials; antimicrobials must be considered only one aspect of the treatment plan, which encompasses a range of concepts, ranging from identifying underlying causes of disease to the use of non-antimicrobial treatment options (chapter 6). Although often overlooked in the context of antimicrobial use, addressing underlying risk factors for disease is a key aspect of infection

prevention and treatment. Failure to properly address underlying factors may prevent adequate response to treat while in others it may result in subsequent re-infection (e.g., failure to address the underlying skin disease in a dog with superficial pyoderma, poor farm management practices), ultimately with decreased clinical success and increased overall use of antimicrobials. Underlying causes cannot always be identified or successfully controlled, but attempts should always be made whenever possible. Investigation of underlying causes should be considered in all cases and be mandatory when recurrent or poorly responsive infections are encountered.

Traditional antimicrobials are not the only, or indeed always the best, option for treatment of infections. A range of alternatives can be considered as complementary therapy or in place of antimicrobials, although scientific evidence is variable. Probiotics are commonly used, particularly for treatment and prevention of gastrointestinal disease; however, there is limited objective evidence of efficacy (or safety) at this point, particularly for companion animals. Quality control of commercial veterinary probiotics appears to be poor (Weese, 2003; Weese and Martin, 2011) and marketing efforts seem to take precedence over development of proper clinical trials.

Immunomodulators and immunostimulants are another approach, particularly for prevention of disease, yet clinical research is lacking and the role of these products in food and companion animals is unclear. Bacteriophage therapy (Biswas et al., 2002) is an appealing approach as a potentially safe, efficacious and non-antimicrobial treatment measure, but until adequate information is available it is not possible to determine whether this will be a viable treatment option. More stringent regulation of complementary therapies and proper research and testing are needed to determine whether these modalities may be useful for the treatment or prevention of disease and the reduction of antimicrobial use and resistance.

The role of vaccination in antimicrobial stewardship should not be overlooked. Decreasing the incidence of infectious disease in animals reduces the need for and use of antimicrobials. This does not apply only to bacterial diseases. Vaccination against viral disease can reduce the incidence of secondary bacterial disease that might require antimicrobial treatment and decrease potential (inappropriate) antimicrobial use in primary viral disease.

Alternative routes of antimicrobial delivery must also be considered (chapter 6). Local, regional or topical therapy, as well as implantation of antimicrobial impregnated materials, can be effective treatment options and potentially lessen the risk of antimicrobial resistance through reduced exposure of the massive commensal bacterial microbiomes of the intestinal tract, respiratory tract, skin and other body sites, along with improved response to treatment.

Infection Control in Promoting Antimicrobial Stewardship

The role of infection control in GSP is critical. There is evidence that the spread of methicillin-resistant staphylococci and extended-spectrum beta-lactamase-producing Enterobacteriaceae in companion animals is partly the result of nosocomially acquired infection (Wieler et al., 2011). Reducing the incidence of infections will obviously reduce the need for antimicrobial therapy (National Health and Medical Research Council, 2010). Good infection control practices on farms and in veterinary hospitals may reduce the need for prophylactic or metaphylactic therapy. Antimicrobials should never be used as a substitute for good animal husbandry and infection control, and veterinarians need to be proactive in preventive medicine and infection control at individual animal, group, farm and hospital levels. The main principles of infection control are straightforward and practical. Basic concepts such as personal hygiene, cleaning and disinfection, identification of potentially infectious cases and use of appropriate physical and procedural barriers form the core of any infection control program (Anderson et al., 2008). In particular, the role of simple hand hygiene must not be overlooked as a tool to decrease antimicrobial use by preventing spread of infectious agents, including resistant bacteria, in many environments. Hand hygiene has been shown to be among the most effective infection control practices, and proper hand hygiene can have significant impacts on infection rates (Boyce et al., 2002; Hirschmann et al., 2001), yet hand hygiene compliance in veterinary facilities tends to be poor (Shaw, 2012). Reliance on disinfectants to control the spread of resistant bacteria should recognize that some disinfectants may themselves select for spread of resistance determinants (chapter 3; Ciusa et al., 2012).

Conclusion

Antimicrobial resistance (chapter 3) will continue to have a marked impact on the practice of human and veterinary medicine, and present new challenges. GSP and proper use of antimicrobials is critical to reduce the incidence of antimicrobial resistance and decrease the emergence of new resistance genotypes and phenotypes, as well as to reduce the versatile genetic elements that capture and move resistance genes in bacterial populations (chapter 3). Antimicrobial stewardship approaches are not a panacea, but are required measures to limit the clinical impact of resistance on human and animal populations, and to permit close contact of individuals and families with their companion animals as well as to maintain a safe, high-quality, and economically viable food production system. GSP ensures veterinarians continued access to required antimicrobials while limiting the impact of animal antimicrobial use on human medicine. GSP maximizes the benefits of antimicrobial therapy both for individual patients and from a public health standpoint.

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Section II

Classes of Antimicrobial Agents

8

Beta-lactam Antibiotics: Penam Penicillins

John F. Prescott

Introduction to Beta-lactam Antibiotics

Alexander Fleming's observation in 1928 that colonies of staphylococci were lysed on a plate contaminated with a *Penicillium* mold was the discovery that led to the development of antibiotics. In 1940, Chain and Florey and their associates were the first to produce sufficient quantities of penicillin from cultures of *Penicillium notatum*. Almost a decade later, penicillin G became widely available for clinical use. In its clinical application, this antibiotic was found to have limitations that included relative instability in gastric acid, susceptibility to inactivation by beta-lactamase (penicillinases), and relative inactivity against clinically important Gram-negative bacteria. This inactivity of Gram-negative rods was subsequently found to result from (1) inability to penetrate the Gram-negative cell wall; (2) lack of available binding sites (penicillin-binding proteins); or (3) enzymatic inactivation. Intensive research led to the isolation of the active moiety, 6-aminopenicillanic acid, in the penicillin molecule. This moiety, which consists of a thiazolidine ring (A) attached to a beta-lactam ring (B) that carries a secondary amino group (R-NH-), is essential for antibacterial activity (Figure 8.1). Isolation of the active moiety has resulted in the design and development of semisynthetic penicillins that overcome some of the limitations associated with penicillin G.

The development of the cephalosporin family, which shares the beta-lactam ring with penicillins (Figure 8.2), led to a remarkable array of drugs with varying ability to penetrate different Gram-negative bacterial species and to resist several beta-lactamase enzymes (chapter 9). Other naturally occurring beta-lactam antibiotics lacking the bicyclic ring of the classic beta-lactam penicillins and cephalosporins have subsequently been described. Many have potent antibacterial activity and are highly inhibitory to beta-lactamase enzymes. Some, such as the carbapenems, oxacephems, penems, and monobactams, have potent antibacterial activity whereas others, such as the oxapenam clavulanic acid, have no intrinsic antibacterial activity but possess potent beta-lactamase inhibitory activity (chapter 10). These latter drugs are combined with older beta-lactams to increase their range of antibacterial activity. Beta-lactam antibiotics are in widespread use because of their selectivity, versatility, and low toxicity.

Chemistry

The penicillins, cephalosporins, carbapenems, monobactams, and penems are referred to as beta-lactam antibiotics. Rupture of the beta-lactam ring, which is brought about enzymatically by bacterial beta-lactamases, results in loss of antibacterial activity. Hypersensitivity reactions appear to be associated with the active moieties of the beta-lactam drugs, so that caution should be exercised when administering cephalosporins to penicillin-sensitive animals because these

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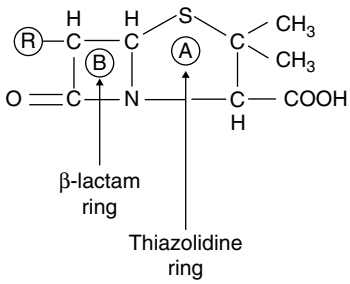


Figure 8.1. Structural formula of penicillin.

drugs are of similar structure. Substitutions can be made on the beta-lactam ring for specific purposes, such as (1) increasing resistance to beta-lactamases of clinically important families or species of bacteria; (2) enhancing activity against selected pathogens; or (3) ensuring favorable pharmacokinetic properties. Thus, some semisynthetic beta-lactam drugs have been designed for specific purposes.

Mechanism of Action

Beta-lactam antibiotics prevent the bacterial cell wall from forming by interfering with the final stage of peptidoglycan synthesis. They inhibit the activity of the transpeptidase and other peptidoglycan-active enzymes called *penicillin-binding proteins* (PBPs; transpeptidases, carboxypeptidases), which catalyze cross-linkage of the glycopeptide polymer units that form the cell wall. The drugs exert a bactericidal action but cause lysis only of growing cells, that is, cells that are undergoing active cell-wall synthesis.

Variation in the activity of different beta-lactams results, in part, from differences in affinity of the drugs for the PBPs. The difference in susceptibility between Gram-positive and Gram-negative bacteria depends on differences in receptor sites (PBPs), on the relative amount of peptidoglycan present (Gram-positive bacteria possess far more), on the ability of the drugs to penetrate the outer cell membrane of Gram-negative bacteria, and on resistance to the different types of beta-lactamase enzymes produced by the bacteria. These differences are summarized in Figures 8.3 and 8.4.

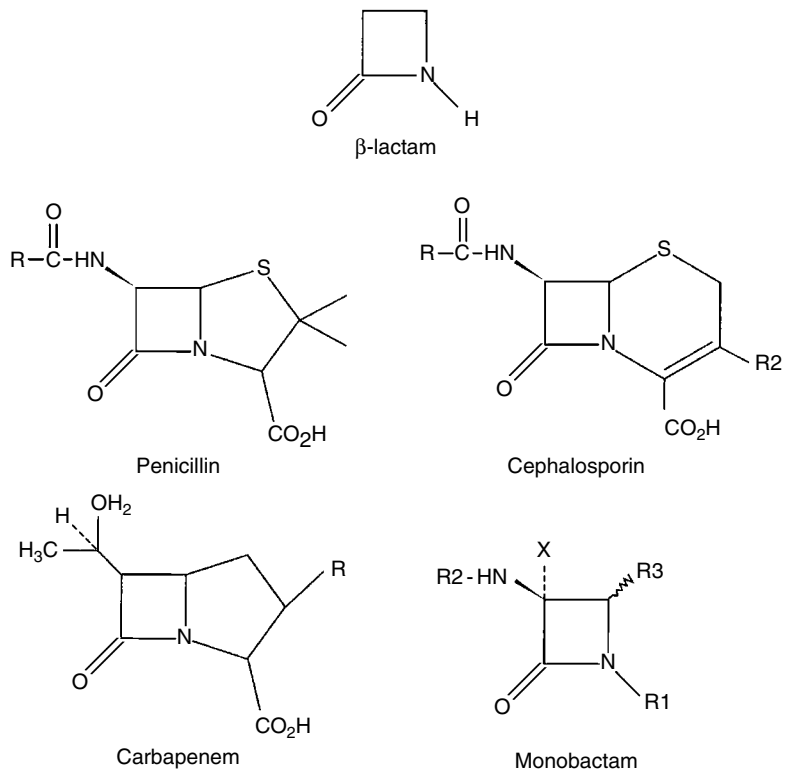


Figure 8.2. Core structures of naturally occurring beta-lactams.

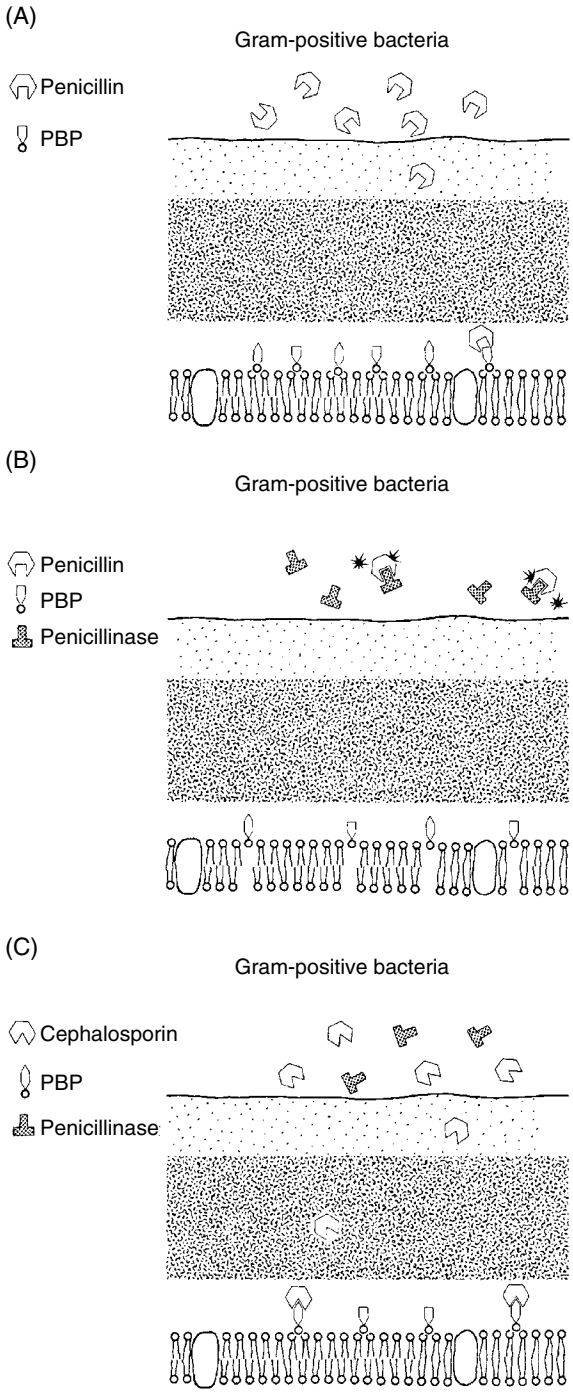


Figure 8.3. Summary of action and resistance to beta-lactam drugs: Gram-positive bacteria. (A) Susceptible bacterium; (B) exogenous beta-lactamase-producing bacterium, e.g., *Staphylococcus aureus*; (C) penicillinase-producing bacterium susceptible to cephalosporin. After R.D. Walker, unpublished, with permission.

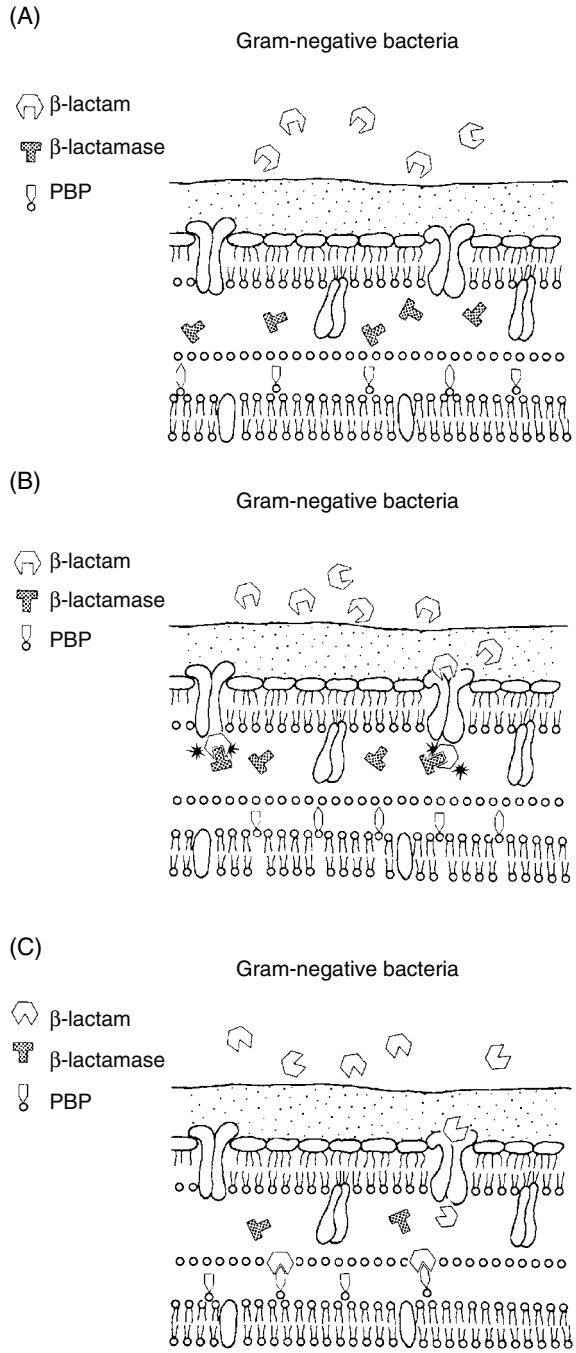


Figure 8.4. Summary of action and resistance to beta-lactam drugs: Gram-negative bacteria. (A) Bacterium constitutively resistant to penetration by beta-lactam; (B) penetration by beta-lactam but destruction by periplasmic beta-lactamase; (C) susceptible Gram-negative bacterium. After R.D. Walker, unpublished, with permission.

Beta-lactam antibiotics are bactericidal drugs with slower kill rates than those exhibited by aminoglycosides or fluoroquinolones. Killing activity starts after a lag period. Against Gram-positive bacteria, all beta-lactams exhibit an *in vitro* post-antibiotic effect. This does not carry over for the streptococci *in vivo*. The beta-lactams do not exhibit a post-antibiotic effect against Gram-negative bacteria, with the possible exception of carbapenems against *Pseudomonas*. Optimal antibacterial efficacy is time- and not concentration-dependent (chapter 5) and therefore requires that serum concentrations exceed MIC of the pathogen for essentially the entire dosing interval, so that these drugs are best administered frequently or by continuous infusion.

Resistance to Beta-lactam Antibiotics

In Gram-positive bacteria, especially *S. aureus*, resistance to penicillin G is mainly through the production of beta-lactamase enzymes that break the beta-lactam ring of most penicillins. *Staphylococcus aureus* secretes beta-lactamase enzymes extracellularly as inducible exoenzymes that are plasmid mediated (Figure 8.3). Inherent resistance to penicillin G of many Gram-negative bacteria results from low permeability of the Gram-negative cell wall, lack of PBPs, and a wide variety of beta-lactamase enzymes (Figure 8.4). Most Gram-negative bacteria inherently express low levels of species-specific, chromosomally mediated beta-lactamase enzymes within the periplasmic space, which sometimes contribute to resistance. These enzymes hydrolyze susceptible cephalosporins more rapidly than penicillin G, but they hydrolyze ampicillin, carbenicillin, and beta-lactamase-resistant penicillins poorly.

Production of plasmid-mediated beta-lactamases is widespread among common Gram-negative primary and opportunist bacterial pathogens. The enzymes are constitutively expressed, present in the periplasmic space, and cause high-level resistance. The majority are penicillinases rather than cephalosporinases (Figure 8.4). The most widespread are those classified on the basis of their hydrolytic activity as TEM-type beta-lactamases, which readily hydrolyze penicillin G and ampicillin rather than methicillin, cloxacillin, or carbenicillin. The less widespread OXA-type beta-lactamases hydrolyze penicillinase-stable penicillins (oxacillin, cloxacillin, and related drugs). More details on beta-lactamases are given in chapter 10. Beta-lactamases probably evolved from PBPs as a protective mechanism for soil organisms exposed to beta-lactams in nature. Because of spread of transferable resistance, beta-lactamase production by pathogens is now both widespread, extensive and increasingly alarming.

A major advance has been the discovery of broad-spectrum beta-lactamase-inhibitory drugs (e.g., clavulanic acid, sulbactam, tazobactam). These drugs have weak antibacterial activity but show extraordinary synergism when administered with penicillin G, ampicillin (or amoxicillin) and ticarcillin, because of the irreversible binding of the beta-lactamase enzymes of resistant bacteria. Other beta-lactamase inhibitors, such as cefotaxime and carbapenems, have potent antibacterial activity in their own right (chapter 10).

Bibliography

Bush K, Macielag MJ. 2010. New β-lactam antibiotics and β-lactam inhibitors. Expert Opin Ther Patents 20:1277.

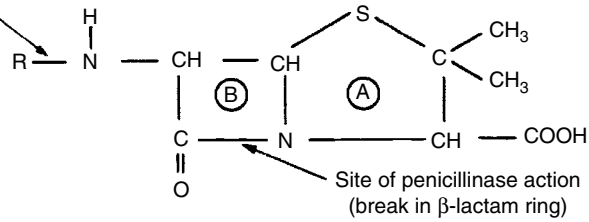
Penam Penicillins

General Considerations

The acidic radical (R), attached to the amino group of 6-aminopenicillanic acid (Figure 8.1), determines the susceptibility of the resulting penicillin to hydrolytic degradation or enzymatic inactivation by bacterial beta-lactamase, and the antibacterial activity of the molecule. Both these factors influence the clinical effectiveness of penicillins, which is also determined by the concentration attained at the site of infection. The nature of the acidic radical has little influence on the rate of elimination of penicillins, but determines the extent of plasma albumin binding and, to a lesser degree, membrane-penetrating ability. The 6-aminopenicillanic acid moiety and structure of the acid radicals of some penicillins are shown in Figure 8.5.

Penam penicillins are readily distinguished on the basis of antimicrobial into six groups ("generations"), which largely correspond to their time of introduction into clinical use (Table 8.1): (1) benzyl penicillin and its long-acting parenteral forms; (2) orally absorbed penicillins similar to benzyl penicillin; (3) staphylococcal penicillinase-resistant isoxazolyl penicillins; (4) extended- or broad-spectrum penicillins; (5) antipseudomonal penicillins; and (6) beta-lactamase-resistant penicillins.

(A) Site of amidase action



6-Aminopenicillanic acid

(B)

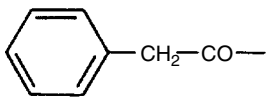
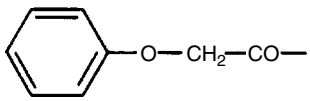
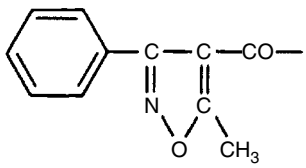
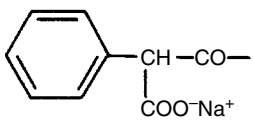
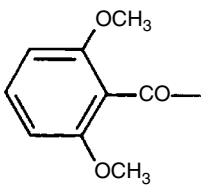
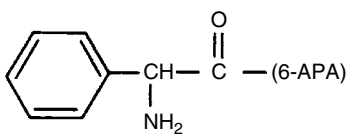
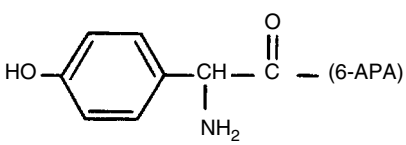
Side chain	Name
	Benzyl penicillin, penicillin G
	Phenoxymethyl penicillin, penicillin V
	Oxacillin
	Carbenicillin
	Methicillin
	Ampicillin
	Amoxicillin

Figure 8.5. Structural formula of some penicillins: (A) basic structure of penicillin G; (B) structures that can be substituted at the R to produce a new penicillin.

Table 8.1. Classification of the six groups of penam penicillins (6-aminopenicillanic acid derivatives).

Group	Important Derivatives	Antimicrobial Advantage
1. Benzyl penicillins	Procaine (long-acting form)	Gram-positive bacteria
2. Orally absorbed benzyl penicillins	Phenoxymethyl penicillin	Gram-positive bacteria
3. Antistaphylococcal isoxazolyl penicillins	Cloxacillin, dicloxacillin, oxacillin, methicillin, nafcillin	Activity against penicillinase-producing (but not methicillin-resistant) *S. aureus* and *S. pseudintermedius*
4. Extended- (broad) spectrum penicillins	Aminobenzylpenicillins (ampicillin, hetacillin, pivampicillin, amoxicillin); amidopenicillins (mecillinam)	Broader spectrum than benzyl penicillins, but beta-lactamase sensitive
5. Antipseudomonal penicillins	Ureidopenicillins (azlocillin, mezlocillin, piperacillin); carboxypenicillins (carbenicillin, ticarcillin)	*P. aeruginosa* activity, reduced Gram-positive activity
6. Beta-lactamase-resistant penicillins	Temocillin	Beta-lactamase resistance (but not methicillin resistance)

Since the 1940s, the progressive development of penicillins for clinical use resulted in derivatives with similar activity to benzyl penicillin but which could be administered orally and/or were resistant to *S. aureus* beta-lactamase (penicillinase). Subsequently, orally administered penicillins with a broader spectrum of activity, which involved greater Gram-negative antibacterial activity, and penicillins active against *P. aeruginosa* were developed. Despite considerable effort at identifying beta-lactamase resistance penam penicillins, however, with the exception of temocillin, extended-spectrum penicillins are susceptible to beta-lactamase producing Gram-negative bacteria. For this reason the use of penicillins against common Gram-negative bacteria is limited in favor of more recently introduced cephalosporin beta-lactams (chapter 9) or combination with beta-lactamase inhibitors (chapter 10).

Mechanism of Action

The targets of all beta-lactam drugs are the PBPs found on the outside of the cytoplasmic membrane that are involved in synthesizing and remodeling the cell wall. Susceptibility of a bacterium to a penicillin depends on a combination of affinity for the PBP, ability to penetrate the cell wall, and ability to resist beta-lactamase enzymes (Figures 8.3 and 8.4). There are usually 4–7 PBPs present in bacteria that are the targets for penicillins. The bactericidal effect in Gram-negative bacteria results from osmotically induced lysis of cells weakened by loss of their peptidoglycan layer. In Gram-positive bacteria, which have considerably greater quantities of peptidoglycan in their cell wall than Gram-negative bacteria, an effect of beta-lactams is not only to prevent the final peptidoglycan cross-linking that gives peptidoglycan its strength but also to release lipoteichoic acid, causing a suicide response by degradation of peptidoglycan by autolysins (endogenous endopeptidase, carboxypeptidase PBPs).

For some Gram-positive cocci, exposure to beta-lactam antibiotics above an optimal killing concentration results in a reduction of killing, which can be considerable (the "Eagle" or paradoxical effect). Its basis appears to be interference of growth by penicillin binding to PBPs other than the major target PBP. Since beta-lactams are effective only against growing, actively cell-wall-synthesizing bacteria, failure to grow results in failure to be killed. The Eagle effect is an important concept, since there may be a tendency to overdose with beta-lactam antibiotics, because they are generally so safe.

Antimicrobial Activity

Benzyl penicillin and orally administered benzyl penicillins (phenoxymethyl penicillin) have outstanding activity against many Gram-positive bacteria, notably beta-hemolytic streptococci, non-resistant staphylococci, *Actinomyces* spp., *Arcanobacterium* spp., *Bacillus* spp., *Clostridium* spp., *Corynebacterium* spp., and *Erysipelothrix rhuseopathiae*. Susceptible Gram-negative species include some *Bacteroides* spp., some *Fusobacterium* spp., and a variety of Gram-negative aerobic bacteria such as *Haemophilus* spp., and many *Pasteurella* sp (Table 8.2). Enterobacteriaceae, *Bacteroides fragilis*, most *Campylobacter* spp., *Nocardia* spp.

Table 8.2. Activity (μg/ml) of penicillins against bacteria of human origin (usual MIC).

	Narrow-spectrum penicillins		Penicillinase-stable penicillins		Broad-spectrum penicillins	
Organisms	Penicillin G	Penicillin V	Methicillin	Cloxacillin	Ampicillin	Carbenicillin
Staphylococcus aureus						
Beta-lactamase⁻	0.02	0.05	1.25	0.1	0.05	1.25
Beta-lactamase⁺	R*	R	2.5	0.25	R	25
Streptococcus agalactiae	0.005	0.01	0.2	0.06	0.02	0.2
Beta-hemolytic streptococci	0.005	0.01	0.2	0.04	0.02	0.2
S. faecalis	3	6	R	R	1.5	50
Clostridium perfringens	0.05	0.1	1	0.5	0.05	0.5
Escherichia coli	50	125	R	R	5	5
Proteus mirabilis	5	50	250	R	1.25	2.5
Proteus, indole⁺	R	R	R	R	R	5
Klebsiella pneumoniae	250	R	R	R	R	250
Enterobacter spp.	R	R	R	R	R	12.5
Pseudomonas aeruginosa	R	R	R	R	R	50

R* = Resistant.
Reprinted with permission from Garrod LP, et al. 1981. Antibiotic and Chemotherapy, 5th ed. New York: Churchill.

and *Pseudomonas* spp. are resistant. Penicillinase-resistant, antistaphylococcal isoxazolyl penicillins (cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin) have activity similar to but slightly less than that of benzyl penicillin, with the exception that they are active against penicillinase-producing *S. aureus* (Table 8.2). Extended-spectrum pencillins (aminobenzylpenicillins such as ampicillin and its esters, and amoxicillin) retain the activity of benzyl penicillin against Gram-positive bacteria but have increased activity against Gram-negative bacteria including *E. coli*, *Proteus* spp., and *Salmonella* spp. They are, however, ineffective against *P. aeruginosa* and are inactivated by beta-lactamases. Mecillinam, another member of the extended penicillin group, differs from aminobenzylpenicillins in its lower activity against Gram-positive bacteria but considerably greater activity against Gram-negative bacteria including a broad spectrum of the Enterobacteriaceae, although it is still inactivated by many beta-lactamases. Penicillins (carboxypenicillins, ureidopenicillins) active against *P. aeruginosa* (carbenicillin, azlocillin, mezlocillin, piperacillin) are effective against both Gram-positive and Gram-negative bacteria, including *P. aeruginosa* (Table 8.2).

Resistance to Penam Penicillins

Most resistance results from production of a beta-lactamase enzyme, although modification of PBPs with reduced drug affinity or reduced bacterial permeability are additional and sometimes concurrent mechanisms of intrinsic or acquired resistance to penam penicillins. Efflux mechanisms and modification of porins in Gram-negative bacteria that prevent entry of penicillins are also recognized. Beta-lactamases are discussed in chapter 10. Resistance because of exogenously produced beta-lactamase is now widespread in *S. aureus*, particularly in clinical isolates, as a result of bacteriophage or plasmid-mediated resistance. Among Gram-negative bacteria, plasmids encoding beta-lactamases have also become widespread and are the cause of extensive acquired resistance. Modification of PBPs is recognized to be increasingly important as another mechanism of resistance to penam penicillins.

The most important type of penam penicillin resistance in human medicine is methicillin (oxacillin)-resistance in *S. aureus* (MRSA), which is widespread in humans in some countries, notably Japan and the United States. Resistance because of this mechanism has emerged dramatically in animals in recent years, notably in dogs, horses, swine, and appears to reflect the incidence of

infection in humans from whom these strains were acquired (Price et al., 2012). The reason(s) for the emergence of MRSA in animals since 2000, and of livestock-associated (LA) MRSA infections in humans, are still unclear but represent host-adaptation of particular clonal types to livestock, with antibiotic resistance developing through selection by antimicrobial use, possibly including zinc compounds (Cavaco et al., 2011). In addition, animal MRSA strains are often hospital-associated and can contaminate veterinary hospital environments to a remarkable extent. Human subclinical and even clinical infections have been acquired from animal sources). MRSA are regarded as resistant to all beta-lactam antimicrobials and are commonly but not always resistant to other antimicrobials. Methicillin-resistant *S. pseudintermedius* (MRSP) are also increasingly isolated from dogs and cats and, like MRSA, are regarded as resistant to all beta-lactam antibiotics (Perreten et al., 2010). They commonly also have other multidrug resistances.

Methicillin-resistance is more frequent in coagulase-negative *Staphylococcus* spp., which may rarely be significant as nosocomial infections in hospitalized animals.

Pharmacokinetic Properties

The penicillins are organic acids that are generally available as the sodium or potassium salt of the free acid. In dry, crystalline form, penicillins are stable but lose their activity rapidly when dissolved. Apart from the isoxazolyl penicillins (cloxacillin, dicloxacillin, oxacillin) and penicillin V, acid hydrolysis in the stomach limits the systemic availability of most penicillins from oral preparations. The penicillins (pK_a 2.7) are predominantly ionized in plasma, have relatively small apparent volumes of distribution (0.2–0.3 L/kg), and have short half-lives (0.5–1.2 hours) in all species of domestic animals. After absorption, they are widely distributed in the extracellular fluids of the body, but cross biologic membranes poorly since they are ionized and poorly lipid soluble. Concentration in milk, for example, is about one-fifth that of serum. Entry across biologic membranes or through the blood-brain or blood-cerebrospinal fluid barrier is enhanced by inflammation, so that inhibitory drug concentrations may be attained at these sites that are normally inaccessible to penicillin.

Penicillins are eliminated almost entirely by the kidneys, which results in very high levels in the urine; nafcillin is an exception, in that it is excreted mainly in bile. Renal excretion mechanisms include glomerular filtration and tubular secretion. The latter is subject to competitive inhibition by other organic acids, such as probenecid. Impaired renal function delays excretion of the penicillins, but the wide margin of safety of this class of drug offsets the absolute need to adjust dosage.

Drug Interactions

Penicillins are usually synergistic with the aminoglycosides against many bacteria, which are susceptible to each drug alone, because they enhance penetration of the aminoglycoside. Such synergism may even occur with penicillinase-producing *S. aureus*. Penicillins are synergistic against these organisms (except MRSA) with drugs that bind beta-lactamase enzymes, such as cloxacillin, clavulanic acid, sulbactam, tazobactam and some cephalosporins. Aminobenzylpenicillins and ureidopenicillins are increasingly combined with beta-lactamase inhibitors (chapter 10).

Toxicity and Adverse Effects

Penicillins and beta-lactam antibiotics generally are remarkably free of toxic effects even at doses grossly in excess of those recommended. The major adverse effects are acute anaphylaxis and collapse; milder hypersensitivity reactions (urticaria, fever, angioneurotic edema) are more common. All penicillins are cross-sensitizing and cross-reacting, but cross-reactions occur in only about 5–8% of human patients treated with cephalosporins. Anaphylactic reactions are less common after oral rather than parenteral administration. Penicillins must not be used in animals known to be sensitive. Less common adverse reactions include haemolytic anemia and thrombocytopenia.

Dosage Considerations

Beta-lactams produce killing and lysis of bacteria at concentrations above MIC. Post-antibiotic effects are observed only for staphylococci *in vivo*, so that dosage requires that drug concentrations exceed MIC for most of the dosage interval ("time-dependent antibiotics"). Excessive drug concentrations may be counterproductive because of the Eagle effect described earlier,

in which sometimes dramatic reduction of killing occurs in the presence of high, supra-MIC concentrations.

Clinical Usage

Penicillins (Table 8.1) are important antibacterial drugs in the treatment of infections in animals. The often exquisite susceptibility of Gram-positive bacteria, such as the beta-hemolytic streptococci, means that benzyl penicillin is often a drug of choice for these infections, because of its high potency and low toxicity. Antistaphylococcal penicillins are in widespread use in the prevention and treatment of staphylococcal infections in cows. The extended-spectrum penicillins, particularly aminobenzylpenicillins, have lost much of their potency against Gram-negative bacteria over the decades, but have been revitalized by their combination with beta-lactamase inhibitors (chapter 10). The antipseudomonal penicillins remain important for this activity but are rivalled by antipseudomonal cephalosporins.

Group 1 Benzyl Penicillin and Long-Acting Parenteral Forms

Sodium benzyl penicillin G is available as the benzyl, the procaine benzyl, and now rarely as the tribenzyl ethylenediamine (benzathine) forms. Frequent dosing of benzyl penicillin is required due to its rapid excretion, so that long acting delayed absorption (procaine, benzathine) forms have been developed, with procaine penicillin being the most extensively used because dosing frequency is usually q 24 h. The principle behind the use of procaine and benzathine penicillin is that both forms delay absorption from the injection site. Thus, while the elimination half-life is the same, the absorption half-life is much longer thus reducing the need for frequent dosing. Delayed absorption also means a lower peak concentration.

Antimicrobial Activity

The activity of penicillin G was originally defined in units. Crystalline sodium penicillin G contains approximately 1,600 units/mg (1 unit = 0.6 μg; 1 million units of penicillin = 600 mg or 0.6 g). Most semisynthetic penicillins are prescribed by weight (mg/kg) rather than units.

- *Good susceptibility (MIC ≤ 0.12 μg/ml)* is shown by many aerobic Gram-positive bacteria including all beta-hemolytic streptococci (such as *Streptococcus agalactiae, S. canis, S. zooepidemicus, S. dysgalactiae*), *S. suis, S. uberis, Bacillus anthracis, Actinomyces* spp., *Arcanobacterium* spp., most corynebacteria (including *C. pseudotuberculosis, C. renale*), *Erysipelothrix rhusiopathiae*, and most *Listeria monocytogenes* (Table 8.2). Susceptible anaerobes include *Clostridium* spp., most *Fusobacterium* spp., and some *Bacteroides*. Susceptible Gram-negative aerobes include *Histophilus somni*.
- *Variable susceptibility* is shown by *S. aureus* and other staphylococci, although in the absence of resistance, staphylococci are highly susceptible.
- *Moderate susceptibility (MIC 0.25–2 μg/ml;* which may sometimes vary because of acquired resistance), is shown by *Actinobacillus* spp., *Borrelia* spp., *Brucella* spp., *Haemophilus* spp., *Leptospira* spp., *Moraxella* spp., *Pasteurella* spp., *Proteus* spp., *Taylorella equigenitalis*, and *Serpulina* spp.
- *Resistance (MIC ≥ 4 μg/ml)* is shown by Enterobacteriaceae (other than some *Proteus* spp.), *Bacteroides fragilis, Bordetella* spp., most *Campylobacter* spp., and *Nocardia* spp.

Antibiotic Resistance

Despite extensive use of penicillin in veterinary medicine for many years, most Gram-positive bacteria remain susceptible to the drug. *Staphylococcus aureus* is a notably exception. The beta-lactamase enzymes of *S. aureus* are mainly active against penicillin G, ampicillin, and carbenicillin but hydrolyze penicillinase-stable penicillins (methicillin, cloxacillin) and cephalosporins poorly. Methicillin-resistant *S. aureus* (MRSA) have increasingly emerged in animals from their reservoir in humans, and have become increasingly problematic, particularly since they are both resistant to all beta-lactams and may also be multiply drug resistant. Resistance in usually susceptible Gram-negative bacteria such as *Haemophilus* and *Pasteurella* is the result of R plasmid-mediated production of beta-lactamases.

Pharmacokinetic Properties

These were discussed earlier under general properties of penam penicillins. Acid hydrolysis in the stomach limits the systemic availability of benzyl penicillin administered orally.

Drug Interactions

Penicillin G is synergistic with the aminoglycosides against many Gram-positive bacteria, except those showing high-level aminoglycoside resistance. Such synergism may be seen even with penicillinase-producing *S. aureus*. Penicillin is synergistic against these organisms with drugs that bind beta-lactamase enzymes (chapter 9). Penicillin G has been combined with streptomycin for use in animals but there is little clinical evidence supporting the clinical value of the combination. For this reason, and more particularly because streptomycin is associated with tissue residues, the combination is no longer available in some countries. In addition, there are significant differences in pharmacokinetic properties between different combined preparations.

Toxicities and Adverse Effects

The parent benzyl penicillin and its numerous derivatives are relatively safe drugs; toxic effects were described under General Considerations. Many of the acute toxicities reported in animals are the result of the toxic effects of the potassium or procaine with which penicillin is combined in the dosage form. To avoid cardiac arrest, care should be taken with the rate at which potassium penicillin G is injected IV; administration of the sodium salt is safer. Procaine penicillin G should never be given by this route. In high doses given IM, the procaine form may cause nervous excitement (incoordination, ataxia, excitability) and death, particularly in horses. It should not be administered to horses within 2 weeks before a race so as to avoid procaine-positive drug test results. Procaine penicillin should be stored in the refrigerator and not used past expiration dates; repeated use of the same injection site should be avoided, especially in horses. Severe, immune-mediated hemolytic anemia with icterus has been reported in horses.

Administration and Dosage

Recommended dosages are shown in Table 8.3.

Because of the relative lack of toxicity of penicillins, their dosage can be tailored, to some extent, to the susceptibility of the infecting bacteria more than with any other class of antibiotic. The effectiveness of penicillin therapy is related to the time that tissue concentration exceeds the MIC of the pathogen. Because of the short half-lives of penicillins, preparations that provide rapid absorption must be administered at short intervals (q 6 h). Low systemic availability from oral forms must be compensated for by increasing the size of the dose.

Penicillin G is available as a potassium or sodium salt that can be administered parenterally as freshly prepared

Table 8.3. Usual dosages of penam penicillins in animals. Note that these uses and dosages do not apply to all species; check species-specific chapters.

Drug	Route	Dose (IU or mg/kg)	Interval (h)	Comment
Penicillin G, sodium aqueous	IM, IV	15–20,000 IU	6–8	
Procaine penicillin G	IM	25,000 IU	24	Every 12 hours for serious infections
Benzathine penicillin	IM	40,000 IU	72	Highly susceptible bacteria only; best avoided
Penicillin V	Oral	10	6–8	Erratic absorption; amoxicillin preferred
Cloxacillin, dicloxacillin, methicillin, oxacillin	Oral	15–25	6–8	Monogastrates only
Ampicillin sodium	IM, IV	10–20	6–8	
Ampicillin (hetacillin)	Oral	10–20	8	Monogastrates only
Amoxicillin	Oral	10–20	8–12	Monogastrates only
Amoxicillin	IM (SC)	10	12	
Amoxicillin, long-acting	IM	15	48	Very susceptible bacteria only
Amoxicillin trihydrate	IM	10–20	12	
Pivampicillin	Oral	25	12	Monogastrates only
Carbenicillin, indanyl sodium	Oral	33	6–8	Urinary tract only
Carbenicillin	IM, IV	33	6–8	
Ticarcillin	IV (IM, SC)	25–40	8	Often used with clavulanic acid
Piperacillin	IV (IM)	50	8	May be used with tazobactam

solutions. Procaine penicillin G is a special form developed to prolong absorption from the IM injection site. A single dose of 25,000 units/kg provides effective serum concentrations against susceptible bacteria for at least 12 hours and generally for up to 24 hours in all species of domestic animals. For moderately susceptible bacteria, high doses of procaine penicillin given once daily may be useful; an example is administration of 45,000 units/kg in the once-daily treatment of bovine *Mannheimia haemolytica* pneumonia but more clinical data is needed on the efficacy of such high dosing, since the Eagle effect may reduce the efficacy of the drug. Oral potassium penicillin G has been used to treat canine urinary tract infections caused by *E. coli* or *Proteus mirabilis*. The response is due to the high concentrations of penicillin that are attained in urine.

Benzathine penicillin is a long-acting, slow release formulation of penicillin G administered every 72 hours. Serum concentrations are usually so low that it can only be recommended for extremely susceptible bacteria; it is best avoided.

Clinical Applications

The general clinical applications of penicillin G are shown in Table 8.4.

Penicillin G is the drug of choice in treating infections caused by Gram-positive bacteria such as streptococci, corynebacteria, *Erysipelothrix*, clostridia, and perhaps of *Listeria*, and some Gram-negative bacteria such as *H. somni, Pasteurella*, and many anaerobes. In addition, it is a drug of choice in treating the spirochetal agent of Lyme disease, *Borrelia burgdorferi*. The advantages of penicillin G are its potent and bactericidal activity against susceptible bacteria and its wide margin of safety; dosage can be tailored to the susceptibility of the pathogen by selecting the form of drug to be administered. Disadvantages are activity only against actively growing bacteria, its need for injection, its narrow-spectrum, widespread resistance in *S. aureus* and Gram-negative bacteria, and the drug's failure to cross biological membranes well, except in acute inflammation.

Cattle, Sheep, and Goats

Penicillin G is the most commonly used antibiotic for food animals. It was initially licensed at an inappropriately low dosage. Parenterally administered penicillin G is the drug of choice for the treatment of disease caused by susceptible bacteria including anthrax, clostridial infections, *Corynebacterium renale* infection, *H. somni* infection, pneumonic pasteurellosis caused by susceptible *Mannheimia* and *Pasteurella*, septicemic pasteurellosis (hemorrhagic septicemia), and infections caused by non-spore-forming anaerobes such as *Fusobacterium necrophorum* and *Porphyromonas asaccharolytica*. Penicillin G's poor activity against slowly multiplying bacteria and relative inability to penetrate biologic membranes may explain its often disappointing effect in treating *A. pyogenes*, actinomycosis, or chronic *S. aureus* mastitis. For most conditions that are penicillin responsive, a dosage of 20–25,000 IU/kg once daily is adequate for procaine penicillin G.

Listeriosis has been successfully treated with a daily dose of 44,000 units/kg of procaine penicillin administered for 7–14 days, but ampicillin is preferred. Penicillin G is effective against acute leptospirosis, although again, ampicillin is probably preferable. Procaine penicillin G (300,000–600,000 units in 1–2 ml) administered

Table 8.4. Applications of penicillin G in clinical infections in animals.

Species	Primary Applications	Secondary Applications
Cattle, sheep, goats	Anthrax, clostridial and corynebacterial infections, *A. pyogenes*, streptococcal mastitis, hemorrhagic septicemia, listeriosis	Actinobacillosis, anaerobic infections, possibly infectious keratoconjunctivitis, leptospirosis
Swine	Streptococcal, clostridial infections, erysipelas, *A. pyogenes, A. suis*	Glasser's disease, pasteurellosis, anaerobic infections
Horses	Streptococcal and clostridial infections	Actinobacillosis, anaerobic infections
Dogs, cats	Streptococcal and clostridial infections	Cat bite abscess, anaerobic infections, leptospirosis

subconjunctivally has been used extensively in the treatment of *Moraxella bovis* keratoconjunctivitis since this maintains therapeutic concentrations for up to 36 hours. One controlled study did not, however, confirm the value of this treatment (Allen et al., 1996).

Pneumonic pasteurellosis has been treated successfully with daily intramuscular or subcutaneous injections of 45,000 units/kg of procaine penicillin. Resistance among *M. haemolytica*, however, is increasing and further increases in dose are not justified. Serious, acute mastitis caused by streptococci or susceptible *S. aureus* can be treated by IM procaine penicillin 20–25,000 IU/kg, q 12 or q 24 h depending on severity, as a probably useful adjunct to frequent stripping of the infected quarter. Penicillin is more commonly administered intramammarily, often combined with streptomycin, and has given excellent results in the treatment of streptococcal infections during lactation, but only modest results against *S. aureus*. Intramammary treatment of susceptible Gram-positive cocci with procaine penicillin G and neomycin showed no advantage over procaine penicillin G alone (Taponen et al., 2003). Penicillin G in fixed combination with streptomycin has been used successfully against severe dermatophilus infection but this combination is no longer available in many countries.

Swine

Penicillin is the parenteral drug of choice in preventing and treating erysipelas, and streptococcal, clostridial, and corynebacterial infections. For acute erysipelas and streptococcal infections, procaine penicillin is preferred, but benzathine penicillin is sometimes used in prophylaxis. *Streptococcus suis* meningitis may be treated successfully with daily injections of procaine penicillin given early in the disease. Penicillin-streptomycin combination (25 mg/kg) administered for 1, 3, or 5 days removed the kidney carrier state in swine infected with *Leptospira pomona* (Allt and Bolin, 1996).

Horses

Penicillin G is used against beta-hemolytic streptococci: in neonatal foals for *S. zooepidemicus* polyarthritis and meningitis, and in adult animals for infections of wounds, lower respiratory and urinary tracts, and of the uterus, where it may be given by parenteral administration and local infusion. It is the drug of choice in strangles, when treatment is required. Penicillin is the preferred antibiotic in tetanus. Injection of procaine penicillin G in the neck or biceps gave higher serum concentrations than injection in the gluteal muscle or SC (Firth et al., 1986). Penicillin should not be administered orally to horses because of its poor absorption and the digestive disturbances it may cause.

Dogs and Cats

Penicillin G is a drug of choice for streptococcal and clostridial infections, for actinomycosis, and for infections caused by susceptible Gram-negative bacteria such as *P. multocida*. Because of penicillin G's activity against anaerobic bacteria, it is particularly suitable in the treatment of periodontal disease, tooth abscesses, wound infections, and perhaps pyometra. However, amoxicillin (and to a lesser extent ampicillin) is preferred for all these uses. Unlike penicillin G, which is erratically absorbed in dogs and cats after oral administration and which therefore is administered parenterally, amoxicillin is well absorbed following oral administration, which increases tissue concentrations and decreases the amount of drug remaining in the gut to cause intestinal disturbance. Because of the very high urinary concentrations attained after administration of penicillin G and amoxicillin by any route, either drug may be used in the treatment of canine urinary tract infections caused by *S. aureus* (even penicillinase-producing), streptococci, *E. coli*, and *P. mirabilis*.

Poultry

Penicillin is used by oral administration in the prevention and treatment of necrotic enteritis, ulcerative enteritis, and intestinal spirochetosis and, in combination with streptomycin, in treating erysipelas in turkeys.

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Group 2 Orally Absorbed Penicillins

Phenoxymethyl penicillin (penicillin V) resists stomach acid hydrolysis and is therefore administered orally. It has a spectrum of activity similar to benzyl penicillin, and is therefore used for the same purposes in monogastrates. Oral administration of penicillin V is used in the effective prophylaxis and treatment of *S. suis* meningitis in swine.

Group 3 Antistaphylococcal Isoxazolyl Penicillins: Cloxacillin, Dicloxacillin, Methicillin, Nafcillin, and Oxacillin

The antistaphylococcal penicillins are resistant to *S. aureus* penicillinase and are used mainly in the treatment or prevention of bovine staphylococcal mastitis. The isoxazolyl penicillins (cloxacillin, oxacillin) are acid stable and may be given orally to monogastric animals, for example, in the treatment of staphylococcal skin infections in dogs. Penicillinase production in *S. aureus* may be detected by the use of nitrocefin-impregnated paper disks.

All are resistant to *S. aureus* penicillinase, although activity against other penicillin-sensitive bacteria is less than that of penicillin G. Activity of the different drugs is similar *in vivo*.

As described earlier, methicillin-resistant *S. aureus* (MRSA) are reported increasingly, particularly in dogs and in horses that are or have been in veterinary hospitals, as well as in farm livestock, notably swine and veal calves (Price et al., 2012). Resistance to methicillin in bovine *S. aureus* isolates is unusual, although are increasingly isolated from veal calves in certain countries. Figures purporting to show extensive resistance in bovine isolates probably reflect inappropriate test conditions or drug inactivity, as methicillin deteriorates readily in storage. As noted earlier, methicillin-resistant *S. pseudintermedius* (MRSP) are also increasingly isolated from dogs and cats and, like MRSA, are regarded as resistant to all beta-lactam antibiotics. With the emergence of MRSA in animals since about 2000, MRSA is an occupational health hazard for veterinarians and veterinary staff, particularly for those who work with horses (Jordan et al., 2011).

Methicillin-resistant (heteroresistant) *S. aureus* may be overlooked. While no single method is ideal, methicillin-resistant *S. aureus* are best detected using oxacillin disks, with *S. aureus* grown 18–24 hours at 30°C or 35°C. Many laboratories now also use PCR to identify the *mecA* gene. Heteroresistant *S. aureus* are often multiply resistant (other beta-lactams, aminoglycosides, macrolides, tetracyclines) but susceptible to rifampin, fluoroquinolones, and trimethoprim-sulfamethoxazole. Methicillin-resistant *S. pseudintermedius* are considered to be resistant (MRSP) if MIC to oxacillin is ≥ 0.5 μg/ml, whereas the breakpoint for MRSA is ≥ 4 μg/ml (Bemis et al., 2009).

Activity of antistaphylococcal isoxazolyl penicillins against streptococci causing mastitis in cows is good. Cure rates approximate those for penicillin-streptomycin combinations. While apparent clinical cure of *S. aureus* mastitis is usual, bacteriologic cure is often disappointing.

In dogs, IV use of nafcillin during surgery to prevent staphylococcal infection has been associated with the development of acute renal failure within 2–4 days of surgery, probably as a result of direct renal damage by the drug (Pascoe et al. 1996). Studies of the pharmacokinetics of dicloxacillin in dogs suggest that IM administration (25 mg/kg, q 8 h) is more reliable than oral administration in achieving serum concentrations of drug consistently ≥ MIC of penicillinase-producing *S. aureus* (Dimitrova et al., 1996).

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Group 4 Extended-Spectrum Penicillins: Aminobenzyl Penicillins: Ampicillin and Amoxicillin

Ampicillin, amoxicillin, and the related esters bacampicillin, hetacillin, pivampicillin, and talampicillin have similar antimicrobial activity, but amoxicillin and possibly pivampicillin have the advantage of achieving higher tissue concentrations because of better absorption from the intestine. The broad-spectrum aminobenzyl penicillins are slightly less active than penicillin G against Gram-positive and anaerobic bacteria and are equally susceptible to staphylococcal penicillinase. These broad-spectrum drugs, however, have considerably greater activity against Gram-negative bacteria such as *E. coli, P. mirabilis*, and *Salmonella*. Nevertheless, acquired resistance has considerably reduced the effectiveness of these drugs. An exciting development has been their combination with beta-lactamase-inhibiting drugs, which increases their effectiveness considerably (chapter 10), and with which these drugs should generally be combined.

Antimicrobial Activity

- *Good susceptibility (MIC ≤ 1 μg/ml)*: As for benzyl penicillin group but includes *Borrelia* spp. and *Leptospira* spp., which are highly susceptible; *Actinobacillus* spp., *Haemophilus* spp., *Moraxella* spp., *Pasteurella* spp. (Tables 8.2 and 8.5).
- *Moderate susceptibility (MIC 2–4 μg/ml)*: As for benzyl penicillin but also *Campylobacter* spp., enterococci. Variable moderate activity (because of acquired resistance) against *E. coli, P. mirabilis*, and *Salmonella*. Acquired resistance in Enterobacteriaceae is widespread.
- *Resistance (MIC > 4 μg/ml, approximately): Bacteroides fragilis, B. bronchiseptica, Citrobacter* spp., *Enterobacter* spp., *Klebsiella* spp., other *Proteus* spp., *P. aeruginosa, Serratia* spp., *Y. enterocolitica*.

Antimicrobial Resistance

Plasmid- or integron-mediated, acquired resistance is common in Gram-negative bacteria and is often multiple, such as that in most enterotoxigenic *E. coli* and *S. typhimurium*. Many *E. coli* that cause bovine mastitis are resistant. Aminobenzylpenicillins are susceptible to *S. aureus* beta-lactamase (Tables 8.2 and 8.5).

Pharmacokinetic Properties

The basic pharmacokinetic properties of penicillins were described under General Considerations. Both ampicillin and amoxicillin are relatively stable in acid. In dogs, the systemic availability of amoxicillin (60–70%) is about twice that of ampicillin (20–40%), so that peak blood concentrations are often twice or more those that occur after the same dose of ampicillin. The absorption of amoxicillin is unaffected by feeding, unlike ampicillin. Hetacillin and pivampicillin are esters of ampicillin developed to increase systemic availability, but it is questionable whether this occurs in dogs. Pivampicillin has significantly better bioavailability in horses than amoxicillin after oral administration. Ampicillin is available as a sodium salt that can be administered parenterally in a freshly prepared solution. The trihydrate salts are less soluble and therefore poorly absorbed from the intestine but form aqueous suspensions that can be injected either IM or SC. These preparations produce low peak concentrations in the serum but they extend the dosing interval to 12 hours. Long-acting preparations

Table 8.5. *In vitro* activity of extended-spectrum and antipseudomonal pencillins against various medically important opportunist bacteria.

	Ampicillin		Mecillinam		Ticarcillin		Azlocillin		Piperacillin	
Organism	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Streptococcus agalactiae	0.06	0.12	2	8	2	4	0.25	1	0.25	1
Escherichia coli	4	128	1	4	16	128	8	128	8	128
Klebsiella pneumoniae	128	128	2	128	128	128	32	128	8	128
Citrobacter diversus	4	128	0.5	4	16	128	4	8	4	4
Enterobacter cloacae	128	128	2	32	8	128	4	32	4	32
Proteus mirabilis	1	4	4	16	0.5	16	0.5	16	0.5	16
Pseudomonas aeruginosa	128	128	128	128	16	128	4	128	4	128
Bacteriodes spp.[a]	1	32	2	16	4	32	2	8	2	4

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[a]Other than *B. fragilis*.

of ampicillin trihydrate, which produce therapeutic serum concentrations for 48 hours, have been introduced. The lower peak plasma concentrations, however, may decrease penetration of the antibiotic to sites of infection.

Drug Interactions

Aminobenzylpenicillins are commonly synergistic with aminoglycosides against Gram-positive bacteria and often also against Gram-negative bacteria, but only if the latter are not resistant to both drugs. The broad-spectrum beta-lactamase inhibitors clavulanic acid and sulbactam, show remarkable synergism with aminobenzylpenicillins against beta-lactamase-producing bacteria (chapter 10).

Toxicities and Adverse Effects

Toxic effects are similar to those described under General Considerations. One hazard with broad-spectrum penicillins is the potential to disturb the normal intestinal flora. In dogs and cats, the effect may be less marked with amoxicillin, which is better absorbed. Ampicillin should not be administered to small rodents (guinea pigs, hamsters, gerbils) or to rabbits since it may produce clostridial colitis (*C. difficile* or, in rabbits, *C. spiroforme*). Administration of pivampicillin in horses was associated with less loose feces or diarrhea than observed in horses given trimethoprim-sulfadiazine (Ensink et al., 1996). Moderate diarrhea has been described in calves after several days of treatment with oral ampicillin, which appears to result from malabsorption caused by a direct effect on intestinal mucosa.

Administration and Dosage

Recommended dosages are shown in Table 8.3.

The soluble sodium salts can be administered parenterally and orally but the poorly soluble trihydrate form should only be administered IM. Reconstituted, aqueous sodium salts are unstable after more than a few hours. Because of their short half-lives, preparations that are rapidly absorbed should be administered every 6 hours to maintain serum drug concentrations over 1 μg/ml for a significant length of time. Amoxicillin is preferred for oral administration because it is better absorbed than ampicillin, and its absorption is unaffected by feeding. Another advantage of oral amoxicillin over ampicillin is that it can be given twice daily to small animals. Long-acting preparations of amoxicillin are available, but it is doubtful whether they maintain therapeutic serum concentrations for the 48-hour recommended dosing interval. Novel controlled-release forms of long-acting amoxicillin are being investigated in dogs (Horwitz et al., 2010).

Clinical Applications

The aminobenzylpenicillins are bactericidal, relatively non-toxic drugs with a broader spectrum of activity than penicillin G and are better distributed in the body. Even with these advantages, relatively high doses are required to treat infections caused by Gram-negative

bacteria. The relatively high prevalence of acquired resistance has limited their place.

Amoxicillin is the best penicillin for the treatment of urinary tract infections and enteric infections caused by susceptible organisms and has similar activity to penicillin G in the treatment of anaerobic infections. Although amoxicillin offers pharmacokinetic advantages over ampicillin, it has some of the same difficulty as ampicillin in attaining concentration in tissues approximating those of susceptible Gram-negative bacteria.

The main clinical applications are similar to those shown in Table 8.4. Amoxicillin is a drug of choice in the treatment of leptospirosis. Ampicillin is preferred to penicillin to treat listeriosis.

In cattle, sheep and goats, oral ampicillin has been used to treat *E. coli* and *Salmonella* infections but acquired resistance markedly now limits their effectiveness for this purpose. Ampicillin is effective against bovine respiratory disease but offers no advantage over penicillin G. Long-acting amoxicillin administered twice at 15 mg/kg IM q 48 h was effective in removing the *Leptospira hardjo* kidney carrier state from the majority of experimentally infected cattle (Smith et al., 1997).

Indications in horses for ampicillin or amoxicillin in horses are few since they offer little advantage over benzyl penicillins, largely because of acquired resistance in Gram-negative bacteria. Oral administration of amoxicillin (or preferably pivampicillin) is appropriate for infections in foals caused by organisms with good susceptibility but cannot be recommended for adult horses.

Ampicillin or amoxicillin are drugs of choice for mixed aerobic-anaerobic infections such as cat-bite infections. Ampicillin or amoxicillin is used in the treatment of canine urinary tract infections, because over 90% of *S. aureus*, streptococci, and *P. mirabilis*, nearly 90% of *E. coli*, and 65% of *Klebsiella* are regarded as susceptible to urinary concentrations of the drug. Nevertheless, treatment results in one study were not conspicuously better than those obtained with penicillin G. The combination of clavulanic acid-amoxicillin is preferred for these purposes, so that the usage of amoxicillin in companion animal practice is about one-third that of the combination (Mateus et al., 2011). Clinical trials in cats showed once-daily dosing with a 50-mg tablet of amoxicillin to be as effective as twice-daily dosing. Field trial comparison in cats of 50-mg amoxicillin twice daily versus 50-mg hetacillin twice daily showed a significant advantage for amoxicillin (Keefe, 1978). Amoxicillin, metronidazole and omeprazole as a triple combination has been used to produce bacteriologic cure in the treatment of *Helicobacter* gastritis in cats but the organism could still be detected by polymerase chain reaction (PCR; Perkins et al. 1996). Triple therapy with amoxicillin, metronidazole and bismuth subcitrate has been used to eradicate gastric *Helicobacter* from dogs. Unfortunately, PCR does not distinguish between viable and non-viable organisms. Amoxicillin produced clinical cure of *B. burgdorferi* infection in the majority of treated dogs but the organism was not eradicated (Straubinger et al., 1997).

In poultry, ampicillin is sometimes administered orally for the prevention or treatment of *E. coli* or *S. aureus* septicemia, or of salmonellosis.

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Group 4 Extended-Spectrum Penicillins: Amidopenicillins: Mecillinam

Mecillinam (amidinopenicillin) is active against a broader range of Enterobacteriaceae than ampicillin, being highly active against *Citrobacter* spp., *Enterobacter* spp.,

E. coli, K. pneumoniae, Proteus spp., and *Yersinia* spp. Unlike aminopenicillins, mecillinam has little activity against Gram-positive bacteria and none against *P. aeruginosa* (Table 8.5). It has high affinity only for PBP2, the enzyme-mediating cylindric growth in Gram-negative rods. Mecillinam is synergistic with many beta-lactamase inhibiting drugs. It is inactivated by many beta-lactamases but many ampicillin-resistant Enterobacteriaceae are susceptible; its efficacy against some beta-lactamase producing bacteria is because of its rapid penetration of cells as well as its low affinity for some of their degradative enzymes. Oral absorption is poor, and in part for this reason this drug has not been used in veterinary medicine. Mecillinam may have potential for use in veterinary medicine for infections caused by susceptible Enterobacteriaceae, at a human dosage in the range of 5–10 mg/kg TID IM.

Group 5 Antipseudomonal Penicillins: Carboxypenicillins: Carbenicillin and Ticarcillin

Carbenicillin was the first penicillin with good activity against *P. aeruginosa* and *Proteus* (Table 8.2) but has now been largely replaced by the more active ticarcillin, azlocillin, and piperacillin. It is administered IV. Two esters (carindacillin, carfecillin) are available for oral administration for urinary tract infections caused by *Proteus* or *P. aeruginosa*. Ticarcillin has a similar spectrum of activity to carbenicillin. It is active against most *E. coli* and *Proteus* and more active than carbenicillin against *P. aeruginosa* (Table 8.5). Most *Klebsiella, Citrobacter*, and *Serratia* are resistant; all *Enterobacter* are resistant. Ticarcillin is generally reserved for *P. aeruginosa* infections but is less active than azlocillin or piperacillin. It is administered IV.

Because of the expense of carbenicillin and ticarcillin, the high dosages required, usually IV administration and general lack of clinical application, it is unlikely that carbenicillin and ticarcillin will be used for parenteral treatment of *Pseudomonas* or other infections in animals. These drugs have potential use in the local treatment of *P. aeruginosa* infections caused by otherwise resistant bacteria, such as otitis externa in dogs, bovine mastitis, ulcerative keratitis, metritis in mares, and possibly, otherwise resistant urinary tract infections. Ticarcillin is licensed in the United States for the treatment of uterine infections in mares caused by beta-hemolytic streptococci (6 g in 250–500 ml by intrauterine infusion at estrus once daily for 3 days). For this purpose, ticarcillin would have no advantage over benzyl penicillin and should be reserved for infections caused by *P. aeruginosa* and other susceptible Gram-negative bacteria. A parenteral (IM) dosage suggested for dogs is 25–40 mg/kg q 6–8 h; IV-administered drug should be given every 4–6 hours. Ticarcillin (15–25 mg/kg, IV, q 8 h) has been used successfully, combined with topical administration, in the treatment of otitis externa in dogs caused by otherwise-resistant *P. aeruginosa* (Nuttall, 1998). Because of the danger of *P. aeruginosa* developing resistance, these agents are probably best used in conjunction with a broad-spectrum aminoglycoside or beta-lactamase inhibitors.

Group 5 Antipseudomonal Penicillins: Ureidopenicillins: Azlocillin, Mezlocillin, and Piperacillin

The expanded spectrum of activity of the antipseudomonal penicillins results from their interaction with PBPs other than those that bind aminopenicillins, their increased penetration of Gram-negative bacteria, and their resistance to some species-specific chromosomal beta-lactamases. Ureidopenicillins bind PBP3, septal murein synthetase. They have increased activity against Gram-negative bacteria compared to carboxy- or aminobenzylpenicllins, notably against *Klebsiella* and *P. aeruginosa* (see Tables 8.2 and 8.5), and increased activity against *B. fragilis*.

Mezlocillin is more active than azlocillin against Enterobacteriaceae, although resistance is not infrequent because the bacteria are susceptible to common beta-lactamases (Table 8.5). Most *Enterobacter* and *Serratia* are resistant. Piperacillin combines the spectrum and is more active than both. It inhibits over 95% of *P. aeruginosa* and many Enterobacteriaceae and is active against many anaerobes, including many *B. fragilis*. Piperacillin is the most active broad-spectrum penicillin but is also susceptible to some common beta-lactamases as well as to the penicillinase of *S. aureus*. Ureidopenicillins may be combined with beta-lactamase inhibitors (e.g., piperacillin with tazobactam, chapter 10) or with aminoglycosides. There is incomplete cross-resistance among ureidopenicillins and carboxypenicillins.

Ureidopenicillins are administered IV although azlocillin may be administered by (painful) IM injection. Expense limits their application. Clinical applications are probably limited to treatment of *P. aeruginosa* infections and, combined with an aminoglycoside or beta-lactamase inhibitor, to serious infections caused by Gram-negative bacteria in immunocompromised hosts.

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Group 6 Beta-lactamase-Resistant Penicillins: Temocillin

Temocillin is ticarcillin modified by the addition of a 6α-methoxy group to increase resistance to beta-lactamase. Temocillin's high activity against Enterobacteriaceae through binding to PBP-3 is at the expense of resistance of *Pseudomonas, B. fragilis*, and Gram-positive bacteria. More than 90% of Enterobacteriaceae are inhibited at ≤ 8 μg/ml. It is, however, stable to expanded-spectrum, plasmid-mediated beta-lactamases and to AmpC enzymes that inactivate third-generation cephalosporins. Temocillin has a long half-life (4.5 hours) in humans, allowing for once-daily dosage. Temocillin has many potential applications but its use, like that of the antipseudomonal penicillins, is limited by expense and the need for IV administration. There are no reports in the veterinary literature of its use in animals.

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9

Beta-lactam Antibiotics: Cephalosporins

John F. Prescott

General Considerations

In cephalosporins, the beta-lactam ring is attached to a 6-membered dihydrothiazine ring with the effect that the cephalosporin nucleus is inherently more resistant to beta-lactamases than the penicillin nucleus (Figure 9.1). The 7-aminocephalosporanic acid molecule also provides more sites than the aminopenicillanic acid molecule for manipulation in the production of semisynthetic drugs. Changes at position 7 (R1) alter beta-lactamase stability and antibacterial properties particularly whereas changes at position 3 (R2) tend to alter metabolic stability and pharmacokinetic properties. True cephalosporins contain the common 7-aminocephalosporanic acid of *Cephalosporium acremonium*, whereas cephamycins are derived from *Streptomyces* species (cefotetan, cefoxitin) or are synthetic derivatives produced by substituting oxygen for sulfur (latamoxef).

Cephalosporins in general have the advantages of beta-lactamase stability, good activity against target proteins (PBPs), and good ability to penetrate bacterial cell walls. Although they may be active against a wide range of organisms, such activities are not uniform and produce often-subtle differences between the different molecules. Pharmacokinetically they are generally similar and have properties typical of the beta-lactams, usually requiring parenteral injection, having short (1- to 2-hour) half-lives, and being excreted usually through the kidneys in the urine. They are bactericidal, relatively non-toxic, and can be used in many penicillin-sensitive individuals.

Classification

Cephalosporins have a wide range of antibacterial activity but show considerable diversity in their antibacterial properties. One approach to their classification has been chronological, with the different cephalosporins introduced since 1975 being described somewhat arbitrarily as "generations" (Tables 9.1 and 9.2). This has implied that each of the generations introduced has added another general level of advantage over the previous generation rather than adding some advantage(s) at the expense of another or others. Differences within the generations often appear subtle but are important. Cephalosporins were originally introduced (first generation) for the treatment of penicillinase-resistant staphylococcal infections with the advantage that these drugs also had a spectrum of activity against Gram-negatives similar to that of the extended-spectrum aminobenzylpenicillins. Alterations of the side-chains on the 7-aminocephalosporanic acid nucleus and the discovery of the cephamycins led to increasing stability to the beta-lactamases of Gram-negative bacteria, including those of *Bacteroides fragilis* and *Pseudomonas aeruginosa*. This increase in stability is, however, usually at the expense of decreasing activity against Gram-positive bacteria and gives pharmacokinetic

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differences. Because of the inadequacies of classification as generations, an expanded classification has been developed on the basis of antimicrobial activity, including beta-lactamase stability and pharmacological properties (Table 9.1). This classification will be followed here.

The "generations" are broadly characterized as follows. First generation: primarily Gram-positive antibacterial activity, administered parenterally (IV, IM, SC) or in some cases orally; second generation: Gram-positive and Gram-negative antibacterial activity, administered by all routes: third generation, decreased Gram-positive but increased Gram-negative antibacterial activity, administered parenterally and in a very few cases orally; fourth generation: increased Gram-positive and Gram-negative antibacterial activity, administered by all routes.

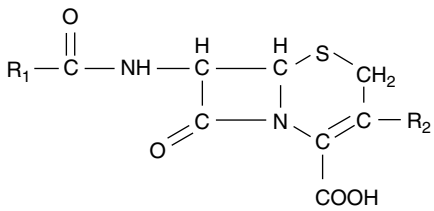

Figure 9.1. Structural formula of the cephalosporin nucleus.

Antimicrobial Activity

The mechanism of action of the cephalosporins is that of beta-lactam antibiotics (chapter 8). For susceptibility testing, cephalothin is the class drug for group 1 and 2, first-generation, cephalosporins. For groups 3–7, second- to fourth-generation cephalosporins, there is no class representative. For susceptibility testing of Enterobacteriaceae, cefotaxime can usually substitute for ceftazidime, ceftizoxime, and ceftriaxone (and vice versa) and cefamandole for cefonicid and cefuroxime (and vice versa). For *P. aeruginosa*, cefoperazone will substitute for ceftazidime (and vice versa) and cefotaxime for ceftriaxone and latamoxef (and vice versa).

Cephalosporins are usually active against beta-hemolytic streptococci and against beta-lactamase producing, but not against methicillin-resistant staphylocci. Most enterococci are resistant. Among Enterobacteriaceae, in the absence of acquired resistance, *E. coli* and *Salmonella* are susceptible, as are some *Proteus* and *Klebsiella* spp. Fourth-generation, group 7, cephalosporins are effective against Enterobacteriaecae and other Gram-negative bacteria resistant to earlier generations of cephalosporins because of acquired beta-lactamase-based resistance. Susceptibility among common Gram-negative aerobic species such as

Table 9.1. Classification of cephalosporins into groups (and generations) based on route of administration and antibacterial activity.

Group	Characteristics	Examples
1 (first generation)	Parenteral; resistant to staphylococcal beta-lactamase; sensitive to enterobacterial beta-lactamase; moderately active	Cephacetrile, cephaloridine, cephalothin, cephapirin, cephazolin
2 (first generation)	Oral; resistant to staphylococcal beta-lactamase; moderately resistant to some enterobacterial beta-lactamase; moderately active	Cefadroxil, cephadrine, cephalexin
3 (second generation)	Parenteral; resistant to many beta-lactamases; moderately active	Cefaclor, cefotetan, cefoxitin, cefuroxime, cefamandole
4 (third generation)	Parenteral; resistant to many beta-lactamases; highly active	Cefotaxime, ceftiofur, ceftriaxone, latamoxef
5 (third generation)	Oral; resistant to many beta-lactamases; highly active	Cefetamet, cefixime, cefpodoxime
6 (third generation)	Parenteral; resistant to many beta-lactamases; active against *Pseudomonas aeruginosa*	Cefoperazone, cefovecin, cefsulodin, ceftazidime
7 (fourth generation); included with group 6 in some classifications	Parenteral; resistant staphylococcal, enterobacterial, and pseudomonal beta-lactamases; highly active	Cefepime, cefquinome, cefpirome

By convention, cephalosporins discovered before 1975 are spelled with a "ph" and after 1975 with a "f."

Table 9.2. Relative activity of cephalosporins against selected opportunist bacteria[a].

Drug	Generation	*S. aureus*[b]	*E. coli, Klebsiella, Proteus*	*Enterobacter*	*Pseudomonas aeruginosa*	*Bacteroides*	Other anaerobes
Cephalothin	1	+++	++	–	–	–	+
Cefuroxime	2	++	+++	–	–	–	+
Cefoxitin	2	+	+++	+	–	++	++
Cefotaxime	3	++	+++	+	–	+	++
Ceftazidime	3	+	+++	++	+++	–	–
Ceftriaxone	3	+	+++	+	–	–	+
Cefepime	4	++	+++	+++	+++	–	+

[a]+++, highly active; ++, moderately active; +, limited activity; –, no clinical activity; susceptibilities for individual isolates may vary.
[b]Methicillin-susceptible *Staphylococcus aureus*. Table adapted from Marshall and Blair, 1999; because of the extensive development of resistance in Enterobacteriaceae since that time, this table should be interpreted as a dated overview.

Haemophilus and *Pasteurella*, including beta-lactamase producers, is usual. Only third-generation antipseudomonal (group 6) and fourth-generation (group 7) cephalosporins are effective against *P. aeruginosa*. Mycobacteria are resistant. Against non-spore-forming anaerobic bacteria, activity is variable and resembles that of aminobenzylpenicillins. Cefoxitin is notably resistant to beta-lactamase producing anaerobes, including *B. fragilis*.

Resistance to Cephalosporins

The three basic mechanisms of resistance to cephalosporins are PBP modification, reduced permeability and increased efflux, and enzymatic inactivation by beta-lactamases. Of these the most important is beta-lactamase production, with more than 1,000 distinct beta-lactamases now recognized. Their importance is both because of the large number of different beta-lactamases that have been selected by the widespread use of extended-spectrum cephalosporins and because genes for these beta-lactamase genes are often transmissible. The topic has been the subject of a number of excellent reviews (Bush and Macielag, 2010; Bush and Fisher, 2011).

Penicillin-Binding Protein (PBP) Modifications

Modification of the PBPs targets can occur after transformation of readily transformable bacteria by fragments of PBP DNA and their homologous recombination with existing PBP genes to produce new "mosaic" PBPs with low affinity for beta-lactams. This has been extensively described for some important human pathogens but is not well described in bacterial pathogens of animals. Other important forms of PBP modification include acquisition of extra "by-pass" (insensitive) PBP genes by methicillin-resistant *Staphylococcus aureus* or by *Enterococcus faecium*, although this has not yet been described in animal pathogens.

Reduced Permeability and Increased Efflux

Reduced production of the porins by which beta-lactams penetrate Gram-negative bacteria has produced resistance to cephalosporins, which in some cases is also the result a periplasmic beta-lactamase enzyme. Such reduced uptake may be mediated by an efflux mechanism that gives broad-spectrum cross-drug class resistance.

Beta-lactamase Inactivation

There has been an astonishing evolution of these enzymes in response to antimicrobial selection with subsequent widespread plasmid- or transposon-mediated dissemination through Gram-negative bacterial populations. Most (class A, C, D molecules) are serine esterases but some (class B) are zinc metalloproteases. Beta-lactamases and their classification are discussed in more detail in chapter 10 (Table 10.1). The two most important classes of beta-lactamases are the extended-spectrum beta-lactamases (ESBLs) and the AmpC cephalosporinases (which included CMY-2 enzymes).

The ability of transposable elements to move beta-lactamases from chromosomes to plasmids (and back again, and between different plasmids), as well as recombination processes involving integrons, means

that the earlier designations of beta-lactamases as either chromosomal or plasmid is increasingly anachronistic. However, the extent or degree of resistance provided by a beta-lactamase is a function both of its activity as well as its quantity, which in turn may depend on plasmid copy numbers or the extent to which chromosomal enzymes can be induced.

First-Generation Cephalosporin Beta-lactamases. The development of aminopenicillins such as ampicillin in the early 1960s importantly broadened the activity of penicillins against Gram-negative bacteria, particularly *Escherichia coli*, but was followed by the development and spread of plasmid-mediated beta-lactamases, notably TEM-1 (now a common feature of *E. coli*), as well as SHV-1 and OXA-1. The first-generation cephalosporins developed at this time were importantly not only resistant to staphylococcal beta-lactamases, which ampicillin was not, but also had a spectrum of activity against Gram-negative aerobes slightly broader than that of aminopenicillins. However, they were susceptible to the same plasmid-mediated beta-lactamases as ampicillin and also lacked its activity against inducible functional group 1 AmpC enzymes.

Second-Generation Cephalosporin Beta-lactamases. In the search for beta-lactams resistant to the beta-lactamases emerging and conferring resistance in the late 1960s, cephalosporins with enhanced beta-lactamase stability were found to be more readily developed than amino- or carboxy-penicillins. These second-generation cephalosporins were more stable to TEM-1 and against some AmpC-inducible enteric bacteria such as *E. coli*. As noted, the first cephamycin, cefoxitin, was also found to be uniquely stable to the chromosomal beta-lactamases of *Bacteroides spp*., including *B. fragilis*. However, these new drugs remained ineffective against important Gram-negative aerobic pathogens such as *P. aeruginosa*.

Third-Generation Cephalosporin Beta-lactamases. The third-generation drugs developed in the 1970s and 1980s in the search for cephalosporins with improved beta-lactamase stability had considerably enhanced activity against Enterobacteriaceae, including TEM-1, TEM-2, and SHV-1 plasmid-containing strains as well as, in some cases, against *P. aeruginosa*. Unlike earlier drugs, they had stability against chromosomal beta-lactamases of *Klebsiella* spp. and against functional group 1 AmpC-inducible enteric bacteria because of their weak induction of these enzymes. These enhanced activities were at the expense of activity against staphylococci.

Unfortunately, resistance has emerged in the Gram-negative bacteria targets of these drugs and, through plasmid and transposon transmission, is becoming increasingly widespread particularly among the *Enterobacteriaceae* (*Enterobacter* spp., *E. coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus* spp., and *Salmonella*). Resistance has also spread to *Burkholderia* spp. and to *P. aeruginosa*. Over 1,000 beta-lactamases now exist. The major types of beta-lactamase that are increasing in global prevalence among opportunist pathogens are the plasmid-encoded functional group 1 cephalosporinases, the group 1e, 2be, 2ber, and 2de extended-spectrum beta-lactamases (ESBLs), the functional groups of 2df, 2de, 2f serine carbapenemases, and the group 3 metallo-beta-lactamases (Table 10.1). Of these, the greatest increase is occurring in the EBSLs.

AmpC Hyperproducers. The hyperproduction of AmpC beta-lactamases occurs most often in bacterial opportunist pathogens that are relatively unusual in animals, notably *Enterobacter* spp. and *Citrobacter freundii*. Paradoxically, although third-generation cephalosporins are weak inducers of these enzymes, they are actually effective in killing organisms producing these enzymes. However, they are ineffective when the enzymes are produced in large amounts by hyperproducers, which are those that have a mutation in the gene for encoding the peptidoglycan recycling enzyme, AmpD. Such "derepressed mutants" resistant to all cephalosporins (and to clavulanic acid and other beta-lactamase inhibitors) may emerge during therapy of infections caused by these two genera (in sites other than the urinary tract) and may be particularly problematic in hospital settings. More seriously, AmpC hyperproduction can become encoded by high copy number plasmids (FOX, MIR, MOX, CMY-beta-lactamase families or types) and mobilized to other Gram-negative bacteria, notably *E. coli* and *Klebsiella* spp. in which the new set of group 1 cephalosporinases may be additive with endogenous non-group 1 beta-lactamases (Bush and Fisher, 2011).

In recent years, there has been increasing spread of a family of CMY2-encoding plasmids in food and

companion animals. For example, hospital acquired infection in multidrug-resistant *E. coli* producing the cephamycinase-encoding gene CMY-2 was described in 23 dogs with nosocomial infections in a veterinary hospital in the United States (Sanchez et al., 2002), with the same isolate being detected in the environment of the intensive care unit and surgical wards. Many of these isolates were also resistant to florfenicol, and the *flo* and bla_{CMY2} genes were found to be transferable, probably by a transposon. Additional resistance to spectinomycin and sulfonamides in the isolates was also provided by integrons (Sanchez et al., 2002). CMY2 AmpC beta-lactamase plasmids appear to be common in, and to move between, *E. coli* and *Salmonella* isolated from food animals and people (Winokur et al., 2001), and appear to have spread recently into *Salmonella* Newport (Zhao et al., 2003). In *Salmonella* isolated from food animals in the United States, ceftiofur resistance has been identified in over 20 serovars, and has increased markedly in serovars such as Heidelberg, Newport, and Typhimurium commonly isolated from human infections (Food and Drug Administration, 2012). In Canada, there was a dramatic rise in CMY-2 producing *S.* Heidelberg in chickens associated with extra-label use of ceftiofur in eggs and day-old poults, with spread of infection into people (Dutil et al., 2010); this fell equally dramatically once ceftiofur was no longer (temporarily) use for this purpose.

Extended-Spectrum Beta-lactamases. ESBLs contain the greatest number of distinct beta-lactamase enzymes that are variants of the broad-spectrum TEM and SHV beta-lactamases, all of which are plasmid or transposon mediated. Currently there are over 200 TEM-type and over 165 SHV-type ESBLs (Table 10.1). These enzymes produce resistance by hydrolyzing the oxyimino-aminothiazole-containing beta-lactams (aztreonam, cefotaxime, ceftazidime, and to some extent cefepime, as well as earlier generation cephalosporins). By contrast, the α-methoxy-cephalosporins (cefoxitin, cefotetan, latamoxef) and imipenem are stable to these enzymes. There are differences between different ESBLs in the rate at which they hydrolyze different cephalosporins. For example, TEM-12 and SHV-2 ESBLs hydrolyze cephalosporins slowly so that infections may respond to third-generation cephalosporin treatment; however, a second single nucleotide mutation in the TEM-12 beta-lactamase gene will produce high-level resistance. Other plasmid-mediated ESBLs not closely related to the TEM and SHV families include the CTX-M family, that preferentially hydrolyze cefotaxime (and cefepime), and number at least 75 distinct enzymes, including the cefotaximases of the SFO-1 and BES-1 types, and the PER, VEB, TLA-1, and GES/IBC types that preferentially hydrolyze ceftazidime (Bonnet, 2004). There is rapidly increasing documentation of third-generation cephalosporin beta-lactamases-producing Enterobacteriaceae infections in animals (Sanchez et al., 2002; O'Keefe et al., 2010; Shaheen et al., 2011). The CTX-M-type ESBLs in particular are expanding among *Salmonella*, in some cases being associated with *sulI*-type integrons associated with complex plasmids (Miriagou et al., 2004).

In human medicine, infection caused ESBL-producing bacteria are seen most often in severely ill hospitalized patients in the intensive care unit, but outbreaks have also been described in nursing homes, pediatric units, and other hospital settings. These outbreaks present very important infection control issues in hospitals. A common approach to control is not only to institute rigorous infection control procedures and monitoring but also to restrict use of extended-spectrum beta-lactams by switching to other drugs classes for empirical therapy of serious infections (see chapter 7).

Most of the third-generation cephalosporin beta-lactamases-producing bacteria described in companion animals have been obtained from veterinary hospitals (Sun et al., 2010; So et al., 2011; Wieler et al., 2011; Haenni et al., 2012), likely reflecting the spread of high-risk tenacious and flexibly resistant clones through this means (Woodford et al., 2011).

The epidemiology of ESBL and AmpC (CMY-2) resistance in *E. coli* or *Salmonella* isolated from food animals such as cattle and swine is complex (Daniels et al., 2009; Agersø et al., 2012; Mollenkopf et al., 2012; Valat et al., 2012) and the link to third-generation cephalosporin use is not always clear. Nevertheless, the emergence and threatening rise of extended-spectrum cephalosporinases reflects the increasing use of third-generation cephalosporins in human and veterinary medicine, as well as the complex ecology of resistance (chapter 6).

Group 3 Metallo-beta-lactamases. Metallo-beta-lactamases have emerged in the last decade as important beta-lactamases particularly of non-fermenting Gram-negative bacteria (*Aeromonas* spp., *P. aeruginosa*). The genes for

these enzymes (IMP, SPM, VIM types) can be transferred through plasmids to Enterobacteriaceae such as *Enterobacter* and *Klebsiella*. Enzymes of the IMP and VIM types can degrade virtually all beta-lactams other than monobactams (Luzzaro et al., 2004). Some of these beta-lactamases are carried on integrons that encode multiple drug resistance genes (Weldhagen, 2004).

Pharmacokinetic Properties

The basic pharmacokinetic and drug disposition characteristics of cephalosporins are typical of beta-lactams (chapter 8), with an elimination half-life of 1–2 hours. Some drugs, however, such as cefotetan and ceftriaxone, have significantly longer half-lives. Group 2 (second-generation) and 5 (third-generation) oral cephalosporins are well absorbed after oral administration, which may be enhanced by formulations as prodrugs that are metabolized to active compound in the body. Some fourth-generation cephalosporins can be administered orally to monogastrates. Clearance is through the kidney in most cases although drugs with high molecular weight and protein binding, such as cefoperazone, are largely excreted in the bile.

Drug Interactions

Cephalosporins are synergistic with aminoglycosides, with which they are sometimes combined in the treatment of infections in neutropenic patients in human medicine.

Toxicity and Adverse Effects

Cephalosporins are among the safest antimicrobial drugs. They have the safety associated with penicillins, although individual drugs may have specific adverse effects. For example, hypoprothrombinamia and platelet abnormalities causing bleeding disorders have been noted with some newer cephalosporins. The broad spectrum of antibacterial activity of second- to fourth-generation drugs may cause overgrowth ("superinfection") of the patients by inherently resistant bacteria including *Clostridium difficile*, which no longer have to compete with susceptible members of the microbial flora. The emergence of multiresistant enterococci as nosocomial infections in human hospital intensive care units is an example of this effect. Gastrointestinal disturbances are therefore also among adverse effects, particularly with drugs excreted through the bile. Human patients allergic to penicillin are sometimes (5–8%) also allergic to cephalosporins. Many second- and third-generation drugs are painful on injection and are usually therefore administered IV, but orally administered third-generation (group 5) cephalosporins are now available.

Dosage Considerations

As with all beta-lactams, the aim of treatment is to maintain serum and tissue concentrations of drug ≥ MIC for the majority of or the entire dosing interval. In recent years, long-acting formulations of third-generation cephalosporins have been introduced for injection in both food and companion animals, which produce serum concentrations exceeding MIC for periods of 4–14 days, depending on the particular formulation and the bacterial pathogen. These have the advantage of efficiency in treating food animals and of ensuring "compliance" in companion animals.

Clinical Usage

Cephalosporins are an important class of antimicrobial agents with widespread potential use.

First-generation cephalosporins have a spectrum of activity and clinical use similar to that of extended-spectrum aminobenzylpenicillins, with the important addition of resistance to staphylococcal beta-lactamase. First-generation oral cephalosporins are therefore used in the treatment of canine *S. intermedius* skin infections and urinary tract infections, as well as bovine *S. aureus* and streptococcal mastitis.

Second- and some third-generation (groups 3, 4) parenteral cephalosporins are used to treat infections caused by bacteria resistant to first-generation drugs. For example, ceftiofur, which has antimicrobial characteristics between group second and third-generation cephalosporins, is used in animals to treat systemic infections caused by Gram-negative aerobes, including *E. coli, Pasteurella* and *Salmonella* infections, but with particular focus on the more susceptible bacteria such as those involved in respiratory disease as well as anaerobic bacteria. Cefovecin is used for treatment of more susceptible bacterial infections in dogs and cats. Cefoxitin has a special place in the treatment of mixed aerobic-anaerobic infections. The antipseudomonal, group 6, cephalosporins are used exclusively in the treatment of *P. aeru*ginosa infections. Other third

(group 5) and the fourth-generation cephalosporins are usually (but not always) reserved in human medicine for the treatment of hospital-based bacterial infections resistant to earlier cephalosporins or alternative antimicrobial drugs. The broad-spectrum and bactericidal activity (at concentrations ≥ 4 x MIC) may be a drawback of newer cephalosporins, since it is associated with the selection of resistant bacterial superinfection and gastrointestinal disturbance. Widespread use of third-generation cephalosporins in human medicine may have been one of the important factors underlying the resistance crisis in medicine, and has been associated with the striking emergence and dissemination of multiple forms of beta-lactamases observed in recent years.

The fourth edition of this book stated that second- and third-generation cephalosporins are not first choice antimicrobial agents in animals but rather should be reserved for use where susceptibility testing indicates that alternatives are not available. This remains the opinion of the author, but these drugs are increasingly widely used in veterinary medicine as first choice antibiotics. There has been a remarkable rise in resistance through ESBLs in Enterobacteriaceae from both food and companion animals (including in foodborne pathogens such as *Salmonella*) associated with the rise of later-generation cephalosporins. The association between ceftiofur use in eggs or day-old broiler chicken poults with CMY-2 beta-lactamase producing *Salmonella* and *E. coli*, and the spread of resistant *S*. Heidelberg into the human population documented in Canada and the United States, suggests that these drugs should not be used for this purpose.

One response to the rise of ESBLs in the United States has been the prohibition in 2012 by the Food and Drug Administration of the extra-label use of cephalosporins in food animals (Food and Drug Administration, 2012). This prohibition extends to use for disease prevention, use at unapproved doses, frequencies, durations, or routes of administration, and use of human or companion animal drugs. The ban does not extend to use of cephapirin products, use to treat an extra-label disease indication, or use in food-producing minor species (e.g., goats, sheep). In Denmark, voluntary discontinuation of cephalosporin use in swine in 2010 was associated with a decline in ESBL-resistant *E. coli* in pigs at slaughter (Agersø et al., 2012).

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Group 1 First-Generation Cephalosporins: Cefacetrile, Cephaloridine, Cefazolin, Cephapirin, Cephradine, and Cephalothin

First-generation, group 1, parenteral cephalosporins share the characteristics of the oral first-generation cephalosporins of high activity against Gram-positive bacteria including beta-lactamase-producing *S. aureus* and *S. pseudintermedius;* moderate activity against certain non-transferable, beta-lactamase-producing, Gram-negative Enterobacteriaceae and fastidious Gram-negatives; and no activity against *Enterobacter* spp., *P. aeruginosa*, and *Serratia* spp., among others. For susceptibility testing, cephalothin is the class drug but cefazolin may also be tested since it is more active against Gram-negative bacteria. Activity is shown for selected bacteria in Tables 9.2 and 9.3.

Acquired resistance is common in Gram-negative but rare in Gram-positive bacteria. Methicillin-resistant *S. aureus* and methicillin-resistant *S. pseudintermedius*, discussed in chapter 8, are resistant to all cephalosporins.

Table 9.3. Activity (μg/ml) of first-generation cephalosporins (cephalothin) against selected animal bacteria.

Organism	MIC_{50}	MIC_{90}
Gram-positive aerobes		
Arcanobacterium pyogenes	0.5	4
Bacillus anthracis	0.25	0.5
Corynebacterium pseudotuberculosis	≤ 1	≤ 1
Erysipelothrix rhusiopathiae	0.25	0.5
Enterococcus spp.	> 32	> 32
Listeria monocytogenes	2	4
Nocardia asteroides	64	> 128
Rhodococcus equi	> 128	> 128
Staphylococcus aureus	0.5	1
Staphylococcus pseudintermedius	0.5	1
Streptococcus agalactiae	≤ 0.12	0.5
Streptococcus canis	≤ 0.12	0.25
Streptococcus uberis	0.5	2
Gram-positive anaerobes		
Actinomyces spp.	0.06	0.12
Clostridium perfringens	0.5	1
Clostridium spp.	0.5	1
Gram-negative aerobes		
Actinobacillus spp.	≤ 1	16
Bordetella avium	≤ 1	≤ 1
Bordetella bronchiseptica	16	64
Brucella canis	8	16
Camylobacter jejuni	≤ 512	≤ 512
Escherichia coli	8	64
Klebsiella pneumoniae	4	≤ 64
Leptospira spp.	1	8
Mannheimia haemolytica	1	8
Pasteurella multocida	1	8
Pseudomonas aeruginosa	> 64	> 64
Salmonella spp.	2	8
Gram-negative anaerobes		
Bacteroides fragilis	> 32	> 32
Bacteroides spp.	16	> 32
Fusobacterium spp.	0.5	≥ 1
Porphyromonas spp.	1	16

Bacteria with MIC ≤ 8 μg/ml are susceptible, 16 μg/ml moderately susceptible, and ≥ 32 μg/ml are resistant.

Pharmacokinetic Properties

IM or SC injection results in rapid absorption with high bioavailability. There is widespread distribution in extracellular fluids in the body but poor penetration across biological membranes (including into the udder) and physiological barriers (such as the cerebrospinal fluid). Cephalothin and cephapirin are metabolized into the less active desacetyl derivatives. The majority of drug is rapidly eliminated in the urine, and tubular secretion (but not glomerular filtration) can be inhibited by probenecid to reduce clearance from the body. The specific mechanism of renal excretion varies with the agent. Half-life is less than 1 hour.

Table 9.4. Parenteral dosage (IV, IM, SC) of group 1 parenteral cephalosporins.

Species	Drug	Dose (mg/kg)	Interval (h)	Comments
Dog, cat	Cephradrine	22	6–8	
	Cephalothin	20–40	6–8	IV only (painful IM)
	Cefazolin	15–30	12	IM, IV
Horse	Cephapirin	20	8	Highly susceptible, e.g., *S. aureus*
	Cefazolin	15–20	8	
	Cephalexin	10	8–12	
Cattle, sheep	Cefazolin	15–20	12	Poor udder penetration
	Cephapirin	10	8–12	As cefazolin

Toxicities and Side Effects

Pain on IM injection of cephalothin means that this drug is rarely used. Non-dose-related hypersensitivity, fever, skin rash, and eosinophilia occur uncommonly. At very high doses, nephrotoxicity caused by acute tubular necrosis may occur. Because of this, cephaloridine is no longer available for clinical use.

Administration and Dosage

Recommended dosage is shown in Table 9.4. Because of the margin of safety, a range of dosage can be used depending on the MIC of susceptible bacteria.

Clinical Applications

Clinical applications of parenteral first-generation cephalosporins have become fewer with the development of beta-lactamase-stable cephalosporins. Applications are as described for oral cephalosporins below, which are used extensively in small animal medicine. These drugs have been used extensively in prophylaxis of surgical wound infections in human patients and are used for this purpose in dogs and cats. Cefazolin has been suggested for administration (20 mg/kg IV) at the time of surgery, repeated SC 6 hours later (Rosin et al., 1993). In dogs and cats, parenteral first-generation drugs might be used to establish high tissue levels rapidly before using an oral cephalosporin. In horses, an important indication would be parenteral treatment of non-MRSA *S. aureus* infections. In the absence of susceptibility testing, their use in treating infections caused by Gram-negative Enterobacteriaceae is not generally recommended since activity is unpredictable (as is the case also for aminobenzylpenicillins). In cattle, different first-generation cephalosporins are in widespread use in treatment and prevention (dry-cow therapy) of mastitis caused by the Gram-positive cocci, as alternatives to pirlimycin, cloxacillin, and penicillin-novobiocin combination. Administration is by the intramammary route.

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Group 2 Oral First-Generation Cephalosporins: Cefadroxil, Cephradine, Cephalexin, and Cephaloglycin

First-generation, group 2, oral cephalosporins share the characteristics of the group 1 parenteral cephalosporins in high activity against Gram-positive bacteria including beta-lactamase-producing *S. aureus;* moderate activity against certain non-transferable, beta-lactamase-producing, Gram-negative Enterobacteriaceae and fastidious Gram-negatives; and no activity against *Enterobacter* spp., *P. aeruginosa*, and *Serratia* spp., among others (Tables 9.2 and 9.4).

Antimicrobial Activity

Antimicrobial activity of oral cephalosporins is similar to that of aminopenicillins with the addition of resistance to the beta-lactamase of *S. aureus*.

- *Good susceptibility (≤ 8 μg/ml)* is shown by many Gram-positive bacteria including *S. aureus*, streptococci (not enterococci), *Actinomyces* spp., *Bacillus* spp., *Corynebacterium* spp., *E. rhusiopathiae*, and most *L. monocytogenes* (Table 9.2). Susceptible anaerobes include some *Bacteroides*, most *Clostridium* spp., and most *Fusobacterium* spp. Susceptible aerobes include fastidious organisms such as *Bordetella avium, Haemophilus* spp., and *Pasteurella* spp.
- *Variable susceptibility*, due to acquired resistance, is shown by *E. coli, Klebsiella* spp., *Proteus* spp., and *Salmonella* spp.
- *Moderate susceptibility (16 μg/ml): Actinobacillus* spp., *Brucella* spp., some *Bacteroides* spp.
- *Resistance (≥ 32 μg/ml): Acinetobacter* spp., *Bacteroides fragilis, Bordetella bronchiseptica, Campylobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Nocardia* spp., *Enterococcus faecalis*, *P. aeruginosa, R. equi, Serratia* spp., and *Yersinia* spp.

Antibiotic Resistance

Acquired resistance occurs in Gram-negative bacteria and is particularly important in Enterobacteriaceae.

Pharmacokinetic Properties

Oral cephalosporins have pharmacokinetic properties similar to penicillin V and the aminobenzylpenicillins. Generally they are rapidly and largely absorbed after oral administration in monogastrates, but not horses; these drugs are unaffected by the presence of food (except for cephradine). Relatively wide distribution occurs in extracellular fluids but penetration across biological membranes is poor. Inflammation enhances passage across barriers. Half-lives are short, usually less than 1 hour although cefadroxil has a longer half-life in dogs. Cephalosporins are largely excreted unchanged in urine. Plasma protein binding is low. Absorption in horses and ruminants is poor and highly erratic.

Drug Interactions

Oral cephalosporins are potentially synergistic with aminoglycosides although indications for such combinations would be unusual.

Toxicities and Side Effects

Cephalosporins are among the safest of antimicrobial drugs. Allergic reactions, including acute, anaphylactic hypersensitivity, are rare. In humans, the majority of allergic reactions are not cross-reactive with penicillin. A small proportion of human patients may develop eosinophilia, rash, and drug-associated fever. Vomiting and diarrhea may occur in a small proportion of monogastrates given oral cephalosporins.

Administration and Dosage

Recommended dosage is shown in Table 9.5. Oral cephalosporins should be administered to monogastrates 3 times daily, although cefadroxil may be administered twice daily at the higher dose. Oral cephalosporins should not be used in herbivores.

Clinical Applications

Oral cephalosporins have similar applications to penicillinase-resistant penicillins and aminobenzylpenicillins in monogastrate animals, so that they are widely used in small animal medicine. The cephalosporins are thus potentially useful in a variety of non-specific infections caused by staphylococci, streptococci, Enterobacteriaceae,

Table 9.5. Recommended oral dosage of oral cephalosporins in animals.

Species	Drug	Dose (mg/kg)	Interval (h)
Dog, cat	Cefachlor	4–20	8
	Cefadroxil	22	12
	Cefixime	5	12–24
	Cefpodoxime proxetil	5–10	24
	Cephalexin	30	12
	Cephadrine	10–25	6–8
Calves (pre-ruminant)	Cefadroxil	25	12
	Cephadrine	7	12
Horse (foals only)	Cefadroxil	20–40	8
	Cefpodoxime proxetil	10	6–12
	Cephadrine	7	12

and some anaerobic bacteria. Long-term use (30 days) in the treatment of chronic *S. aureus* pyodermas in dogs is one useful application. Prophylactice use on 2 consecutive days a week prevented recurrence of German Shepherd recurrent furunculosis (Bell, 1995). Cephalexin has been described as the drug of choice for *K. pneumoniae* urinary tract infections, although a fluoroquinolone is now a better choice. Apart from skin and urinary tract infections caused by susceptible organisms, other applications include the treatment of abscesses and wound infections caused by susceptible organisms in dogs and cats. Despite widespread in for the treatment of canine pyoderma caused by *S. aureus* and *S. pseudintermedius*, resistance in *S. aureus* has not until recently become a problem. An interesting recent report suggested that oral cephalexin treatment in dogs might enhance fecal shedding of CMY-2 positive *E. coli* (Damborg et al., 2011).

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Group 3 Second-Generation Parenteral Cephalosporins: Cefaclor, Cefoxitin, Cefmetazole, Cefotetan, Cefuroxime, and Cefamandole

Second-generation, group 3, parenteral cephalosporins have a wide spectrum of antibacterial activity largely because of their stability to a broad range of beta-lactamases. They are moderately active against Gram-positive bacteria. Cephamycins (cefotetan, cefoxitin) are products of *Streptomyces* rather than of *Cephalosporium* species and differ from cephalosporins in the presence of a methoxy group in the 7 position of the cephalosporin nucleus. Cephamycins are very stable to beta-lactamases, including those of *Bacteroides fragilis*, but like other second-generation drugs are not active against *P. aeruginosa*.

Antimicrobial Activity

Cefoxitin is resistant to most bacterial beta-lactamases, although it penetrates Gram-negative bacteria relatively poorly. Antimicrobial activity is slightly broader and greater than that of cefazolin and other first-generation cephalosporins for Gram-negative bacteria and includes *Enterobacter* spp. and *Serratia* spp. Activity against Gram-positive bacteria is slightly less. Cefoxitin is stable to the beta-lactamase of *B. fragilis* and has good activity against this and other *Bacteroides*, *Porphyromonas* and *Prevotella* spp. *Pseudomonas aeruginosa*, enterococci, and some Enterobacteriaceae are resistant (Table 9.2). Cefotetan has the greatest activity of the 7-methoxy cephalosporins against Gram-negative bacteria but *P. aeruginosa* is resistant. A proportion of *Citrobacter*, *Enterobacter*, and *Serratia* spp. are resistant. Activity against anaerobes is similar to cefoxitin but a proportion of *B. fragilis* are resistant. Cefmetazole has a spectrum of activity similar to cefoxitin but it is more active against Enterobacteriaceae.

Resistance

Stable derepression of inducible beta-lactamases associated with hyperproduction of AmpC beta-lactamases in certain Gram-negative pathogens is an important mechanism of resistance. Cefoxitin is a powerful beta-lactamase inducer and can therefore antagonize the effects of other beta-lactams. As described earlier, in recent years there has been increasing spread of a family of cephamycinase (CMY2)-encoding plasmids in animals, noted not only in hospital-acquired *E. coli* infections in companion animals but also in *Salmonella*.

Certain strains of methicillin-resistant *S. pseudintermedius* may be falsely reported as susceptible to cefoxitin by laboratories because of its poor induction of the *mecA* gene (Weese et al., 2009). This is why an oxacillin disk is preferable to cefoxitin in testing methicillin resistance (Bemis et al., 2009).

Pharmacokinetic Properties

Pharmacokinetic properties and toxicities are similar to those of first-generation parenteral cephalosporins. With one exception, they are not absorbed following oral administration. Excretion, which can be delayed by probenecid, is largely renal. Half-lives in cattle and horses are about 1 hour. The 3-hour half-life of cefotetan in humans allows twice-daily dosing. Cefuromine axetil is an ester of cefuroxime that is hydrolyzed in the intestinal mucosa and liver to yield active drug, producing good bioavailability after oral administration.

Toxicities and Adverse Effects

Second-generation cephalosporins cause pain on IM injection and may cause thrombophlebitis when administered IV. Cefoxitin may cause hypoprothrombinemia and a tendency to bleed in human patients. Cefamandole in humans produces alcohol intolerance by blocking liver acetaldehyde dehydrogenase and may cause a coagulopathy associated with hypoprothrombinemia, which is reversible by vitamin K. For this latter reason, cefamandole is rarely if ever used in human medicine. Use in animals has been too limited to describe toxicities, but their broad antibacterial activity may lead to gastrointestinal disturbances and superinfection by resistant microorganisms, including yeasts. This has been particularly marked with cefuroxime axetil administered orally to human patients.

Administration and Dosage

Administration is usually IV because of pain associated with IM dosage. Dosage in animals, which in some cases is empirical, is shown in Table 9.6. Cefuroxime axetil is administered orally in monogastrates.

Clinical Applications

Clinical applications in animals are limited by the expense of these drugs, but may be similar to those identified in human medicine where cefoxitin is valued particularly for its broad activity against anaerobes, especially *B. fragilis*, as well as against Enterobacteriaceae. Indications are thus treatment of severe mixed infections with anaerobes (aspiration pneumonia, severe bite infections, gangrene, peritonitis, pleuritis) and prophylaxis in colonic surgery or ruptured intestine. Cefuroxime is available and effective for short-lasting dry-cow therapy and for treatment of clinical mastitis in lactating cows. Cefuroxime axetil is used by the oral route in human medicine for the treatment of *otitis media* and upper respiratory infections caused by susceptible bacteria. The widespread use of cephalosporins for this purpose may have been largely responsible for the extensive emergence of penicillin resistance in *Streptococcus pneumoniae*, an important human pathogen, in recent years.

Table 9.6. Dosage of groups 3 and 4 parenteral cephalosporins in animals.

Species	Drug	Dose (mg/kg)	Interval (h)
Dog, cat	Cefotaxime IM (SC)	20–40	8 (SC 12)
	Cefoperazone IV, IM	20–25	6–8
	Cefovecin	8	14 days
	Cefoxitin (IV, IM, SC)	15–30	6–8
	Cefiofur IM	2.2	24
	Ceftizoxime IV, IM	25–40	8–12
	Ceftriaxone IV, IM	25	12–24
	Cefuroxime IV	10–15	8–12
Cattle, sheep, goats	Ceftiofur IM (SC)	1.1–2.2	24
Cattle	Ceftiofur crystalline free acid, posterior ear	6.6	5 days
Horses	Cefotaxime IV	20–30	6–8
	Cefoxitin IV, IM	20	8
	Ceftiofur IM	2.2–4.4	12–24
	Ceftiofur IV	5	12 (foals only)
	Ceftiofur crystalline free acid IM (2 sites)	6.6	96 hours
	Ceftriaxone IV, IM	25	12 (not adults)
Swine	Ceftiofur IM	3–5	24
	Ceftiofur crystalline free acid IM	5.0	5 days

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Group 4 Third-Generation Parenteral Cephalosporins: Cefmenoxime, Cefotaxime, Cefovecin, Ceftizoxime, Ceftriaxone, Ceftiofur, and Latamoxef

Third-generation, group 4, parenteral cephalosporins are distinguished by their high antibacterial activity and their broad resistance to beta-lactamases; they have particularly good activity against most Enterobacteriaceae. Exceptions include *Enterobacter* and *Serratia*. Streptococci are highly susceptible, staphyloccci moderately susceptible, and enterococci are resistant. Latamoxef (moxalactam) is an oxacephem with an oxygen atom replacing the sulfur at the C_1 position of the cephalosporin nucleus. Its wide anti-Enterobacteriaceae activity is similar to that of others in the group but latamoxef is more active against *B. fragilis, Citrobacter* spp., and *Enterobacter* spp., and less active against *S. aureus* (Table 9.7). Some *P. aeruginosa* are resistant.

- *Good susceptibility (MIC ≤ 2 μg/ml):* Highly active against streptococci, including *Streptococcus suis* (not enterococci). Good activity against many other Gram-positive bacteria (benzyl penicillin sensitive; Tables 9.2 and 9.7). Fastidious Gram-negative bacteria (*Actinobacillus* spp., *Haemophilus* spp., *Pasteurella* spp.) including beta-lactamase producers are all highly susceptible. *Clostridium* spp., and *Fusobacterium* spp. are susceptible but *Bacteroides* spp. are often resistant. Among Gram-negative bacteria, *E. coli, Klebsiella* spp., *Proteus* spp. and *Salmonella* spp. are susceptible.
- *Moderately susceptible (4 μg/ml): S. aureus;* some *Citrobacter* spp., *Enterobacter* spp., some *P. aeruginosa*, and *Serratia* spp.,
- *Resistance (MIC ≥ 8 μg/ml Acinetobacter* spp., *Bordetella* spp., some *Enterobacter* spp. and *Serratia* spp., some *P. aeruginosa*, enterococci, and methicillin-resistant *S. aureus* and *S. pseudintermedius*.

Table 9.7. Susceptibility (MIC_{90}, μg/ml) of selected animal pathogens to ceftiofur.

Organism	MIC_{90}
Gram-positive aerobes	
Rhodococcus equi	≤ 1
Staphylococcus aureus	1
Streptococcus dysgalactiae	≤ 0.004
Streptococcus equi	≤ 0.004
Streptococcus hyicus	1
Streptococcus suis	0.12
Streptococcus uberis	0.03
Streptococcus zooepidemicus	≤ 0.12
Gram-negative aerobes	
Actinobacillus pleuropneumoniae	≤ 0.06
Escherichia coli	0.5
Haemophilus parasuis	0.06
Histophilus somni	≤ 0.03
Mannheimia haemolytica	≤ 0.03
Moraxella bovis	0.25
Pasteurella multocida	≤ 0.004
Salmonella spp.	1
Anaerobic bacteria	
Bacteroides fragilis	≥ 16
Bacteroides spp.	4
Fusobacterium necrophorum	≤ 0.06
Peptostreptococcus anaerobius	0.12

Antibiotic Resistance

Transferable resistance to third-generation cephalosporins as a result of AmpC hyperproduction, extended-spectrum beta-lactamases, and to a lesser extent beta-lactamase group 3 metallo-beta-lactamases (Table 9.1), has been discussed earlier and is an important threat to the continued use of these cephalosporins in animals, particularly in food animals because of public health considerations. In recent years, multidrug resistance plasmids carrying the bla_{CMY2} encoding resistance to ceftiofur and ceftriaxone have been identified in *Salmonella enterica* serovars Newport and Typhimurium, among others, and is often found in strains with concomitant resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (Doublet et al., 2004). The *cmy-2* gene appears to have been mobilized into different plasmid backbones that have spread through *E. coli* and *Salmonella* through conjugation (Carattoli et al., 2002).

In human medicine, the breakpoint for resistance used to be 64 ≥ μg/ml, so that there was confusion between resistance reported for animal isolates as it relates to resistance in human isolates. For example, in the Canadian Integrated Program for Antimicrobial Resistance Surveillance report for 2003 data (CIPARS, 2005), ceftiofur resistance (breakpoint ≥ 8 μg/ml) was particularly high in *Salmonella* isolated from chicken in Quebec but not as high for human *Salmonella* isolates tested for ceftriaxone (breakpoint ≥ 64 μg/ml); when the same 8 μg/ml breakpoint was applied to both drugs, percentage resistance was the same. As discussed further under beta-lactamases in chapter 10, the human medical CLSI breakpoints for ceftriaxone for Enterobacteriaceae were revised in 2010 to ≤ 1 μg/ml for susceptible and ≥ 4 μg/ml for resistance.

Pharmacokinetic Properties

Third-generation, group 4, parenteral cephalosporins are not absorbed after oral administration but are rapidly and well absorbed after IM or SC administration, giving peak serum levels in 0.5–1 hour. While data are often lacking, the half-life is about 1 hour following IV infection. In cattle, the half-life of ceftiofur is about 2.5 hours. By contrast, the half-lives for many of these cephalosporins are 1–2 hours for humans, with the marked exception of ceftriaxone that, because of extensive protein-binding, has a half-life of 8 hours giving it the potential for once-daily dosing. Distribution into tissues in extracellular fluid is widespread but passage across membranes or physiological barriers is poor. Meningeal inflammation significantly enhances otherwise poor CSF penetration so that, because of exceptional antibacterial activity, these cephalosporins are drugs of choice for bacterial meningitis caused by Enterobacteriaceae. Cefotaxime is metabolized in the body to the less active desacetyl-cefotaxime. Excretion is largely through the urinary tract, with cefotaxime being excreted through tubular mechanisms and the others through glomerular filtration. Probenecid administration delays tubular excretion. Biliary elimination also occurs, notably for ceftriaxone and latamoxef. These drugs should therefore be avoided in species with expanded large intestines. Cetriaxone has a long elimination half-life, giving this drug the advantage of twice-daily dosing.

Ceftiofur hydrochloride form is more stable than ceftiofur sodium, though both are rapidly metabolized to desfuroylceftiofur, the primary metabolite. Their pharmacokinetic properties are similar. A crystalline free acid formulation of ceftiofur has the advantage of delayed absorption, so that for highly susceptible bacteria dosing frequency can be reduced to 96–120 hours apart, depending on the species and route of administration. For example, the crystalline free acid formulation administered as a single subcutaneous injection into the ear of cattle at 6.6 mg/kg is slowly absorbed and gives plasma concentrations exceeding the MIC of common respiratory tract bacterial pathogens for about 6 days. Similarly, this formulation administered intramuscularly in swine also has a long half-life, with plasma concentrations after intramuscular injection of 5 mg/kg exceeding the MIC of common respiratory tract pathogens for about 5 days.

Drug Interactions

Group 4 cephalosporins are synergistic with aminoglycosides, with which they often need to be combined in the treatment of febrile illness in neutropenic human patients.

Toxicities and Side Effects

Toxicities and side effects are similar to those described for groups 1–3 cephalosporins, but the nephrotoxic potential is low. Because of the broad antibacterial activity of these cephalosporins, gastrointestinal disturbances and superinfection by resistant microorganisms, including yeasts, may be anticipated, although there does not seem to be a specific link between use of these drugs in horses and development of colitis. In human medicine, there is a strong association between group 4 and 6 cephalosporin use and *C. difficile* diarrhea. Anecdotal reports suggest that there may be a link between ceftiofur use in neonatal piglets and the development of *C. difficile* infection. In horses, IM administration has been occasionally associated with gastrointestinal disturbance, including severe colitis. Gastrointestinal disturbances were noted in 4 of 6 mares administered ceftriaxone IV (Gardner and Aucoin, 1994), probably because of its biliary excretion, so this drug should be used cautiously if at all in horses. Cutaneous drug reaction to ceftiofur, characterized by hair loss and pruritus, has been described in a cow.

Cefmenoxime in humans produces alcohol intolerance by blocking liver acetaldehyde dehydrogenase and a coagulopathy associated with hypoprothrombinemia,

which is reversible by vitamin K. Clinically important bleeding disorders caused by hypoprothrombinemia or disorders of platelet function are more common with latamoxef than with any other cephalosporin in human patients (about 20%), so that this drug is not generally recommended for clinical use. Vitamin K prophylaxis is suggested if the drug is used.

Administration and Dosage

Recommended dosages, which in some cases are empirical, are shown in Table 9.6. To some extent, dosage can be tailored to the susceptibility of the organism, with the aim to maintain drug concentrations ≥ MIC throughout the majority of the dosing interval. For example, dosage of ceftiofur sodium or hydrochloride for highly susceptible organisms associated with lower respiratory disease is usually 1.1–2.2 mg/kg q 24 h, but for *E. coli* infections caused by susceptible organisms the dose might be as high as 2.2–4.4 mg/kg q 12 h. Dosage of the crystalline free acid formulation in food animals and horses, and of cefovecin in companion animals, is less frequent. Enterobacteriaceae are, however, on an edge of susceptibility for formulations of ceftiofur sodium or hydrochloride used in animals, so that dosage should be higher. Ceftriaxone has the advantage that dosage is twice daily whereas dosage of other group 4 cephalosporins (other than ceftiofur) is usually q 8 h.

Clinical Applications

Because of expense, the availability of cheaper alternatives, and the potential to select for resistant bacteria, third-generation group 4 cephalosporins should be reserved for serious, probably life-threatening, infections caused by Gram-negative bacteria, especially Enterobacteriaceae. Despite the recommendations to reserve these drugs for serious infections, and only for infections where susceptibility testing indicates that alternatives are not available, there is an increasing and unfortunate tendency to use these drugs as first choice in animals. As noted earlier, because of resistance concerns, in 2012 there was a prohibition in the United States by the Food and Drug Administration on the extra-label use of cephalosporins in food animals (Food and Drug Administration, 2012).

These cephalosporins are drugs of choice in meningitis caused by *E. coli* or *Klebsiella* spp. They are recommended, in combination with an aminoglycoside, in severe infections caused by multiply resistant bacteria in compromised hosts, such as neutropenic hosts. These drugs have potential application in septicemia, serious bone and joint infections, some lower respiratory tract infections, intra-abdominal infections caused by Enterobacteriaceae, and some soft tissue infections where cheaper alternative drugs are not available. There is increasing interest in their value in treating systemic complications of human salmonellosis (bacteremia, meningitis, osteomyelitis). The poor activity of some of these cephalosporins against Gram-negative anaerobes is a drawback; ceftiofur, however, has good activity against anaerobes. Although not well documented in many animal species, they have a tendency to select for *Clostridium difficile* infections.

Cattle, Sheep, and Goats

Ceftiofur sodium and hydrochloride is used extensively for the treatment of acute, undifferentiated bovine pneumonia with the advantage of a low recommended dose (1.1–2.2 mg/kg, q 24 h) and zero drug withdrawal time in milk. Treatment is for 3–5 days and has proved as effective as treatment with sulbactam-ampicillin or potentiated sulfonamides for this purpose. In one study of treatment of relapse of undifferentiated fever/bovine respiratory disease in feedlot cattle, ceftiofur was less effective than enrofloxacin (Abutarbush et al., 2012). Dosage IM of 3 mg/kg q 12 h was inadequate for the parenteral treatment of mastitis caused by *E. coli* (Erskine et al., 1995). Treatment of severe coliform mastitis with ceftiofur was, however, shown to reduce death or culling (Erskine et al., 2002). Intramammary treatment of moderate coliform mastitis with ceftiofur hydrochloride produced a significant increase in bacteriological cure compared to untreated controls (Shukken et al., 2011). Ceftiofur sodium and hydrochloride is also used in the treatment of acute bovine interdigital necrobacillosis and the hydrochloride form is approved in the United States for the treatment of post-parturient metritis. Overall, procaine penicillin may be a better choice than ceftiofur for treatment of these latter infections, because of its equivalent or better antibacterial activity and narrower spectrum, with less likelihood of producing resistance in "by-stander" bacteria. Ceftiofur has also been experimentally at the extra-label dose of 5 mg/kg q 24 h in the treatment of *Salmonella* infection of calves

(Fecteau et al., 2003). Multiple treatments with ceftiofur sodium have been used to eliminate *Leptospira* from the kidneys of cattle although tetracycline and tilmicosin were equally effective (Alt et al., 2001) and may be preferred as less likely to induce important resistance in "bystander" bacteria.

The crystalline free acid formulation of ceftiofur administered into the ear at 6.6 mg/kg gives plasma concentrations exceeding the MIC of respiratory tract pathogens (*H. somni*, *M. haemolytica*, *P. multocida*) for over 5 days. It has application for the treatment of respiratory disease caused by these highly susceptible bacteria, as well as for treatment of interdigital necrobacillosis. Administration by routes other than SC in the ear can lead to violative residues, and should be avoided. The advantage of this formulation is that most animals with susceptible infections will respond within 3–5 days.

Horses

Ceftiofur sodium and the crystalline free acid are suitable for use in horses in treating bacterial infections caused by susceptible bacteria (Table 9.7). The crystalline free acid is indicated specifically for treatment of lower respiratory tract infections caused by *S. zooepidemicus* (MIC ≤ 0.25 mg/kg), with the advantage that a second dose at 96 hours should give concentrations in serum exceeding the MIC of this highly susceptible bacterium for a further 6 days. At 2.2 mg/kg IM q 24 h, ceftiofur sodium has been shown to be as effective as ampicillin in the treatment of respiratory infections in adult horses. Overall, procaine penicillin is a better choice than ceftiofur for treatment of such infections, because of its equivalent or better antibacterial activity for *S. zooepidemicus* and narrower spectrum, with the disadvantage of more frequent dosing compared to the crystalline free acid formulation. Intramuscular rather than oral administration is a drawback. The drug has potential application for treatment of septicemia in foals, perhaps combined with an aminoglycoside. A suggested intravenous dose for foals with septicemia caused by susceptible bacteria, which included Enterobacteriaceae, was 5 mg/kg every 12 hours (Meyer et al., 2008). Ceftiofur sodium has been used successfully to treat pleuritis and peritonitis caused by susceptible organisms.

Cefotaxime has been used effectively in the treatment of neonatal septicemia and meningitis caused by *Acinetobacter* spp., *Enterobacter* spp., and *P. aeruginosa*. Ceftriaxone may be particularly suitable for the treatment of meningitis in foals because it crosses the healthy blood-cerebrospinal fluid barrier. A dosage suggested for Gram-negative bacterial meningitis was 25 mg/kg q 12 h (Rinnger et al., 1998). This drug should, however, be used with caution in adult horses because of its hepatic excretion.

Swine

Ceftiofur sodium is available for use in swine in the treatment of respiratory or systemic infections caused by susceptible bacteria such as *P. multocida*, beta-lactamase producing *Actinobacillus* spp., *Haemophilus parasuis* and *Streptococcus suis*. The crystalline free acid formulation of ceftiofur administered at 5 mg/kg gives plasma concentrations exceeding the MIC of respiratory tract pathogens (*A. pleuropneumoniae*, *H. parasuis*, *P. multocida, S. suis*) for over 5 days, so that it has the advantage of single-dose treatment of infections caused by such susceptible bacterial infections. Ceftiofur has also been used in the control of *Salmonella choleraesuis* infections. It also has application for IM administration in the treatment of neonatal colibacillosis. Anecdotally, the practice of routine injection of neonatal pigs with ceftiofur may, however, predispose them to infection with *Clostridium difficile*, which has emerged as a significant problem in some swine farms in recent years. Narrower-spectrum drugs are often effective and should be preferred for the clinical applications outlined above.

Dogs and Cats

Cefovecin has been introduced as a long-acting subcutaneous formulation for dogs and cats, with the remarkable property that it produces serum concentrations ≥ 0.25 mg/ml (MIC_{90} of *S. pseudintermedius*) for most of 14 days. It is thus used for the single treatment of infections caused by highly susceptible bacteria including those commonly involved in skin infections, bite wounds and abscesses (*S. pseudintermedius*, *S. canis*, *P. multocida*). Because of urinary excretion, it is also effective against enteric bacteria causing urinary tract infections. Treatment can be repeated at 14-day intervals on two to four occasions in cats and dogs, respectively, depending on the susceptibility and clinical considerations. The advantage claimed, notably in cats,

is that administration by this route enhances the chance of compliance in comparison to owners trying to administer amoxycillin-clavulanic acid pills twice daily by mouth, and thus of enhancing the likelihood of cure. In one study, treatment failure estimated to be associated with non-compliance was 14% (van Vlaenderen et al., 2011). Cefovecin has a similar spectrum of activity clinical efficacy to amoxycillin-clavulanic acid (Stegemann et al., 2007). Serious adverse clinical effects have not been reported, with any hypersensitivity effects lasting 3–5 days.

Many companion animal practices use amoxycillin-clavulanic acid as a "first-line" antibiotic (Mateus et al., 2011; Murphy et al., 2012). Cefovecin has a similar spectrum of activity although dosage might give slightly lower serum concentrations against Enterobacteriaceae than amoxycillin-clavulanic acid. However, antibiotics should be chosen that have the narrowest spectrum of activity. For example, most cat bite infections can be successfully treated with amoxycillin and staphylococcal skin infections with cephalexin, so that these antibiotics should be preferred over a potentiated aminopenicillin or third-generation cephalosporin. The rapid rise and dissemination of broad-spectrum beta-lactamase resistance in Enterobacteriaceae of companion animals supports the enhanced stewardship of these drugs (Shaheen et al., 2010).

Poultry

Ceftiofur is administered SC to day-old chicken and turkey poults for the control of *E. coli* infections and navel infections, and has been injected in ovo for the same purpose. As described earlier, as a result of extra-label use for egg injection, CMY-2 beta-lactamase resistant strains have developed in *E. coli* and *Salmonella* in broilers, with spread of resistant *Salmonella* to humans to cause serious infections. This has been best documented in Canada (Dutil et al., 2010) but is also recognized in the United States (M'ikanatha, et al., 2010), and in other countries. There is evidence that these resistant *E. coli* infections are also reaching and causing disease in humans or, if not themselves disease causing, that they may be a source of resistance genes (Johnson et al., 2009). The use of third-generation cephalosporin drugs in poultry has important public health considerations that suggest that they should not be used in broiler production.

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Group 5 Third-Generation Oral Cephalosporins: Cefetamet, Cefixime, and Cefpodoxime

Third-generation, group 5, oral cephalosporins are highly active cephalosporins resistant to many beta-lactamases and available for oral administration. Cefixime is structurally related to cefotaxime and ceftizoxime and shares their antibacterial activity. Cefetamet pivoxil is a prodrug hydrolyzed to the active cefetamet and largely shares the antibacterial spectrum of cefixime and other group 4 parenteral cephalosporins. Cefpodoxime proxetil is also a prodrug that is absorbed from and de-esterified in the gastrointestinal tract to release the active metabolite cefpodoxime.

Antimicrobial Activity

Similar to that of group 4, third-generation parenteral cephalosporins. Among Gram-positive aerobes, third-generation oral cephalosporins are relatively inactive against *S. aureus* (MIC_{90} canine *S. aureus* 2 μg/ml), active against pyogenic streptococci but inactive against enterococci. Good activity against many other benzyl-penicillin sensitive Gram-positive bacteria (Tables 9.2 and 9.7). They have broad activity against Enterobacteriaceae that may exclude some *Citrobacter* and *Enterobacter*. *Pseudomonas* spp. are resistant. Fastidious Gram-negative bacteria (*Actinobacillus* spp., *Haemophilus* spp., *Pasteurella* spp.) including beta-lactamase producers are all highly susceptible. Among human pathogens, they are active against beta-lactamase producing *Haemophilus* spp. but inactive against penicillin-resistant *Streptococcus pneumoniae*. *Clostridium* spp. and *Fusobacterium* spp. are susceptible but *Bacteroides* spp. are often resistant. Proposed breakpoints for cefpodoxime for use in dogs are: Susceptible ≤2 μg/ml, Intermediate 4 μg/ml, and Resistant ≥8 μg/ml.

Antibiotic Resistance

Similar to that of group 4, third-generation parenteral cephalosporins.

Pharmacocokinetic Properties

The pharmacokinetic properties of group 5 cephalosporins are typical of those of beta-lactams generally. Cefpodoxime has a relatively long half-life in dogs, half-life of about 5.6 hours, so that plasma concentrations exceed 1 μg/ml for about 24 hours after a dose of 10 mg/kg.

Drug Interactions

Group 5 cephalosporins are synergistic with aminoglycosides, with which they often need to be combined in the treatment of febrile illness in neutropenic human patients.

Toxicities and Side Effects

Adverse effects of group 5 cephalosporins in humans relate mainly to gastrointestinal disturbance (diarrhea, nausea, vomiting), that occur in about 10% of human patients. Similar effects might be anticipated in animals. Like all broad-spectrum antimicrobial drugs, they should not be administered to herbivores with expanded large intestines. Cefpodoxime administered orally to dogs has been associated with no adverse effects.

Administration and Dosage

Dosage recommendations are given in Table 9.5. Cefixime's long elimination half-life allows once-daily administration in people. Dosage recommended for cefetamet in children is 20 mg/kg q 12 h. Cefpodoxime has been approved in the United States for dosage to dogs at 5–10 mg/kg administered once daily, with twice-daily administration in refractory infections. The upper dose is

preferable for susceptible *S. aureus* or *S. pseudintermedius* infections. A suggested dosage of cefpodoxime in foals was 10 mg/kg every 6–12 hours (Carrillo et al., 2005).

Clinical Applications

Cefetamet is used in the treatment of upper respiratory and urinary tract infections in people. Cefixime is used in people for the same purposes as cefetamet and has been advocated as an orally administered "follow-up" to a group 4 parenteral cephalosporin. Cefpodoxime has been approved for use in dogs in the United States for skin infections (wounds and abscesses) caused by susceptible organisms. It has the advantage over cephalexin of once-daily administration for this purpose (Cherni et al., 2006). Second- and third-generation cephalosporins are not first choice antimicrobial agents in animals but rather should be reserved for use where susceptibility testing indicates that alternatives are not available.

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Group 6 Antipseudomonal Parenteral Cephalosporins: Cefoperazone, Cefsulodin, and Ceftazidime

Antipseudomonal, group 6, parenteral cephalosporins are distinguished by the high activity against *P. aeruginosa*. Cefsulodin has otherwise a very narrow spectrum of activity. Ceftazidime and cefoperazone have a spectrum of activity almost identical to group 4 cephalosporins but with approximately 10 and 3 times greater activity against *P. aeruginosa*, respectively (Table 9.2). Resistance to ceftazidime is rare in *P. aeruginosa*. The group 6 drugs are otherwise slightly less active than group 4 drugs against most organisms. Antipseudomonal cephalosporins are synergistic with aminoglycosides, with which they are often combined in the treatment of *P. aeruginosa* infections in neutropenic human patients. Resistance, because of AmpC beta-lactamases, has been described in *Enterobacter, Citrobacter*, *Serratia*, and other genera of the Enterobacteriaceae, and through ceftazidime-specific PER type extended-spectrum beta-lactamases has been described (Table 10.1).

Pharmacokinetic properties are similar to those described for other parenteral cephalosporins. One exception is the largely hepatic elimination of cefoperazone, which therefore tends to be relatively often associated with gastrointestinal disturbance in humans. Thus, this drug is contraindicated in horses and other herbivores with an expanded large bowel. Cefoperazone, but not ceftazidime, elimination in urine is reduced by probenecid. There has been little study of the pharmacokinetic properties in animals.

Toxicities and side effects are the same as for other cephalosporins generally. Cefoperazone is likely contraindicated in those herbivore species with an expanded large bowel.

Empirical dosage is shown in Table 9.8.

These drugs are largely reserved in human medicine for *P. aeruginosa* and other Gram-negative septicemias in neutropenic human patients, in which efficacy is considerably enhanced by combination with an aminoglycoside. Cephalosporins have slow bactericidal activity compared to aminoglycosides. Subcutaneous injection of 30 mg/kg q 4 h or constant IV infusion of

Table 9.8. Empirical IM dosage of group 6 antipseudomonal parenteral cephalosporins.

Species	Drug	Dose (mg/kg)	Interval (h)
Dog, cat	Cefoperazone	20	6–8
	Ceftazidime	25–50	8–12
Cattle	Cefoperazone	30	6–8
	Ceftazidime	20–40	12–24
Horse (caution)	Cefoperazone	30	6–8
	Ceftazidime	25–50	8–12

4.1 mg/kg/hour were estimated to produce serum concentrations exceeding the MIC of canine clinical isolates of *P. aeruginosa* (Moore et al., 2000).

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Group 7 Fourth-Generation Parenteral Cephalosporins: Cefepime, Cefpirome, and Cefquinome

Although sometimes considered as part of the group 6 parenteral cephalosporins, the "fourth-generation," group 7, parenteral cephalosporins have high activity against Enterobacteriaceae, moderate activity against *P. aeruginosa*, and enhanced activity against staphylococci. They are stable to hydrolysis by many plasmid- or chromosomally mediated beta-lactamases and are poor inducers of group 1 beta-lactamases.

Antimicrobial Activity

Cefepime is an enhanced potency, extended-spectrum cephalosporin, the zwitterionic nature of which gives it rapid ability to penetrate through the porins of Gram-negative bacteria to the cell membrane. Both cefepime and cefpirome have higher affinity for essential PBPs and greater resistance to hydrolysis by beta-lactamases than other cephalosporins. In particular, they are resistant to, and a poor inducer of, group 1 beta-lactamases. There are no reports of activity against specific animal pathogens, however.

- *Good susceptibility (MIC ≤ 8 μg/ml*: Methicillin-susceptible *Staphyloccus* spp., *Streptococcus* spp., Enterobacteriaceae including *Citrobacter* spp., *Enterobacter* spp. *E. coli*, and *Serratia* resistant to group 4 cephalosporins; *P. aeruginosa*, including isolates resistant to group 6 cephalosporins; beta-lactamase producing *Haemophilus* spp.; *C. perfringens*, *Peptostreptococcus* spp. (Table 9.9).
- *Resistance (MIC ≥ 32 μg/ml: Enterococcus* spp., *L. monocytogenes*, *Bacteroides* spp., *C. difficile*.

Table 9.9. Activity (MIC_{90}, μg/ml) of cefepime and cefpirome.

Organism	Cefepime	Cefpirome
S. aureus	2	0.5
S. agalactiae	0.13	0.06
Enterococcus faecalis	16	4
E. coli	0.12	0.12
Proteus mirabilis	0.06	0.06
Pseudomonas aeruginosa	4	8
Acinetobacter spp.	8	4

Pharmacokinetic Properties

Pharmacokinetic properties of these parenterally administered cephalosporins are typical of those of other parenteral cephalosporins generally. Most drug is excreted through the urine.

Drug Interactions

Combination of cefepime with aztreonam is synergistic against *P. aeruginosa* with derepressed cephalosporinases, since aztreonam protects cefepime against these enzymes in the extracellular environment (Lister et al. 1998).

Toxicities and Adverse Effects

Toxicities and adverse effects in people are those of cephalosporins generally, with the major effect being gastrointestinal disturbance. Treatment was withdrawn in about 5% of patients treated with cefpirome and 1–3% of patients treated with cefepime because of adverse effects. Gastrointestinal effects must be anticipated if these drugs are used in animals, and have been observed in horses administered cefepime by the oral or IM route (Guglick et al., 1998).

Administration and Dosage

These drugs are administered IV or IM twice daily to human patients; dosage can to some extent be tailored to the nature and severity of the infection. In horses, a

dosage recommendation of cefepime was 2.2 mg/kg q 8 h (Guglick et al., 1998). This is a very low dosage based on extrapolation from the empirical dose of 50 mg/kg q 8 h in children. By contrast, an IV dose of cefepime estimated for treatment of susceptible bacteria in neonatal foals was 11 mg/kg q 8 and 40 mg/kg q 6 h for dogs (Gardner and Papich 2001). Recommended dosage of cefquinome is adult horses with *S. zooepidemicus* respiratory disease is 1 mg/kg once daily for 5–10 days and in foals with *E. coli* septicemia 1 mg/kg q 12 h. In Europe, approved dosage of cefquinome for respiratory disease in cattle caused by susceptible bacteria, foot rot or acute *E. coli* mastitis is 1 mg/kg q 24 h, and in calves with *E. coli* septicemia is 2 mg/kg q 24 h.

Clinical Applications

Fourth-generation cephalosporins are used in human medicine in the treatment of nosocomial or community acquired lower respiratory disease, bacterial meningitis, urinary tract infections and uncomplicated skin or skin-related infections. They have shown no advantage in clinical trials comparing them to cefotaxime or ceftazidime in treatment of infections in people. These drugs are valuable extended-spectrum cephalosporins for the treatment of serious infections in people. Cefquinome is used in Europe and Japan in treatment of bovine respiratory disease and, by intramammary or IM administration, in the treatment of coliform and other bacterial mastitis. In general, its efficacy in field studies of treatment of infections in cattle and swine has been similar to and slightly superior to that of ceftiofur (Lang et al., 2003). It is approved in Europe for the treatment of equine respiratory disease caused by *S. zooepidemicus* or foal septicemia caused by *E. coli*. Second- and third-generation cephalosporins should be reserved for use where susceptibility testing indicates that alternatives are not available.

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10

Other Beta-lactam Antibiotics: Beta-lactamase Inhibitors, Carbapenems, and Monobactams

John F. Prescott

The continuing development of beta-lactam antibiotics by changes of atoms within the basic beta-lactam ring or its attachment to the thiazolidine ring has produced compounds with significantly different activity from penam penicillins and the cephalosporins and cephamycins. Carbapenem and monobactam class antibiotics (Figure 8.1) have been introduced into human medicine but none have been approved for use in veterinary medicine. By contrast, some beta-lactamase inhibitors (clavulanic acid, sulbactam) have been successfully introduced into veterinary medicine in combination with aminobenzylpenicillins, producing broad-spectrum antibacterial drugs that overcome the limitations some of the acquired resistance had placed on the older extended-spectrum penicillins. Resistance, however, increasingly continues to threaten the efficacy of these beta-lactams, as beta-lactamase resistance genes evolve and then expand and spread through mobile genetic elements among Gram-negative bacteria, in part spearheaded by the expansion of certain "high-risk" bacterial clones, with subsequent dissemination of their resistance genes into the broader Gram-negative enteric and other Gram-negative bacterial populations.

Beta-lactamases and Beta-lactamase Inhibitors: Clavulanic Acid, Sulbactam, and Tazobactam

Introduction

Beta-lactamase production is a major factor in constitutive or acquired resistance of bacteria to beta-lactam antibiotics. The clinical importance of beta-lactamases has been associated particularly with the rapid ability of plasmid-mediated resistance to spread through bacterial populations. Such resistance has considerably reduced the value of what were once important drugs, such as amoxicillin. Three beta-lactamase inhibitors, clavulanic acid, sulbactam and tazobactam (Figure 10.1), have considerably enhanced the activity of penicillins against bacteria with acquired plasmid-mediated resistance. Although possessing weak antibacterial activity on their own, their irreversible binding to susceptible beta-lactamases (Table 10.1) allows the active beta-lactam antibiotic, with which they are combined, to bind to the penicillin-binding proteins (PBPs) resulting in lysis of the bacterial pathogen. Antibiotics combined for clinical use with clavulanic acid or sulbactam, which both have a similar spectrum of

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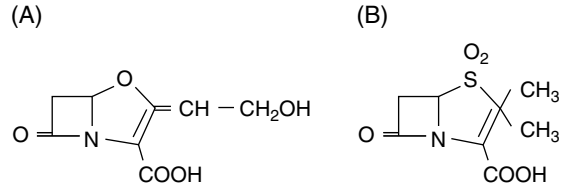

Figure 10.1. Structural formulas of clavulanic acid (A) and sulbactam (B).

Table 10.1. Functional and molecular characteristics of the major groups of beta-lactamases.[a]

Bush-Jacoby group	Molecular Class	Attributes of Beta-lactamases in Functional Group (Examples)	Inhibited by Clavulanic Acid
1	C	Often chromosomal enzymes in Gram-negative bacteria. Confer resistance to all classes of beta-lactams, except carbapenems. Plasmid-encoded include LAT, MIR, ACT, FOX, CMY family beta-lactamases, including FOX-1, CMY-2, MIR-1.	–
1e	C	Increased hydrolysis of ceftazidime (CM-37).	–
2a	A	Staphylococcal and enterococcal penicillinases included. High resistance to penicillins.	+
2b	A	Broad-spectrum beta-lactamases, primarily Gram-negative bacteria (TEM-1, SHV-1).	+
2be	A	Extended-spectrum beta-lactamases conferring resistance to oxyimino-cephalosporins (cefotaxime, ceftiofur, ceftazidime) and monobactams (CTX-M, includes CTX-M15, PER, SHV, some OXA, TEM, VEB).	+
2ber	A	Extended-spectrum cephalosporinases, monobactamases (CMTs, TEM-50, TEM-89).	–
2br	A	Inhibitor-resistant TEM (IRT) beta-lactamases; one inhibitor-resistant SHV-derived enzyme (TEM-30, SHV-10).	±
2c	A	Carbenicillin-hydrolyzing enzymes (PSE-1).	+
2d	D	Cloxacillin-hydrolyzing enzymes; modestly inhibited by clavulanic acid (OXA family).	±
2de	D	Extended-spectrum cephalosporinases (OXA-11, OXA-15).	±
2df	D	Carbapenemases (OXA-23, OXA-48).	±
2e	A	Cephalosporinases (CepA).	±
2f	A	Hydrolysis of carbapenems, cephalosporins, cephamycins, penicillins, weak inhibition by clavulanic acid (KPC-2, IMI-1).	±
3a	B	Broad spectrum of all beta-lactams except monobactams (IMP-1, IND-1, NDM-1, VIM-1).	–
3b	B	Preferential hydrolysis carbapenems (CphA, Sfh-1).	–

Table adapted from Bush and Fisher, 2011.

[a]See www.lahey.org/Studies/ for current list of TEM, SHV, and OXA beta-lactamases, and links to websites for other beta-lactamases.

beta-lactamase-inhibiting activities, have included amoxicillin, ampicillin, and ticarcillin. Clavulanic acid and sulbactam are synergistic with a number of penicillins and cephalosporins that are readily hydrolyzed by plasmid-mediated beta-lactamases, including benzyl- and aminiobenzylpenicillins and third-generation cephalosporins. Introduction of clavulanic acid and sulbactam has been a significant advance in antimicrobial therapy of infections in animals. The beta-lactamase inhibitors should be used with caution in herbivores with expanded large intestines because of potential for disrupting normal flora resulting in diarrheic illness.

Beta-lactamases: Classification

Beta-lactamases are enzymes that degrade beta-lactam drugs by opening the beta-lactam ring (Figure 8.2). As described in chapter 9, there has been a remarkable evolution of these enzymes in response to antimicrobial selection and widespread dissemination through Gram-negative bacterial populations through plasmids and transposons. The beta-lactamases of clinically important pathogens have been studied in exquisite detail. They consist of a wide variety of related proteins, hundreds of which have been fully characterized. They may be chromosomally mediated (inducible or constitutive) or plasmid-mediated, with transferable spread causing the greatest chaos and threat to the continued use of these drugs for certain infections. Numbers appear to be rising almost exponentially (Bush and Fisher, 2011). Beta-lactamases of Gram-positive bacteria may be exported extracellularly whereas beta-lactamases of Gram-negative bacteria are usually found in the periplasmic space but may be found extracellularly when the bacterium lyses (Figures 8.3 and 8.4). Certain "high-risk" clones of *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* have had an important role globally in human medicine in spreading resistance because of their ability to survive in humans in hospital settings and to flexibly accumulate and change resistance, thus acting as a reservoir of resistance genes for other bacteria (Woodford et al., 2011).

Classification is based on a combination of molecular characterization (nucleotide, amino acid sequence) and functional characterization (substrate, inhibition profile; Table 10.1), although these do not account for other changes that can affect the susceptibility of a bacterium. Although there is general correlation with molecular-based typing approaches, a functional approach to classification is preferred because very fine differences in molecular character may cause dramatic differences in function (Bush and Jacoby, 2010. Functional groups are identified by their inhibition by clavulanic acid and EDTA as well as according to substrate hydrolysis profiles (eg, benzyl penicillin, ceftazidime, cefotaxime, imipenem). The four major groups of beta-lactamases are: penicillinases, AmpC-type cephalosporinases, extended-spectrum beta-lactamases (ESBLs), and carbapenemases (Table 10.1). ESBLs, cephalosporinases that hydrolyze extended-spectrum cephalosporins form the largest group but carbapenemases are increasing rapidly (Bush, 2010).

Part of the increasing complexity of beta-lactamase resistance is that bacteria may not only acquire and maintain multiple distinct beta-lactamase enzymes but this resistance may add to resistance mediated through changes in porin function and efflux mechanisms.

The genes for beta-lactamases are found in the chromosome or on plasmids, and may be moved from these sites by transposons. Transfer of some of these genes has been widespread within and between species, genera and families. The evolution of beta-lactamases has occurred at a dramatic rate among bacteria, probably in response to selection by the extensive use of beta-lactam antibiotics, especially those with an increasing spectrum of activity. Plasmid-mediated beta-lactamases are centrally important in beta-lactamase resistance. For example, plasmid-mediated TEM-1 beta-lactamase, which encodes ampicillin resistance, has become widespread in *E. coli*. More recently, plasmid-mediated ESBLs, discussed below, have emerged among Enterobacteriaceae, although many remain sensitive to cefoxitin and imipenem, and usually also to the beta-lactamase inhibitors clavulanic acid and tazobactam. However, some TEM variants resistant to beta-lactamase inhibitors have been described (Table 10.1).

All Gram-negative bacteria produce beta-lactamases, usually functional group 1, from genes located on their chromosomes. In some genera (eg, *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Serratia*), as described in Chapter 9 in the section "Resistance to Cephalosporins," these AmpC hyperproducers are inducible, producing high concentrations of enzyme that overwhelm local concentrations of beta-lactamase inhibitors. In some cases, therefore, mutants with derepressed inducible beta-lactamases have emerged among the genera listed that are resistant to the beta-lactams that previously were effective against them. More seriously, however, as described in chapter 9, AmpC hyperproduction may become plasmid encoded by high copy number plasmids (CMY2, FOX, MIR, MOX). The dissemination of CMY2 AmpC beta-lactamase plasmids among *E. coli* and *Salmonella*, discussed in chapter 9, is a particular current concern.

The most rapidly expanding family of beta-lactamases in the group 2 serine beta-lactamases are the ESBLs,

which contain the functional groups 1e, 2be, 2ber, and 2de (Table 10.1; Bush and Fisher, 2011). The expansion of the CTX-family of ESBLs has displaced the early TEM- and SHV-derived ESBLs, with CTX-M15 being the globally most widely distributed ESBL in human medicine (Bush, 2010; Johnson et al., 2012). This beta-lactamase, and the *E. coli* or *K. pneumoniae* clones that harbor it, is being identified in infections in companion animals (O'Keefe et al., 2010; Wiler et al., 2011; Haenni et al., 2012), likely as a result of infection originally acquired from humans and then amplified in and spread from veterinary hospitals.

Identification of ESBLs can be problematic, and is an area of intense current debate. Most guidelines recommend screening based on reduced susceptibility to extended-spectrum cephalosporins as a primary screen, followed by use of a second test (such as use of double different cephalosporin ± clavulanic acid disks spaced at specific distances) to confirm ESBL production, although the latter will not always be necessary if an obvious ESBL is identified. Recently, however, screening and confirmation tests have been replaced by recommended breakpoints. In the Committee for Clinical Laboratory Standards Institute (CLSI) human guidelines (CLSI, 2010), breakpoints for Enterobacteriaceae were changed to $\leq 1\,\mu g/ml$ and $\leq 4\,\mu g/ml$ for ceftazidime or cefotaxime, to be reported as found. Previously, resistant bacteria (which had higher breakpoints) were reported as resistant to cephalosporins, regardless of the MIC. The reason for this earlier system was treatment failures in patients infected with ESBLs (Livermore et al., 2012). There is continued argument as to the best way of reporting and interpreting susceptibility data. Susceptibility testing is markedly affected by *in vitro* conditions such as innoculum concentration, as well as the potential for increase in MIC due to beta-lactamase hyperproduction. The equivalent European susceptibility testing advisory committee (EUCAST, 2011) has similar but marginally different breakpoints. The current breakpoint recommendations will be revised to revert to the earlier recommendations (Livermore et al., 2012).

The current consensus about the definition of ESBLs excludes the serine carbapenemases (functional groups 2df, 2de, 2f; Table 10.1), which, however, also represent a rapidly expanding group of beta-lactamases. The carbapenameses include the *K. pneumoniae* carbapenemases (KPCs) that have disseminated through transposons to other *Enterobacteriaceae* and other Gram-negatives such as *Acinetobacter* spp. and *P. aeruginosa* (Bush, 2010).

Group 3 beta-lactamases are metalloenzymes that hydrolyze most beta-lactams including carbapenems and resist beta-lactamase inhibitors. Genes for these enzymes have been identified on plasmids, many of which carry multiple beta-lactamases and other antibiotic resistance genes, among opportunist bacteria isolated from human patients. They thus represent virtually untreatable infections. Although clonal spread of the host bacterium was originally responsible for dissemination of resistant bacteria, spread to less virulent clones has been through conjugative plasmids aided by the transposon or integron carriage of the resistance genes. NDM-1 (New Delhi Metallo-beta-lactamase) is a recently emerged novel functional group 3 enzyme that has reached multiple enteric bacteria. It is an example of the rapid ability of highly resistant bacteria to disseminate globally in human hospital settings.

Beta-lactamase Inhibitors

The concept behind the use of beta-lactamase inhibitors is that they have little antibacterial activity in their own right but have a high affinity for beta-lactamases, and they can be administered with a beta-lactam that would be highly active against the pathogen if it were not for its beta-lactamases. In other words, the inhibitors (clavulanic acid, sulbactam, tazobactam) have high substrate specificity for a wide variety of beta-lactamases. Their binding to these inhibitors is irreversible, thus allowing the active beta-lactam (amoxycillin, piperacillin, etc.) to kill the organism since beta-lactamase is effectively absent. The spectrum of activity of these inhibitors is described in Table 10.1; clavulanic acid and tazobactam have a similar spectrum.

The increasing complexity and dissemination of beta-lactamases has resulted in some of the most resistant Gram-negative bacterial pathogens observed in human medicine containing a multiple repertoire of beta-lactamases, including those resistant to inhibitors. Future developments to counteract this may include development of the monobactams, some of which will target to bacteria through siderophore-mediated uptake by bacterial iron-transport systems (Bush and Fisher, 2011). It is likely that effective beta-lactams, if they can be developed, will contain several different components.

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Clavulanic Acid

Clavulanic acid is a synthetic compound, the bi-cyclic nucleus of which has similarities to a penicillin, apart from the oxygen in place of the sulfur and a missing acylamino side chain at position 6. It has good affinity for many plasmid-mediated beta-lactamases (Table 10.1) and all chromosomally mediated penicillinases, but little for chromosomal cephalosporinases. This latter group of enzymes, however, usually hydrolyze amoxicillin and ticarcillin, with which clavulanic acid is combined, poorly. Clavulanic acid is combined with amoxicillin in the ratio of 2:1 and with ticarcillin in the ratio of 15:1. The combinations are usually bactericidal at one or two dilutions below the MIC of amoxicillin or ticarcillin used alone.

Clavulanic Acid–Amoxicillin

Antibacterial Activity. Amoxicillin–clavulanic acid has a spectrum of activity similar to that of a first- or second-generation cephalosporin.

- *Good susceptibility (MIC ≤ 8/4 μg/ml, S. aureus, S. pseudintermedius ≤ 4/2)* is shown with several bacteria: excellent susceptibility of Gram-positive bacteria, including beta-lactamase-producing *S. aureus*. Fastidious Gram-negative bacteria (*Actinobacillus* spp., *Bordetella* spp., *Haemophilus* spp., *Pasteurella* sp.) are susceptible, including strains resistant to amoxicillin. Enterobacteriaceae such as *Escherichia coli, Klebsiella* spp., *Proteus* spp., and *Salmonella* spp. are usually susceptible; most anaerobes, including *Bacteroides fragilis*, are susceptible (Table 10.2).
- *Variable susceptibility* is found in some *E. coli* and *Klebsiella* spp.

Table 10.2. Activity of amoxicillin–clavulanic acid (MIC_{90}, μg/ml) against selected veterinary pathogens.

Organism	MIC_{90}	Organism	MIC_{90}
Gram-positive cocci			
S. aureus	0.5	*S. dysgalactiae*	≤ 0.13
S. intermedius	0.25	*S. suis*	≤ 0.13
S. agalactiae	≤0.13		
Gram-positive rods			
A. pyogenes	0.25	*L. monocytogenes*	0.25
Gram-negative aerobes			
A. pleuropneumoniae	0.5		
B. bronchiseptica	2	*P. multocida*	0.25
E. coli	8	*Pseudomonas* spp.	≥ 32
H. somni	0.06	*P. mirabilis*	0.5
M. bovis	0.06	*Salmonella*	2
M. haemolytica	0.13		
Anaerobic bacteria			
B. fragilis	0.5	*P. asaccharolytica*	1.0
C. perfringens	0.5	*Fusobacterium* spp.	≥ 32

After Mr. C. Hoare, Smith Kline Beecham (unpublished observations, with permission), with additions.

- *Resistance (MIC ≥ 32/16 μg/ml)* is shown among *Citrobacter* spp., *Enterobacter* spp., *P. aeruginosa, Serratia* spp., and methicillin-resistant *S. aureus* and *S. pseudintermedius*.

Antibiotic Resistance. Clavulanic acid may induce beta-lactamases in susceptible *Providencia* and *Enterobacter*. Until recently, emergence of resistance to clavulanic acid had not been a problem in bacteria isolated from animals. However, a variety of resistance mechanisms have rapidly emerged in recent years (Table 10.1), in both food animals (CMY-2 especially) and companion animals (ESBLs). These include plasmid-encoded functional group 1 CMY, FOX and other families of beta-lactamases that do not bind to clavulanic acid, the ESBLs, and the carbapenemases (Table 10.1).

Pharmacokinetic Properties. Clavulanic acid is well absorbed after oral administration and has pharmacokinetic properties similar to amoxicillin. Tissue distribution in extracellular fluids is widespread but penetration into milk and into uninflamed cerebrospinal fluid is relatively poor. Half-life is about 75 minutes. The drug is largely eliminated unchanged in the urine. Interesting, in dogs, higher doses than those recommended for treatment appear to show an inhibitory effect of amoxicillin on the absorption of the clavulanate component (Vree et al., 2003), but the significance of this observation is unclear.

Toxicity and Side Effects. The combination is well tolerated. The major side effect reported in about 10% of human patients has been gastrointestinal effects after oral administration, of nausea, vomiting, and diarrhea. This is associated with a direct effect on gastrointestinal motility of the clavulanic acid component so that recommended oral doses should not be exceeded. Mild gastrointestinal upset has been reported in dogs and cats. Other side effects are those of penicillins generally. The combination should not be used in penicillin- or cephalosporin-sensitive animals. The drug should not be administered orally to herbivores or by injection to horses. It should also not be used in rabbits, guinea pigs, hamsters, or gerbils.

Administration and Dosage. Recommended dosage is shown in Table 10.3. The recommendations by the manufacturers for once-daily dosing of parenterally administered drug in food animals likely represents underdosing, with twice-daily or more frequent administration taking advantage of the time-dependent pharmacodynamic requirement for efficacy of beta-lactam drugs. Clinical trials comparing dosage in food animals might confirm this deduction.

Clavulanic acid is highly moisture sensitive, so precautions must be taken to ensure dryness during storage.

Clinical Applications. Clavulanic acid–amoxicillin is a valuable addition as an orally administered antibiotic in monogastrates. It extends the range of amoxicillin against beta-lactamase-producing common opportunist pathogens, including fastidious organisms, Enterobacteriaceae, and an aerobic bacteria. It is not effective against *P. aeruginosa*. Some *E. coli, Proteus*, and *Klebsiella* are only susceptible to urinary concentrations of the

Table 10.3. Suggested dosage of clavulanic acid, sulbactam, or tazobactam potentiated penicillins.

Drug	Species	Route	Dose (mg/kg)	Interval
Clavulanate-amoxoicillin	Dogs, cats	PO	12.5–20	8–12
		SC	10	8
	Cattle	IM	7	12–24
	Pre-ruminant calves	PO	5–10	12
	Sheep	IM	8.75	12–24
Clavulanate-ticarcillin	Dogs, cats	IV	40–50	6–8
	Horses	IV	50	6
Sulbactam-ampicillin	Cattle	IM	10	24
Penicillin-tazobactam	Dogs, cats	IV	4	6

combination so that the combination can be recommended for empirical treatment of urinary tract infections in dogs and cats. Activity against anaerobes is a particularly useful attribute.

The combination is a valuable addition as a parenterally (IM) administered drug in food-producing animals, particularly for lower respiratory tract infections of cattle and pigs caused by beta-lactamase-producing *Actinobacillus, Haemophilus*, and particularly *Pasteurella*. Its potential in the treatment of *E. coli* diarrhea and of salmonellosis needs to be explored in clinical trials, although currently available ceftiofur formulations have similar activity and application.

The drug should not be administered orally to herbivores or by injection to horses, rabbits, guinea pigs, hamsters, or gerbils.

Cattle, Sheep, and Goats. Clavulanic acid–amoxicillin has been introduced for use in cattle; its application is similar to that of ceftiofur (chapter 9). Uses include the treatment of lower respiratory tract infections, particularly of soft tissue infections including anaerobic infections, plus neonatal calf diarrhea caused by *E. coli* and *Salmonella*. Dosage recommended for oral treatment of *E. coli* diarrhea in calves was 12.5 mg combined drug/kg q 12 h for at least 3 days (Constable, 2004). The drug has been used parenterally in conjunction with intramammary administration to treat clinical mastitis in cows, where combined use gave improved results over intramammary use alone (Perner et al., 2002). In sheep, the combination can be recommended in the treatment of pasteurellosis. There are few published data on pharmacokinetic behavior of the drug in ruminants but the dosing rate recommended by the manufacturer (Table 10.3) appears low. There may therefore be advantage to at least twice-daily injection of the recommended dose. Plasmid, CMY2 beta-lactamase, mediated resistance to the combination has been described in multidrug-resistant *Salmonella* of several serovars derived from cattle including Newport (Zhao et al., 2001, 2003).

Swine. The combination has potential application in the treatment of a variety of infections in swine caused by plasmid-mediated beta-lactamase-producing bacteria, possibly including neonatal diarrheal *E. coli*. The combination would be expected to have similar activity to that of ceftiofur products currently used in swine (chapter 9).

Dogs and Cats. Clavulanic acid–amoxicillin has many applications in dogs and cats, with the advantage of twice-daily oral administration for medication by owners. It is a very widely used antibiotic in companion animal practice (Mateus et al., 2011; Murphy et al., 2012). Among other applications are skin and soft tissue infections caused by *S. aureus* and infections following bite wounds that involve mixed bacteria including anaerobes, upper and lower respiratory tract infections, anal sacculitis, gingivitis, and urinary tract infections involving common opportunist bacteria (*S. aureus, E. coli, Proteus, Klebsiella*). Apart from urinary tract infections, the drug is not recommended for serious infections caused by *S. aureus, E. coli, Proteus*, or *Klebsiella* since tissue concentrations may not exceed the MIC for some strains for a sufficient part of the dosing interval. Interestingly, however, doubling the dose was not associated with increased cure in the treatment of canine pyoderma (Lloyd et al., 1997). The drug was not as effective as clindamycin for treatment of superficial pyoderma (Littlewood et al., 1999), and this combination should not be a first choice for pyoderma. First-generation cephalosporins have proven efficacy and a narrower spectrum less likely to select for important resistance in *S. aureus* and other pathogens. For treatment of *Bordetella* infections, the combination would be preferred to amoxicillin alone because isolates are less likely to be resistant to the combination (Speakman et al., 2000). The drug may have particular value in the treatment of peritonitis associated with intestinal content spillage, because of its activity against enteric bacteria including anaerobes.

In view of the poor ability of beta-lactams to penetrate membranes, this combination showed unexpected efficacy in treating *Chlamydophila psittaci* infection in cats, which exceeded that of doxycycline (Sturgess et al., 2001). However, unlike doxycycline treated cats, infection in some cats treated with clavulanic acid–amoxicillin recurred. Treatment for 4 weeks is therefore recommended although it is likely that amoxicillin alone would have the same efficacy.

The emergence of ESBLs in companion animals (So et al., 2010; Shaheeen et al., 2011; Sun et al., 2012), and the increase in methicillin-resistant *S. aureus* and *S. pseudintermedius* in companion animals is a rising threat to inhibitor-potentiated beta-lactams as well as to third-generation cephalsporins.

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Clavulanic Acid–Ticarcillin

Clavulanic acid–ticarcillin is available as a parenteral (usually IV) drug for use in human medicine. It offers the advantage over clavulanic acid–amoxicillin of the greater activity of ticarcillin against *Enterobacter* and *P. aeruginosa*. The combination has good activity against the majority of ticarcillin-resistant Enterobacteriaceae, *S. aureus*, anaerobes including *B. fragilis*, and many *P. aeruginosa*. However, the MIC_{90} of bacterial isolates from disease processes, especially *Enterobacter, E. coli*, and *Klebsiella*, is on the high end of the susceptibility range (MIC ≤ 16 μg/ml) or in the moderately susceptible range (MIC 32–64 μg/ml; Sparks et al., 1988). No potentiating activity occurs with the combination for *Enterobacter, P. aeruginosa*, and *Serratia*, and results of treatment of human clinical infections caused by these organisms have sometimes been disappointing, possibly because of induction of beta-lactamases by the clavulanate component. The combination has the disadvantage in animals of requiring frequent (6- to 8-hour) IV dosage (Table 10.3), although a 12-hour dosing interval may be used in neonatal foals. In human medicine, it may have application in the empirical treatment of serious infections in immunocompromised patients, when combined with an aminoglycoside. Because of need for IV dosage, applications of clavulanic acid–ticarcillin in veterinary medicine are few.

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Sulbactam

Sulbactam (penicillinic acid sulfone) is a synthetic derivative of 6-aminopenicillanic acid. It is poorly absorbed orally, but a double ester linkage of sulbactam with ampicillin has been developed to produce the prodrug, sultamicillin, which is well absorbed orally and releases the two drugs in the intestinal wall. Sulbactam has no antibacterial activity by itself but irreversibly binds the same groups of beta-lactamases as clavulanic acid, though sulbactam's affinity is several times lower. It also binds beta-lactamases of *Citrobacter, Enterobacter, Proteus*, and *Serratia* that clavulanic acid does not.

The same level of inhibition as clavulanic acid can, however, be achieved by increasing the concentration of sulbactam (2:1) for clinical use. It is combined with ampicillin in part because of pharmacokinetic similarities but has also been combined with cefoperazone.

Sulbactam-Ampicillin

Antibacterial activity is slightly broader but is marginally lower than that of clavulanic acid–amoxicillin (Table 10.1, 10.2). Sulbactam-ampicillin's lower affinity for beta-lactamases may limit its activity against some potent beta-lactamase-producing bacteria.

Pharmacokinetic properties are similar to those of amoxicillin–clavulanic acid, but sulbactam is poorly absorbed orally. It is available for use in human medicine as the orally absorbed prodrug sultamicillin. The combination is well absorbed after IM injection, distributes well into tissues in the extracellular space, and penetrates CSF through inflamed meninges. Penetration into milk is modest. Elimination is largely in the urine. The half-life is about 1 hour. Pharmacokinetic studies in calves (Fernández-Varón et al., 2005) and in sheep (Escudero et al., 1999) have suggested that the ampicillin concentration could be raised since sulbactam was more slowly eliminated than ampicillin.

The combination used for parenteral injection is well tolerated, and the side effects are those of penicillins generally, without the diarrhea that may occur with the orally administered clavulanic acid–amoxicillin. Intramuscular injection may be painful. The combination should not be used in herbivores with expanded large intestines (horses, rabbits, hamsters, guinea pigs), although adverse effects were not observed in foals (Hoffman et al., 1992).

Clinical Applications. Like amoxicillin-clavulanic acid, sulbactam-ampicillin restores and extends the antibacterial activity of ampicillin to include common bacteria that have acquired beta-lactamases. Its application is similar to that of ceftiofur (chapter 9). Sulbactam-ampicillin has been introduced into food animal medicine for the treatment of bovine respiratory disease for its activity against *Pasteurella* (including beta-lactamase-producing strains), *Histophilus somni, Arcanobacterium pyogenes*, and opportunist bacteria, including *E. coli*. The efficacy and superiority of the combination to ampicillin alone has been demonstrated in experimental and field studies. It was as efficacious as ceftiofur in the treatment of bovine respiratory disease in one study (Schumann and Janzen, 1991). Its advantage over ampicillin in the parenteral treatment of undifferentiated diarrhea in neonatal calves has been demonstrated. Once-daily dosage in cattle, while clinically effective, appears to represent underdosing based on pharmacokinetic and pharmacodynamic considerations, and there may be advantage to more frequent dosing. The combination might be useful, given at high dosage, for *E. coli* meningitis in calves. This combination may also see extra-label use for diseases such as salmonellosis; however, there have been no clinical trials reporting its use for diseases other than undifferentiated bovine respiratory disease and enteric colibacillosis. Others of the many potential clinical applications are described for cattle under benzyl penicillin and clavulanic acid–amoxycillin, with the combination clearly having the advantage over benzyl penicillin for many applications. Suggested dosing is shown in Table 10.3.

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Tazobactam

Tazobactam is a beta-lactamase inhibitor with activity similar to but broader than clavulanic acid and sulbactam. For example, it resists hydrolysis by Bush group 1 and group 3 beta-lactamases in addition to beta-lactamases inhibited by clavulanic acid (Table 10.1). Unlike clavulanic acid, it is also only a poor to moderate inducer of beta-lactamases. Combined with piperacillin in an 8:1 ratio (piperacillin:tazobactam), it has considerably enhanced the activity of this group 5 (antipseudomonal) penicillin against beta-lactamase producing bacteria generally.

The combination possesses broad-spectrum activity against many Enterobacteriaceae and other Gram-negative bacteria. Minor exceptions include *Enterobacter* spp. and *Xanthomonas maltophila*. Activity against anaerobic bacteria such as *B. fragilis*, including cefoxitin-resistant *B. fragilis*, is an important feature of the combination. It is active against a wide range of Gram-positive bacteria. Pharmacokinetic properties are typical of beta-lactam drugs generally.

Indications in human medicine are generally those where third-generation cephalosporins are indicated, with an emphasis on the additional beneficial effects of this combination against anaerobic bacteria. It rivals imipenem in breadth of antibacterial activity. The drug is, therefore, used in treatment of intra-abdominal infections (where mixed aerobic-anaerobic infections are likely to be present) and other polymicrobial infections. It is as effective for this purpose as clindamycin-gentamicin combination or as imipenem. It is also used in the treatment of fever in neutropenic patients (in combination with an aminoglycoside). Its advantage over ticarcillin/clavulanate combination in the treatment of human community acquired lower respiratory infection has been convincingly demonstrated. While indications for use in animals of this broad-spectrum drug are few, empirical dosage is suggested in Table 10.3.

Carbapenems: Imipenem-Cilastatin, Meropenem, and Biapenem

Carbapenems (Figure 8.2) are derivatives of *Streptomyces* spp. that differ from penam penicillins by the substitution of a CH_2 group for the sulphur in the five-membered ring attached to the beta-lactam ring. They have the widest activity of any antibiotic, except possibly trovafloxacin, being highly active against a wide variety of Gram-positive and Gram-negative bacteria and resistant to many beta-lactamases. N-formimidoyl thienamycin (imipenem) is stable to bacterial beta-lactamases other than the Bush-Jacoby functional groups of carbapenemases 2df and 2e group and the group 3 beta-lactamases (Table 10.1). Its hydrolysis by a dihydropeptidase in the kidney is overcome by 1:1 combination with cilastatin, a dihydropeptidase inhibitor. Other semisynthetic carbapenems, meropenem and biapenem, have activity similar to imipenem but resist degradation by the renal dihydropeptidase.

Antibacterial Activity

Carbapenems are active against almost all clinically important aerobic or anaerobic Gram-positive or Gram-negative cocci or rods. Individual species may be resistant. They offer the advantages of broad antimicrobial activity and, by comparison to third- and fourth-generation cephalosporins, resistance to Bush groups 1 and most 2 beta-lactamases, although the situation is changing. Biapenem and meropenem are slightly less active than imipenem against Gram-positive bacteria but equivalent or slightly more active against Gram-negative aerobes. Breakpoints have been slightly reduced for human medicine, with the new and old shown below. Detection of carbapenem-resistant Enterobacteriaceae is challenging because of the notorious heterogeneity in resistance levels that vary with the enzyme and bacterial host (Gazin et al., 2012; Livermore et al., 2012). Rapid detection based on molecular methods such as multiplex real-time PCR appears to be sensitive and specific and is being assessed in tertiary-care human hospitals for its value in implementing infection control measures.

- *Good susceptibility (MIC ≤ 1–4 μg/ml)* is shown by most pathogenic bacteria, which includes most Gram-positive bacteria; imipenem is highly active against Gram-positive cocci (including most enterococci), similar to that of benzyl penicillin. *Mycobacterium avium-intracellulare*, *Nocardia* spp., *Brucella* spp. are susceptible. These drugs are highly active against anaerobic bacteria, including *B. fragilis*. These drugs are the most active of the beta-lactam antibiotics against Gram-negative bacteria. Their activity includes beta-lactamase-producing fastidious organisms, Enterobacteriaceae including beta-lactamase-producing isolates, and most *P. aeruginosa*. They are slightly less active against *Proteus* spp. than against other enteric organisms.
- *Resistance (MIC ≥ 8–16 μg/ml)* is shown by methicillin-resistant *S. aureus*, *Burkholderia cepacia*, and by some Enterobacter spp., *Aeromonas* spp., *P. aeruginosa*, *P. maltophilia*, and *Enterococcus faecium*.

Antibiotic Resistance

Carbapenems are regarded as "last resort" drugs in human medicine, but the last few years have seen a marked rise and dissemination of carbapenemase and

metallo-beta-lactamase-resistant Gram-negative enteric (*E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Salmonella*) and other bacteria (*Acinetobacter* spp., *P. aeruginosa*) and their genes (e.g., KPC, IMI, IMP, NDM, VIM; Table 10.1) in human medicine, so that they almost rival the ESBLs in their emergence but are more serious (Bush, 2010; Bush and Fisher, 2011). These same bacteria may also carry multidrug resistance to non-beta-lactam antibiotics making their possessors virtually untreatable. Mutations in porins and PBPs may combine with beta-lactamases to enhance resistance. The resistance genes are often found on integrons that are embedded in plasmids or transposons, and are therefore highly mobilizable, particularly if the plasmids are promiscuous. Resistance during therapy with imipenem has been commonly reported in *P. aeruginosa* and attributed to alterations in outer-membrane proteins, which reduce permeability; many of these isolates are susceptible to meropenem.

Pharmacokinetic Properties

These carbapenems are not absorbed after oral administration, although orally administered carbapenems are being developed. Following IV administration, they are widely distributed to extracellular fluid throughout the body and reach therapeutic concentrations in most tissues in humans. There is poor penetration into cerebrospinal fluid even with inflammed meninges. They have the low volume of distribution typical of beta-lactam drugs. Imipenem is almost exclusively eliminated through the kidneys, being metabolized in renal tubules by a dihydropeptidase enzyme. Addition of cilastatin prevents this metabolism. This increases the elimination half-life and allows the drug to be excreted in large amounts in active form into urine. Meropenem by contrast is stable to dihydropeptidase. Half-life of these carbapenems is about 1 hour.

Toxicity and Side Effects

The most common side effects in human patients have been gastrointestinal disturbance (nausea, vomiting, diarrhea) in about 4% of patients, hypersensitivity reactions (rash) in about 3% of patients, and, for imipenem, seizures in about 0.5% of patients have been associated with high doses, renal failure, or underlying neurological abnormalities. Hypersalivation was noted in dogs given rapid IV infusion and vocalization presumably indicating pain was noted in 1 and 2 of 6 dogs administered the drug IM or SC respectively (Barker et al., 2003). Liver enzymes may rise transiently during treatment. Meropenem use in people is associated with a lower incidence of gastrointestinal disturbance than imipenem, and does not cause seizures.

Drug Interactions

Carbapenems may be synergistic with aminoglycosides against *P. aeruginosa*. Rapid emergence of resistance in *P. aeruginosa* (about 20%) during treatment with imipenem suggests that it should be combined with an aminoglycoside for infections with this organism, although the combination may not prevent the emergence of resistance.

Administration and Dosage

Imipenem is administered IV (over 20–30 minutes) or by deep IM injection, q 8 h. Dosage in dogs and cats, for which it is used occasionally, is largely empirical, in the range 5–10 mg/kg q 8 h. The drug may be given SC as well as IM in dogs (Barker et al., 2003), although this may be painful.

Meropenem is usually administered IV; an empiric dosage is 5–10 mg/kg q 8 h. Bidgood and Papich (2002) did not observe painful effects of SC administration in dogs and suggested a dosage of 8–12 mg/kg SC q 8 or q 12 h depending on the susceptibility of the organism being treated. In horses, Orsini et al. (2005), however, recommended a higher IV dosage of 10–20 mg/kg q 6 h for treatment of susceptible infections.

Clinical Applications

These extraordinary antimicrobial drugs are used in human medicine in the treatment of hospital-acquired infections caused by multiply resistant Gram-negative bacteria, or mixed aerobic and anaerobic infections, particularly including infections in immunocompromised patients. Purposes for which they are used successfully in human patients include a variety of serious infections including: intra-abdominal infections (less effective than piperacillin-tazobactam but equivalent to clindamycin-tobramycin or cefotaxime-metronidazole), severe lower respiratory tract infections (as or more effective than third-generation cephalosporin-amikacin treatment), septicemia (equivalent to ceftazidime-amikacin in febrile neutropenic patients), life-threatening soft tissue

infections, osteomyelitis. Imipenem is not recommended for the treatment of bacterial meningitis or of *P. aeruginosa* infection. Meropenem is as effective as cefotaxime or ceftriaxone in treatment of bacterial meningitis in people.

Carbapenems should be reserved for the treatment of infections caused by cephalosporin-resistant Enterobacteriacea and for empirical treatment of febrile illness in neutropenic patients (chapter 21). They should only be used rarely in veterinary medicine. The potential for emergence of *P. aeruginosa* resistant to imipenem suggests that administration of imipenem with an aminoglycoside would be prudent. The growing tendency of small animal intensive care units to use imipenem as a first line antibacterial drug in seriously ill animals with undiagnosed infection will result in progressive development of resistant nosocomial infections in these settings (Shimada et al., 2012). The problem with their use is that they have such broad-spectrum bactericidal action that bacterial superinfections with resistant bacteria are likely, leading to contamination of the environment with such naturally resistant bacteria.

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Monobactams: Aztreonam

Monobactams possess the simple beta-lactam ring without the attached thiazolidine ring (Figure 8.2). Aztreonam was the first monobactam introduced into human medicine. Other monobactams such as tigemonam, which can be administered orally, are in clinical trials in human medicine. Aztreonam is a synthetic analogue of an antibiotic isolated from a *Streptomyces* species. It binds mainly to PBP3, disrupting cell-wall synthesis, and is stable to most beta-lactamases. Comments below are largely confined to aztreonam.

Antibacterial Activity

- *Good susceptibility (MIC ≤ 8 μg/ml)* is limited by PBP3 binding to almost all Gram-negative aerobic bacteria, particularly fastidious organisms (*Haemophilus* spp., *Pasteurella* spp.) and Enterobacteriaceae. The susceptibility of *P. aeruginosa* is variable.
- *Resistance (MIC ≥ 32 μg/ml)* is shown in Gram-positive bacteria and anaerobic bacteria; other *Pseudomonas* spp., *B. cepacia*, *Citrobacter* spp., and *Enterobacter* spp. are often resistant because of ESBLs.

Antibiotic Resistance

Aztreonam is hydrolyzed by ESBLs and carbapenemases but are resistant to Bush group 1 cephalosporinases.

Pharmacokinetic Properties

Aztreonam is not absorbed after oral administration. It is rapidly absorbed after IM injection in human patients and distributes widely in extracellular fluid throughout the body. Penetration into the cerebrospinal fluid of human patients with meningitis has achieved concentrations that should eliminate infections with Enterobacteriaceae. Half-life is about 1.6 hours in people; elimination is mainly renal.

Toxicity and Side Effects

Toxicity is similar to that of benzyl penicillin, with no apparent cross-allergy in human patients allergic to penicillins or cephalosporins. These drugs do not cause the gastrointestinal disturbances associated with carbapenems and other broad-spectrum beta-lactam antibiotics. Their inactivity against Gram-positive bacteria may lead to superinfection with yeasts and with Gram-positive aerobes, including *Enterococcus* spp. and *S. aureus*.

Drug Interactions

Aztreonam is often synergistic with aminoglycosides, including aminoglycoside-resistant Gram-negative bacteria and *P. aeruginosa*. This may have little advantage since aztreonam is often used clinically as a substitute for an aminoglycoside. Aztreonam may have advantage combined with beta-lactams susceptible to Bush group 1 cephalosporinases, since it is poorly inactivated by these enzymes.

Administration and Dosage

Aztreonam is administered IV (over 3–5 minutes) or IM. An empirical dose in animals is 30–50 mg/kg q 8 h.

Clinical Applications

The narrow spectrum of aztreonam precludes its use in human medicine for empirical treatment of infections, except possibly for urinary tract infections. Its potential lies in the possibility to substitute for the more toxic aminoglycosides in combination therapy, for example with clindamycin or metronidazole in serious, mixed anaerobic infections or with erythromycin in mixed infections where Gram-positive bacteria may be present. Aztreonam is used on its own in a wide variety of infections involving Gram-negative bacteria (urinary tract, lower respiratory tract, septicemia) with success as a relatively non-toxic drug in human medicine, including in seriously ill, immunocompromised patients infected with multiply resistant Gram-negative aerobes. Its place in veterinary medicine appears to be slight but might include treatment of meningitis in neonatal animals.

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Tribactams

Tribactams have a tricyclic structure related to that of carbapenems. Sanfetrinem cilexetil is the prodrug of sanfetrinem and is administered orally in people. It has high stability to many beta-lactamases and a broad spectrum of activity similar to that of carbapenems.

11

Peptide Antibiotics: Polymyxins, Glycopeptides, Bacitracin, and Fosfomycin

Patricia M. Dowling

Polymyxins, glycopeptides, bacitracin, and fosfomycin are peptide antibiotics with a variety of actions against bacteria. Streptogramins are also peptides but are discussed in chapter 11 because of their common mechanism of action with lincosamides. Glycopeptides are important, particularly in human medicine, because of their activity against Gram-positive bacteria, including multidrug-resistant enterococci and staphylococci. The clinical development of polymyxins, bacitracin, and fosfomycin has not been pursued since their discovery early in the antibiotic era. But because of the worldwide increase in multidrug-resistant bacterial infections, these drugs are being re-evaluated for clinical use against multidrug resistance. There is increasing use of these "last resort" drugs in veterinary medicine as well as human medicine.

Polymyxins

Polymyxins are antibiotic products of *Bacillus polymyxa* subspecies *colistinus*. Polymyxin E (colistin) and polymyxin B are the only polymyxins used clinically. When first developed in the 1940s they were of great interest for their activity against *Pseudomonas aeruginosa*. They were limited mainly to oral (colistin) or topical (polymyxin B) use due to their systemic toxicity. But more recent studies suggest that they are far less toxic than previously considered and there is great interest in using these antibiotics in the treatment of carbapenem-resistant Gram-negative bacterial infections (Lim et al., 2010). In horses, dogs and cats, there is interest in their systemic use at subantimicrobial doses for binding and inactivating endotoxin.

Chemistry

Polymyxins are basic cyclic decapeptides. Colistin is polymyxin E and is chemically related to polymyxin B. Colistin is available as the sulfate for oral or topical administration and as the less toxic sulfomethate (colistin methanesulphonate sodium) for parenteral use. Dosages are given in International Units or metric units depending on the source; 10 units of polymyxin B = 1 μg, 10 units of colistin sulphate or colistin methanesulphonate = 0.5 μg. They are stable, highly water-soluble drugs.

Mechanism of Action

Polymyxins are cationic, surface-active agents that displace Mg^{2+} or Ca^{2+} and disrupt the structure of cell membrane phospholipids and increase cell permeability by a detergent-like action. Polymyxins disorganize the outer membrane of Gram-negative bacteria by binding lipopolysaccharides (LPS, endotoxin) through direct interaction with the anionic lipid A region. This action neutralizes the endotoxin capacity of LPS (Coyne and Fenwick, 1993). The bactericidal activity of polymyxin B is concentration dependent and related to the ratio of the area under the concentration-time curve to the MIC (AUC:MIC; Guyonnet et al., 2010; Tam et al., 2005).

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Antimicrobial Activity

Polymyxin B and colistin are similarly rapidly bactericidal and highly active against many species of Gram-negative organisms, such as *Escherichia coli, Salmonella*, and *Pseudomonas aeruginosa*, but not against *Proteus, Serratia*, or *Providencia* (Table 11.1). Susceptible bacteria have an MIC of ≤ 4 μg/ml. Gram-positive and anaerobic bacteria are resistant. Activity against *P. aeruginosa* is reduced *in vivo* by the presence of physiologic concentrations of calcium. To widen the range of antimicrobial activity, neomycin and bacitracin are combined with polymyxin B in topical preparations (e.g., Polysporin®). Neomycin and polymyxin B are also available combined in a bladder irrigation solution designed for local treatment of *E. coli* cystitis in women.

Resistance

Gram-negative bacteria may develop resistance through common mechanisms for both colistin and polymyxin B. Acquired resistance is rare but can occur in *P. aeruginosa*. Veterinary isolates of *P. aeruginosa* remain routinely susceptible to polymyxin B (Hariharan et al., 2006). The most important mechanism of resistance involves modifications of the bacterial outer membrane, mainly through the alteration of LPS (Falagas et al., 2010). Other resistance mechanisms include further modifications of the bacterial outer membrane and development of an efflux pump/potassium system. Like the aminoglycosides, first-exposure adaptive resistance occurs (Tam et al., 2005).

Table 11.1. Activity of polymyxin B and colistin (MIC_{90}, μg/ml) against selected Gram-negative aerobes.

Organism	Polymyxin B MIC_{90}	Colistin MIC_{90}
Actinobacillus spp.	0.5	0.4
A. pleuropneumoniae	–	1
Bordetella bronchiseptica	0.5	0.12
Brucella canis	100	16–32
Campylobacter jejuni	32	8
Escherichia coli	1	8–16
Histophilus somni	2	0.1
Klebsiella pneumoniae	1	4–8
Pasteurella multocida	4	–
Proteus spp.	128	> 128
Pseudomonas aeruginosa	8	8
Salmonella spp.	128	–
Serratia spp.	20	–
Taylorella equigenitalis	2	0.5

Pharmacokinetic Properties

The polymyxins are not absorbed from the gastrointestinal tract. Colistin sulphate is administered orally for a local antibiotic effect. Colistin methanesulphonate sodium or polymyxin B can be administered intravenously or intramuscularly. Colistin methanesulphonate causes less pain at the injection site and less renal toxicity than polymyxin B, but polymyxin B has greater local activity. Polymyxins bind moderately to plasma proteins but extensively to muscle tissue, diffuse poorly through biologic membranes, and attain low concentrations in transcellular fluids and in milk. Because of tissue binding, accumulation occurs with chronic dosing. The strong affinity of the polymyxins to the muscle tissue results in persistent drug residues (Ziv et al., 1982). When administered IV, CSF concentrations of colistin methanesulphonate sodium reach 25% of plasma concentrations. The polymyxins are slowly excreted unchanged by glomerular filtration into urine. High concentrations will accumulate in patients with renal insufficiency.

Drug Interactions

Polymyxins are synergistic with a variety of antimicrobial drugs through their disorganizing effects on the outer and cytoplasmic membranes. Colistin is synergistic *in vivo* with rifampin or ceftazidime against multidrug-resistant *P. aeruginosa* (Giamarellos-Bourboulis et al., 2003). *In vitro* studies indicate synergy between colistin and carbapenems for colistin-susceptible/carbapenem-resistant Gram-negative bacteria (Yahav et al., 2012).

Toxicity and Adverse Effects

Polymyxins are well tolerated after oral or local administration, but systemic use causes nephrotoxic, neurotoxic, and neuromuscular blocking effects. Colistin is less toxic than polymyxin B, but colistin methanesulphonate has reduced antimicrobial activity compared to colistin sulfate.

In humans, reversible peripheral neuropathy, with paresthesia, numbness around the mouth, blurring of

vision, and weakness occur in about 7% of treated patients; neuromuscular blockade causing respiratory insufficiency occurs in about 2% of patients, particularly in those treated with high doses. The polymyxins have been considered highly nephrotoxic, causing damage to the renal tubular epithelial cells. Risk factors for nephrotoxicity include age (geriatric), preexisting renal insufficiency, hypoalbuminemia, and concomitant use of non-steroidal anti-inflammatory drugs or vancomycin. Renal failure appears dose dependent, with some studies identifying the total cumulative dose predictive of renal failure, and others the daily dose (Yahav et al., 2012).

Calves treated with 5 mg/kg IM polymyxin B showed lethargy and apathy 2–4 hours after injection, and some developed transient ataxia. A dose of 5 mg/kg of polymyxin B or colistin methanesulphonate sodium was highly nephrotoxic, but 2.5 mg/kg had minimal effects. In sheep, 1 of 3 ewes died of respiratory failure within 2 hours of an IM dose of 10 mg/kg of polymyxin B (Ziv, 1981). A new formulation of colistin sulfate for IM use showed minimal toxicity in mice, rabbits and pigs (Lin et al., 2005).

Topical application of polymyxins B-containing ophthalmic formulations have been associated with anaphylactic reactions in cats (Hume-Smith et al., 2011). Topical application of polymyxin B ear drops was associated with development of pemphigus vulgaris in a dog (Rybnicek and Hill, 2007).

Administration and Dosage

Because of toxicity, parenteral polymyxins have not used routinely in animals. For treatment of enteric infections, oral colistin at 50,000 IU/kg q 12 h or intramuscular at 2.5–5 mg/kg have been recommended. The usual parenteral dose of colistin methanesulphonate is 3 mg/kg administered IM or IV at 12-hour intervals. A new formulation of colistin sulfate is recommended for use in piglets at 2.5 mg/kg IM every 12 hours (Lin et al., 2005). For endotoxemia in horses, a dose of polymyxin B at 5,000–10,000 IU/kg IV every 8–12 hours is suggested (Barton et al., 2004; Morresey and Mackay, 2006). A dose of polymyxin B at 1,000 IU/kg IV appears safe and efficacious for endotoxemia in cats (Sharp et al., 2010). For treatment of endotoxic shock in dogs, a colistin dose of 12,500 U/kg every 12 hours was safe and efficacious (Senturk, 2005).

Clinical Applications

The low incidence of antimicrobial resistance and their endotoxin-neutralizing properties have renewed interest in the polymyxins. Concerns regarding nephrotoxicity are the main limitations for systemic use, so careful patient selection and close renal function monitoring is advised. In countries where these drugs are used in an extra-label manner, slow tissue residue depletion is an important consideration.

Cattle

Polymyxins are used in some countries for the treatment of colibacillosis and salmonellosis in calves.

The potential of polymyxin B to inactivate endotoxin may be useful in the treatment of coliform mastitis. An IM dose of 5.0 mg/kg of polymyxin B produces milk concentrations exceeding 2 μg/ml for 4 hours, which is sufficient to eliminate the more susceptible coliforms. The anti-endotoxin effect is seen only in the early stages of coliform mastitis, experimentally within 2–4 hours of infusion of endotoxin (Ziv, 1981). Since about 100 μg of polymyxin B inactivates only 0.2 μg of endotoxin, and endotoxin concentrations may reach 10 μg/ml in coliform mastitis, even intramammary doses are inadequate to neutralize all the endotoxin. In an experimental model of coliform mastitis, intramammary infusion of polymyxin B after endotoxin was infused prevented the increase in plasma lactate dehydrogenase activity and moderated the decrease in plasma zinc concentration, but otherwise did not alter the clinicopathologic course of endotoxin-induced acute mastitis (Ziv and Schultze, 1983). Polymyxin B is available in an intramammary mastitis formulation in Canada in combination with penicillin G procaine, novobicin, dihydrostreptomycin and hydrocortisone (Special Formula 17900) and as a single agent treatment in Europe.

Swine

Colistin has been used extensively in pigs (outside of North America) as an oral treatment for neonatal colibacillosis. Pharmacokinetic/pharmacodynamic integration suggests a dosage regimen of 100,000 IU/kg body weight per day or 50,000 IU/kg administered at 12-hour intervals (Guyonnet et al., 2010). An IM injectable formulation of colistin sulfate from China appears promising for the treatment of *E. coli* infections in swine (Lin et al., 2005).

Horses

Polymyxins are used locally to treat bacterial keratitis or metritis caused by *Klebsiella* spp. or *P. aeruginosa*. Polymyxin B is formulated as "triple antibiotic" ophthalmic ointment or solution, in combination with bacitracin and neomycin.

Polymyxin B has been evaluated for its endotoxin-binding activity in horses. In foals challenged with LPS, it reduces fever, respiratory rate and serum activities of tumour necrosis factor (TNF) and interleukin-6 (Durando et al., 1994). In adult horses, it ameliorates clinical signs and decreases plasma TNF activity (Barton et al., 2004). Conversely, polymyxin B was ineffective in ameliorating the endotoxemia associated with carbohydrate overload (Raisbeck et al., 1989). If used, treatment should begin as soon as possible, as the LPS scavenging effects are only beneficial in the first 24–48 hours, after which tolerance to LPS develops. In equine models of endotoxemia, neuromuscular blockade and apnea were not observed, and nephrotoxicity was only observed at very high dosages. Therefore, the anti-endotoxin dose is administered to horses as a slow IV bolus.

Dogs and Cats

Polymyxins are used in the local treatment of bacterial keratitis, otitis externa, and other skin infections caused by susceptible Gram-negative bacteria. In an endotoxic dog model, colistin administration improved capillary refill time and hydration and significantly reduced serum tumour necrosis factor concentrations (Senturk, 2005).

Poultry

Colistin is widely used in China for the treatment of Gram-negative infections in chickens, turkeys and ducks (Zeng et al., 2010).

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Glycopeptides: Vancomycin, Teicoplanin, and Avoparcin

Vancomycin, teicoplanin, and avoparcin are glycopeptides antibiotics with activity against Gram-positive bacteria and particularly against Gram-positive cocci. Vancomycin and teicoplanin are currently available as formulations for human use in various parts of the world, whereas avoparcin is only available for veterinary use in some countries. Because of their outstanding activity against a broad spectrum of Gram-positive bacteria, vancomycin and teicoplanin have often been considered the drugs of "last resort" for serious staphylococcal and enterococcal infections. Glycopeptides had been in clinical use for almost 30 years before high-level resistance first emerged in enterococci. More recently, there have been disturbing reports of low- and intermediate-level resistance to vancomycin in strains of *Staphylococcus aureus*. Avoparcin had been used extensively as an antibiotic growth promoter for chickens and pigs in Europe. It was withdrawn for use in Europe because it was associated with selection for vancomycin-resistant enterococci (VRE) in farm animals, which then were a source of infection for humans. Under the Animal Medicinal Use Clarification Act of 1996, the extra-label use of glycopeptides is banned in animals in the United States. The expense of treatment with vancomycin and teicoplanin effectively limits the use of these drugs in countries where their use is not specifically banned.

Vancomycin

Chemistry

Vancomycin is a high molecular weight glycopeptide, a fermentation product of *Streptomyces orientalis*. The generic name *vancomycin* derives from the term *vanquish*. It is available as the stable and highly soluble hydrochloride.

Mechanism of Action

The glycopeptides, including vancomycin and teicoplanin, are large, rigid molecules that inhibit bacterial cell wall peptidoglycan synthesis. Their three-dimensional structure contains a cleft into which peptides of only a highly specific configuration can fit (D-alanyl-D-alanine). This configuration is found only in Gram-positive bacteria cell walls, hence glycopeptides are selectively toxic. Glycopeptides interact with cell wall D-Ala-D-Ala by hydrogen bonding and forming stable complexes. As a result, glycopeptides inhibit the formation of the backbone glycan chains (catalyzed by peptidoglycan polymerase) from the simple wall subunits as they are extruded through the cytoplasmic membrane. The subsequent transpeptidation reaction necessary for rigidity of the cell wall is also inhibited.

Antimicrobial Activity

Vancomycin is bactericidal to most Gram-positive aerobic cocci and bacilli, but the majority of Gram-negative bacteria are resistant. Organisms with an MIC ≤ 2–4 μg/ml are regarded as susceptible, those with 8–16 μg/ml as intermediate, and those with ≥ 32 μg/ml as resistant (Table 11.2).

The best pharmacodynamic predictor of vancomycin efficacy is area under the concentration time curve over the MIC (AUC:MIC)of ≥ 400 (Craig, 2003). Because of the pharmacokinetic limitations of vancomycin, the desired AUC:MIC ratio is not achievable for any pathogen with an MIC value > 1 μg/ml.

Resistance

Antibiotic resistance is generally uncommon but occurs with some frequency in *Enterococcus* spp., especially *E. faecium*, in which it has been extensively characterized. VanA resistance encodes resistance to all glycopeptides and is associated with a plasmid-mediated transposable element Tn*1546*. The VanA gene changes the D-alanyl-D-alanine part of the pentapeptide side chain of N-acetylmuramic acid to D-alanyl-D-lactate, preventing glycopeptide binding and thus evading inhibition of cell wall synthesis. VanB resistance affects vancomycin but not teicoplanin. It is chromosomal in origin and not

Table 11.2. Activity of vancomycin (MIC_{90} μg/ml) against selected bacteria.

Organism	MIC_{90}	Organism	MIC_{90}
Actinomyces spp.	8	*Listeria monocytogenes*	1
Arcanobacterium pyogenes	1	*Nocardia*	256
Clostridium difficile	1	*Rhodococcus equi*	0.25
C. perfringens	1	*Staphylococcus aureus*	2
C. septicum	2	Beta-hemolytic streptococci	2
Enteroccus spp.	4		

usually transferable, but acts in a similar manner to VanA. VanC resistance is a non-transferable lower-level resistance observed in *E. gallinarum*. Cross-resistance may occur within drugs of the glycopeptide class but not with other drug classes. Semisynthetic glycopeptides are being developed to overcome the problem of VanA and VanB resistance.

The world-wide emergence of vancomycin-resistant enteroccoci (VRE) is a serious human health concern. Isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to glycopeptides are increasingly cultured from clinical patients, including animals. Some strains isolated from people exhibit frank resistance to vancomycin. The demonstrated in-human transmission of vancomycin resistance from VRE to MRSA at the same infection site underscores the potential danger of a coexisting reservoir of both pathogens (Witte, 2004). There is increasing concern that food and companion animals are a source of these highly resistant pathogens (Freitas et al., 2011; Ghosh et al., 2011; Ghosh et al., 2012; Ramos et al., 2012). Linkage of vancomycin resistance genes with macrolide resistance genes on the same plasmids has been implicated as the cause of VRE persistence in countries that banned the use of avoparacin but continued the use of tylosin in animal feed (Aarestrup et al., 2001). It is predicted that acquired resistance determinants in commensal enterococcal populations will persist for decades, even in the absence of glycopeptide use (Johnsen et al., 2011).

Pharmacokinetic Properties

Vancomycin is poorly absorbed after oral administration, so is only administered by this route for a local effect, as in the treatment of *Clostridium difficile* colitis. Penetration into tissues is relatively poor, although the drug enters CSF when the meninges are inflamed. The half-life in humans is about 6 hours, 2 hours in dogs, and nearly 3 hours in horses (Orsini et al., 1992; Zaghlol and Brown, 1988). Most of the drug is excreted through the kidneys by glomerular filtration, with a small proportion excreted in bile. Vancomycin hydrochloride causes marked tissue damage, so is administered by IV infusion over a 60-minute period. Dosage adjustment is required for patients with renal impairment. Plasma concentrations can be monitored with dose intervals adjusted to give trough concentrations approximating the MIC of susceptible organisms.

Drug Interactions

Vancomycin is synergistic with aminoglycosides against Gram-positive cocci. It appears to be synergistic *in vivo* with rifampin against *Staphylococcus aureus*. It is antagonistic *in vitro* with many other drugs, so must be carefully administered.

Toxicity and Adverse Effects

Vancomycin is highly irritating to tissues on injection and must be administered slowly IV in dilute form. Rapid IV injection produces a histamine-like reaction in humans (red-neck syndrome). The drug is ototoxic in humans, particularly in patients treated with large doses or in those with renal insufficiency. Vancomycin is also potentially nephrotoxic; nephrotoxicity is exacerbated by high doses and concurrent use of nephrotoxic drugs. There is some information on toxicity in laboratory animals but no reports of toxicity in companion animals.

Administration and Dosage

Dosage recommendations are largely empirical. For treating enteric infections, 5–10 mg/kg PO every 12 hours has been recommended. In dogs, Zaghlol and Brown (1988) recommended a parenteral dosage of 15 mg/kg q 6 h and in horses Orsini and others (1992) recommended 4.3–7.5 mg/kg given as a 1-hour IV infusion every 8 hours. Vancomycin was dosed in a cat at 19.4 mg/kg every 12 hours for 10 days (Pressel et al., 2005). It has been administered by intravenous or intramedullary regional perfusion in horses (Rubio-Martinez et al., 2005) Vancomycin can be formulated as antimicrobial impregnated polymethylmethacrylate (AIPMM) or plaster of Paris beads, dextran polymer matrix or in bone cement for local therapy of musculoskeletal infections (Atilla et al., 2010; Joosten et al., 2005; Liu et al., 2002; Thomas et al., 2011).

Clinical Applications

There are few indications for the use of vancomycin in animals, particularly since this is a "last resort" drug in human medicine. In humans, it is primarily used to treat infections caused by multiresistant Gram-positive bacteria when there are no other treatment options. It may be used to treat patients allergic to penicillins and cephalosporins. It is also the drug of choice in people for the oral treatment of *Clostridium difficile* colitis because of its activity and narrow bactericidal spectrum. While

C. difficile causes toxic enteritis in horses and companion animals, reported isolates are susceptible to metronidazole.

There are few reports of the clinical use of vancomycin in veterinary medicine. The decision to use vancomycin to treat a highly resistant pathogen in a veterinary patient should only be made after consideration of the health risks to in-contact humans and other animals. An effective infection control program is mandatory for such cases. Vancomycin therapy resolved clinical signs of cholangiohepatitis in cats, but did not necessarily produce a microbiological cure (Jackson et al., 1994; Pressel et al., 2005). Practitioners have erroneously administered oral vancomycin in the treatment of systemic infections in dogs (Weese, 2008). Vancomycin has been administered IV alone or in combination with an aminoglycoside, to treat methicillin-resistant staphylococcal and enterococcal infections in horses (Orsini et al., 2005). Vancomycin AIPMMA beads were used in conjunction with systemic vancomycin therapy at 6 mg/kg IV every 8 hours in a post-surgical infection from a methicillin-resistant *Staphylococcus epidermidis* in a horse (Trostle et al., 2001).

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Teicoplanin

Teicoplanin has a molecular structure similar to that of vancomycin and is also a derivative of an actinomycete. It is a complex of five closely related antibiotics. Teicoplanin has activity similar to and slightly greater than vancomycin, being restricted also in activity to Gram-positive bacteria. It has excellent activity against

S. aureus (including methicillin-resistant strains) and against streptococci (in which it is more active than vancomycin), *L. monocytogenes, C. difficile, C. perfringens*, and other Gram-positive bacteria. *Enterococcus faecalis* are somewhat less susceptible than other cocci. *Nocardia* are resistant to teicoplanin. Susceptible organisms are those with an MIC ≤4 μmg/ml. Activity *in vitro* is more affected by test conditions than the activity of vancomycin. Like vancomycin, the 24-hour AUC:MIC correlates with efficacy (Craig, 2003).

Also like vancomycin, development of resistance to teicoplanin is uncommon and these drugs have been regarded as resistance-resistant. Nevertheless, VanA resistance (causing cross-resistance to teicoplanin) occurs in enterococci and resistance may develop in coagulase-negative staphylococci, either as a result of selection of mutants with progressive increases in MIC occurring in bacteria during treatment or, less commonly, by plasmid-mediated mechanisms.

In humans, teicoplanin is not absorbed after oral administration. Absorption after IM injection is excellent and the drug distributes widely into tissues in extracellular fluid. The elimination half-life is remarkably prolonged in humans, between 45 and 70 hours after IV injection. Penetration into cerebrospinal fluid is poor because of high molecular weight and poor lipid solubility. Elimination is almost entirely renal. Pharmacokinetic information is only available for sheep (Naccari et al., 2009). In sheep given an IV dose of 6 mg/kg, teicoplanin was characterized by a low volume of distribution and a plasma elimination half-life of 5 hours. With IM injection, the bioavailability was 100% and "flip-flop" kinetics occurred as evidenced by an increase in plasma elimination to 9 hours.

Teicoplanin is synergistic with aminoglycosides against some Gram-positive cocci, including penicillin-tolerant enterococci, is indifferent or additive with rifampin, and may be synergistic with imipenem against Gram-positive cocci.

In humans, teicoplanin is usually well tolerated. Adverse effects reported, in order of frequency, include: hypersensitivity skin reactions (rash, pruritus, urticaria), pain (IM) or phlebitis (IV) at injection sites, and rarely nephrotoxicity or ototoxicity (usually in patients also receiving aminoglycosides). Teicoplanin, unlike vancomycin, can be administered by rapid IV injection. No information on toxicity in domestic animals is available.

Teicoplanin is used in human medicine for the treatment of serious infections caused by Gram-positive bacteria where a bactericidal drug is needed, where there is resistance to first line drugs, or where synergism with an aminoglycoside for broad-spectrum or enhanced activity is required. Uses include septicemia, endocarditis, bone and joint infections, and cystitis caused by multiresistant enterococci. Use in veterinary medicine has been limited. In 19 sheep treated with a single IM dose, teicoplanin was clinically and microbiologically effective against mastitis caused by strains of *S. aureus*, coagulase-negative staphylococci and *S. agalactiae*. No adverse reactions were observed. Teicoplanin (40 mg/day IM) was effective in the treatment of an encrusted cystitis caused by *Corynebacterium urealyticum* in a dog after treatment with rifampin failed (Gomez et al., 1995).

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Avoparcin

Avoparcin was used extensively as an antibiotic growth promoter in poultry and swine in Europe. The recognition that it selected for vancomycin-resistant enterococci (VRE) in animals, and VRE contaminated a high proportion of meat products derived from these animals, led to its withdrawal from use in Europe (Casewell et al., 2003). Avoparcin was not used in animal agriculture in North America. With the avoparacin ban in 1995, there was an immediate decrease in VRE isolated from poultry, but not in pigs until tylosin was also banned from feed use (Aarestrup et al., 2001). However, VREs have continued to cause significant problems in human hospitals not only in Europe but also in North America, where avoparcin has never been used in animals. Recent studies conclude that animal-associated VRE probably reflect the former use of avoparcin in animal production in Europe, whereas VRE in human-associated samples may be a result of antimicrobial use in hospitals (Kuhn et al., 2005).

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Bacitracin

Bacitracin is a polypeptide product of *Bacillus subtilis*. Bacitracin was first discovered in 1943 and named after the *Bacillus* that was isolated from wound of a 7-year-old American girl named Margaret Tracey. It inhibits the formation of bacterial cell-wall peptidoglycan by complexing directly with the pyrophosphate carrier and inhibiting the dephosphorylation reaction required for its regeneration. It is bactericidal to Gram-positive bacteria but has little activity against Gram-negative organisms. Resistance develops slowly. One unit of bacitracin = 26 μg of the USP standard.

Because bacitracin is highly nephrotoxic after parenteral administration, it is generally only used orally for a local effect or topically in the treatment of superficial infections of the skin and mucosal surfaces, particularly where activity against Gram-positive bacteria is required. Bacitracin is not absorbed from the gastrointestinal tract, so no residues are found in meat when the product is administered orally. Because beta-lactam antibiotics are potent contact sensitizers, they are not administered topically; bacitracin replaces them for Gram-positive coverage in topical products. However, allergic reactions and fatal anaphylaxis have been described in humans (Jacob and James, 2004). Bacitracin is often combined with neomycin and polymyxin B for broad-spectrum activity in treating minor skin wounds or bacterial keratitis. While frequently used as a first line treatment in horses, a review of equine bacterial keratitis found only 64% of *S. zooepidemicus* isolates were sensitive to bacitracin, suggesting that previous triple antibiotic therapy encourages antimicrobial resistance (Keller and Hendrix, 2005).

Bacitracin is administered orally in poultry and swine in North America, as bacitracin methylene disalicylate or zinc bacitracin, for growth promotion and for prevention and treatment of enteritis (Butaye et al., 2003). Bacitracin, along with other antibiotic growth promoters, has been banned for use in the European Union since 2006 (Castanon, 2007). Necrotic enteritis caused by *C. perfringens* in chickens is prevented by the addition of bacitracin at doses of 55–110 ppm to the feed. Incorporation in feed may prevent proliferative adenomatosis in swine although *Lawsonia intracellularis* is resistant *in vitro* (Kyriakis et al., 1996). Zinc bacitracin was used in the treatment of colitis induced by tetracycline-contaminated sweet feed in a herd of horses (Keir et al., 1999).

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Fosfomycin

Fosfomycin (L-[*cis*]-1,2 epoxypropyl phosphonic acid) is a phosphoenolpyruvate analogue that irreversibly inhibits pyruvyl transferase, the enzyme catalyzing the first step of peptidoglycan biosynthesis. It is produced by various *Streptomyces* spp. It is available for human use as fosfomycin tromethamine, as a single oral dose treatment for urinary tract infections. In some countries outside of North America, fosfomycin calcium for oral use and fosfomycin disodium for intravenous use are available. It has a broad spectrum of activity against a wide

range of Gram-positive and Gram-negative bacteria. It is highly active against Gram-positive pathogens such as *Staphylococcus aureus* and *Enterococcus*, and against Gram-negative bacteria such as *P. aeruginosa* and *Klebsiella pneumonia* (Michalopoulos et al., 2011). Its unique mechanism of action may provide a synergistic effect to other classes of antibiotics including beta-lactams, aminoglycosides, and fluoroquinolones. Oral fosfomycin is mainly used in the treatment of urinary tract infections, particularly those caused by *Escherichia coli* and *Enterococcus faecalis* (Falagas et al., 2010). Fosfomycin is considered a time-dependent antimicrobial. Activity is reduced by alkaline pH and the presence of glucose, sodium chloride or phosphates in culture media. Resistance, which can be chromosomal or plasmid-mediated, is uncommon. There is no cross-resistance with other antibacterial drugs.

Fosfomycin has a low volume of distribution (0.2 L/kg) and minimal protein binding. Bioavailability of fosfomycin disodium from SC and IM injections is variable (38–85%). Oral bioavailability in dogs is only 30%. Fosfomycin is rapidly eliminated, with a plasma half-life of 1.23 hours in horses, 1.3 hours in dogs, 2.2 hours in cattle, 2 hours in broilers, and 1.5 hours in swine (Gutierrez et al., 2010; Gutierrez et al., 2008; Soraci et al., 2011; Sumano et al., 2007; Zozaya et al., 2008).

Studies performed in rats showed that fosfomycin had a protective effect against nephrotoxicity due to aminoglycosides, by inhibiting aminoglycoside-induced histamine release from mast cell destruction (Michalopoulos et al., 2011). However, cats given fosfomycin for only 3 days developed acute renal insufficiency, while no adverse effects were seen in dogs (Fukata et al., 2008; Gutierrez et al., 2008).

There is increasing interest in the use of fosfomycin in the treatment multidrug-resistant Gram-negative infections in veterinary species. Therapeutic options for *E. coli* infections in dogs or cats are limited with the increase in resistance to third-generation cephalosporins and fluoroquinolones. In a study where 275 clinical (from dogs and cats, predominantly urinary tract isolates) and experimental isolates were tested, 272 (98.9%) were susceptible (MIC ≤ 2 μg/ml), 2 were intermediate, and only 1 was resistant to fosfomycin (MIC ≥ 256; Hubka and Boothe, 2011). For multidrug-resistant clinical isolates, 97.2% were evaluated as susceptible. All isolates exhibiting extended-spectrum beta-lactamase production were susceptible to fosfomycin. While the availability of an oral formulation is attractive for administration to dogs and cats, further studies are needed, particularly regarding nephrotoxicity in cats, before fosfomycin can be recommended for clinical use. Oral fosfomycin was also efficacious in the control of experimental *E. coli* infection in broiler chickens (Fernandez et al., 1998), but because of its value in treating multidrug resistant bacteria in humans, fosfomycin is unlikely to be approved for use in food animal species.

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12

Lincosamides, Pleuromutilins, and Streptogramins

Steeve Giguère

Lincosamides, pleuromutilins, and streptogramins are structurally distinct but share many common properties. They are basic compounds characterized by high lipid solubility, wide distribution in the body, and capacity to penetrate cellular barriers. In addition, along with macrolides, they share overlapping binding sites on the 50S subunit of the ribosome.

Lincosamides: Lincomycin, Clindamycin, and Pirlimycin

Chemistry

Lincomycin, the parent compound, was isolated in 1963 from the fermentation of *Streptomyces* spp. Many modifications of the lincomycin molecule have been developed in an attempt to produce an improved antibiotic. Of these, only clindamycin showed distinct advantages over lincomycin. Pirlimycin, a clindamycin analog, is also approved as an intramammary infusion for the treatment of mastitis in cattle. The chemical structure of lincomycin and clindamycin are displayed in Figure 12.1.

Mechanism of Action

The lincosamides inhibit protein synthesis by binding to the 50S ribosomal subunit and inhibiting peptidyl transferases. The ribosomal binding sites are the same as or closely related to those that bind macrolides, streptogramins, and chloramphenicol. Lincosamides can be bactericidal or bacteriostatic, depending on the drug concentration, bacterial species, and inoculum of bacteria. Many Gram-negative bacteria are resistant because of impermeability and methylation of the ribosomal binding site of lincosamides.

Antimicrobial Activity

Lincosamides are moderate-spectrum antimicrobial drugs. Clindamycin is several times more active than lincomycin, especially against anaerobes and *S. aureus*. Lincosamides are active against Gram-positive bacteria, anaerobic bacteria, and some mycoplasma (Table 12.1). They lack activity against most Gram-negative bacteria.

- *Good susceptibility (MIC ≤ 2.0 μg/ml)*: Gram-positive aerobes: *Bacillus* spp., *Corynebacterium* spp., *Erysipelothrix rhusiopathiae*, staphylococci, streptococci (but not enterococci). Gram-negative bacteria: *Campylobacter jejuni*. Anaerobes: many anaerobes including *Actinomyces* spp., *Bacteroides* spp. (including *B. fragilis*), and *C. perfringens* (but not all other *Clostridium* spp.). *Fusobacterium* spp., and anaerobic cocci are particularly susceptible to clindamycin. Activity of clindamycin against anaerobes is similar to chloramphenicol, and metronidazole. Clindamycin has activity against some protozoa such as *Toxoplasma gondii* and *Plasmodium falciparum*. It is has some activity against *Pneumocyctis jiroveci*. The breakpoint

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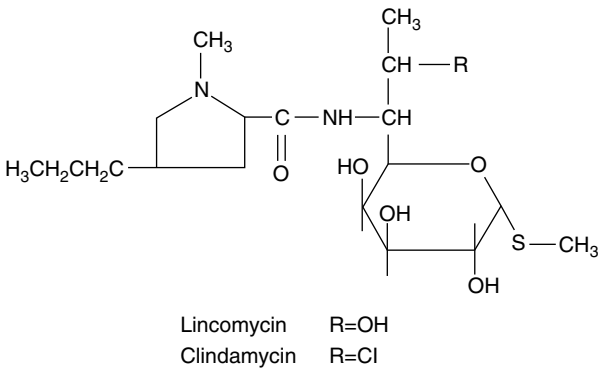

Figure 12.1. Chemical structure of lincomycin and clindamycin.

set by the CLSI for susceptibility to clindamycin in *Staphylococcus* spp. isolates from dogs with skin and soft tissue infection is ≤ 0.5 μg/ml. The breakpoint set by the CLSI for susceptibility to pirlimycin in *Staphylococcus aureus* and *Streptococcus* spp. isolates from cows with mastitis is ≤ 2 μg/ml.

- *Resistant (MIC ≥ 4 μg/ml)*: All aerobic Gram-negative rods, *Nocardia* spp., and *Mycobacterium* spp. Lincosamides are also inactive against *Enterococcus faecalis* and *E. faecium*.

Resistance

Resistance can develop to the lincosamides alone but more commonly cross-resistance occurs among macrolides, lincosamides, and streptogramin group B antibiotics (MLSB resistance). In some instances, cross-resistance may also include ketolides (phenotype referred to as MLSK resistance) and oxazolidinones (MSLKO) antimicrobials. Cross-resistance is not always present and its occurrence depends on the mechanism of cross-resistance.

Lincosamide-resistant strains generally have the MLSB resistance phenotype. This can occur by spontaneous point mutations in the genes coding for the ribosomal peptidyltransferase loop. However, in most strains, resistance is the result of methylation of adenine residues in the 23S ribosomal RNA of the 50S ribosomal subunit, which prevents drug binding to the target site. The rRNA methylases are encoded by a series of structurally related erythromycin-resistant methylase (*erm*) genes. The *erm* genes are acquired through mobile elements and can be located on the bacterial chromosome or on plasmids.

This cross-resistance is of two types: (1) constitutive resistance ($MLSB_c$), where bacteria show high-level resistance to all MLSB antibiotics; and (2) dissociated inducible cross-resistance ($MLSB_i$), in which bacteria resistant to macrolides but initially fully susceptible to clindamycin rapidly develop resistance to lincosamides when exposed to macrolides. Constitutive resistant mutants are rapidly selected from the inducible strains during treatment with either lincosamides or macrolides. Constitutive resistance may be more common among bacteria isolated from food animals fed tylosin or virginiamycin as growth promoters. $MLSB_c$ isolates are readily recognized during *in vitro* susceptibility testing as being resistant to both macrolides and clindamycin. However, $MLSB_i$ resistance is not detected by standard *in vitro* susceptibility testing methods. Such isolates appear resistant to macrolides but susceptible to clindamycin under standard testing conditions. As a result, isolates that are resistant to macrolides but susceptible to clindamycin should also be tested for methylase-mediated clindamycin resistance by an additional assay, the D-zone test. Isolates that demonstrate inducible clindamycin resistance based on a D-zone test should be reported as clindamycin resistant (Lewis and Jorgensen, 2005).

Other mechanisms of resistance to lincosamides involve enzymatic inactivation and active efflux of the drug from the periplasmic space. Inactivation is mediated by nucleotidyltransferases encoded by *lnu*(A-F).

Pharmacokinetic Properties

Lincosamides are basic compounds with pK_a values of about 7.6. They have high lipid solubility and consequently large apparent volumes of distribution. They are well absorbed from the intestine of non-herbivores and eliminated mainly by hepatic metabolism, although about 20% is eliminated in active form in the urine. Clindamycin is hydrolyzed in the liver in to at least 7 metabolites. All metabolites but one are devoid of antibacterial activity. Tissue concentrations consistently exceed serum concentrations by several times because of passage across cell membranes. Because of the lincosamide's basic character, ion trapping also occurs in tissues, such as the udder and prostate where pH is lower than blood. Extensive binding to plasma

Table 12.1. *In vitro* activity (MIC_{90}) of lincosamides and pleuromutilins antibiotics (µg/ml) against selected bacterial and mycoplasmal pathogens.

Organisms	Clindamycin/ Lincomycin[a]	Pirlimycin	Tiamulin	Valnemulin
Gram-positive aerobes				
Arcanobacterium pyogenes	< 0.06*	–	0.03	–
Erysipelothrix rushiopathiae	1	–	4	–
Rhodococcus equi	4	–	64	–
Staphylococcus aureus	0.25	1	0.03	–
Streptococcus equi	16	8	0.5	–
S. agalactiae	4	0.5	–	–
S. dysgalactiae	16	1	–	–
S. uberis	> 32	8	–	–
Enterococcus faecalis	16	2	> 32	–
Gram-negative aerobes				
Actinobacillus pleuropneumoniae	> 32[a]	–	8	–
Histophilus somni	–	–	2	–
Mannheimia haemolytica	–	–	4	–
Pasteurella multocida	> 25	–	32	–
Escherichia coli	> 32	> 32	32	–
Klebsiella spp.	> 32	> 32	> 128	–
Enterobacter spp.	> 32	> 32	> 32	–
Anaerobes				
Dichelobacter nodosus	0.25	–	–	–
Bacteroides fragilis	0.5	0.06	–	–
Fusobacterium necrophorum	0.5	0.5	0.016	–
Brachyspira hyodysenteriae	4	–	0.25	0.06
Brachyspira pilosicoli	8	–	0.5	0.5
Clostridium perfringens	4	0.5	–	–
Mycoplasma				
Mycoplasma bovis	> 256	–	0.25	–
M. hyorhinis	2[a]	–	0.25	–
M. hyopneumoniae	0.12[a]	–	0.25	< 0.005
M. hyosynoviae	–	–	0.06	< 0.005
M. mycoides mycoides	–	–	0.5	–
Ureaplasma spp.	–	–	0.06	–
Leptospira spp.	0.2	–	4	–
Lawsonia intracellularis	32[a]	–	4	2

[a] MIC values indicated by[a] are for lincomycin, the others are for clindamycin.
* Some reports show resistance to clindamycin

proteins and relatively rapid elimination prevent concentrations in cerebrospinal fluid (CSF) from exceeding 20% of serum concentrations. Clindamycin achieves therapeutic concentrations in bone, although levels are relatively low, around 10–30% of serum concentrations.

Drug Interactions

Combination with spectinomycin appears to give marginally enhanced activity against mycoplasmas *in vitro*. Clindamycin is commonly combined with an aminoglycoside or a fluoroquinolone in human medicine to treat or prevent mixed aerobic-anaerobic bacterial infections,

particularly those associated with intestinal spillage into the peritoneum. The combination generally has additive or synergistic effects *in vitro* against a wide range of bacteria. Clindamycin has synergistic effects with metronidazole against *B. fragilis* but only additive effects with trimethoprim-sulfamethoxazole combination against common Gram-negative or Gram-positive aerobes. Combination with macrolides or chloramphenicol is antagonistic *in vitro*.

Toxicity and Adverse Effects

The major toxic effect of the lincosamides is their ability to cause serious and fatal diarrhea in humans, horses, rabbits, and other herbivores.

In humans, mild diarrhea follows the use of lincosamides in up to 10% of patients, but in some (0–2.5% of those treated) this may become severe, resulting in pseudomembranous colitis with profound shock, dehydration, and death. The disease is caused by the rapid colonic growth of lincosamide-resistant *Clostridium difficile* through destruction of competing anaerobic microflora of the colon. Treatment with vancomycin or metronidazole is often successful. Less serious toxic effects in humans include depressed neuromuscular transmission and post-anesthetic paralysis, depression of cardiac muscle after rapid IV injection, mild liver damage, drug rashes, and urticaria.

In cattle, oral administration of lincomycin at concentrations as low as 7.5 parts/million (ppm) in feed has resulted in inappetence, diarrhea, ketosis, and decreased milk production. Inadvertent contamination of feed with 8–10 ppm of lincomycin and 40 ppm of metronidazole caused some affected cows to develop severe diarrhea and to lose consciousness. In horses, lincosamides administered by parenteral or oral route can cause severe enterocolitis, which may be fatal. In one inadvertent mixing of lincomycin in horse feed, a dose of 0.5 mg/kg caused an outbreak of diarrhea in which one horse died. Anal swelling, diarrhea, irritable behavior and skin reddening have been reported in pigs but these signs are generally self-limiting within 5–8 days.

Lincosamides are highly toxic to rabbits, guinea pigs, and hamsters. Concentrations as low as 8 ppm accidentally added to feed have been followed by severe and fatal cecocolitis in rabbits. In rabbits the effect is the result of bacterial overgrowth in the large bowel of *C. difficile* or *Clostridium spiroforme*.

Lincomycin is relatively non-toxic to dogs and cats. Anorexia, vomiting, and diarrhea have sometimes occurred especially with oral use. Administration of clindamycin capsules without food or water has resulted in esophagitis and esophageal ulcerations sometimes progressing to stricture in cats (Beatty et al., 2007). Anaphylactic shock has been reported after IM injection. Because of their peripheral neuromuscular blocking and cardiac depressive effects, lincosamides should not be given with anesthetics or by rapid IV injection. Clindamycin given IM is very painful.

Administration and Dosage

Usual dosages are shown in Table 12.2.

After oral administration to monogastric animals, lincomycin is generally absorbed well and clindamycin is absorbed almost completely. Food significantly reduces absorption of both drugs, especially lincomycin. Complete absorption occurs from IM injection sites. Clindamycin palmitate, available as a syrup for oral administration, is rapidly hydrolyzed in the intestine before absorption. The drug is also available in capsules as the hydrochloride for oral administration and as the phosphate for IM, SC, or IV injection. The SC route is superior to the IM route in terms of local tolerance and serum concentrations. Lincomycin is available as the hydrochloride for PO, IM, and IV administration. The dosage should be reduced in patients with hepatic insufficiency.

Table 12.2. Usual dosages of lincosamides and pleuromutilins in animals.

Species	Drug	Dosage (mg/kg)	Route	Interval (h)
Dog/cat	Clindamycin	5–11	PO, IV, IM, SC	12–24
	Lincomycin	10–20	PO, IV, IM	12–24
Ruminants[a]	Lincomycin	5–10	IM	12–24
	Tiamulin	20	IM	24
Swine	Lincomycin	10	IM	24
	Tiamulin	10–15	IM	24
		8–23	PO, feed	24
	Valnemulin	1.5–4	PO, feed	24

[a]These drugs are not approved for use in ruminants and have few, if any, indications.

Clinical Applications

Lincosamides are used in the treatment of staphylococcal infections (dermatitis, osteomyelitis) caused by penicillin G–resistant organisms, for other Gram-positive aerobic infections in penicillin-sensitive individuals, and in the treatment of anaerobic infections. In general, clindamycin is preferred to lincomycin. Clindamycin has excellent activity against anaerobes, equivalent to alternatives such as cefoxitin, chloramphenicol, and metronidazole. Clindamycin may be combined with an aminoglycoside or a fluoroquinolone in the treatment of mixed anaerobic infections. Clindamycin may be preferable to penicillin G or ampicillin in the treatment of streptococcal toxic shock syndrome, since it better inhibits superantigen synthesis (Sriskadan et al., 1997). Lincosamides penetrate well into the prostate and eyes. There are some doubts about the efficacy *in vivo* of clindamycin in the treatment of toxoplasmosis, although combination with pyrimethamine may enhance efficacy. Clindamycin may be useful in treating *Pneumocystis jiroveci* infection, in combination with primaquine. In swine, lincomycin is used extensively in the prevention and treatment of dysentery and sometimes for mycoplasma infections.

Cattle, Sheep, and Goats

There are no formulations of lincosamides labeled for systemic use in ruminants. The major indication for the use of lincosamides in cattle is intramammary infusion in cases of mastitis. Pirlimycin is labeled and commercially available for that purpose. Intramammary pirlimycin been proven effective against mastitis caused by *Staphylococcus* species such as *Staphylococcus aureus* and *Streptococcus* species such as *Streptococcus dysgalactiae* and *Streptococcus uberis* (Gillespie et al., 2002; Olivier et al., 2004). Prepartum treatment of dairy heifers with pirlimycin reduces the prevalence of early lactation mastitis caused by coagulase-negative staphylococci (Middleton et al., 2005).

There are few, if any, indications for the other lincosamides in ruminants because of the availability of approved alternatives. Subconjunctival injection of clindamycin was effective in the treatment of naturally occurring infectious bovine keratoconjunctivitis (Senturk et al., 2007). A single IM injection of the combination (5 mg/kg lincomycin, 10 mg/kg spectinomycin) cured over 90% of sheep with acute or chronic foot rot and was almost as effective as the same dose given on each of 3 days (Venning et al., 1990). Lincomycin (8 g/L) administered as a spray once daily for 5 days was effective in the control of papillomatous digital dermatitis in cattle (Shearer and Elliott, 1998). The combination has also been used in the treatment of rams to prevent ureaplasma contamination of semen (Marcus et al., 1994). The successful treatment of arthritis and pedal osteomyelitis usually associated with *A. pyogenes* with parenteral lincomycin was also reported (Plenderleith 1988). Oral administration of lincomycin to ewes at a dosage of 225 mg/day resulted in severe enterocolitis leading to death in 2,000 of 3,000 exposed animals (Bulgin 1988).

Swine

Lincomycin is largely used in pigs to control dysentery and mycoplasma infections; control of erysipelas and streptococcal infections may be incidental benefits to incorporating the drug in feed for the principal purposes. Lincomycin is used in feed or water (33 mg/L) to treat (100 ppm feed) or prevent (40 ppm feed) swine dysentery. Lincomycin can also be administered at 11 mg/kg IM for 3–7 days. A drawback has been failure to sterilize *B. hyodysenteriae*, so that withdrawal of drug is followed by recrudescence of infection. Nevertheless, whole herd medication has apparently eradicated swine dysentery from closed herds even in some cases with infection caused by apparently resistant organisms. Lincomycin is effective in reducing losses from *Mycoplasma hyosynoviae* and *Mycoplasma hyorhinis*. Pleuromutilins are considerably more effective than lincomycin in control of swine dysentery and mycoplasma infections in swine. Lincomycin delivered in the drinking water has also been shown to be effective for the treatment of proliferative enteropathy both in a field study and following experimental infection (Bradford et al., 2004; Alexopoulos et al., 2006). Lincomycin may be given in feed, water, or by IM injection.

Horses

Lincomycin and clindamycin have been used experimentally to induce enterocolitis in horses. These drugs should not be used in horses, although there are rare reports of apparently successful use in the treatment of osteomyelitis by IM injection without apparent adverse effects.

Dogs and Cats

Lincosamides are used in the treatment of abscesses, osteomyelitis, periodontal disease, and soft tissue or wound infections that involve Gram-positive cocci or anaerobic bacteria. In experimentally induced *Staphylococcus aureus* osteomyelitis in dogs, a dosage of 11 mg/kg of clindamycin administered q 12 h for 28 days effectively resolved the infection. Dosage of 5.5 mg/kg q 12 h was less effective. Clindamycin has been administered at low daily oral dosage in the successful prophylaxis of recurrent staphylococcal skin infections. Field trials have demonstrated the 94–100% efficacy of single-daily dosing with 11 mg/kg orally (average duration 45 days) in the treatment of deep pyoderma in dogs (Harvey et al., 1993; Scott et al., 1998). Lincomycin (22 mg/kg, q 12 h) orally is equally effective in the treatment of staphylococcal skin disease in dogs (Harvey et al., 1993).

In a study of experimental anaerobic infections in dogs, clindamycin at 5.5 or 11 mg/kg administered twice daily IM, was highly efficacious and gave better results than lincomycin, 22 mg/kg twice daily. Clindamycin is used effectively in the treatment of dental infections in dogs, when combined with dental surgery or cleaning (Johnson et al., 1992). Anecdotally, its routine use in periodontal surgery has been associated with problems of salmonellosis in veterinary hospitals. Clindamycin is useful for prostatic infections caused by Gram-positive bacteria. Dosing of 11 mg/kg once daily orally appears to be appropriate, but the same dose could be administered twice daily in serious infections (e.g., osteomyelitis).

Clindamycin has been used successfully in the treatment of toxoplasmosis in a dog and in cats, although it was unsuccessful in treating feline chorioretinitis or anterior uveitis in all cases. Clindamycin administered to cats experimentally infected with toxoplasmosis did not prevent ocular lesions and was associated with increased morbidity and mortality from hepatitis and interstitial pneumonia (Davidson et al., 1996). In contrast, clindamycin completely prevented shedding of *T. gondii* in experimentally infected cats even after severe immunosuppression (Malmasi et al., 2009). Combination with pyrimethamine was less effective in long-term treatment of toxoplasmic encephalitis in human patients than the combination of pyrimethamine-sulfadiazine (Katlama et al., 1996). Clindamycin was successful in resolving clinical signs caused by *Neosporum caninum* in dogs although the pathogen was not necessarily eradicfated (Dubey et al., 1995; Dubey et al., 2007). Clindamycin was also successful for the treatment of dogs experimentally infected with *Babesia gibsoni* (Wulansari et al., 2003). Clindamycin in combination with diminazene and imidocarb was more effective at eradicating *B. gibsoni* in naturally infected dogs than a combination of atovaquone and azithromycin (Lin et al., 2012)

Poultry

Lincomycin-spectinomycin combination is administered orally to young chickens for the control of mycoplasmal air sacculitis and complicated chronic respiratory disease caused by *M. gallisepticum* and *E. coli* Lincomycin has also been used in the control of necrotic enteritis caused by susceptible pathogens such as *C. perfringens*.

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Pleuromutilins: Tiamulin and Valnemulin

Tiamulin and valnemulin are semisynthetic derivatives of the naturally occurring diterpene antibiotic pleuromutilin. Pleuromutilins have outstanding activity against anaerobic bacteria and mycoplasma and are used almost exclusively in animals, largely in swine.

Mechanism of Action

Pleuromutilin antibiotic derivatives inhibit protein synthesis by binding to the 50S subunit of the bacteria. Tiamulin and valnemulin are strong inhibitors of peptidyl transferase. They can bind concurrently with the macrolide erythromycin but compete with the macrolide carbomycim for binding to the ribosome (Poulsen et al., 2001).

Antimicrobial Activity

Tiamulin and valnemulin have outstanding activity against anaerobic bacteria and mycoplasma (Table 12.1). They are active against some Gram-positive aerobic bacteria including *Staphylococcus* spp., *A. pyogenes*, and some streptococci. Tiamulin is active against only a few Gram-negative aerobic species and inactive against Enterobacteriaceae (Table 12.1) although subinhibitory concentrations may reduce adhesive properties of enterotoxigenic *E. coli* (Larsen, 1988). Activity against anaerobic bacteria and mycoplasma is better than that of macrolide antibiotics. Swine respiratory pathogens with a MIC ≤ 8 μg/ml are considered susceptible and ≥ 32 μg/ml are considered resistant to tiamulin. Valnemulin is about twice as active as tiamulin against bacteria and over 30 times more active against swine mycoplasma *in vitro* (Aitken et al., 1999).

Resistance

As with the macrolides, chromosomal mutation to resistance of pleuromutilins emerges readily on *in vitro* passage of bacteria in the presence of the drug. The rate of emergence is significantly lower than with tylosin. There is one-way cross-resistance with tylosin: mycoplasma isolates resistant to tylosin have slightly increased resistance to tiamulin, but mycoplasma isolates resistant to tiamulin are completely resistant to tylosin. There is isolate variation in bacterial cross-resistance with the other macrolides and lincosamide antibiotics, which may include modest increases in resistance to spectinomycin and chloramphenicol. Mutations at the peptidyl transferase center associated with reduced susceptibility to tiamuline have been characterized in *Brachyspira* spp. isolates (Pringle et al., 2004). A significant increase in the MIC of tiamulin and valnemulin for *Brachyspira hyodysenteriae* isolates over time has been documented (Lobova et al., 2004; Hidalgo et al., 2011).

Pharmacokinetic Properties

Tiamulin is used as the hydrogen fumarate in the oral preparation but as the tiamulin base in the parenteral product. Valnemulin is available as a hydrocloride premix for medicated feed. Little information has been published on the pharmacokinetic characteristics of pleuromutilins. Tiamulin is rapidly absorbed after oral administration to pre-ruminant calves and has a half-life of 25 minutes following parenteral administration. Tiamulin is a lipophilic, weak organic base, with a pK_a of 7.6. The drug penetrates cells well and is concentrated several-fold in milk. Concentration in other tissues is several times that in serum. The half-life in dogs after IM administration was 4.7 hours, and serum concentrations were higher and maintained for longer when the drug was given by SC injection (Laber, 1988). Tiamulin is almost completely absorbed after oral administration in monogastric species but would be expected to be inactivated by rumen flora if administered orally to ruminants. Administration of tiamulin to swine in medicated feed, rather than by direct oral administration, strongly decreases its rate and extent of absorption and consequently serum concentrations. The bioavailability of valnemulin in pigs and in broiler chickens is around 90%. Similar to what has been described for tiamulin, valnemulin concentrations in the colonic content and tissues exceed serum concentrations. Dosage recommendations are presented in Table 12.2.

Drug Interactions

Drug interactions have not been studied extensively but are likely to be similar to those described for lincosamides and macrolides. Tiamulin and valnemulin have been shown to interact with ionophores such as monensin, salinomycin, lasalocide, and narasin. Animals should not receive these products during at least 5 days before or after treatment with pleuromutilins. Severe growth depression, ataxia, paralysis or death may result (Miller, 1986).

Toxicity and Adverse Effects

Tiamulin should not be fed at therapeutic concentrations with ionophores such as monensin, narasin, and salinomycin to animals (pigs, poultry) because of the dose-dependent fatal effects of such combinations, which results from tiamulin's potent inducer-inhibiting activity against cytochrome P-450 in the liver.

Intramuscular injection of certain preparations may be irritating but formulations of tiamulin base in sesame oil are not. Intravenous injection of calves resulted in severe neurotoxicity and death. Orally administered tiamulin is transiently unpalatable and irritating in calves.

Acute dermatitis with cutaneous erythema and intense pruritus has been described in pigs following oral administration of tiamulin (Laperle, 1990), where it was associated with poor hygiene and overcrowding. It was suggested that metabolites of tiamulin in urine had a directly irritant effect on the skin.

Medication of pigs with valnemulin in the European Union has resulted in adverse effects characterized by inappetence, pyrexia, ataxia, and sometimes recumbency. The majority of cases occurred in Denmark and Sweden. In these countries, the incidence of these adverse effects ranged from 0.03% to 1.8% of all pigs treated. On some farms, up to one third of treated pigs became affected with a mortality rate of 1%. An epidemiological study has suggested an association between susceptibility to these adverse reactions and the Swedish and Danish Landrace breed.

Pleuromutilins should not be administered to horses because of the potential danger for disruption of the colonic microflora and predisposition to enterocolitis.

Clinical Applications

Tiamulin or valnemulin are used extensively in swine against mycoplasma pneumonia, swine dysentery, and proliferative illeitis. Less commonly, tiamulin has been used against leptospirosis, and to a lesser extent against bacterial pneumonia. Tiamulin is preferred over macrolides for many infections.

Cattle, Sheep, and Goats

Pleuromutilins are not approved for use in ruminants. There are few reports of the use of tiamulin or valnemulin in cattle. Tiamulin has been used successfully to prevent *Mycoplasma bovis* fibrinous polyarthritis and synovitis in veal calves after administration in milk at 400 ppm for the fattening period (Keller et al., 1980). In sheep, tiamulin had a beneficial effect on the course of field cases of infectious rickettsial keratoconjunctivitis (Konig, 1983). Ball and McCaughey (1986) found that a single SC injection of aqueous tiamulin eliminated ureaplasma from the genital tract of 18 of 22 sheep.

Valnemulin administered orally was effective in the control of *Mycoplasma bovis* infections in calves under both experimental and field conditions. In one study, Valnemulin resulted in a more rapid reduction of clinical scores and eliminated *M. bovis* from the lungs more effectively than enrofloxacin (Stipkovits et al., 2005). Valnemulin topical spray has a similar efficacy as lincomycin in the treatment of digital dermatitis in cattle (Laven and Hunt, 2001).

Swine

Tiamulin is labeled in the United States as a growth promoter and for the treatment of swine dysentery associated with *B. hyodysenteriae* and pneumonia due to *A. pleuropneumoniae* susceptible to tiamulin. It has good activity against *E. rhusiopathiae, Leptospira*, and streptococci and moderate activity against *A. pleuropneumoniae*. Tiamulin is used in strategic medication in pig production to prevent and treat common infections. Its activity *in vitro* against *M. hyopneumoniae* requires confirmation *in vivo*.

The drug is highly effective in preventing and treating swine dysentery. Concentrations of 60 ppm in water for 3–5 days apparently eradicated experimental infections; relapses occurred when lower concentrations were used. Tiamulin at 30 ppm in feed has prevented dysentery. Incorporation into water (45 ppm for 5 days, 60 ppm for 3 days) effectively treated swine dysentery and was better than tylosin (Pickles, 1982). A single IM dose of 10–15 mg/kg has successfully treated clinical cases of dysentery (Burch et al., 1983). Tiamulin may be used to eradicate swine dysentery from herds using a variety of approaches. These have included daily injection of carriers with 10 mg/kg IM for 5 consecutive days, combined with management changes and rodent control (Blaha et al., 1987), or oral administration to grower pigs for 10 days followed by carbadox for 42 days (Moore, 1990).

Tiamulin has been effective in treating field cases of enzootic pneumonia and other mycoplasma infections. In one study, treatment with 200 ppm in the feed for 10 days at weaning significantly reduced lung lesions (Martineau et al., 1980). Administration in the drinking water at 3 mg/kg to pigs with enzootic pneumonia markedly improved average daily weight gain and feed efficiency (Pickles, 1980). In another study, tiamulin was as effective as tulathromycin and florfenicol for reducing fever and attenuating clinical signs during natural outbreaks of respiratory disease in swine. The most common pathogens isolated from affected pigs were *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, and *Mycoplasma hyopneumoniae* (Najiani et al., 2005). Tiamulin has proved superior to tylosin in treating experimental mycoplasma and bacterial pneumonia in swine (Hannan et al., 1982). Tiamulin has been used with apparent success in eradicating *A. pleuropneumoniae* infection from herds (Larsen et al., 1990) and also in reducing lesions in pigs treated for chronic *A. pleuropneumoniae* infection (Anderson and Williams, 1990).

Tiamulin fed at 200 ppm in feed for 10 days cured chronic kidney carriage of experimental *L. pomona* infection. Tiamulin administered in drinking water significantly reduced the effects of experimentally induced *S. suis* type 2 infection (Chengappa et al., 1990). Tiamulin is effective in the prevention and treatment of proliferative enteropathy (McOrist et al., 1996).

Valnemulin is approved in the European Union for the treatment and prevention of enzootic pneumonia (*M. hyopneumoniae*), swine dysentery (*B. hyodysenteriae*), colonic spirochaetosis (*B. pilosicoli*) and proliferative enteropathy (*L. intracellularis*) in pigs. Valnemulin has been shown to be effective for the treatment or prevention of both experimentally induced and naturally acquired infection with *M. hyopneumoniae*, *B. hyodysenteriae*, *B. pilosicoli* and *L. intracellularis* (Burch, 2004b). Although valnemulin significantly reduces lung lesions in cases of enzootic pneumonia, *M. hyopneumoniae* is not completely eliminated.

Poultry

Valnemulin and tiamulin in the drinking water have been shown to be effective in the control of *Mycoplasma gallisepticum* infections (Jordan, 1998). Tiamulin was also shown to be effective for the treatment of *B. pilosicoli* infections (Stephens and Hampson, 2002).

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Streptogramins

Streptogramins are a group of natural (virginiamycin, pristinamycin) or semisynthetic (quinupristin/dalfopristin) cyclic peptides. The natural streptogramines are produced as secondary metabolites by *Streptomyces* spp. Streptogramins are unique among antibiotics since each member of the class consists of at least two structurally unrelated molecules: group A streptogramins (macrolactones) and group B streptogramins (cyclic hexadepsipeptides). Virginiamycin has been developed largely as a growth promoter, but pristinamycin and quinupristin/dalfopristin have been developed for clinical use in human medicine, the former for oral administration and the latter for parenteral use. Only virginiamycin has

been studied in veterinary species. New streptogramins with improved *in vitro* activity are currently being investigated (Eliopoulos et al., 2005).

Mechanism of Action

Streptogramins inhibit bacterial protein synthesis by undergoing strong irreversible binding to the 50S ribosomal subunit. The group A and B streptogramins bind to separate sites on the 50S subunit of the bacterial ribosome. Binding of group A streptogramins to the ribosome induces a conformational change that increases affinity of the ribosome for group B compounds. Group A streptogramins prevent peptide bond formation during the chain elongation step, while group B components cause the release of the incomplete peptide chains from the 50S ribosomal subunit. The group B streptogramins share an overlapping binding site with macrolides and lincosamides on the ribosome even tough these antimicrobials are structurally unrelated to each other. Individually, the A and B compounds are bacteriostatic, whereas in combination they are bactericidal. Their synergistic activity tends to reduce the emergence of bacteria resistance to either drug.

Resistance

Since group A and B streptogramins are chemically unrelated and have different binding sites, the mechanisms of resistance to these two compounds are different. Resistance may be chromosomal or plasmid mediated. The first and most common mechanism of resistance to streptogramins B is the acquisition of rRNA methylases encoded in the erythromycin-resistant methylase (*erm*) genes. These enzymes add one or two methyl groups to a single adenine in the 23S rRNA moity. This gives the host bacteria resistance to macrolides, lincosamides, and streptogramins B (MLSB) antibiotics. The second and less common mechanism for resistance to streptogramins B is linearisation of the hexadepsipeptide ring by specific lyases.

Resistance to class A streptogramins is mediated by 2 mechanisms. The first mechanism is active efflux due to ABC transporter proteins. These proteins pump the drug out of the cell or the cellular membrane, keeping intracellular concentrations low and allowing the ribosome to function. The second mechanism is inactivation of the drug by acetyltransferases.

Virginiamycin

Virginiamycin is an antibiotic mixture of virginiamycin S (group B) and virginiamycin M (group A), produced as a fermentation product of *Streptomyces virginiae*. The drug is mainly active against Gram-positive aerobic and anaerobic bacteria (such as *Clostridium perfringens*). Most Gram-negative bacteria are resistant: *Histophilus*, *Lawsonia intracellularis*, *Leptospira* spp., and *B. hyodysenteriae* are exceptions. *Mycoplasma* spp. are often susceptible.

There are few data available on the pharmacokinetic properties of virginiamycin in animals. The drug is not absorbed after oral administration. It is safe if administered orally. Virginiamycin is still used in some countries to promote growth in animals at the level of 5–20 ppm (chapter 22). The use of virginiamycin for this indication was been banned by the European Union in 1999 because of resistance in enterococcal isolates. It is administered to swine at 110 ppm in feed to control swine dysentery, but results have sometimes been poor. The drug does not eradicate infection, and duration of treatment should be several weeks. Virginiamycin (Founderguard) has been used to control cecal fermentation and prevent laminitis in horses fed high concentrate rations. Dietary supplementation with virginiamycin may also lessen some behavioral problems associated with management of stabled horses and the intake of grain.

There is little information on the development and prevalence of resistance to virginiamycin. Studies of *C. perfringens* isolated from turkeys and pigs have not identified resistant isolates. In a recent study, horses receiving virginiamycin to prevent pasture-associated laminitis were not significantly more likely to shed streptogramin-resistant *E. faecium* compared to non-exposed horses. However, the high frequency of resistance within both groups was alarming. Use of virginiamycin as a feed additive may result in the selection of resistant fecal enterococci with cross-resistance to a related streptogramin antibiotic, quinupristin-dalfopristin (Synercid), which has been used in human medicine for the treatment of vancomycin-resistant enterococci and other infections (see below).

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Pristinamycin and Quinupristin/Dalfopristin

Pristinamycin was isolated from *Streptomyces pristinaespiralis*. Pristinamycin has 2 components: 30–40% is pristinamycin IA (group B) and 60–70% is pristinamycin IIA (group A). Pristinamycin has been used as an oral antibiotic for humans in Europe since 1968. It is active against Gram-positive bacteria, especially *Staphylococcus* and *Streptococcus* spp., and a few Gram-negative bacteria such as *Haemophilus*, *Neisseria*, and *Legionella* spp. It is also active against *Mycoplasma* spp.

Quinupristin/dalfopristin consists of a mixture of semisynthetic water-soluble derivatives of pristinamycins IA (quinupristin) and IIA (dalfopristin). Its water solubility allows IV administration, making it the first injectable streptogramins available for clinical use. The combination has a wide distribution in most tissues. In humans, both components are highly protein bound and are cleared rapidly from plasma via biliary excretion by hepatic conjugaison. Phlebitis at the site of infusion is the most common adverse effect. Arthralgia and myalgia, both of which are reversible upon discontinuation of therapy, occur in up to 5% of treated patients.

Quinupristin/dalfopristin is bactericidal against many Gram-positive bacteria, with selective activity against some fastidious Gram-negative aerobes and Gram-negative anaerobes. Gram-positive bacteria with acquired resistance to macrolides and lincosamides commonly develop resistance to the streptogramin B rather than to the A component of the combination. These features, as well as the properties of high susceptibility among Gram-positive bacteria, make this combination of considerable interest in human medicine for the treatment of susceptible multiresistant bacteria. Examples include methicillin-resistant *S. aureus* (MRSA) and penicillin- or erythromycin-resistant pyogenic streptococci. An important feature is the activity of the combination against vancomycin-resistant *Enterococcus faecium*. Since virginiamycin is used extensively as a growth promoter in animals, there is considerable concern that continued use of this drug in food-producing animals may interfere with the efficacy of the combination for the treatment of vancomycin-resistant enterococcal infections in people. Quinupristin/dalfopristin is also active *in vitro* against *Streptococcus pneumoniae*, *Neisseria* spp., *Mycoplasma* spp., *Legionella* spp., *Haemoplilus* spp., and *Chlamydia* spp. Among the anaerobes, *Clostridium perfringens* and *C. difficile* are the most susceptible. The combination is also active against many other anaerobes including *Fusobacterium* spp. and peptostreptococci.

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13

Macrolides, Azalides, and Ketolides

Steeve Giguère

Macrolides (*macro* meaning large and *olide* meaning lactone) are characterized by having a central 12- to 16-membered lactone ring that has few or no double bonds and no nitrogen atoms to which two or more sugar moieties are attached. The efficacy of this group of drugs against important human pathogens, including *Campylobacter, Chlamydia, Legionella*, and *Mycobacterium* species, has resulted in development of semisynthetic members with increased antibacterial activity, improved pharmacokinetic parameters, and reduced adverse reactions.

The macrolides are classified according to the number of atoms comprising the lactone ring e.g., 12-, 13-, 14-, 15-, or 16- (Figure 13.1). The 12-member ring macrolides are no longer used in clinical practice. Tulathromycin, a semisynthetic macrolide approved for use in swine and cattle, consists of an equilibrated regioisomeric mixture of a 13-membered ring (10%) and a 15-membered ring (90%). The unique structural feature of this antimicrobial places it in a novel category of macrolides termed *triamilides*. The 14-member ring group contains compounds of natural origin (erythromycin and oleandomycin) and semisynthetic derivatives (clarithromycin, roxithromycin, dirithromycin). The 15-member ring is represented by azithromycin, gamithromycin, and one isomer of tulathromycin. The 15-membered ring macrolides are termed *azalides* as they have a nitrogen atom in the lactone ring. The 16-member group also contains both compounds of natural origin (spiramycin, josamycin, midecamycin) and semisynthetic derivatives (tilmicosin, tildipirosin).

As a class, the macrolides exhibit broad distribution in tissues and, in the case of some of the newer drugs, prolonged half-lives. They also have excellent activity against many important bacterial pathogens of animals. The macrolides are also known for their intracellular accumulation within phagocytes. However, the precise pharmacodynamic relationships between intracellular concentrations and bacterial killing remain to be defined.

Mechanism of Action

Macrolides inhibit protein synthesis by reversibly binding to 50S subunits of the ribosome. They inhibit the transpeptidation and translocation process, causing premature detachment of incomplete polypeptide chains. Their binding sites on the 23S rRNA of the 50S ribosomal subunit overlap with that of lincosamides, streptogramins, ketolides and oxazolidinones but are different from those of chloramphenicol. Macrolides are generally bacteriostatic agents but they may be bactericidal at high concentrations and against a low inoculum of some highly susceptible bacteria.

Resistance

Three different mechanisms account for most bacterial resistance to the action of macrolides: (1) rRNA methylation; (2) active efflux; and (3) enzymatic inactivation.

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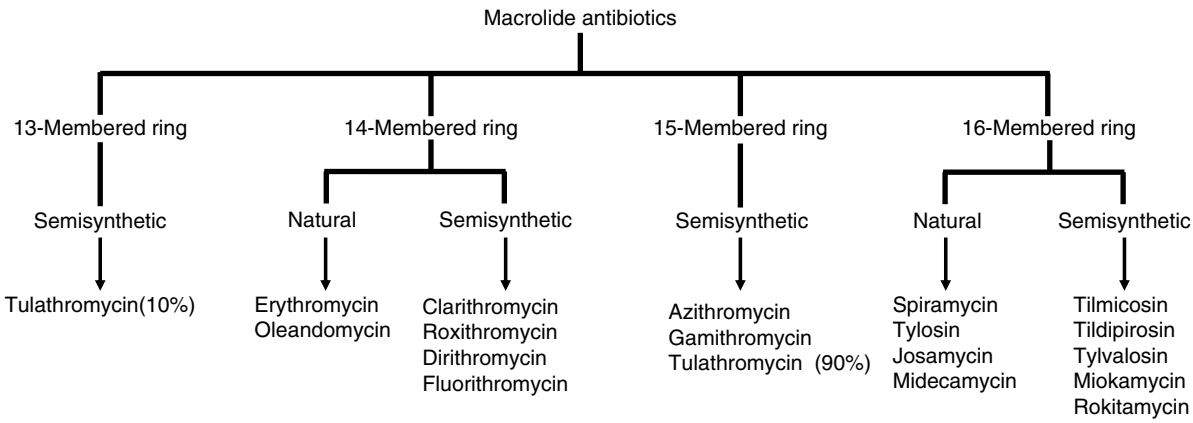

Figure 13.1. Classification of macrolide antimicrobials according to the size of the macrocyclic lactone ring.

rRNA methylation and active efflux are the mechanisms responsible in the majority of resistant isolates. Most macrolide resistance genes are associated with mobile elements and thus have the capacity to spread between strains, species, and bacterial ecosystem.

rRNA methylation, encoded by erythromycin-resistant methylase (*erm*) genes, results in cross-resistance to the macrolides, lincosamides, and streptogramin B (MSLB resistance). To date, 35 different rRNA methylases have been characterized. These methylase genes are widely distributed in both Gram-positive and Gram-negative bacteria, and can be located on plasmids or transposons. The expression of *erm* genes can be constitutive or inducible. Constitutive resistance occurs when the methylase enzyme is inherently produced. Inducible resistance occurs when enzyme induction is effected by exposure of the microorganism to 14- or 15-member ring macrolides, but not to 16-member ring macrolides.

Efflux of macrolide antimicrobial agents is mediated by members of the ATP binding cassette family of proteins or by major facilitator superfamily transporters. These proteins pump antimicrobial agents out of the cell or cellular membrane, thereby allowing the bacterial ribosomes to function again. Currently, 20 different efflux genes have been recognized. Some of these genes confer resistance to 14- and 15-member ring macrolides while not interfering with susceptibility to 16-member ring macrolides, ketolides, lincosamides, and streptogramin B. Other efflux genes lead to a variety of different resistance patterns including resistance to all macrolides, lincosamides, and streptogramins. The efflux genes have been found in a variety of Gram-positive and Gram-negative bacteria.

The third and less common mechanism of resistance is due to enzymatic inactivation. There are currently 2 esterase and 6 phosphorylase inactivating enzymes known to be involved in macrolide resistance. The clinical significance of this last mechanism has not been clearly established.

Between 1% and 4% of macrolide-resistant Gram-positive bacteria do not carry any of the known acquired macrolide resistance genes described above. These isolates typically have mutations in their rRNA genes and/or ribosomal protein genes, which confer macrolide resistance.

Drug Interactions

There have been relatively few studies of the interactions of macrolide antibiotics with other antimicrobial drugs. Combinations of erythromycin with other macrolides, lincosamides, and chloramphenicol are antagonistic *in vitro*. Erythromycin has been used alone or with an aminoglycoside to prevent or treat peritonitis after intestinal spillage, but it is not as effective as clindamycin or metronidazole in combination with an aminoglycoside. Combination of a macrolide and a fluoroquinolone or aminoglycoside may be synergistic, antagonistic, or indifferent depending on the microorganism studied. Combination of a macrolide with rifampin is synergistic against *Rhodococcus equi*.

Erythromycin and many other macrolides lead to inactivation of the cytochrome P450 enzyme complex. Thus, concurrent administration of erythromycin increases concentrations of drugs that are primarily dependent upon CYP3A enzyme metabolism such as theophylline, midazolam, carbamazepine, omeprazole, and ranitidine. Clarithromycin and roxithromycin have lower affinity for the P450 system than erythromycin and other classic macrolides (except spiramycin). Azithromycin, dirithromycin and spiramycin do not interact with the hepatic cytochrome P450 system and are not associated with the drug interactions observed with erythromycin and other macrolides.

Anti-inflammatory and Prokinetic Activities of Macrolides

Macrolides have immunomodulatory effects that are beneficial for humans suffering from many inflammatory pulmonary diseases such as cystic fibrosis, idiopatic bronchiectasis, and chronic obstructive pulmonary disease (Friedlander and Albert, 2010). These effects are likely independent of the antibacterial activity of these drugs. Erythromycin, azithromycin, clarithromycin, and roxithromycin inhibit chemotaxis and infiltration of neutrophils into the airway and, subsequently, decrease mucus secretion. The mechanisms of action for the anti-inflammatory properties of the macrolides are multifactorial and still under investigation (Altenburg et al., 2011). Macrolides inhibit the production of many proinflammatory cytokines including interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-alpha by suppressing the transcription factor nuclear factor-kappa B or activator protein-1. Macrolides also inhibit formation of leukotriene B4, which attracts neutrophils and inhibit superoxide anion release by neutrophils that may be present in the airway. In addition, macrolides block formation of adhesion molecules necessary for neutrophil migration. Recent studies suggest an effect of macrolide on adaptive immunity as well. These anti-inflammatory and immunomodulatory effects have been described in foals receiving erythromycin (Lakritz et al., 1997), and in cattle and pigs administered tilmicosin or tulathromycin (Fischer et al., 2011; Lakritz et al., 2002; Nerland et al., 2005).

Macrolides with 14- or 16-member ring have prokinetic effects on the gastrointestinal tract by acting as motilin receptor agonists. These effects have been demonstrated for erythromycin in horses (Lester et al., 1998) and dogs (Cowles et al., 2000), and for erythromycin, tylosin, and tilmicosin in cattle (Nouri and Constable, 2007).

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Macrolides Approved for Veterinary Use: Erythromycin, Tylosin, Spiramycin, Tilmicosin, Tulathromycin, Gamithromycin, Tildipirosin, and Tylvalosin

Erythromycin

Erythromycins are produced as a complex of six components (A to F) by *Saccharopolyspora erythraea* (formerly *Streptomyces erythraeus*). Only erythromycin A has been

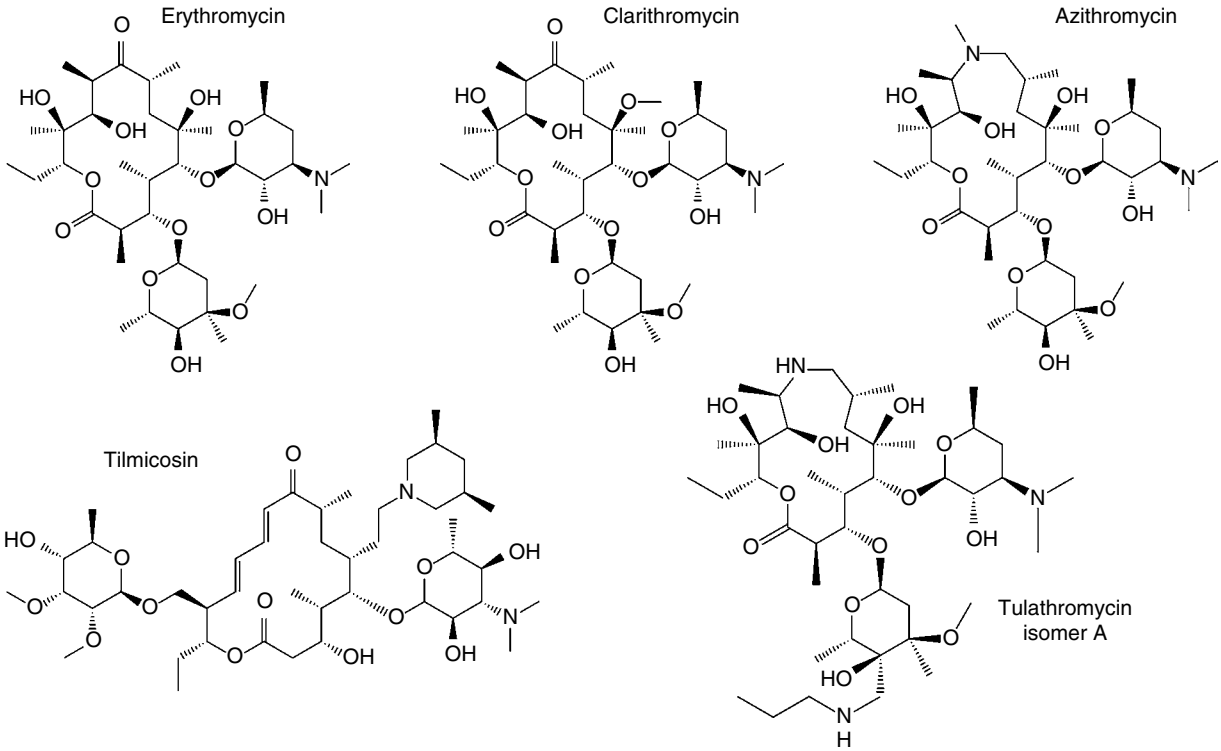

Figure 13.2. Structural formulas of macrolides. Courtesy of Jérôme del Castillo.

developed for clinical use. Erythromycin has a macrocyclic lactone nucleus to which ketones and amino sugars are attached (Figure 13.2). Its base has a pK_a of 8.8, is poorly soluble in water, and is unstable in gastric acid.

Antimicrobial Activity

- *Good susceptibility (MIC ≤ 0.5 μg/ml)* is generally seen in the following Gram-positive aerobes: *Bacillus* spp., *Corynebacterium* spp., *Erysipelothrix rhusiopathiae, Listeria* spp., *Rhodococcus equi*, staphylococci, streptococci. Among Gram-negative aerobes: *Actinobacillus* spp., *Brucella* spp.; *Campylobacter* spp., *Leptospira* spp. Anaerobic bacteria: *Actinomyces* spp., *Bacteroides* spp. (except *B. fragilis*), *Clostridium* spp., some *Fusobacterium* spp., and anaerobic cocci. Erythromycin is also active against some *Chlamydia*/*Chlamydophila* spp. and *Mycoplasma* spp. (Table 13.1).
- *Moderate susceptibility (MIC 1–4 μg/ml)* occurs in enterococci, *Arcanobacterium pyogenes*, some *Bordetella* spp., *Haemophilus* spp., *Legionella* spp., *Ehrlichia* spp., *Pasteurella* spp.
- *Resistant (MIC ≥ 8 μg/ml)* bacteria include all Enterobacteriaceae, *Pseudomonas* spp., *Nocardia* spp., *Mycobacterium* spp. (other than *M. kansasii*), and some *Mycoplasma* spp.

Pharmacokinetic Properties

The erythromycin base is highly susceptible to degradation from gastric acids. To circumvent this, orally administered erythromycin requires an enteric coating. However, this leads to considerable individual variation in absorption. Erythromycin is available for oral administration as the free base, the stearate or phosphate salts, and as estolate or ethylsuccinate esters. The stearate is hydrolyzed in the intestine to the active base, and the ethylsuccinate and estolate esters are absorbed as such and hydrolyzed in the body to the active base. Feeding interferes quite markedly with oral absorption. Like all

Table 13.1. *In vitro* activity (MIC_{90}) of veterinary macrolides (μg/ml) against selected bacterial and mycoplasmal pathogens.

Organisms	Erythromycin	Tylosin	Spiramycin	Tilmicosin	Gamithromycin	Tulathromycin	Tildipirosin
Gram-positive aerobes							
Arcanobacterium pyogenes	2	2	4	0.05*		8	
Erysipelothrix rhusiopathiae	0.13	< 0.13	0.25	< 0.13			
Rhodococcus equi	≤ 0.25	64	128	32	1	> 64	
Staphylococcus aureus	0.25	2	8	1			
Streptococcus agalactiae	≤ 1	1		4			
Streptococus uberis	≤ 0.5	1	0.5*				
S. equi subsp. zooepidemicus	≤ 0.25				0.125		
Gram-negative aerobes							
Actinobacillus pleuropneumoniae	8	32	32	2		32	8
Histophilus somni	2	8	128	8	0.5	4	4
Mannheimia haemolytica	16	128		4	1	2	1
Pasteurella multocida	16	128		16	1	1	1
Bordetella bronchiseptica				16		8	4
Haemophilus parasuis	2*			8*		2	1
Moraxella bovis	1	16		4		0.5	
Moraxella bovoculi		16		≤ 4		4	
Anaerobes							
Dichelobacter nodosus	0.25	1	1				
Bacteroides fragilis	32	0.25*	> 64				
Fusobacterium necrophorum	8	4	64	4		64	
Brachyspira hyodysenteriae	> 128	> 128	> 128	> 64			
Clostridium perfringens	4	2		4			
Mycoplasma							
Mycoplasma bovis	0.5	0.5	4	> 128	4	1	
Mycoplasma hyorhinis	128	1	0.5	4		> 32	
Mycoplasma hyopneumoniae	4	1	1	0.5		> 32	
Mycoplasma mycoides subsp. *mycoides*	0.06	0.06	0.5	0.06			
Ureaplasma spp.		0.13	0.5				
Leptospira spp.	0.06	0.06					
Lawsonia intracellularis	0.5	64		2			

*Some reports show resistance.

macrolides, erythromycin is well distributed in the body, being concentrated in tissues, although penetration into cerebrospinal fluid is low. Prostatic fluid concentrations are approximately half that of serum concentration. The drug is metabolized and excreted largely in the bile and, although some intestinal reabsorption occurs, most is lost in feces. Urinary excretion is only 3–5% of the total administered dose.

Erythromycin is available for parenteral injection as the base, glucoheptonate, or lactobionate. Parenteral administration causes tissue irritation at the site of administration.

Toxicity and Adverse Effects

The incidence of serious adverse effects is relatively low and depends upon the animal species. One problem shared with all macrolides is their irritating nature, which leads to severe pain on IM injection, thrombophlebitis and periphlebitis after IV injection, and an inflammatory reaction after intramammary administration. Dose-related gastrointestinal disturbances (nausea, vomiting, diarrhea, intestinal pain) occur in most animals species treated with erythromycin, either as a result of disruption of the normal intestinal microflora, or as a result of stimulatory

effects on smooth muscle because erythromycin binds motilin receptors. These adverse effects are not life threatening except in adult horses, where macrolides, because they are largely excreted in the bile, can lead to serious diarrheic illness. Deaths have occurred due to *Clostridium difficile* in adult horses administered erythromycin (Gustafsson et al., 1997). Interestingly, severe *C. difficile* diarrheal illness has also developed in the mares of foals treated orally with erythromycin and rifampin for *Rhodococcus equi* infection. This may be a direct effect of mares ingesting small quantities of antibiotic from the feces of their foals or an indirect effect of mares acquiring erythromycin-resistant *C. difficile* infection from their foals, or a combination of these circumstances (Båverud et al., 1998). Deaths from typhlocolitis have also been reported in rabbits. Oral administration of erythromycin has caused severe diarrhea in ruminating calves. Because of this effect combined with poor absorption, oral administration of erythromycin to cattle is not recommended. The drug appears safe in dogs and cats. The estolate form has been associated with self-limiting cholestatic hepatitis and jaundice with abdominal pain, especially with repeated and prolonged use or in patients with preexisting hepatic disease.

Other adverse effects of erythromycin in foals include hyperthermia and respiratory distress that may be more marked in foals kept in high environmental temperatures (Traub-Dargatz et al., 1996).

Administration and Dosage

Dosages of erythromycin are shown in Table 13.2. When administered IV, erythromycin must be diluted and administered by slow infusion to prevent adverse reactions.

Table 13.2. Usual dosages of selected macrolides in animals.

Species	Drug	Dosage (mg/kg)	Route	Interval (h)
Dog/cat	Erythromycin	10–20	PO	8–12
	Clarithromycin	5–10	PO	12
	Azithromycin	5 (cat), 10 (dog)	PO	24
	Tylosin	10–20	PO	12
		5–10	IM	12
Ruminants	Erythromycin	1.1–2.2	IM	24
	Tylosin	4–10	IM	24
	Tilmicosin[a]	10	SC	Single dose
	Tulathromycin	2.5	SC	Single dose
	Gamithromycin	6	SC	Single dose
	Tildipirosin	4	SC	Single dose
Horses[b]	Erythromycin	25	PO	6–8
	Erythromycin	5	IV*	6
	Clarithromycin	7.5	PO	12
	Azithromycin	10	PO, IV*	24–48
Swine	Erythromycin	2–20	IM	12–24
	Tylosin	9	IM	12–24
	Tilmicosin	200–400 g/ton of feed		
	Tulathromycin	2.5	IM	Single dose
	Tildipirosin	4	IM	Single dose
	Tylvalosin	50–100 g/ton of feed		
	Tylvalosin	50 ppm	Water	

*Slow iv infusion.
[a]Cattle and sheep only.
[b]Mainly indicated in foals.

Clinical Applications

Erythromycin is a drug of choice to prevent or treat *Campylobacter jejuni* diarrhea or abortion. Erythromycin is also used as an alternative to penicillin in penicillin-allergic animals in the treatment of infections caused by susceptible Gram-positive aerobes, a less useful alternative to clindamycin or metronidazole in anaerobic infections, an alternative to ampicillin or amoxycillin in the treatment of leptospirosis, and an alternative to tetracyclines in rickettsial infections. The generally bacteriostatic nature of the drug is a disadvantage of erythromycin and other macrolides.

Cattle, Sheep, and Goats. Erythromycin has limited use in respiratory disease, as *H. somni, A. pyogenes*, and anaerobic bacteria are often moderately susceptible, and some mycoplasma and most *Mannheimia haemolytica* isolates are resistant. Due to the extreme pain associated with parenteral injection, it should be avoided when other antimicrobial drugs are available. Erythromycin is perhaps most useful in its intramammary infusion form for lactating and dry-cow therapy of mastitis where it has a short milk withdrawal time (36 hours). A single IM injection of 10 mg/kg was effective in the treatment of virulent footrot in sheep (Ware et al., 1994).

Swine. Erythromycin has little place in the treatment of swine infections with the possible exceptions of leptospirosis (Alt and Bolin, 1996).

Horses. Erythromycin is an alternative to penicillin G or trimethoprim-sulfonamide in the treatment of staphylococcal and streptococcal infections. The potential for inducing diarrhea limits its use in adult horses. Erythromycin is a drug of choice in the treatment of *Rhodococcus equi* pneumonia in foals, and should be used in combinations with rifampin, both for the synergistic effect and to reduce the risk of emergence of resistant mutants. Intramuscular injection causes severe local irritation in horses. The combination of orally administered erythromycin and rifampin has been used successfully to treat experimentally induced *Neorickettsia risticii* infection and may represent an alternative to tetracyclines (Palmer and Benson, 1992). Erythromycin, alone or in combination with rifampin, is also the treatment of choice for *Lawsonia intracellularis* infections in foals (Lavoie et al., 2000).

Dogs and Cats. Erythromycin may be a second choice for infections caused by Gram-positive cocci and anaerobic bacteria. It is the drug of choice in treating *C. jejuni* enteritis (Monfort et al., 1990).

Poultry. Erythromycin is administered in water for the prevention and treatment of staphylococcal or streptococcal infection, necrotic dermatitis, infectious coryza, and *M. gallisepticum* infection.

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Tylosin

Tylosin is a macrolide antibiotic isolated from *Streptomyces fradiae*. Its chemical structure and its mechanism of action are similar to other macrolide antibiotics.

Antimicrobial Activity

Tylosin has a similar spectrum of activity to erythromycin. It is less active against bacteria, except for *B. hyodysenteriae*, but more active against a broad range of *Mycoplasma* spp. (Table 13.1).

Pharmacokinetic Properties

The pharmacokinetic properties of tylosin are characteristic of the macrolides in general. Tylosin is a weak base (pK_a 7.1) and is highly lipid soluble. The elimination half-life in dogs and cattle is about 1 hour with apparent volumes of distribution of 1.7 and 1.1 L/kg, respectively. The half-life is considerably longer in sheep, goats and pigs, at approximately 4 hours.

Toxicity and Adverse Effects

Tylosin is a relatively safe drug. Its toxic effects are generally similar to those reported for erythromycin. The drug is irritating to tissue when administered IM or SC. Pigs have been reported to react to injection by developing edema, pruritus, edema of rectal mucosa, and mild anal protrusion. These effects may in part be attributed to the drug vehicle. Tylosin has been reported to cause fatal diarrhea in a horse. Inadvertent feeding of dairy cows with a concentrate contaminated with 7–20 ppm of tylosin resulted in ruminal stasis, inappetance, foul-smelling feces, and decreased milk production. Many of the cows became hyperesthetic and some became recumbent (Crossman and Poyser, 1981). Intravenous administration in cattle has produced shock, dyspnea, and depression. Tylosin and spiramycin have induced contact dermatitis in veterinarians.

Administration and Dosage

Tylosin is administered by IM injection (Table 13.2), by the intramammary route, or for feed incorporation in swine. Tylosin tartrate is readily absorbed from the intestine, but tylosin phosphate is relatively poorly absorbed.

Clinical Applications

Tylosin is not as active as erythromycin against most bacteria but has greater activity against *Mycoplasma* spp. In pigs, where it is also used as a growth promoter, its use in the prevention and treatment of swine dysentery and *Mycoplasma* infections is being replaced by the more active tiamulin. Apart from its use against *Mycoplasma* tylosin is, like erythromycin, a second-choice antibiotic in most clinical situations.

Cattle, Sheep, and Goats. Tylosin is used in cattle primarily to treat pneumonia associated with *Mycoplasma bovis* and otitis media and interna in calves. Other possible indications include treatment of foot rot, metritis, pinkeye, and mastitis caused by Gram-positive cocci. Tylosin may be administered at low concentrations to feedlot cattle on high-concentrate diets to improve weight gain and feed efficiency, and to prevent liver abscesses. Because of the availability of newer macrolide antibiotics, this is now the major use of tylosin.

In cattle, tylosin (7.5–15 mg/kg IM twice a day) has been successful in controlling and eliminating experimental *Mycoplasma mycoides* pneumonia. In calves the drug has been used effectively to treat *Mycoplasma bovis* pneumonia and arthritis. However, in studies where tylosin was dosed IM at 10 mg/kg twice a day it delayed, but did not prevent, experimentally induced *M. bovis* arthritis (Stahlheim, 1976). In goats, tylosin is a drug of choice for treating *Mycoplasma* pneumonia, such as that caused by *M. mycoides* spp. *capri*. A high dosage of 25–35 mg/kg IV at 8- to 12-hour intervals is recommended.

Swine. Tylosin is used in some countries to promote growth and improve weight gain. For the treatment of atrophic rhinitis, injection of piglets for variable periods has reduced frequency of the disease, suggesting that tylosin inhibits *Pasteurella multocida* (or its production of Pmt toxin), despite the bacteria's relatively high MIC. Injection of neonatal pigs has reduced the frequency of *M. hyopneumoniae* lesions (Kunesh, 1981). Tylosin was not as effective as tiamulin in controlling an experimental mixed *Mycoplasma* and bacterial pneumonia (Hannan et al., 1982). Tylosin, 8.8 mg/kg twice a day IM, or tylosin-sulfonamide, 100 ppm of each drug in feed, was effective in treating pigs with experimentally induced *P. multocida* and *A. pyogenes* pneumonia (Matsuoka et al., 1983).

Control of swine dysentery by tlosin is hampered by the development of resistance; the *in vivo* effect of the drug varies with the MIC, which ranges from 4 to > 32 μg/ml. Derivatives of tylosin may have greater activity against resistant organisms (Jacks et al., 1986). Tylosin (100 ppm) is effective in preventing or treating proliferative enteropathy (McOrist et al., 1997). Other potential

uses include parenteral treatment of erysipelas and infections involving *A. pyogenes* and anaerobes. Tylosin (44 mg/kg IM once daily for 5 days) effectively treated experimentally induced leptospirosis in swine (Alt and Bolin, 1996).

Horses. Injection of tylosin has been fatal to horses. There is no experience with its oral administration but no indication for such use, which might be likely to result in enterocolitis.

Dogs and Cats. Tylosin has been used successfully in dogs to treat abscesses, wound infections, tonsillitis, tracheobronchitis, and pneumonia caused by pathogens such as staphylococci, streptococci, anaerobes, and *Mycoplasma*. Occasional pain and swelling at the injection site and vomiting after oral administration have been reported. A tylosin-sulfonamide combination is licensed for the treatment of upper respiratory tract infections in dogs. Tylosin is often effective in the treatment of the upper respiratory tract infection complex of cats, possibly because of its effect against *Chlamydophila* and *Mycoplasma*. Tylosin administered orally has been shown to be effective in the treatment of *Staphylococcus intermedius* pyoderma in dogs (Scott et al., 1994; Harvey, 1996); a dose of 10 mg/kg q 12 h was shown to be almost as effective as 20 mg/kg q 12 h (Scott et al., 1996). Therapy with oral tylosin has been successful for the attenuation of diarrhea in dogs with chronic enteropathies for which specific causes have been ruled out (Westermark et al., 2005). In a recent randomized double-blinded prospective clinical trial, tylosin at 25 mg/kg q 24 h resulted in normal fecal consistency in 17 of 20 (85%) dogs whereas administration of a placebo improved fecal consistency in only 2 of 7 dogs (Kilpinen et al., 2011)

Poultry. Tylosin has been used by IM injection for the control of *Mycoplasma* infections and added to the water o control of avian spirochetosis. Resistance in some *M. gallisepticum* isolates may reduce the efficacy of tylosin (Migaki et al., 1993). In one study, tylosin was found to be almost as effective as danofloxacin in control of infection caused by *Mycoplasma gallisepticum* in broiler chickens (Jordan et al., 1993). Administration of tylosin in drinking water for 5 days resulted in an immediate resolution of eggshell abnormalities associated with *Mycoplasma synoviae* infection (Catania et al., 2010).

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Spiramycin

Spiramycin is several times less active against bacteria than erythromycin. Its spectrum of activity is similar to that of the other macrolides, but it is not as effective against *Mycoplasma* as tylosin or tiamulin. Resistance, antimicrobial drug interactions, and toxic properties are similar to those of the other macrolides.

Despite relatively poor activity *in vitro*, spiramycin has quite exceptional ability to concentrate in tissues, in part by tissue binding. This results in concentrations in organs reaching 25–60 times those of serum. The drug persists even when serum concentrations are negligible. Thus, spiramycin has the paradoxical effect of being less active than erythromycin *in vitro* but as or more active *in vivo*. Like other macrolides, it also has a direct effect on phagocytic cells and as such, has particular potential against intracellular organisms. In humans, it is used in the treatment of toxoplasmosis (Hotop et al., 2012). In calves, Schilferli et al. (1981) found that a parenteral administration of 50 mg/kg twice a day for 5 days resulted in lung concentrations of approximately 100 μg/g. Not all this drug is active; in mammary tissue about 75% is inactive. One result of its tissue concentration is the persistence of drug residues for prolonged periods, a particular problem in the treatment of mastitis in lactating cows but also more generally in food animals. Spiramycin is used extensively in France for the treatment of infections in farm animals. It has the same applications as tylosin.

Spiramycin was used extensively in Europe as a broiler chicken growth promoter prior to the ban on these products by the European Union. Resistance in bacteria isolated from chickens fed spiramycin is widespread in Europe (Aarestrup et al., 1998).

Cattle, Sheep, and Goats

Spiramycin has similar applications to tylosin. The drug has been used successfully to treat contagious bovine pleuropneumoniae when administered at 25 mg/kg IM at 48-hour intervals for 3 doses (Provost, 1974). In one field trial of the treatment of bovine respiratory disease, spiramycin was considerably less effective than florfenicol (Madelenat et al., 1997). In another study, a dose of 20 mg/kg resulted in spiramycin concentrations in mastitic milk of greater than 2.5 μg/ml for 48 hours after IM injection. Intramuscular injection of this dose after the last milking gave effective milk drug concentrations for 6–8 days (Ziv, 1974). In lactating cows, a single intramammary dose of 600 mg resulted in effective concentrations for 36–48 hours, but persistent residues limit the use of the drug. Parenteral administration of spiramycin for 3–5 days did not give satisfactory results in mastitis caused by penicillin-resistant *S. aureus* (Pyorala and Pyorala, 1998). Spiramycin administered orally at a dose of 100 mg/kg in the last third of gestation to ewes effectively prevented experimental *Toxoplasma* abortion. Bioavailability after oral administration is limited in ruminants. Spiramycin, administered at 20–30 mg/kg IM, successfully treated ovine infectious rickettsial keratoconjunctivitis; in serious cases the drug should be repeated 5 and 10 days after the first injection (Konig, 1983). One interesting potential application is the use of a single injection of the parenteral dosage form of spiramycin to treat endometritis in sheep and cattle, because of the extraordinarily long half-life of the drug (Cester et al., 1990).

Swine and Poultry

Spiramycin has the same applications as tylosin in pigs and poultry.

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Tilmicosin

Tilmicosin, 20-deoxo-20-(3,5-dimethylpiperidin-1-yl) desmycosin, is a semisynthetic derivative of tylosin.

Antimicrobial Activity

Tilmicosin has antibacterial and antimycoplasma activity between that of erythromycin and tylosin (Table 13.1). Typical of macrolides, it inhibits Gram-positive bacteria including *Clostridium* spp., *Staphylococcus* spp., and *Streptococcus* spp., Gram-negative bacteria including *Actinobacillus* spp., *Campylobacter* spp., *Histophilus* spp., and *Pasteurella* spp. All Enterobacteriaceae are resistant. *Mycoplasma* susceptibility can be quite variable. *Mannheimia haemolytica* isolates from cattle with respiratory disease are regarded as susceptible if their MIC is ≤ 8 μg/ml, intermediate if MIC is 16 μg/ml, and resistant if their MIC is ≥ 32 μg/ml. Tilmicosin resistance among *M. haemolytica* (6 of 745 isolates or 0.8%) and *P. multocida* (16 of 231 isolates or 6.9%) isolates is uncommon (McClary et al., 2011). *Pasteurella multocida and Actinobacillus pleuropneumoniae* isolates from swine with respiratory disease are regarded as susceptible if their MIC is ≤ 16 μg/ml and resistant if their MIC is ≥ 32 μg/ml.

Pharmacokinetic Properties

The pharmacokinetic properties of tilmicosin are similar to that of macrolides in general, and are characterized by low serum concentrations but large volumes of distribution (> 10 L/kg), with accumulation and persistence in tissues including the lung, which may concentrate the drug 20-fold compared to serum. Subcutaneous tilmicosin is 100% bioavailable in cattle and has a half-life ranging between 21 and 35 hours (Lombardi et al., 2011). Cows administered 10 mg/kg SC as a single dose maintained milk concentrations > 0.8 μg/ml for 8–9 days (Ziv et al., 1995). Tilmicosin is rapidly absorbed and slowly eliminated (elimination half-life of 25 hours) after oral administration to pigs (Shen et al., 2005). In contrast, tilmicosin is not absorbed after oral administration to horses. After IM or SC administration to horses, tilmicosin accumulates in phagocytic cells and lung tissue (Womble et al., 2006; Clark et al., 2008).

Toxicity and Adverse Effects

Tilmicosin is potentially toxic to the cardiovascular system, which varies to some extent with species. The drug is fatal to swine when administered by IM injection at doses ranging between 10 and 20 mg/kg. Care should be taken to avoid accidental injection of people, which can be fatal. The toxic dose for goats is only about 30 mg/kg SC, or ≥ 2.5 mg/kg IV. In horses, SC or IM administration of tilmicosin has resulted in severe reactions at the injection site and in development of diarrhea in some horses (Womble et al., 2006; Clark et al., 2008). The toxic effects of tilmicosin are mediated through its effects on the heart, possibly via rapid depletion of calcium (Main et al., 1996).

Administration and Dosage

Administration is summarized in Table 13.2.

Clinical Applications

Cattle, Sheep, and Goats. Tilmicosin has been developed as a long-acting formulation for use in bovine respiratory disease. A single SC dose of 10 mg/kg results in lung concentrations exceeding the MIC of *M. haemolytica* for 72 hours. Experimental and field data support the value of single-dose SC tilmicosin prophylaxis on arrival of cattle in feedlots and in the treatment in pneumonia of cattle (Ose and Tonkinson, 1988; Schumann et al., 1991; Young, 1995; Musser et al., 1996; Rowan et al., 2004). Doses of 20 mg/kg appeared slightly more effective than 10 mg/kg (Gorham et al., 1990). Repeat injections after 3 days are necessary in some animals (Laven and Andrews, 1991; Scott, 1994). Tilmicosin is not approved for use in lactating cattle because of the prolonged period (2–3 weeks) during which milk residues can be detected. Intramammary tilmicosin at drying-off has been shown to be efficacious in curing some existing *S. aureus* infection (Dingwell et al., 2003). However, tilmicosin should not be administered by the intramammary route in lactating dairy cows because of the persistence of residues. Milk from all glands of any lactating

cows accidentally treated should be discarded for a minimum of 82 days following intramammary administration (Smith et al., 2009).

Tilmicosin is approved for single-dose SC treatment of ovine respiratory disease associated with *M. haemolytica*. Administration of tilmicosin may be fatal in goats.

Swine. Tilmicosin has been shown by experimental and clinical studies to be useful as an oral medication in swine (200–400 ppm) in the control of *Actinobacillus* spp. or *P. multocida* pneumonia (Paradis, 2004). It may also be useful in the control of atrophic rhinitis. In feed, treatment with 400 ppm of tilmicosin phosphate significantly reduced the presence of *A. pleuropneumoniae* on the surface of tonsils but was unable to completely eliminate the organism from deeper tonsillar tissues nor to prevent bacterial shedding by carrier animals (Fittipaldi et al., 2005). There is no information on its effect against *Mycoplasma* pneumonia. Tilmicosis is effective *in vitro* against *Lawsonia intracellularis* and would likely control proliferative enteropathy. Tilmicosin should only be administered orally to swine as IM administration causes vomiting, tachypnea, convulsions, and sometimes death.

Rabbits. Tilmicosin at 25 mg/kg SC was effective in treating pasteurellosis in rabbits; this dose may need to be repeated after 3 days to further enhance a clinical cure (McKay et al., 1996).

Poultry. Tilmicosin is effective in the treatment of experimentally induced *Mycoplasma gallisepticum* infection when administered at 50 mg/l of drinking water for 3 or 5 days (Charleston et al., 1998). At 300–500 g/ton it prevented infection; interestingly, use of the pellet binder bentonite inhibited the effect of tilmicosin in a concentration-dependent manner (Shryock et al., 1994).

Horses. Because of severe injection site reactions and the risk of colitis, tilmicosin is rarely if ever indicated for use in horses.

Other Species. Tilmicosin is not approved or recommended for use in species other than those described above because of toxicity.

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Tulathromycin

Tulathromycin, a semisynthetic macrolide, consists of an equilibrated regioisomeric mixture of a 13-membered ring (10%) and a 15-membered ring (90%) macrolide. The unique structural feature of this antimicrobial places it in a novel category of macrolides termed *triamilides*.

Antimicrobial Activity

The antimicrobial activity of tulathromycin appears similar to that of tilmicosin. The drug is active *in vitro* against many Gram-negative pathogens including *M. haemolytica*, *P. multocida*, *H. somni*, *Moraxella bovis*, *Fusobacterium necrophorum*, *A. pleuropneumoniae*, *Haemophilus parasuis* (MIC_{90} 2 μg/ml), and *Bordetella bronchiseptica* (MIC_{90} 8 μg/ml). Tulathromycin is active *in vitro* against most *Mycoplasma* spp. although resistance is not uncommon. The activity of tulathromycin against Gram-positive bacterial pathogens has not yet been studied extensively. Based on a small number of isolates, tulathromycin is active against *Arcanobacterium pyogenes* (MIC_{90} 1 μg/mL) but poorly active against *Rhodococcus equi* (MIC_{90} > 64 μg/mL; Table 13.1). *Mannheimia haemolytica*, *Pasteurella multocida and Histophilus somni* isolates from cattle with respiratory disease are regarded as susceptible if their MIC is ≤ 16 μg/ml and resistant if their MIC is ≥ 64 μg/ml.

Pharmacokinetic Properties

The pharmacokinetics of tulathromycin in cattle, swine, goats, and horses are characterized by rapid absorption from the injection site, extensive distribution into tissues, and slow elimination that collectively contribute to high and sustained lung concentrations. The bioavailability of tulathromycin following SC (cattle) and IM (swine) administration is approximately 90% and the elimination half-life is about 90 h. The apparent volume of distribution following IV administration to cattle is 12 L/kg. Peak lung concentrations are approximately 4 μg/g. Lung concentrations are 25–180 times higher than concurrent serum concentrations. Elimination half-life from lung tissue of cattle is approximately 11 days (Nowakoski et al., 2004; Benchaoui et al., 2004; Cox et al., 2010). Tulathromycin concentrates in bronchoalveolar cells of cattle and horses and is eliminated slowly from these cells (Scheuch et al., 2007; Cox et al., 2010). Oral bioavailability in pigs is approximately 50% (Wang et al., 2011).

Toxicity and Adverse Effects

Tulathromycin is safe to use in swine and cattle. No serious adverse events were noted during the clinical development program of the drug. At 10 times the recommended dosage, the most significant adverse effects were associated with pain, swelling and discoloration at the injection site. Based on limited data, the drug appears to be safe in goats (Clothier et al., 2010) and foals (Venner et al., 2007). Safety has not been assessed in other species.

Administration and Dosage

Administration is summarized in Table 13.2.

Clinical Applications

Cattle. Tulathromycin is approved for the treatment or control of bovine respiratory disease caused by *M. haemolytica*, *P. multocida*, *H. somni*, and *Mycoplasma bovis*. Additional approved indications include the treatment of infectious bovine keratoconjunctivitis associated with *Moraxella bovis*, and bovine foot rot (interdigital necrobacillosis) associated with *Fusobacterium necrophorum* and *Porphyromonas levii*. In multiple studies, tulathromycin was more effective than florfenicol or tilmicosin in the prevention or treatment of undifferentiated respiratory disease in cattle (Nutsch et al., 2005a; Rooney et al., 2005; Skogerboe et al., 2005). Tulathromycin was also effective in the treatment of calves experimentally infected with *M. bovis* (Godinho et al., 2005). Interestingly, the drug was as effective regardless of the MIC of the challenge strain (1 or > 64 μg/ ml). Although this is not an approved

indication, tulathromycin has been shown to clear *Leptospira borgpetersenii* serovar hardjo type hardjo-bovis from the urine and kidneys of experimentally infected cattle (Cortese et al., 2007).

Swine. Tulathromycin is indicated for the treatment of swine respiratory disease caused by *A. pleuropneumoniae*, *P. multocida*, *B. bronchiseptica*, *H. parasuis*, or *Mycoplasma hyopneumoniae*. The drug is also approved for the control of *A. pleuropneumoniae*, *P. multocida*, *or Mycoplasma hyopneumoniae* in groups of pigs where swine respiratory disease has been diagnosed. Tulathromycin was as at least as effective as ceftiofur, florfenicol or tiamulin for the treatment of undifferentiated respiratory disease in swine (McKelvie et al., 2005; Nutsch et al., 2005b). A single dose of tulathromycin was as effective as three daily administrations of enrofloxacin for the treatment of pigs inoculated experimentally with *M. hyopneumoniae* (Nanjiani et al., 2005).

Sheep and Goats. Although not labeled for use in small ruminants, tulathromycin would be a reasonable alternative for the treatment of respiratory disease in small ruminants. In an uncontrolled study of sheep and goats with caseous lymphadenitis, closed system lavage in combination with either intralesional or SC administration of tulathromycin resulted in resolution of the abscesses in the majority of cases (Washburn et al., 2009). However, the *in vitro* activity of tulathromycin against *Corynebacterium pseudotuberculosis* has not been studied.

Horses. Tulathromycin was compared to azithromycin-rifampin for the treatment of foals with subclinical pneumonia as identified by ultrasonographic screening on a farm with a high cumulative incidence of *R. equi* infections. Although differences in survival were not statistically significant, pulmonary abscesses 1 week after initiation of treatment with tulathromycin were significantly larger and duration of therapy was significantly longer, indicating that tulathromycin is not as effective as standard therapy with azithromycin-rifampin (Venner et al., 2007). These results might be explained by the fact that tulathromycin is poorly active against *R. equi in vitro* with an $MIC_{90} > 64$ μg/mL (Carlson et al., 2010).

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Gamithromycin

Gamithromycin is a semisynthetic azalide approved for the treatment and control of bovine respiratory disease. Gamithromycin differs from most other macrolides approved for veterinary use in its structural composition by having a 15-membered semisynthetic lactone ring with a uniquely positioned alkylated nitrogen atom at the 7a-position.

Antimicrobial Activity

The antimicrobial activity of gamithromycin appears similar to that of other azalides such as azithromycin. The drug is active *in vitro* against *M. haemolytica*, *P. multocida*, *H. somni, Mycoplasma bovis, Streptococcus equi* subspecies *zooepidemicus* and *Rhodococcus equi* (Table 13.1). The activity of gamithromycin against other bacterial pathogens has not been studied.

Pharmacokinetic Properties

The pharmacokinetics of gamithromycin in cattle are characterized by rapid absorption from the injection site, extensive distribution into tissues, and slow elimination, which collectively contribute to high and sustained concentrations in pulmonary epithelial lining fluid, bronchoalveolar cells, and lung tissue (Giguère et al., 2011). The bioavailability of gamithromycin after SC administration to cattle is nearly 100% (Huang et al. 2010). The apparent volume of distribution after IV administration is 25 L/kg (Huang et al., 2010). Peak lung concentrations are approximately 28 μg/g after administration of an SC dose of 6 mg/kg. Lung concentrations are 16–650 times higher than concurrent plasma concentrations. Lung elimination half-life values for cattle are 6–7 days (Huang et al., 2010; Giguère et al., 2011).

Toxicity and Adverse Effects

Gamithromycin is safe to use in cattle. No serious adverse events were noted during the clinical development program of the drug. Transient discomfort and mild to moderate injection site swelling may be seen in some treated animals. Safety has not been assessed in other species.

Administration and Dosage

Administration and dosages are summarized in Table 13.2.

Clinical Applications

Cattle. Gamithromycin is approved for the treatment or control of bovine respiratory disease associated with *M. haemolytica*, *P. multocida*, *Mycoplasma bovis*, or *H. somni* in beef and non-lactating dairy cattle. The efficacy of gamithromycin for the treatment and control of bovine respiratory disease has been documented in multiple studies (Baggott et al., 2011; Lechtenberg et al., 2011).

Sheep and Goats. Although not licensed for use in small ruminants, gamithromycin would be a reasonable alternative for the treatment of respiratory disease in sheep and goats. Subcutaneous injection of gamithromycin at a dose of 6 mg/kg was apparently effective in the treatment of footrot-like lesions associated with *Bacteroides melaninogenicus* in a herd of ewes (Sargison et al., 2011).

Horses. Intramuscular administration of gamithromycin to foals at a dosage of 6 mg/kg maintains pulmonary epithelial lining fluid concentrations above the MIC_{90} for *S. equi* subspecies *zooepidemicus* and phagocytic cell concentrations above the MIC_{90} for *R. equi* for approximately 7 days (Berghaus et al., 2012). However, treatment of foals with gamithromycin is not recommended until the clinical efficacy and the safety of the drug have been established.

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Tildipirosin

Tildipirosin is a semisynthetic 16-membered macrolide derived from the naturally occurring tylosin. The chemical structure of tildiprosin is characterized by two piperidine substituents on C20 and C23, and a basic mycaminose sugar moiety at C5 of the macrocyclic lactone ring. Owing to three nitrogen atoms accessible to protonation, tildipirosin is a tribasic molecule.

Antimicrobial Activity

Tildipirosin is active *in vitro* against *M. haemolytica*, *P. multocida*, *A. pleuropneumoniae*, *B. bronchiseptica*, *H. somni, and H. parasuis* (Table 13.1). The activity of tildipirosin against other bacterial pathogens of veterinary importance has not been studied.

Pharmacokinetic Properties

The pharmacokinetics of tildipirosin in cattle and swine are characterized by rapid absorption from the injection site, extensive distribution into tissues and slow elimination, which collectively contribute to high and sustained concentrations in bronchial fluid, and lung tissue (Rose et al., 2012; Menge et al., 2012). The bioavailability of tildipirosin after SC administration to cattle is approximately 80% (Menge et al., 2012). The apparent volume of distribution after IV administration to cattle is 49 L/kg (Menge et al., 2012). Peak lung concentrations are approximately 15 (Menge et al., 2012; Rose et al., 2012;) and 4 μg/g after administration of a dose of 4 mg/kg to cattle and swine, respectively (Menge et al., 2012; Rose et al., 2012).

Toxicity and Adverse Effects

Tildipirosin is safe to use in cattle. No serious adverse events were noted during the clinical development program of the drug. Mild to moderate injection site swelling and pain on palpation of the injection site are common in treated cattle and swine. During clinical trials in swine, treatment with tildipirosin caused shock symptoms in 2 of 1048 treated animals. Safety has not been assessed in other species.

Administration and Dosage

Administration and dosages are summarized in Table 13.2.

Clinical Applications

Cattle. Tildipirosin is approved for the treatment or control of bovine respiratory disease associated with *M. haemolytica*, *P. multocida*, or *H. somni* in beef and non-lactating dairy cattle.

Swine. In some countries, tildipirosin is approved for the treatment of respiratory disease associated with *A. pleuropneumoniae*, *P. multocida*, *B. bronchiseptica*, and *H. Parasuis*.

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Tylvalosin

Tylvalosin (acetylisovaleryltylosin) is a new 16-membered lactone ring macrolide antibiotics recently approved in some countries for use in swine and poultry.

Antimicrobial Activity

Tylvalosin is highly active *in vitro* against *Mycoplasma synoviae* (Cerdá et al., 2002), *M. hyopneumoniae*, and *M. gallisepticum*. It is also active against some but not all isolates of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* (Pringle et al., 2012). *In vitro* susceptibility data on a limited number of isolates indicate that the drug is active against some obligate anaerobes (e.g., *Bifidobacterium* spp. *Clostridium* spp., *Eubacterium* spp. *Peptostrectococcus* spp. and *Bacteroides* spp.). Tylvalosin is not active against enteric Gram-negative bacteria. The activity of tylvalosin against many bacterial pathogens of veterinary importance has not been studied.

Pharmacokinetic Properties

Tylvalosin tartrate is rapidly absorbed after oral administration to pigs and chicken. Tylvalosin is rapidly metabolized to 3-O-acetyltylosin, which possesses equivalent microbiological activity to the parent compound.

In pigs, plasma concentrations are below the limit of quantification after administration of the recommended dose.

In chickens, peak plasma concentrations are achieved approximately 1 hour after a single oral dose. Tylvalosin is rapidly distributed to the major organs. In pigs, highest concentrations are found in bile, spleen, lung, kidney and liver. Tylvalosin concentrations in the lung are detected for up to 12 hours after administration. Part of the overall efficacy of the product might be due to the activity of the metabolites rather than to tylvalosin alone.

Toxicity and Adverse Effects

No adverse reactions related to the drug were observed during clinical or target animal safety studies.

Administration and Dosage

Administration and dosages are summarized in Table 13.2.

Clinical Applications

Poultry. In some countries, tylvalosin tartrate is approved for the prevention and treatment of Mycoplasmosis (*M. gallisepticum*, *M. synoviae* and other *Mycoplasma* spp.) and diseases associated with *Clostridium perfringens* in chickens and turkeys. The drug is also indicated for prevention and treatment of Mycoplasmosis in pheasants.

Swine. In the United States, tylvalosin tartrate is approved for the control of porcine proliferative enteritis associated with *Lawsonia intracellularis* infection. In many other countries, the drug is also approved for the treatment and prevention of porcine proliferative enteropathy (Guedes et al., 2009), swine enzootic pneumonia caused by susceptible strains of *M. hyopneumoniae*, and swine dysentery caused by *B. hyodysenteriae*.

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Other Classic Macrolides

Uncommon macrolide antibiotics (oleandomycin, josamycin, kitasamycin, rosaramicin) have activity similar to erythromycin, spiramycin, and tylosin. There is little reported experience with their use in veterinary medicine, although kitasamycin is used in Japan. The agents appear to have nothing to offer over the commonly used classic macrolide antibiotics.

Advanced-Generation Macrolide Antibiotics: Roxithromycin, Clarithromycin, and Azithromycin

Interest in the macrolides has been stimulated by their activity against traditional and emerging human pathogens, including *Campylobacter* spp., *Helicobacter* spp., *Legionella* spp., as well as against intracellular organisms that have emerged through the AIDS epidemic, such as *Bartonella* spp. and *Mycobacterium* spp. Newer erythromycin derivatives with enhanced pharmacokinetic and in some cases broader antibacterial activities include roxithromycin, dirithromycin, clarithromycin, and azithromycin.

Roxithromycin is an acid-stable derivative of erythromycin with similar activity to erythromycin and complete cross-resistance with erythromycin. Roxithromycin differs from erythromycin by an improved pharmacological profile characterized by enhanced oral bioavailability and longer half-life, allowing for once- or twice-daily administration. It is a well-tolerated alternative to erythromycin for daily oral administration. Dirithromycin has similar *in vitro* activity as erythromycin but offers the advantage of once-daily dosage. Dirithromycin is no longer available in the United States.

Clarithromycin, a 6-0-methyl derivative of erythromycin, is approximately twice as active as erythromycin against bacteria on a weight basis, has a half-life about twice that of erythromycin, and includes good activity against *Mycobacterium avium*. Azithromycin, an acid-stable 15-membered ring azalide, is more active than erythromycin against Gram-negative bacteria and also has a considerably lengthened half-life relative to erythromycin. The application of these and other newer macrolides for veterinary use will likely take advantage of their long half-lives, which may allow for a single administration in the treatment of infections caused by

pathogens such as *Campylobacter* and *Mycoplasma*, and of infections caused by intracellular bacteria.

Antimicrobial Activity

Bacteria with MIC ≤ 2 μg/ml are generally regarded as susceptible and ≥ 8 μg/ml as resistant to newer macrolides. All these macrolides approved for use in human medicine share similar antibacterial spectrum of activity against Gram-positive isolates with clarithromycin being the most active against *Rhodococcus equi* (Table 13.3). Azithromycin has the broadest *in vitro* spectrum against Gram-negative bacteria, including moderate activity against *Salmonella enterica*, but the others also have activity against important human upper respiratory tract Gram-negative pathogens (*Bordetella pertussis*, *Haemophilus influenzae*, *and Moraxella catarrhalis*). Other important antibacterial effects includes excellent activity against the genera *Bartonella*, *Borrelia*, *Brucella*, *Campylobacter*, *Chlamydia* (*trachomatis*), *Legionella*, *Leptospira*, *Mycoplasma*, members of the *Spirochetaceae*, and *Ureaplasma*. Mycobacteria such as *M. avium* are often moderately susceptible. Activity against anaerobic bacteria is variable (Table 13.3).

Pharmacokinetic Properties

In comparison to erythromycin, from which they have been developed, newer macrolides are acid stable, produce fewer gastrointestinal adverse effects, have higher bioavailability following oral administration, have considerably lengthened serum half-lives, and produce higher tissue concentrations, so that single or twice daily dosing is appropriate. Oral bioavailability of azithromycin is approximately 97% in dogs and about 50% in cats and foals. The oral bioavailability of clarithromycin in dogs is lower, ranging between 60 and 80%. The bioavailability of clarithromycin in dogs is not significantly influenced by feeding (Vilmanyi et al., 1996). Azithromycin but not clarithromycin, is also available as an

Table 13.3. *In vitro* activity (MIC_{90}) of erythromycin and newer macrolides (μg/ml) against selected bacterial pathogens.

Organisms	Erythromycin	Roxithromycin	Clarithromycin	Azithromycin
Gram-positive aerobes				
Arcanobacterium pyogenes	≤ 0.016	≤ 0.03	≤ 0.016	≤ 0.016
Erysipelothrix rhusiopathiae	0.03	0.13	0.06	0.03
Listeria monocytogenes	0.25	0.5	0.13	1
Rhodococcus equi	0.5*	0.25*	0.06*	1*
Staphylococcus aureus	0.25	0.25	0.25	0.25
Streptococcus agalactiae	0.13	0.13	0.06	0.13
S. equi (subsp. *equi* and *zooepidemicus*)	≤ 0.25		≤ 0.06	≤ 0.12
Gram-negative aerobes				
Escherichia coli	> 4		> 4	> 8
Klebsiella spp.	> 4		> 4	> 8
Salmonella enterica	> 4		> 4	4
Pasteurella multocida	4	4	2	1
Pasteurella spp. (equine)	1		1	0.25
Brucella spp.	16	16	8	2
Gram-negative: other				
Bartonella henselae	0.13	0.13	0.03	0.016
Campylobacter spp.	2	2	2	0.5
Helicobacter pylori	0.5	0.125	1	0.25
Anaerobes				
Bacteroides fragilis	> 8		16	4
Clostridium perfringens	4		4	4
Fusobacterium necrophorum	16	16	8	1
Peptostreptococcus spp.	> 32	> 32	> 32	> 32

*Resistance has been documented.

IV formulation. Serum elimination half-lives are 20 hours and 35 hours for azithromycin in foals and cats, respectively. The elimination half-life of clarithromycin in foals (4.8 hours) is shorter than that of azithromycin but longer than that of erythromycin (1 hour). The long half-lives of these newer drugs, which is particularly marked for azithromycin, apparently results from extensive uptake by, and slow release from, tissues rather than resulting from delayed metabolism. The major route of excretion is the bile and intestinal tract, although clarithromycin is more markedly excreted through the kidney. About half the administered azithromycin is excreted unchanged in the bile in dogs and cats. Tissue half-lives in cats vary from 13 hours in fat to 72 hours in heart muscle (Hunter et al., 1995). Concentrations of azithromycin in the lung and spleen of cats exceeded 1 μg/ml 72 hours after a single oral dose of 5.4 mg/kg (Hunter et al., 1995). Tissue concentrations of azithromycin are generally 10–100 times those achieved in serum. The extensive tissue distribution of azithromycin appears to result from its concentration within macrophages and neutrophils. The half-life of azithromycin in foal neutrophils is 49 hours (Davis et al., 2002). Bronchoalveolar cells and pulmonary epithelial lining fluid concentrations in foals are 15- to 170-fold and 1- to 16-fold higher than concurrent serum concentrations, respectively (Jacks et al., 2001). In foals, clarithromycin achieves considerably greater concentrations in pulmonary epithelial lining fluid and alveolar macrophages than either erythromycin or azithromycin. However, the half-life of clarithromycin at these sites is much shorter than that of azithromycin (Suarez-Mier et al., 2007).

Toxicity and Adverse Effects

In humans, newer macrolides are typically well tolerated and cause less gastrointestinal disturbances than erythromycin. The limited experience in dogs and cats suggest the same to be true in these species. As with earlier macrolides, these drugs occasionally can induce enterocolitis in foals. Adult horses appear to have a higher incidence of enterocolitis associated with administration of macrolides than foals. Clarithromycin may be fetotoxic and should not be administered to pregnant animals.

Administration and Dosage

Dosage recommendations for dogs, cats, and foals are summarized in Table 13.2.

Clinical Applications

There is limited experience with the use of newer macrolides in veterinary medicine, but these drugs offer the advantage for monogastrates of better oral bioavailability, potentially fewer adverse effects, and less frequent administration compared to erythromycin. Their particular efficacy against intracellular organisms is a considerable advantage. Potential applications include those described for erythromycin. For example, as an alternative to penicillin in penicillin-allergic animals for the treatment of infections caused by susceptible Gram-positive aerobes, an alternative to ampicillin or amoxycillin in the treatment of leptospirosis, and an alternative to tetracyclines in treatment of *Rickettsia* and *Coxiella* infections. Newer macrolides may have advantage in the treatment of intracellular infections in monogastrates, including *Bartonella*, *Chlamydophila psittaci* and atypical mycobacterial infections. Clarithromycin is effective in the treatment of atypical *Mycobacterium* infections, when combined with other antibiotics. Other areas that need to be investigated are use against *Mycoplasma* infections in animals, since medically important *Mycoplasma* are highly susceptible to clarithromycin *in vitro*.

Dogs and Cats

Azithromycin in combination with atovaquone was effective in eliminating *Babesia gibsoni* from persistently infected dogs (Birkenheuer et al., 2004). Administration of azithromycin to dogs with experimental Rocky Mountain spotted fever resulted in improvement of most of the clinical signs but was not as effective as doxycyline or trovafloxacin in decreasing vascular injury to the eye and clearing viable circulating rickettsiae (Breitschwerdt et al., 1999). Azithromycin prevented or resolved episodes of acute arthritis and reduced the bacterial load but failed to eliminate *Borrelia burgdorferi* in infected dogs (Straubinger, 2000). Azithromycin, given at a dose of 10–15 mg/kg daily for 3 days and then twice weekly, provided a similar rapid resolution of clinical signs when compared to doxycycline in cats with *Chlamydophila felis* infections. However, as opposed to doxycycline, azithromycin was ineffective in clearing infection (Owen et al., 2003). In a prospective, randomized, placebo-controlled clinical trial, azithromycin at a dose of 10 mg/kg PO once daily was found to be safe and effective for the treatment of papillomatosis in dogs

(Yağci et al., 2008). Clarithromycin, in combination with amoxicillin and a proton pump inhibitor, has been used successfully for the treatment of gastric ulcers associated with *Helicobacter* spp. in dogs (Anacleto et al., 2011).

Horses

The main indication for the use of azithromycin or clarithromycin in the horse is for the treatment of *Rhodococcus equi* infections in foals. The combination of clarithromycin-rifampin is more effective than erythromycin-rifampin or azithromycin-rifampin especially in severely affected foals (Giguère et al., 2004). The incidence of diarrhea in foals treated with clarithromycin is similar to that observed with erythromycin. In most cases, diarrhea is mild and self-limiting. However, diarrheic foals should be monitored carefully because some may develop depression and severe diarrhea, leading to dehydration and electrolyte loss. Clarithromycin and azithromycin, just like erythromycin, should only be used when no other alternatives are available in adult horses because of the potential for severe enterocolitis. Concurrent administration of rifampin considerably reduces absorption of clarithromycin in foals possibly by inhibition of an unknown intestinal uptake transporter (Peters et al., 2011; Peters et al., 2012).

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Ketolides

Ketolides are members of a new semisynthetic 14-membered ring macrolide, with a 3-keto group instead of an α-L-cladinose on the erythronolide A ring. The two most widely studied ketolides are telithromycin and cethromycin. Both have been developed for oral use. Their spectrum of activity is similar to that of the newer-generation macrolides. However, they offer the advantage of overcoming some, but not all, of the current mechanisms of resistance to standard macrolides within Gram-positive cocci. In general, *Staphylococcus aureus* and *Streptococcus pyogenes* strains with inducible MLSB resistance are susceptible to ketolides whereas strains with constitutive expression of MLSB are resistant. Conversely, constitutively resistant *Streptococcus pneumoniae* retains high susceptibility to ketolides. Ketolides are also active against most Gram-positive isolates that are resistant to macrolides because of macrolide efflux (*mef*) genes. The pharmacokinetics properties of ketolides include a long half-life as well as extensive tissue distribution and uptake into respiratory tissues and fluids, allowing for once-daily dosing. Adverse effects of ketolides in humans are similar to those of macrolides and usually related to the gastrointestinal tract with

diarrhea, nausea, and abdominal pain being the most frequently reported. Albeit rare, cases of fulminant hepatitis and hepatic necrosis have been reported during therapy with telithromycin in humans. The major indication for the use of ketolides in human medicine is in the treatment of community-acquired pneumonia caused by erythromycin-resistant Gram-positive isolates. Clinical trials focusing on respiratory infections indicate bacteriological and clinical cure rates similar to comparators, even in patients infected with macrolide-resistant strains.

Horses

The recent increase in resistance to macrolides amongst isolates of *R. equi* has led to the investigation of the pharmacokinetics of telithromycin in foals and of its *in vitro* activity against macrolide-susceptible and macrolide-resistant *R. equi* isolates. The pharmacokinetic profile of telithromycin in foals is similar to that of clarithromycin and azithromycin with accumulation in pulmonary epithelial lining fluid and bronchoalveolar cells. Telithromycin was significantly more active than traditional macrolides against macrolide-resistant *R. equi* (Javsicas et al., 2010). However, the MIC_{90} of telithromycin for macrolide-resistant *R. equi* isolates (8 μg/mL) was significantly higher than that of macrolide-susceptible isolates (0.25 μg /mL), indicating that at least 1 macrolide-resistance mechanism in *R. equi* likely confers resistance to ketolides as well.

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14

Aminoglycosides and Aminocyclitols

Patricia M. Dowling

General Considerations

The aminoglycosides and aminocyclitols are bactericidal antibiotics primarily used to treat serious infections caused by aerobic Gram-negative bacteria and staphylococci. Amikacin and tobramycin have excellent activity against *Pseudomonas aeruginosa*. However, the use of aminoglycosides and aminocyclitols has been eclipsed by the development of fluoroquinolones, which have better safety profiles and better distribution kinetics. Renal accumulation of aminoglycosides results in detectable drug residues for prolonged periods, so their extra-label use in food animals is strongly discouraged. Nevertheless, they remain important drugs in the treatment of severe Gram-negative sepsis, although their highly cationic, polar nature means that distribution across membranes is limited. Single daily dosing is now recommended for most dosage regimens as it maximizes efficacy and reduces toxicity.

Chemistry

The aminoglycoside antibiotics—streptomycin, dihydrostreptomycin, kanamycin, gentamicin, tobramycin, amikacin, and neomycin—are large molecules with numerous amino acid groups, making them basic polycations that are highly ionized at physiological pHs. Their polarity largely accounts for the pharmacokinetic properties that are shared by all members of the group. Chemically, they consist of a hexose nucleus to which amino sugars are attached by glycosidic linkages. This is why these molecules are also referred to as aminocyclitols or aminoglycosidic aminocyclitols. The aminoglycosides can be divided into 4 groups on the basis of the type and substitution pattern of their aminocyclitol molecule: derivatives containing the aminocyclitol streptidine (e.g., streptomycin and dihydrostreptomycin), derivatives containing the aminocyclitol streptamine (e.g., spectinomycin), derivatives containing a 4,5-disubstituted deoxystreptamine moiety (e.g., neomycin), and derivatives containing a 4,6-disubstituted deoxystreptamine moiety (e.g., gentamicin, kanamycin, amikacin, tobramycin).

Mechanism of Action

Aminoglycosides must penetrate bacteria to assert their effect. Penetration can be enhanced by the presence of a drug that interferes with cell wall synthesis, such as a beta-lactam antibiotic. Susceptible, aerobic Gram-negative bacteria actively pump the aminoglycoside into the cell. This is initiated by an oxygen-dependent interaction between the antibiotic cations and the negatively charged ions of the bacterial membrane lipopolysaccharides. This interaction displaces divalent cations (Ca^{++}, Mg^{++}), which effects membrane permeability. Once inside the bacterial cell, aminoglycosides bind to the 30S ribosomal sub-unit and cause a misreading of the genetic code, interrupting normal bacterial protein synthesis. This leads to changes in the cell membrane permeability, resulting in additional antibiotic uptake, further cell disruption, and ultimately, cell death.

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The extent and types of misreading vary because different members of the group interact with different proteins. Streptomycin acts at a single site but the other drugs act at several sites. Other effects of aminoglycosides include interference with the cellular electron transport system, induction of RNA breakdown, inhibition of translation, effects on DNA metabolism, and damage to cell membranes. The bactericidal effect is through the formation of abnormal cell membrane channels by misread proteins.

Aminoglycoside action is bactericidal, and dose (concentration) dependent. For example, gentamicin concentrations in the range of 0.5–5.0 μg/ml are bactericidal for Gram-positive and some Gram-negative bacteria. At 10–15 μg/ml, gentamicin is effective against the more resistant bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Proteus mirabilis*. The clinical implication is that high initial doses increase ionic bonding, which enhances the initial concentration-dependent phase of rapid antibiotic internalization and leads to greater immediate bactericidal activity. Human clinical studies demonstrate that proper initial therapeutic doses of aminoglycosides are critical in reducing mortality from Gram-negative septicemia. For antimicrobials whose efficacy is concentration-dependent, high plasma concentration levels relative to the MIC of the pathogen (C_{max}:MIC ratio, also known as the inhibitory quotient or IQ) and the area under the plasma concentration-time curve that is above the bacterial MIC during the dosage interval (area under the inhibitory curve, AUIC = AUC/MIC) are the major determinants of clinical efficacy. For the aminoglycosides, a C_{max}:MIC ratio of 10 is suggested to achieve optimal efficacy (McKellar et al., 2004).

The aminoglycosides have a significant post-antibiotic effect (PAE); the period of time where antimicrobial concentrations are below the bacterial MIC, but the antimicrobial-damaged bacteria are more susceptible to host defenses (Gilbert, 1991). The duration of the PAE tends to increase as the initial aminoglycoside concentration increases.

Antimicrobial Activity

The antibacterial action of the aminoglycosides is directed primarily against aerobic, Gram-negative bacteria. Because bacterial uptake is oxygen-dependent, they are not active against facultative anaerobes or aerobic bacteria under anaerobic conditions. They are active against some Gram-positive bacteria, such as *Staphylococcus* spp. Emerging strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudintermedius* (MRSP) typically retain susceptibility to gentamicin and/or amikacin. They are often effective against enterococci, but therapy against streptococci is more effective when combined with a beta-lactam antibiotic. *Salmonella* and *Brucella* spp. are intracellular pathogens and are often resistant. Some mycobacteria, spirochetes and mycoplasma are susceptible. In potency, spectrum of activity, and stability to enzymes from plasmid-mediated resistance, amikacin > tobramycin ≥ gentamicin > neomycin = kanamycin > streptomycin. Amikacin was developed from kanamycin and has the broadest spectrum of activity of the aminoglycosides. It is effective against Gram-negative strains not susceptible to other aminoglycosides because it is more resistant to bacterial enzymatic inactivation. It is also considered the least nephrotoxic, but it is less efficacious against streptococci than gentamicin. Streptomycin and dihydrostreptomycin are the most active of these drugs against mycobacteria and *Leptospira* and the least active against other organisms. The activities of selected aminoglycosides against selected bacteria and mycoplasma are shown in Table 14.1.

The bactericidal action of the aminoglycosides on aerobic Gram-negative bacteria is markedly influenced by pH, being most active in an alkaline environment. Increased local acidity secondary to tissue damage or bacterial destruction may explain the failure of aminoglycosides to kill usually susceptible pathogens. Another factor affecting activity is the presence of purulent debris, which ionically binds to aminoglycosides and inactivates them. When using an aminoglycoside to treat purulent infections (e.g., abscesses), surgical debridement and/or drainage increases efficacy.

Resistance to Aminoglycoside Antibiotics

Most clinically important resistance to aminoglycosides is caused by plasmid-mediated enzymes, broadly classified as phosphotransferases, acetyltransferases, and adenyltransferases. At least 11 enzymes have been identified that can inactivate aminoglycosides. These enzymes modify the aminoglycosides at their exposed hydroxyl or amino groups to prevent ribosomal binding. They are present in the periplasmic space of bacteria,

Table 14.1. Activity (MIC_{90}) of selected aminoglycosides (μg/ml) against bacterial pathogens.

Organism	Streptomycin	Neomycin	Kanamycin	Gentamicin
Gram-positive cocci				
Staphylococcus aureus	32	0.5	4	1
Streptococcus agalactiae	128		128	64
S. uberis	64	32	32	
Gram-positive rods				
Arcanobacterium pyogenes	>128	128	64	8
Bacillus anthracis	<8		0.5-4	≤4
Corynebacterium pseudotuberculosis				4
C. renale	64		2	≤0.25
Erysipelothrix rhusiopathiae			>64	≤64
Listeria monocytogenes	32	4	16	16
Mycobacterium tuberculosis	0.5			
Nocardia asteroides	16	4	128	16
Rhodococcus equi	4	≤0.25	2	≤0.25
Gram-negative rods				
Actinobacillus spp.	≤1		4	1
Bordetella bronchiseptica	256		8–16	2
Brucella canis	0.25		0.5	0.12
Campylobacter jejuni			4	0.5
Escherichia coli	>64	>64	>64	2
Histophilus somni	8	16–32	8	8
Helicobacter pylori			2	
Klebsiella pneumonia	256	256	8	4
Leptospira spp.	0.5		4	
Moraxella bovis	16	0.12	0.12	0.5
Mannheimia haemolytica	>128	32	32	8
Pasteurella multocida				
Cattle	>128	32	32	8
Pigs	16–32		8–16	8
Proteus spp.	>16	16	4	2
Pseudomonas aeruginosa	>16	64	128	8
Salmonella spp.	>128	4	>32	8
Taylorella equigeniatlis	>128	2	1	0.5

Note: Some reports are higher because of resistance. This table is designed partly to illustrate the differences in quantitative susceptibility among different aminoglycosides.

so that extracellular inactivation of drug does not occur. Plasmid-mediated resistance to aminoglycosides is transferable between bacteria. A single type of plasmid may confer cross-resistance to multiple aminoglycosides and to other unrelated antimicrobials. A single bacterial isolate may have any one of a variety of combinations of resistance to different antibiotics conferred by the particular plasmid it carries. For example, one *E. coli* strain may be simultaneously resistant to ampicillin, apramycin, chloramphenicol, gentamicin, kanamycin, sulfonamide, streptomycin, tetracycline, and trimethoprim (Pohl et al., 1993). Antimicrobial resistance in organisms such as *E. coli* and *Salmonella* species is a focus of international research due to potential transference of antimicrobial resistance from animal to human pathogens. Because there are few alternative treatment options, aminoglycosides are increasingly considered in the treatment of MRSA and MRSP infections in companion animals (Papich, 2012).

Strains with reduced permeability and consequently two- to four-fold increases in MIC may be selected during treatment with aminoglycosides. Such strains show cross-resistance to all other drugs within the group. Chromosomal mutation resulting in resistance is relatively unimportant except for streptomycin and dihydrostreptomycin, where it occurs readily, even during treatment, as a result of a single-step mutation to high-level resistance. For the other drugs, chromosomal resistance develops slowly, because there are many 30S ribosomal binding sites. Resistance to aminoglycosides is increasingly important in limiting their effectiveness.

Both subinhibitory and inhibitory aminoglycoside concentrations produce resistance in bacterial cells surviving the initial ionic binding (Barclay and Begg, 2001). This first-exposure adaptive resistance is due to decreased aminoglycoside transport into the bacteria. Exposure to 1 dose of an aminoglycoside is sufficient to produce resistant variants of an organism with altered metabolism and impaired aminoglycoside uptake. *In vitro*, animal and clinical studies show that the resistance occurs within 1–2 hours of the first dose. The duration of adaptive resistance relates directly to the half-life of elimination of the aminoglycoside. With normal aminoglycoside pharmacokinetics, the resistance may be maximal for up to 16 hours after a single dose, followed by partial return of bacterial susceptibility at 24 hours and complete recovery at 40 hours. The clinical significance of this phenomenon is that frequent dosing or constant infusion of an aminoglycoside is less effective than high-dose, once-daily dosing.

Pharmacokinetic Properties

Aminoglycosides are poorly absorbed from the normal gastrointestinal tract, but are well absorbed after IM or SC injection. Following parenteral administration, effective concentrations are obtained in synovial, perilymph, pleural, peritoneal, and pericardial fluid. When given to neonates or animals with enteritis, oral absorption may be significantly increased and result in violative tissue residues in food animals. When given by intrauterine or intramammary infusion to cows, gentamicin is well absorbed and results in prolonged tissue residues. Aminoglycosides bind to a low extent to plasma proteins (less than 25%). As they are large molecules and highly ionized at physiological pHs, they are poorly lipid soluble and have limited capacity to enter cells and penetrate cellular barriers. These drugs do not readily attain therapeutic concentrations in transcellular fluids, particularly cerebrospinal and ocular fluid. The milk-to-plasma equilibrium concentration ratio is approximately 0.5. Their apparent volumes of distribution are relatively small (< 0.35 L/kg) and their plasma elimination half-lives are short (1–2 hours) in domestic animals. Even though these drugs have a small volume of distribution, selective binding to tissues including kidney cortex occurs, so that kidney residues persist in animals for extensive periods. Gentamicin is distributed into synovial fluid in normal horses and local inflammation may increase drug concentrations in the joint and concentrations may increase with repeated doses. Regional perfusion techniques and aminoglycoside-impregnated polymethyl methacrylate beads are excellent methods of local delivery that avoid the adverse effects of systemic therapy.

Elimination is entirely by renal excretion (glomerular filtration), and unchanged drug is rapidly excreted in the urine. Impaired renal function decreases rate of excretion and makes it necessary to adjust the dosage interval to prevent accumulation and toxicity. The significant individual variation in pharmacokinetic parameters between animals of the same species exacerbates problems of toxicity with this drug class (Brown and Riviere, 1991).

Drug Interactions

Aminoglycosides are commonly additive and sometimes synergistic with beta-lactam drugs. Synergism does not usually occur in the presence of high-level plasmid-mediated or chromosomal resistance. The aminoglycosides are synergistic against streptococci, enterococci, *Pseudomonas* and other Gram-negative bacteria if combined with beta-lactam antibiotics due to disruption of the bacterial cell wall by the beta-lactam antibiotic (Winstanley and Hastings, 1989). Combinations of newer beta-lactam drugs with newer aminoglycosides provide optimal therapy in seriously ill, neutropenic patients with bacterial infections. Aminoglycosides are physically incompatible with a number of drugs including many beta-lactams, so they should never be mixed in the same syringe. If administered sequentially through an infusion set, care should be taken to flush well between drugs.

Toxicity and Adverse Effects

All aminoglycosides can cause varying degrees of ototoxicity and nephrotoxicity (Table 14.2). Nephrotoxicity (acute tubular necrosis) is the most common adverse effect of aminoglycoside therapy. Neomycin is the most nephrotoxic and streptomycin and dihyrostreptomycin are the least nephrotoxic. Amikacin is often recommended in critical patients over gentamicin as it is considered less nephrotoxic. Uptake and accumulation of aminoglycosides into renal tubular epithelium demonstrates saturable kinetics. The aminoglycosides enter the renal tubule after filtration through the glomerulus. From the luminal fluid, the cationic aminoglycoside molecules bind to anionic phospholipids on the proximal tubular cells. The aminoglycoside is taken into the cell via carrier-mediated pinocytosis and translocated into cytoplasmic vacuoles, which fuse with lysosomes. The drug is sequestered unchanged in the lysosomes. With additional pinocytosis, drug continues to accumulate within the lysosomes. The accumulated aminoglycoside interferes with normal lysosomal function and eventually the overloaded lysosomes swell and rupture. Lysosomal enzymes, phospholipids, and the aminoglycoside are released into the cytosol of the proximal tubular cell, disrupting other organelles and causing cell death (Brown et al., 1991; Figure 14.1).

The risk factors for aminoglycoside toxicity include prolonged therapy (>7–10 days), multiple doses per day, acidosis and electrolyte disturbances (hypokalemia, hyponatremia), volume depletion (shock, endotoxemia), concurrent nephrotoxic drug therapy, age (neonates, geriatrics), preexisting renal disease, and elevated trough concentrations (Mattie et al., 1989).

Table 14.2. Relative risks of toxicity of different aminoglycosides at usual dosage.

Drug	Vestibular Toxicity	Cochlear Toxicity	Renal Toxicity
Streptomycin	+++	++	(+)
Dihydrostreptomycin	++	+++	(+)
Neomycin	+	+++	+++
Kanamycin	+	++	++
Amikacin	(+)	+	++
Gentamicin	++	+	++
Tobramycin	(+)	(+)	(+)

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Calcium supplementation reduces the risk of nephrotoxicity. Nephrotoxicity can also be decreased by feeding the patient a high-protein diet/high-calcium diet such as alfalfa to large animals and diets higher than 25% protein to small animals, as protein and calcium cations compete with aminoglycoside cations for binding to renal tubular epithelial cells (Behrend et al., 1994; Schumacher et al., 1991). High dietary protein also increases glomerular filtration rate and renal blood flow, thereby reducing aminoglycoside accumulation.

Because nephrotoxicity is related to aminoglycoside accumulation in the renal proximal tubular cells, it is logical that peak concentrations are not related to toxicity and that longer dosage intervals result in less total drug contact with the renal brush border membrane. High-dose, once-daily dosing of aminoglycosides is now common in human and veterinary medicine; it takes advantage of the concentration-dependent killing and long PAE of these drugs and avoids first exposure adaptive resistance and nephrotoxicity (Gilbert, 1991).

Serum concentrations of aminoglycosides can be monitored to reduce toxicity and to confirm therapeutic concentrations (Bucki et al., 2004). To allow for the distribution phase, blood sampling for the peak concentration is done at 0.5–1 hour after administration and the trough sample is usually taken prior to the next dose. The peak and trough concentrations can then be used to estimate the elimination half-life for the individual patient. An increase in the elimination half-life during therapy is a very sensitive indicator of early tubular insult. If using a once-daily regimen, a blood sample just prior to the next dose may be below the recommended trough concentrations and may even be below the limit of detection of the assay. For these patients, an 8-hour post-dose sample will provide a more accurate estimate of the elimination half-life. Serum concentrations of drug should be 0.5–2 μg/ml before the next dose (gentamicin, tobramycin) or less than 6 μg/ml for amikacin.

If therapeutic drug monitoring is unavailable, then nephrotoxicity is detected by an increase in urine gamma glutamyl transferase (GGT) enzyme and an increase in the urine GGT:urine creatinine (Cr) ratio (van der Harst et al., 2005). The UGGT:UCr may increase to 2–3 times baseline within 3 days of a nephrotoxic dose. If these tests are not available, the development of proteinuria is the next best indicator of nephrotoxicity and it is easily determined in a practice

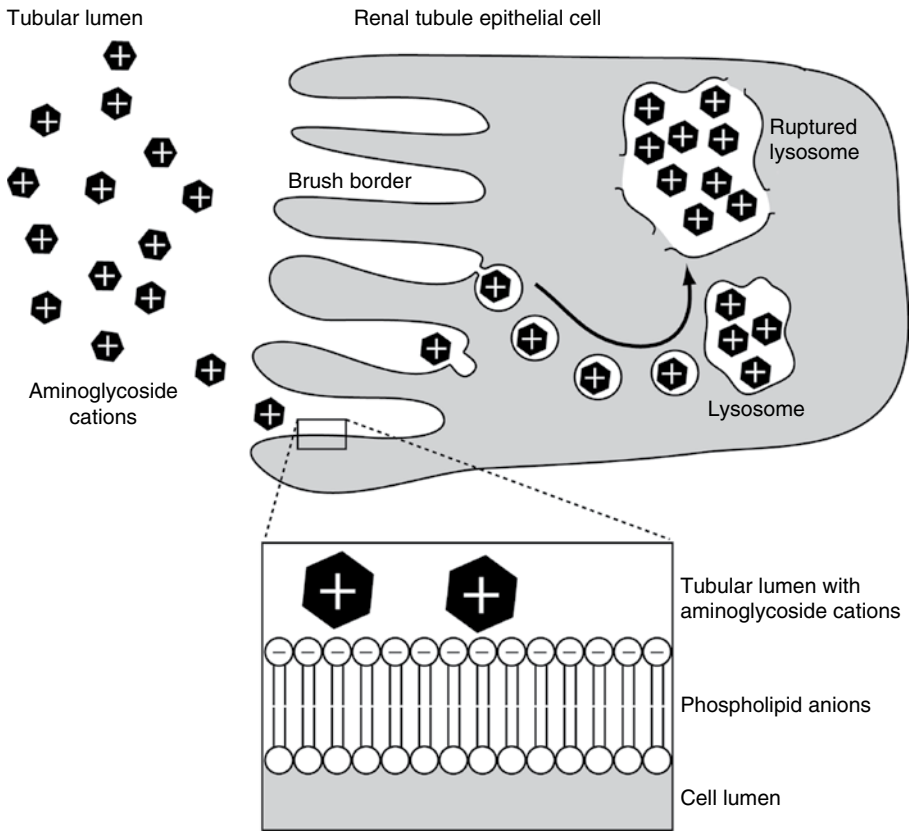

Figure 14.1. Aminoglycoside cations interact with phospholipid anions on the brush border of renal tubule epithelial cells. Then they are pinocytosed and accumulate in lysosomes until they cause the lysosome to rupture, which destroys the cell.

setting. Elevations in serum urea nitrogen and Cr confirm nephrotoxicity, but are not seen for 7 days after significant renal damage has occurred. Elimination half-lives of 24–45 hours have been reported in horses with renal toxicity, further prolonging the toxic exposure to the drug. While peritoneal dialysis is useful in lowering creatinine and serum urea nitrates, it may not be effective in significantly increasing the elimination of the accumulating aminoglycoside. The animal's ability to recover most likely depends on the type of medication exposure and the amount of healthy renal tissue remaining to compensate.

Aminoglycoside ototoxicity occurs from the same mechanisms as nephrotoxicity. The tendency to produce vestibular damage (streptomycin, gentamicin) or cochlear damage (amikacin, kanamycin, neomycin) varies with the drug. Tobramycin appears to affect both vestibular (balance) and cochlear (hearing) functions equally. This drug-specific toxicity may be due to the distribution characteristics of each drug and concentration achieved in each sensory organ. The ototoxic effect of aminoglycosides is potentiated by the loop diuretics furosemide and ethacrynic acid and probably other diuretic agents.

All aminoglycosides given rapidly IV cause bradycardia, reduce cardiac output, and lower blood pressure through an effect on calcium metabolism. These effects are of minor significance (Hague et al., 1997). Neuromuscular blockade is a rare effect, related to blockade of acetylcholine at the nicotinic cholinergic receptor. Is most often seen when anesthetic agents are administered concurrently with aminoglycosides. Affected patients should be treated promptly with parenteral calcium chloride at 10–20 mg/kg IV or calcium gluconate at

30–60 mg/kg IV or neostigmine at 100–200 µg/kg to reverse dyspnea from muscle response depression. Edrophonium at 0.5 mg/kg IV will also reverse neuromuscular blocking effects.

Dosage Considerations

Aminoglycosides produce rapid, concentration-dependent killing of Gram-negative aerobes and a prolonged PAE (McKellar, et al., 2004). Therefore, the maximum plasma concentration (C_{max}) to MIC ratio determines efficacy. A C_{max}:MIC ratio of 8–12:1 optimizes bactericidal activity. Higher initial serum concentrations may also be associated with a longer PAE. Traditionally, aminoglycosides were administered every 8–12 hours. If the aminoglycoside is dosed multiple times a day or the drug concentration remains constant, as with a continuous infusion, first exposure adaptive resistance persists and increases and the risks of nephrotoxicity and ototoxicity increases. Dose administration at 24-hour intervals, or longer, may increase efficacy by allowing time for adaptive resistance to reverse. Some clinicians have expressed reservations about once-daily dosing when intestinal damage allows continued exposure to bacteria that may replicate during the prolonged periods of subtherapeutic aminoglycoside concentrations, but this has not been documented clinically. Studies in human and veterinary patients support high-dose, once-daily therapy of aminoglycosides (Albarellos et al., 2004; Godber et al., 1995; Magdesian et al., 1998; Martin Jimenez et al., 1998; Nestaas et al., 2005). However, the optimal doses and the ideal therapeutic drug monitoring strategy are still unknown. All dosage regimens should take into account the patient's renal function, the exclusive renal excretion route of aminoglycosides, and their toxicity potential. Neonates typically have a high percentage of extracellular water than adults; therefore, the volume of distribution of aminoglycosides is higher and they typically require higher dosages.

Clinical Usage

The toxicity of aminoglycosides has largely restricted their use to the treatment of severe infections. The more toxic aminoglycosides (neomycin) are largely restricted to topical or oral use for the treatment of infections caused by Enterobacteriaceae. The less toxic aminoglycosides are usually reserved for the parenteral treatment of severe sepsis caused by Gram-negative aerobes and increasingly, the treatment of methicillin-resistant staphylococcal infections. Of these, gentamicin is usually the first choice followed by amikacin, which due to expense is reserved for sepsis caused by organisms resistant to gentamicin. But even the expensive aminoglycosides can be used for local therapy of musculoskeletal infections. Antimicrobial impregnated polymethyl methacrylate beads, collagen sponges and regional perfusion (intravenous or intraosseous) provide high local concentrations with less expense and less risk of systemic toxicity.

Because aminoglycoside residues persist in renal tissues for prolonged periods, the extra-label use in food animals should be avoided. A voluntary resolution against the extra-label administration of aminoglycosides has been adopted by the American Association of Bovine Practitioners, the Academy of Veterinary Consultants, the National Cattlemen's Beef Association and the American Veterinary Medical Association.

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Streptomycin/Dihydrostreptomycin

Streptomycin and dihydrostreptomycin are members of the streptidine group. Dihydrostreptomycin has very similar properties to streptomycin but is more likely to cause deafness. Streptomycin was the earliest aminoglycoside introduced for clinical use.

Antimicrobial Activity

Streptomycin and dihyrdrostreptomycin are active against mycobacteria, some mycoplasma, some Gram-negative rods (including *Brucella*), and some *Staphylococcus aureus*. With the exception of mycobacteria, streptomycin is the least active of the aminoglycosides. Among susceptible bacteria are *Leptospira, Francisella tularensis, Yersinia pestis*, and most *Campylobacter fetus* ssp. *venerealis* (Table 14.1). Organisms with MIC $\leq 4\,\mu g/ml$ are regarded as susceptible.

Antimicrobial Resistance

Acquired resistance to streptomycin and dihyrdrostreptomycin is widespread in veterinary pathogens and has virtually eliminated the use of these drugs except for special applications. Even agricultural use of streptomycin selects for multidrug-resistant nasal and enteric bacterial flora, including extended-spectrum beta-lactamase-producing *E. coli* (Scherer et al., 2012). Most clinically important resistance is caused by plasmid-specified enzymes, certain of which specifically inactivate only streptomycin. Plasmid-mediated resistance is commonly linked with sulfonamide, ampicillin, and tetracycline resistance genes. Chromosomal mutations to resistance arise commonly *in vitro* and often *in vivo* within a few days of treatment, although such mutants are sometimes less viable than their parents.

Drug Interactions

Streptomycin or dihydrostreptomycin are commonly combined with other drugs either to prevent the emergence of chromosomal resistance or for a synergistic effect. They are commonly synergistic with cell wall active antibiotics such as penicillin, and combination formulations were once available. This synergism occurs against Gram-positive bacteria such as streptococci, which are otherwise impermeable to the drug, and in bacteria with chromosomal mutation to low-level resistance. Synergism does not usually occur in the presence of high-level plasmid or chromosomal resistance or in Gram-negative bacteria.

Toxicity and Adverse Effects

Besides resistance, toxicity limits the use of streptomycin and dihyrdrostreptomycin. They cause vestibular damage—an effect that increases with the daily and cumulative dose, with the height of peak serum concentrations, and with preexisting renal disease. In general, no toxic effects occur if streptomycin is used at recommended doses for up to 1 week. Streptomycin can cause permanent vestibular damage, producing ataxia that progresses to incoordination, nystagmus, loss of righting reflex, and death. The effects are dose related. Daily IM injections of doses 5–10 times those recommended produce this effect in cats in about 10 days. Cats are particularly sensitive to streptomycin and usual doses may produce nausea, vomiting, salivation, and ataxia.

Neuromuscular blockade is produced when streptomycin is given at high doses. Although this effect is insignificant at normal doses, deaths have occurred in dogs and cats given high doses of penicillin-streptomycin combinations for prophylaxis of surgical infection after general anesthesia, since general anesthetics and muscle relaxants potentiate the neuromuscular blocking effects.

Table 14.3. Common dosages of aminoglycosides and aminocyclitols in animals.

Drug	Route	Dosage (mg/kg)	Interval (h)/Duration	Comments
Amikacin	IU	2 g	24 × 3 days	Metritis in mares
	IV, IM, SC	10	24 × 5–7 days	Gram-negative infections in adult horses
	IV, IM, SC	20–25	24 × 5–7 days	Gram-negative infections in neonatal foals
Apramycin	PO	12.5	24 × 7 days	Colibacillosis in swine
	IM	20	12–24 × 5 days	Salmonellosis in calves
Dihydrostreptomycin	IM	12.5–15	24 × 3–5 days	Leptospirosis in cattle, swine, and dogs
Gentamicin	IU	2.0–2.5 g	24 × 3–5 days	Metritis in mares
	IV, IM, SC	4–6	24 × 5–7 days	Gram-negative infections in adult horses, dogs, cats
	IV, IM, SC	10–14	24 × 5–7 days	Gram-negative infections in neonatal foals
Kanamycin	PO	10 mg/kg	8 × 5 days	Enteric infections in dogs
Neomycin	PO	4.5–12	24 × 3–14 days	Enteric infections. Efficacy limited due to resistance
Spectinomycin	PO	20–40	24 × 3–5 days	Colibacillosis in swine, chronic respiratory disease in chickens
	SC	11–22	Once	Fowl cholera in turkeys
	SC	10	24 × 3–5 days	Bovine respiratory disease
Spectinomycin with lincomycin	SC	20	12–24 × 3–21 days	Bacterial infections in dogs and cats
Streptomycin	PO	10 mg/kg	24 × 3–5 days	Enteric infections in chickens, swine, and calves. Efficacy limited due to resistance

Administration and Dosage

Streptomycin is only available in the United States as an oral sulfate solution administered in drinking water of chickens, swine and calves. In the United States and Canada, dihydrostreptomycin is only available in intramammary formulations along with procaine penicillin G. In Europe, streptomycin is available as an injectable product alone, and in combination with dihydrostreptomycin. Dosages of streptomycin and dihyrdrostreptomycin are shown in Table 14.3.

Clinical Applications

Dihyrdrostreptomycin or streptomycin is used in the treatment of leptospirosis in cattle, swine and dogs. Streptomycin is rarely used alone for infections in animals because of widespread resistance, particularly in Gram-negative bacteria and the penicillin combination products are no longer available. The newer aminoglycosides are more active against a greater number of organisms and are less toxic.

Cattle, Sheep, and Goats

Leptospirosis in cattle can be successfully treated by administration of dihydrostreptomycin-penicillin G (Alt et al., 2001). Oxytetracycline, tilmicosin, and ceftiofur also were effective for resolving leptospirosis and may be useful substitutes for dihydrostreptomycin, as it is no longer available for use in food-producing animals in the United States or Canada as a parenteral product. An experimental and a field study showed that either single or 5-day treatment of streptomycin in cows experimentally infected with *L. hardjo* was effective in stopping shedding for at least 70 days after treatment (Gerritsen et al., 1994; Gerritsen et al., 1993). But injectable streptomycin is no longer available in the United States or Canada.

Swine

Dihydrostreptomycin/penicillin G is effective for treatment of acute and persistent leptospirosis in swine when given at higher than label doses (Alt and Bolin, 1996). This regimen may be useful for treatment of breeding stock or animals destined for import/export.

Dogs and Cats

There seems to be little place for streptomycin in infections of dogs, other than as part of combination therapy in for brucellosis (Ledbetter et al., 2009). Streptomycin should not be used in cats.

Poultry

Streptomycin is sometimes used in oral treatment of nonspecific enteritis in chickens and, combined with penicillin, in the parenteral treatment of erysipelas in turkeys.

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Dihydrosteptamine Aminoglycosides: Neomycin Group

Neomycin is the isomeric mixture of neomycin B and C. Framycetin is identical to neomycin B. Paramomycin (aminosidine) is closely related to neomycin.

Antimicrobial Activity

Neomycin has similar activity to kanamycin on a weight basis and is several times more active than streptomycin; it is less active than gentamicin, tobramycin, and amikacin. Activity against *Staphylococcus aureus* is good but is generally low against other Gram-positive bacteria (Table 14.1). Many opportunist Gram-negative pathogens are susceptible to neomycin, although the prevalence of susceptible strains is slightly less than for kanamycin and far less than for gentamicin. Bacteria with an MIC ≤ 8 μg/ml are regarded as susceptible.

Resistance

Plasmid-mediated resistance occurs through a variety of enzymes. Such resistance, which often confers multiple drug resistance, is relatively common in enteric commensals and pathogens but less common among other opportunist pathogens.

Drug Interactions

Neomycin shows *in vitro* synergistic activity with beta-lactam antibiotics and bacitracin against Gram-positive bacteria, and it is commonly included in topical, ophthalmic (also as framycetin) and intramammary products. The combination of EDTA-Tris and neomycin is synergistic against the microorganisms associated with otitis externa in dogs (Sparks et al., 1994).

Toxicity and Adverse Effects

Neomycin is the most toxic of the aminoglycosides and readily causes nephrotoxicity and deafness. It should never be used parenterally for this reason. Toxic effects are generally not produced when administered orally or applied locally, but severe adverse effects of deafness and tubular necrosis have occurred in humans after oral administration.

Cats given high IM doses (100 mg/kg) daily showed nephrotoxic effects and became deaf in a few days; dogs are about equally susceptible. When treated for infectious enteritis with paramomycin, cats have developed acute renal failure, deafness and cataracts (Gookin et al., 1999). Total deafness was described in a dog after administration of 500 mg SC for 5 days (Fowler, 1968). In cattle, parenterally administered neomycin causes nephrotoxicity and deafness, which may be enhanced by dehydration. In pigs, transient posterior paresis and apnea immediately after injection have resulted from neuromuscular blockade. In horses, IM administration of 10 mg/kg caused renal tubular injury (enzymuria and cylindriuria) within 4 days of neomycin administration (Edwards et al., 1989).

Administration and Dosage

Neomycin is reserved for local treatment of infections, often combined with bacitracin and polymixinB ("triple antibiotic") for broad-spectrum synergistic activity. Framycetin is also used in topical ophthalmologic preparations. Neomycin is routinely incorporated into "over-the-counter" oral formulations for enteritis in animals. These formulations are largely ineffective due to widespread resistance in the Enterobacteriaceae. In some countries, neomycin is used as a parenteral injection for food animals and horses. Because of toxicity, such usage is strongly discouraged. It is also incorporated into

combination formulations for intramammary treatment of mastitis in dairy cows.

Clinical Applications

Neomycin is used for the local treatment of intestinal infections, of wound, otic, or skin infections, and of mastitis. Its relatively broad spectrum of activity and the bactericidal effect made the drug popular in some countries for parenteral use in farm animals as an inexpensive "alternative" to gentamicin. However, safer, considerably less toxic, and more efficacious alternate drugs are now readily available. Framycetin is found in some veterinary ophthalmic formulations.

Cattle, Sheep, and Goats

Neomycin is used in the oral treatment of enteric infections in ruminants, though resistance increasingly limits its effectiveness (Constable, 2004; Constable, 2009). Shull and Frederick (1978) found that routine addition of neomycin to milk powder of neonatal calves increased the frequency of diarrhea, possibly through a suppressive effect on normal intestinal microflora or through an irritant effect on the mucosa and it may increase shedding of *E. coli* O157:H7 (Alali et al., 2004). Neomycin is absorbed after oral administration (approximately 3%) and may lead to kidney residues in cattle, especially veal calves with enteritis (Pedersoli et al., 1994; Wilson et al., 1991). Routine intrauterine administration of neomycin boluses to postparturient cattle significantly increased the number of services per conception compared to controls (Fuquay et al., 1975). Neomycin is commonly incorporated into intramammary formulations for mastitis. But the use of a penicillin G-neomycin combination did not increase the efficacy of the treatment over that achieved by using penicillin G alone in bovine clinical mastitis caused by penicillin-susceptible, Gram-positive bacteria (Taponen et al., 2003).

Paromomycin is available in some countries for parenteral use for the treatment of bovine respiratory disease. Paramomycin is used in the treatment of acute cryptosporidiosis caused by *Cryptosporidium parvum* (Fayer and Ellis, 1993; Grinberg et al., 2002) but may be less effective than azithromycin for this purpose.

Swine

Neomycin is used in the oral treatment of *E. coli* enteritis in swine, although resistance increasingly limits its use.

Horses

Neomycin may be used in the local treatment of infections caused by susceptible bacteria but is too toxic to consider for parenteral administration (Edwards et al., 1989). Oral administration is suggested for selective intestinal decontamination in horses with hepatic encephalopathy.

Dogs and Cats

Neomycin is used in combination formulations for the local treatment of infections in dogs and cats, such as otitis externa, bacterial keratitis and anal sac infections. Paramomycin has been used in the treatment of cryptosporidiosis in cats (Barr et al., 1994) and leishmaniasis in dogs (Oliva et al., 2004; Oliva et al., 1998).

Poultry

Neomycin is sometimes administered orally to chickens and turkeys in the treatment of *Salmonella* infections.

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Kanamycin Group

The kanamycin group contains the kanamycins and semisynthetic derivatives such as amikacin, the nebramycins such as tobramycin and apramycin, and gentamicin, netilmicin, and sisomicin.

Kanamycin

Antimicrobial Activity

Kanamycin (Figure 14.2) has similar activity to neomycin. It is active against many species of mycobacteria and mycoplasma but is inactive against *Pseudomonas aeruginosa* and anaerobes (Table 14.1).

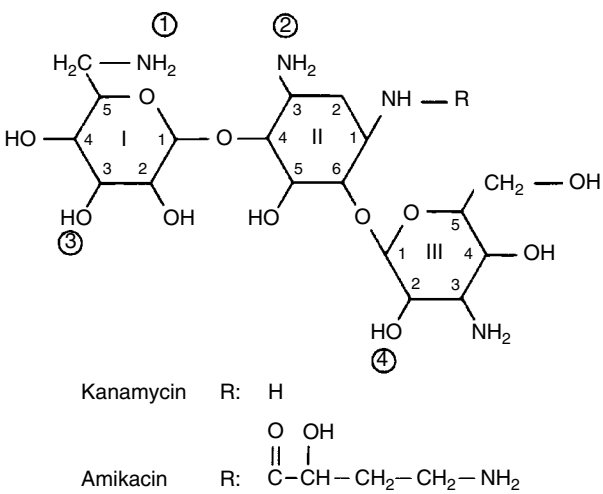

Figure 14.2. Chemical structure of kanamycin.

Bacteria with an MIC ≤ 16 μg/ml are regarded as susceptible, of 32 μg/ml as intermediate, and of ≥ 64 μg/ml as resistant.

Resistance

Plasmid-mediated resistance can occur through a variety of enzymes. Chromosomal resistance develops slowly but is far less important. Cross-resistance occurs with neomycin and one-way cross-resistance with streptomycin. Acquired resistance of *Escherichia coli* and other Gram-negative rods occurs frequently.

Toxicity and Adverse Effects

Kanamycin has a larger therapeutic index than neomycin but is less toxic on a weight basis. Although excessively high doses are toxic to dogs and cats, cats given 100 mg/kg daily SC over 30 days showed no ill effects, and neither did dogs given the same dose over 9 months (Yeary, 1975).

Clinical Applications

Kanamycin has been largely replaced for parenteral administration by more active aminoglycosides. For local applications it offers no advantage over neomycin. Kanamycin is only available in the United States as an oral product for bacterial enteritis in dogs in combination with antidiarrheals, but some injectable formulations are still available in Europe.

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Amikacin

Amikacin is a chemical modification of kanamycin (Figure 14.2), with greater activity than kanamycin, but with similar activity to gentamicin or tobramycin. Amikacin is remarkable in its resistance to most of the enzymes that inactivate the other aminoglycosides. This makes amikacin particularly valuable in the treatment of *Pseudomonas aeruginosa* infections.

Antimicrobial Activity

Susceptible bacteria (MIC ≤ 16 μg/ml) are the Enterobacteriaceae including gentamicin-resistant

Enterobacter spp., *E. coli, Klebsiella* spp., *Proteus* spp., and *Serratia* spp. Among Gram-positive bacteria, *Nocardia* spp. and staphylococci are susceptible (Table 14.4). Veterinary isolates of methicillin-susceptible staphylococci and MRSA and MRSP are typically susceptible (Rubin et al., 2011). Amikacin is typically more active than gentamicin against *P. aeruginosa*, but less active against streptococci. Resistant bacteria (MIC ≥ 64 μg/ml) include anaerobes, many streptococci and enterococci, and some *Pseudomonas* spp.

Antimicrobial Resistance

Emergence of resistance to amikacin has been uncommon compared to gentamicin and other newer aminoglycosides but hospital-associated plasmid-mediated resistance in Gram-negative bacteria has been described (Orsini et al., 1989). Resistance in *E. coli* isolates is more common in companion animals than food animals (Davis et al., 2011; Lei et al., 2010).

Pharmacokinetic Properties

Amikacin's pharmacokinetic properties are typical for an aminoglycoside. Reported volumes of distribution range from 0.15 to 0.3 L/kg and plasma elimination half-lives range from 1 to 2 hours in adult animals (Pinto et al., 2011). Protein binding is low. Elimination half-lives are prolonged in neonates, especially if they are septic or hypoxic (Green and Conlon, 1993; Green et al., 1992; Wichtel et al., 1992). Bioavailability from IM or SC injection is high (90–100%). Amikacin distributes into peritoneal fluid and synovial fluid in horses.

Table 14.4. Activity (MIC_{90}) of tobramycin, amikacin, and apramycin (μg/ml) against selected bacteria.

Organism	Tobramycin	Amikacin	Apramycin
Gram-positive aerobes			
Nocardia spp.	>32	2	
Rhodococcus equi	1	≤0.25	
Staphylococcus aureus	8	4	1
Streptococcus pyogenes	64	256	32
Gram-negative aerobes			
Actinobacillus spp.	2	8	16
Bordetella bronchiseptica	2	8	16
Campylobacter jejuni	2	2	
Escherichia coli	0.5	2	8
Klebsiella pneumoniae	8	4	4
Pasteurella multocida	2	8	16
Proteus spp.	1	4	8
Pseudomonas aeruginosa	8	16	16
Salmonella spp.	2	4	8

Drug Interactions

Amikacin is synergistic with beta-lactams (e.g., azlocillin or ticarcillin) against *P. aeruginosa*. Synergistic activity is seen when amikacin is combined with EDTA-Tris plus amikacin against canine otitis isolates of *S. pseudintermedius*, *Proteus mirabilis*, *P. aeruginosa*, and *E. coli* (Sparks et al., 1994). Combinations of amikacin and erythromycin were antagonistic against *Rhodococcus equi* isolates *in vitro* (Giguère et al., 2012).

Toxicity and Adverse Effects

Amikacin may be slightly less nephrotoxic and ototoxic than kanamycin. In animals with normal renal function, amikacin administered at recommended doses for 2–3 weeks rarely causes toxic effects. Monitoring renal function during treatment is recommended. Concerns that decreased glomerular filtration in neonatal foals might lead to a need to reduce dosage to prevent nephrotoxicity were reported to be unfounded by Adland-Davenport et al. (1990), since renal clearance was greater in foals than in adult horses. Dosage should be adjusted in cases of preexisting renal impairment, preferably guided by therapeutic drug monitoring.

Administration and Dosage

Suggested drug dosages are shown in Table 14.3. Amikacin is labeled for intrauterine use in mares and IM or SC use in dogs. It is frequently administered IV, SC, IM, by intra-articular injection, or by local venous or intraosseous perfusion in many species.

Clinical Applications

Amikacin is a broad-spectrum, bactericidal drug. It is useful for severe infections in animals, such as Gram-negative septicemia caused by gentamicin-resistant

organisms and multidrug-resistant staphylococcal infections. In human medicine, it is often combined with anti-pseudomonal penicillins in the treatment of *P. aeruginosa* infections in neutropenic patients.

Horses. Amikacin is approved for use in the United States and Canada for the intrauterine treatment of bacterial endometritis of mares and should be reserved for *P. aeruginosa* and *K. pneumoniae* infections as activity against *Streptococcus zooepidemicus* is poor. Pharmacokinetic studies support the use of 2 g intrauterine infusions once daily rather than IM treatment (Orsini et al., 1996).

Amikacin is used in neonatal foals in the treatment of septicemia or pneumonia. Magdesian and others (2004) found that a once-daily dose of 21 mg/kg in foals did not cause nephrotoxicity and suggested that once-daily dosing might be more efficacious than divided daily dosing, for reasons discussed earlier. As efficacy correlates to the C_{max}:MIC ratio, an initial dosage of 25 mg/kg q 24 h is suggested for foals to achieve peak concentrations of > 40 µg/ml (Bucki et al., 2004).

Amikacin is also used in the treatment of musculoskeletal infections caused by *Staphylococcus* spp. and Gram-negative bacteria. Due to the expense of systemic therapy, it is often administered by intra-articular injection, or by regional intravenous or intraosseous perfusion to the distal limbs. Such local administration results in high amikacin concentrations in joints and tendon sheaths and avoids systemic toxicity (Butt et al., 2001; Kelmer et al., 2012; Parra-Sanchez et al., 2006; Taintor et al., 2006). When performing intra-articular injections with corticosteriods or chondroprotective drugs (e.g., hyaluronate), because of the catastrophic consequences of sepsis, a small amount of amikacin is frequently added to the therapy (Dabareiner et al., 2003).

Dogs and Cats. Amikacin is approved for parenteral use in dogs in the United States. It is also used in cats. Indications include serious Gram-negative infections (pyelonephritis, skin or soft tissue infections) caused by otherwise resistant Enterobacteriaceae or *P. aeruginosa*, for which alternate drugs are not available or appropriate. There is increasing interest in the use of amikacin for treatment MRSA and MRSP infections (Frank and Loeffler, 2012; Papich, 2012)

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Apramycin

Apramycin, like tobramycin, is a nebramycin isolated from the fermentation of *Streptomyces tenebrans*. It has not been developed for clinical use in humans but has been used in the oral treatment of Gram-negative bacterial enteritis of farm animals.

Apramycin is active against *S. aureus*, many Gram-negative bacteria, and some mycoplasma (Table 14.4). Additional studies are required to define its spectrum of activity. Bacteria with an MIC ≤ 16 μg/ml are regarded as susceptible.

The unique chemical structure of apramycin resists most of the plasmid-mediated degradative enzymes. Resistance is rare among Gram-negative bacteria, so that many pathogenic *E. coli* and *Salmonella* isolated from animals are susceptible. The emergence of carbapenemases in Enterobacteriaceae is driving a search for therapeutic alternatives, and there is interest in apramycin for its ability to evade of rRNA methylases (Livermore et al., 2011). Livestock-associated methicillin-resistant *S. aureus* containing the apramycin resistance gene apmA have been detected (Kadlec et al., 2012). Most of these genes are located on multiresistance plasmids, which enable their co-selection and persistence.

Apramycin is approved in some countries as an injectable product for the treatment of colibacillosis in calves. In swine, apramycin is highly effective in prophylaxis and treatment of colibacillosis (Andreotis et al., 1980). The drug is incorporated into water so that pigs consume sufficient amounts to obtain 12.5 mg/kg daily for 7 days. Efficacy has been reported against naturally acquired *E. coli* infections in broilers (Cracknell et al., 1986). Enteritis significantly increases oral absorption in chickens, which may be problematic for tissue residues (Thomson et al., 1992). Tissue residues would be expected to be typical of aminoglycosides in general.

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Gentamicin

Gentamicin is one of the fermentation products of *Micromonospora purpurea;* because it is not a *Streptomyces* product, it is spelled "gentamicin," not "gentamycin."

Antimicrobial Activity

Gentamicin is one of the most active aminoglycosides (Table 14.1). The drug is active against most Gram-negative aerobic rods including many *Pseudomonas aeruginosa*, against some Gram-positive bacteria, and against mycoplasma. It is usually more active against streptococci than amikacin. Gentamicin has little activity against mycobacteria or *Nocardia* and none against anaerobic bacteria or against aerobic bacteria under anaerobic conditions. Like all aminoglycosides, it is a bactericidal, concentration-dependent killer and penetrates phagocytic cells poorly. There is widespread susceptibility among veterinary pathogenic bacteria although resistance is sometimes a problem in veterinary hospital settings (Peyrou et al., 2003; Sanchez et al., 2002).

In human hospitals, there have been explosive outbreaks of nosocomial infection caused by gentamicin-resistant bacteria of many species.

- *Susceptible bacteria (MIC ≤ 2 μg/ml [dogs and horses] or ≤ 4 μg/ml [other species])* are most Enterobacteriaceae including *Enterobacter* spp., *E. coli, Klebsiella* spp., *Proteus* spp., *Serratia* spp., *Yersinia* spp., *Brucella* spp., *Campylobacter* spp., *Haemophilus* spp., and *Pasteurella* spp. Most strains of *P. aeruginosa* are susceptible. Among Gram-positive bacteria, *S. aureus* are typically susceptible but susceptibility of streptococci and many other Gram-positive aerobes can be variable. *Prototheca zopfii* are generally susceptible. *Rhodococcus equi* is susceptible *in vitro*, but clinical efficacy is poor due to poor penetration and activity in abscesses.
- *Resistant bacteria (MIC ≥ 8–16 μg/ml)* include many Gram-positive aerobes, some *Pseudomonas* spp., and anaerobes. Strains of gentamicin-resistant *P. aeruginosa* are commonly susceptible to amikacin or tobramycin.

Pharmacokinetic Properties

Like amikacin, reported values of distribution for gentamicin range from 0.15 to 0.3 L/kg and plasma elimination half-lives range from 1 to 2 hours in adult animals. Protein binding is low. The larger volume of distribution in neonates means that the dose in should be higher than in adults, but dosage intervals need to be extended (Burton et al., 2012). Gentamicin transfers across the placenta and can achieve therapeutic concentrations in the allantoic fluid in pony mares (Murchie et al., 2006).

Drug Interactions

Gentamicin is commonly synergistic with beta-lactam antibiotics against a wide variety of Gram-negative rods, including *P. aeruginosa*. It is commonly synergistic with beta-lactam antibiotics against Gram-positive bacteria such as *Listeria monocytogenes*. Gentamicin is synergistic with trimethoprim-sulfonamide combinations against *E. coli* and *K. pneumoniae*. Antagonism may occur with chloramphenicol, tetracycline, and erythromycin. Combinations of gentamicin and rifampin are antagonistic against *Rhodococcus equi* (Giguère et al., 2012).

Injectable beta-lactam formulations are incompatible with gentamicin, so they should not be mixed in the same syringe. Care must be taken when both drugs are administered through the same intravenous line to flush thoroughly between drugs.

Halothane anesthesia causes significant changes in the pharmacokinetics of gentamicin in horses; total body clearance and volume of distribution decrease while half-life of elimination increases (Hague et al., 1997; Smith et al., 1988). In horses, concurrent administration of phenylbutazone with gentamicin decreases the elimination half-life of gentamicin by 23% and decreases the volume of distribution by 26%; while the phenylbutazone pharmacokinetics are not affected (Whittem et al., 1996).

Toxicity and Adverse Effects

Gentamicin causes the expected aminoglycoside toxic effect of neuromuscular blockade, that is exacerbated by anesthetics. It causes minor cardiovascular depressive effects; so it should not be given rapidly IV. Gentamicin is potentially ototoxic, but the major toxic effect is nephrotoxicosis, which limits prolonged use. High trough concentrations are associated with nephrotoxicity due to gentamicin accumulation in renal tubular epithelial cells. Because of the nephrotoxic potential of gentamicin, it is best reserved for severe infections. Ideally, serum drug concentrations should be monitored in treated animals. Otherwise, renal function must be carefully monitored.

Subclinical renal damage, which occurs with most therapeutic regimens, is generally reversible and clinically insignificant. Risk factors for gentamicin-induced nephrotoxicity include immaturity or old age, acidosis, concurrent use of diuretics such as furosemide, daily and total dose, fever, dehydration, previous aminoglycoside treatment, concurrent treatment with amphotericin B and non-steroidal anti-inflammatory drugs and, in the dog, pyometra. Fever decreases clearance and the volume of distribution, thus increasing plasma gentamicin concentrations.

Currently, high-dose, once-daily gentamicin therapy is recommended to maximize antimicrobial efficacy and minimize nephrotoxicity. Monitoring peak and trough serum concentrations to detect changes in the elimination half-life is the most proactive way to detect the onset of nephrotoxicity, but may be difficult to do in a clinical setting. The next best indicator is an increase in urine GGT and an increase in the urine GGT:urine

Cr ratio. Elevations in serum urea nitrogen and Cr confirm nephrotoxicity, but are not seen for 7 days after significant renal damage has occurred. Elimination half-lives of 24–45 hours have been reported in horses with renal toxicity, further prolonging the toxic exposure to the drug (Sweeney et al., 1988). While peritoneal dialysis is useful in lowering creatinine and serum urea nitrogen, it may not be effective in significantly increasing the elimination of the accumulating aminoglycoside. Nomograms based on age and renal function are used in calculating gentamicin dosage in human patients but are not available in veterinary medicine. Recent studies of population pharmacokinetic studies of gentamicin in horses showed that a considerable proportion of the individual variability recognized in gentamicin disposition could be explained by differences in body weight and serum creatinine (Martin Jimenez et al., 1998), and such data can be used to estimate the dosage for once-daily dosing. Renal damage in dogs administered the recommended dosage of gentamicin is usually mild or moderate and reversible (Albarellos et al., 2004).

Nephrotoxicity can be decreased by feeding treated animals a high-protein diet/high-calcium diet such as alfalfa to large animals and a diet higher than 25% protein to small animals, as protein and calcium cations compete with aminoglycoside cations for binding to renal tubular epithelial cells (Behrend et al., 1994; Schumacher et al., 1991). High dietary protein also increases glomerular filtration rate and renal blood flow, thereby reducing aminoglycoside accumulation. The sparing effect of the diet may be related to the competitive inhibition by protein at the proximal tubule or the nephrotoxic-sparing effect of calcium (Brashier et al., 1998).

Cats are particularly susceptible to gentamicin toxicosis, which manifests initially as loss in vestibular function, followed by nephrotoxicity. Therapeutic doses are usually safe in cats treated for reasonable periods (5 days; Hardy et al., 1985; Short et al., 1986; Waitz et al., 1971). Monitoring of renal function or therapeutic drug monitoring is advised in seriously ill cats, for which the drug should be reserved. Nephrotoxicosis in a cat associated with excessive infusion of gentamicin into an abscess has been described (Mealey and Boothe, 1994).

Antimicrobial-associated diarrhea (AAD) is the most common adverse effect of antimicrobial therapy in horses. While causality cannot be established, in a review of 5251 horses treated with antimicrobials for non-gastrointestinal signs, 32 were diagnosed with probable AAD, the most frequently used antimicrobials in horses with AAD were gentamicin in combination with penicillin (n = 7; Barr et al., 2012).

Administration and Dosage

Administration and dosages for major use species are shown in Table 14.3. Gentamicin is labeled for intrauterine use in horses (and cattle in some countries), IM or PO use in piglets, SC use in day-old poults and chicks, and IM or SC use in dogs. It is frequently administered IV, SC, IM, and by intra-articular injection, and by intravenous or intraosseous perfusion. It is used extra-label in many other species as well.

Clinical Applications

Clinical uses of gentamicin are shown in Table 14.5. Gentamicin is bactericidal against aerobic bacteria, especially Gram-negative bacteria, and is particularly useful for its activity against Enterobacteriaceae and

Table 14.5. Applications of gentamicin to clinical infections in animals.

Species	Primary Application	Comments
Horses	Gram-negative septicemia in foals, pleuropneumonia, and surgical prophylaxis for colic surgery. Metritis in mares. Infectious keratitis	Nephrotoxcity limits use
Dogs, cats	Gram-negative septicemia. Infectious keratitis. Otitis externa	Nephrotoxcity and ototoxicity limits use
Cattle, sheep, goats	Labeled for metritis in cattle in some countries. Gram-negative septicemia	Not recommended due to prolonged kidney residues
Pigs	Neonatal colibacillosis.	
Poultry	Gram-negative septicemia in poults and chicks	Labelled to treat day-old birds but is also administered *in ovo*

Pseudomonas aeruginosa. It is a drug of choice in the treatment of severe sepsis caused by Gram-negative aerobic rods, but the fluoroquinolones have a similar spectrum of activity with better tissue distribution and safety profiles.

Cattle, Sheep, and Goats. Gentamicin is of limited value in these species because of cost and prolonged tissue residues. Gentamicin is not recommended for extra-label use in ruminants in the United States or Canada. Due to renal accumulation, detectable residues may persist for years following treatment (Chiesa et al., 2006; Dowling, 2006). It has been used extra-label in the treatment of coliform mastitis in dairy cows. One well-conducted field study of cows suffering from coliform mastitis showed no beneficial effects of systemic administration of the drug (Jones and Ward, 1990). The benefit of intramammary infusion has been questioned and, experimentally, intramammary gentamicin had no beneficial effect on the course of *E. coli* mastitis in cows (Erskine et al., 1992).

Swine. Gentamicin is used to treat neonatal colibacillosis in piglets from day 1 to day 3 of age, with either a single IM injection or an oral dose of 5 mg. If multiple doses are given or if administered to older piglets, a significantly increased withdrawal time should be followed.

Horses. Gentamicin is widely used in horses because of its relatively broad spectrum of activity, the prevalence of susceptible bacteria, and the sentimental value of horses treated compared to most farm animals. Gentamicin is extensively used in horses for the treatment of pneumonia and pleuropneumonia (Mair, 1991; Raidal, 1995; Sweeney et al., 1991). It is often combined with a beta-lactam antibiotic for synergistic activity. Metronidazole is often added to the combination for treatment of pleuropneumonia in horses to extend the spectrum to beta-lactam-resistant anaerobes such as *Bacteroides fragilis*.

Gentamicin is frequently administered with a beta-lactam antibiotic to horses undergoing colic surgery (Traub-Dargatz et al., 2002). Endotoxemia increases the elimination half-life of gentamicin in these horses (Sweeney et al., 1992; van der Harst et al., 2005a,b), but gentamicin pharmacokinetics are not altered by fluid administration (Jones et al., 1998) or peritoneal lavage (Easter et al., 1997). The risk of nephrotoxicity can be reduced by providing a diet high in protein and calcium, such as alfalfa hay (Schumacher, et al., 1991).

In foals, gentamicin is often used in the treatment of Gram-negative septicemia, but because of its poor penetration of the blood-brain barrier it is ineffective in the treatment of meningitis. The drug should not be used for more than 5–7 days without monitoring renal toxicity and trough serum concentrations (Raisis et al., 1998).

Gentamicin approved for intrauterine use in mares is used in the treatment of infectious metritis in mares caused by susceptible *S. zooepidemicus, K. pneumoniae* or *P. aeruginosa*. Gentamicin should not be used routinely at or before service or insemination to avoid promoting resistance and destroying normal vaginal microflora. Stallions with *Klebsiella* or *Pseudomonas* infections of the genital tract have been successfully treated with gentamicin at 4.4 mg/kg twice daily IM or IV (Hamm, 1978).

Gentamicin is often a first-line topical therapy for bacterial ulcerative keratitis, as *S. zooepidemicus* and *P. aeruginosa* are the most frequent pathogens isolated. Susceptibility testing should be done however, as increasing resistance to gentamicin has been observed for these pathogens and ineffective therapy may be catastrophic (Keller and Hendrix, 2005;Sauer et al., 2003).

Gentamicin is administered by intra-articular injection for the treatment of septic arthritis in horses, as concentrations in synovial fluid achieved by this route exceed those achieved by parenteral administration by up to 100 times, thus exceeding the MIC of susceptible pathogens for 24 hours (Lescun et al., 2006;Meijer et al., 2000). Intraosseous or intravenous regional perfusion also achieves high local concentrations for the treatment of septic arthritis or osteomyelitis (Mattson et al., 2004;Werner et al., 2003). High dosage may cause toxic osteonecrosis (Parker et al., 2010). Gentamicin-impregnated polymethyl methacrylate beads are also successfully used to treat septic arthritis (Booth et al., 2001; Farnsworth et al., 2001; Haerdi-Landerer et al., 2010). Gentamicin-impregnated collagen sponges implanted in the tarsocrural joint of horses provides peak concentrations > 20 times the minimum inhibitory concentrations reported for common pathogens causing septic arthritis (Ivester et al., 2006).

Dogs and Cats. The widespread susceptibility of common bacterial pathogens of dogs and cats makes

gentamicin a popular drug in small animal practice, where it is used with excellent efficacy in the treatment of respiratory tract, skin and soft tissue, ocular (superficial infections), and gastrointestinal tract infections. Post-surgical infections in dogs typically involve gentamicin-susceptible organisms (Gallagher and Mertens, 2012). Gentamicin-impregnated polymethyl methacrylate beads and regional intravenous gentamicin perfusion can be used for local therapy of musculoskeletal infections (Vnuk et al., 2012). Local implantation of gentamicin-impregnated collagen sponges in dogs also appears safe and effective (Delfosse et al., 2011; Renwick et al., 2010). Gentamicin's activity against *Staphylococcus pseudintermedius* and *P. aeruginosa* has made it especially useful for topical treatment of canine otitis externa (Zamankhan Malayeri et al., 2010). But unless predisposing factors are corrected, *P. aeruginosa* often becomes resistant in chronic cases (Hariharan et al., 2006). When applied topically to clinically normal dogs with intact or ruptured tympanic membranes, gentamicin does not cause detectable cochlear or vestibular damage (Strain et al., 1995).

Poultry. Gentamicin is administered SC to 1- to 3-day old turkey poults and 1-day old chicks in the prevention and treatment of *E. coli, P. aeruginosa, Arizona paracolon* and *Salmonella* infections. It is also injected *in ovo* to eggs in hatcheries to prevent infection prior to hatching.

Camelids. Gentamicin is used in camelids for treatment of Gram-negative infections. Camelids appear susceptible to gentamicin-induced nephrotoxicty (Hutchison et al.; 1993). A pharmacokinetic study in normal adult llamas demonstrated high peak concentrations and prolonged elimination times, suggesting that gentamicin should be administered at lower doses and long dosing intervals (Dowling et al., 1996).

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Spectinomycin

Spectinomycin (Figure 14.3) is a product of *Streptomyces spectabilis*. It is an aminocyclitol antibiotic that lacks most of the toxic effects of the aminoglycoside antibiotics but, unfortunately, is limited in application by the ready development of resistance. There are discrepancies between resistance to the drug *in vitro* and apparent efficacy in some cases clinically, which have not been explained. For example, Goren et al. (1988) observed high efficacy of orally administered spectinomycin or lincomycin-spectinomycin in treating experimentally

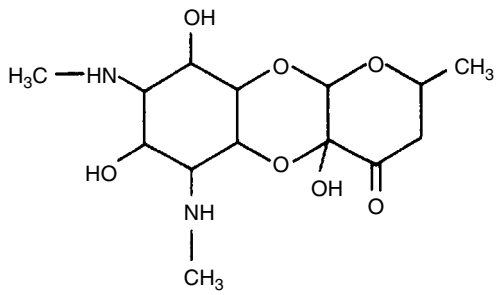

Figure 14.3. Chemical structure of spectinomycin.

induced *E. coli* infections in chickens, despite the absence of any antimicrobial activity in the serum of these chickens. To explain this discrepancy, they suggested that a metabolite or degradation product of the drug might reach the respiratory tract and interfere with bacterial attachment. This explanation is speculative since it has not been shown that the drug undergoes metabolism in any species. In humans, all of an administered dose is recovered in the urine within 48 hours after injection.

Antimicrobial Activity

Spectinomycin is a usually bacteriostatic, relatively broad-spectrum drug that can be bactericidal at concentrations 4 times MIC. It is not particularly active on a weight basis (Table 14.6). Bacteria are usually regarded as susceptible if their MIC is ≤ 20 μg/ml. Susceptibility among aerobic Gram-negative rods is unpredictable because of the presence of naturally resistant strains. *Mycoplasma* spp. are susceptible but *P. aeruginosa* is resistant.

Resistance

Natural resistance to spectinomycin in many enteric bacteria is widespread. Chromosomal one-step mutation to high-level resistance develops readily *in vivo* and *in vitro*, in a manner similar to streptomycin resistance. Chromosomally resistant strains do not show cross-resistance with aminoglycosides. Plasmid-mediated resistance is uncommon. Vaillancourt et al. (1988) reported a marked drop (from 91%–24%) of *in vitro* susceptibility of *Actinobacillus pleuropneumoniae* isolated over a 5-year period, associated with the widespread use of the drug to treat pleuropneumonia in swine. They noted, however, discrepancies between *in vitro* resistance and apparent field efficacy. Susceptibility of Gram-negative pathogens involved in bovine respiratory disease is variable (Welsh et al., 2004). *Mycoplasma bovis* isolates can acquire resistance to spectinomycin (Francoz et al., 2005).

Table 14.6. Activity (MIC_{90}) of spectinomycin (μg/ml) against selected bacteria and mycoplasma.

Organism	MIC_{90}(μg/ml)
Gram-positive aerobes	
Rhodococcus equi	8
Staphylococcus aureus	64
Streptococcus pyogenes	64
Gram-negative aerobes	
Actinobacillus pleuropneumoniae	32
Bordetella avium	>128
B. bronchiseptica	>256
Brucella canis	1
Escherichia coli	>400
Histophilus somni	25
Klebsiella pneumoniae	32
Ornithobacterium rhinotracheale	≤64
Pasteurella multocida	32
Proteus spp.	>128
Pseudomonas aeruginosa	>256
Salmonella spp.	≤64
Taylorella equigenitalis	4
Mycoplasmas	
M. bovis	4
M. bovigenitalium	4
M. hyopneumoniae	1
M. hyorhinis	1
M. hyosynoviae	4

Drug Interactions

Combination with lincomycin may marginally enhance spectinomycin's activity against mycoplasma and *Lawsonia intracellularis*.

Toxicity and Adverse Effects

Spectinomycin seems to be relatively non-toxic in animals; it does not induce ototoxicity or nephrotoxicity but may, like the aminoglycosides, cause neuromuscular blockade. The apparent lack of reported toxic effects may reflect lack of long-term usage. Administration of lincomycin-spectinomycin oral preparations, by parenteral injection to cattle, has produced heavy losses associated with severe pulmonary edema. Similar problems have

been noted with misuse of spectinomycin, and attributed to endotoxin contamination (Genetsky et al., 1994).

Pharmacokinetic Properties

Pharmacokinetic properties are similar to those of the aminoglycosides.

Administration and Dosage

Administration and dosages are shown in Table 14.3.

Clinical Applications

Spectinomycin has been largely abandoned in human medicine because of the rapid development of resistance and unpredictable antibiotic susceptibility. The drug is used in animals in the treatment of mycoplasma infections, of diseases caused by Enterobacteriaceae (*E. coli*, diarrhea, septicemia), and of respiratory disease caused by Gram-negative bacteria. The development of resistance in bacteria limits its long-term use. It is sometimes combined with lincomycin to give a broad-spectrum combination with activity against Gram-positive aerobic as well as anaerobic bacteria.

In cattle, spectinomycin was approved in the United States and Canada for SC injection (daily for 3–5 days) to treat bovine respiratory disease caused by *Mannheimia hemolytica* and *Pasteurella multocida*, but it is no longer available. It has been used successfully to treat *Salmonella dublin* infection in calves at a dosage of 22 mg/kg SC on the first day and 0.5 g PO twice daily for an additional 4 days (Cook, 1973). Combined the lincomycin, the drug was effective in treating *Ureaplasma* infection in rams (Marcus et al., 1994).

In pigs, spectinomycin is available as an oral solution for the treatment of colibacillosis. It is also administered IM for the treatment of respiratory disease, including *A. pleuropneumoniae*. Resistance has limited use for this latter purpose. While not approved for this use, IM injection of 10 mg/kg BID for 3 days has been used successfully to treat pigs severely affected with proliferative intestinal adenomatosis. The MIC of spectinomycin against *Lawsonia intracellularis* (32 μg/ml) is the lowest among the aminoglycosides but suggests that the organism is barely susceptible, at least *in vitro* (McOrist et al., 1995). Spectinomycin is available combined with lincomycin for the oral treatment of swine dysentery and the combination is effective therapy for porcine proliferative enteropathy (McOrist et al., 2000).

In dogs, spectinomycin has been administered by IM injection for a variety of infections from Gram-negative bacteria but no reports of efficacy are available. It is available combined with lincomycin and approved for the treatment of streptococcal, staphylococcal, *Mycoplasma* and *Pasteurella* infections, in dogs and cats with dosage based on 20 mg/kg IM of the spectinomycin component administered once or twice daily. The combination is effective for the treatment of tonsillitis, conjunctivitis, laryngitis, and pneumonia in dogs.

In poultry, spectinomycin is used parenterally in young poults as a single injection to control salmonellosis, pasteurellosis (fowl cholera), *E. coli*, and *Mycoplasma synoviae*. Spectinomycin can be administered in the water to control mortality associated with chronic respiratory disease and infectious synovitis in chickens. The activity of spectinomycin against mycoplasma is a particularly useful attribute but it is surprising that the drug administered orally would have any effect on systemic infections, since it is at best poorly absorbed from the intestine.

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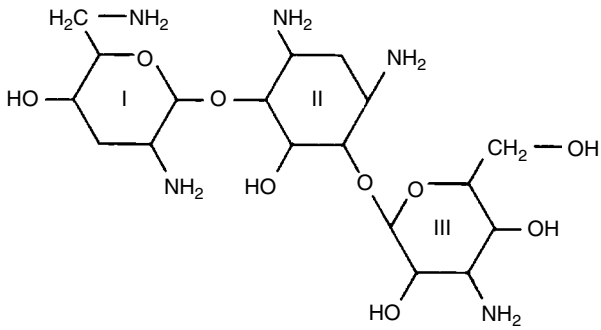

Figure 14.4. Chemical structure of tobramycin.

Tobramycin

Tobramycin is a naturally occurring deoxykanamycin (Figure 14.4) with antimicrobial and pharmacokinetic properties similar to gentamicin. Tobramycin is structurally related to kanamycin and has 4 times the activity of gentamicin against *Pseudomonas* spp., but resistance can emerge in canine isolates (Lin et al., 2012). Tobramycin is generally not effective against gentamicin-resistant strains of Enterobacteriaceae. For treatment of serious *P. aeruginosa* infections, tobramycin should be combined with an antipseudomonal penicillin. Tobramycin is less nephrotoxic than gentamicin, although ototoxic properties are similar. In a study of tobramycin pharmacokinetics in cats, Jernigan et al. (1988) found persistent elevations of blood urea nitrogen and serum creatinine, suggesting possible renal damage 3 weeks after a single dose (5 mg/kg) of tobramycin. The authors suggested that this high dose may have occupied and saturated binding sites in the kidneys from which the drug was only slowly released. Blood urea nitrogen concentrations rose in fewer cats after a lower dose (3 mg/kg). Besides evidence of renal toxicity, there was also evidence of dose-dependent differences in pharmacokinetics, suggesting that further studies of toxicity and pharmacokinetics are required in multiple-dosing studies before tobramycin can be recommended in cats. After intravenous administration to horses, tobramycin pharmacokinetics are similar to other aminoglycosides (Hubenov et al., 2007). Currently, due to the expense of systemic therapy, tobramycin use in veterinary medicine is mainly limited to the ophthalmic formulation in the treatment of bacterial keratitis due to *P. aeruginosa*. Emerging resistance to tobramycin has been documented in equine corneal infections (Sauer et al., 2003) Tobramycin has also been used in antibiotic-impregnated polymethyl methacrylate beads for the treatment of septic arthritis or osteomyelitis in horses (Holcombe et al., 1997). Tobramycin-impregnated calcium sulfate beads appear safe and effective in the treatment of staphylococcal osteomyelitis in dogs (Ham et al., 2008).

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15

Tetracyclines

Jérôme R.E. del Castillo

The tetracyclines are the class of antibiotics with the highest use in veterinary medicine. They are first-line drugs in food animals, including aquaculture species, exotic animals, and honeybees, but their use is much lower in companion animals, horses, and humans. They were the first discovered broad-spectrum antibiotics, acting against Gram-positive and Gram-negative bacteria, mycoplasmas, some mycobacteria, most pathogenic alpha-proteobacteria, and several protozoan and filarial parasites. The molecular structures of chlortetracycline and oxytetracycline were elucidated shortly after their approval. This achievement spawned a second generation of semisynthetic congeners (e.g., doxycycline) with even better pharmacokinetic and pharmacodynamic properties. But the spread of tetracycline resistance and the introduction of new large-spectrum antibiotics limited their medical use between the 1970s and the 2000s. In the last 20 years, the discovery of their beneficial non-antibiotic properties, and the emergence of multiresistant nosocomial pathogens, has spurred the development of a new generation of tetracyclines that evade most of their resistance mechanisms, or are anti-inflammatory drugs devoid of anti-infective properties.

Chemistry

The tetracyclines are substituted 2-naphtacene carboxamides (Figure 15.1). All first-generation congeners are produced by *Streptomyces* strains that possess aromatic polyketide synthases. Until recently, the newer tetracyclines were obtained by chemically modifying the first-generation molecules (i.e., semisynthesis), but a high-yield enantioselective synthesis route may now produce several second- and third-generation molecules (e.g., glycylcyclines). Structurally, the carboxamide group flanked by a β-keto-enol group (carbons 1–3), the α-oriented dimethylamine (carbon 4), and the oxygenated groups on the lower half of the tetracyclines (carbons 10–12a) are required to retain antibiotic activity. The β-keto-enol group of carbons 11, 11a, and 12 is a chelation site for multivalent cations (e.g., Ca^{2+}), and carbons 5–9 are sites for facultative chemical substitutions that change the liposolubility of the molecule (Figure 15.1). The latter two properties greatly influence their pharmacokinetic and pharmacodynamic properties.

The tetracyclines are amphoteric drugs that are ionized at all pH values. In solution, they form a mixture of zwitterions, cations, and anions, respective proportions of which depend on the pH of the medium. At pH values ranging between 4 and 7, the zwitterionic form predominates; its null net charge favors its passage across cell membranes. As the tetracyclines are sparingly soluble in water, they are formulated as acid or basic salts that may be administered orally or parenterally. This class of drug molecules is fairly stable at physiological pH values with the exception of chlortetracycline, which degrades in basic mediums at a rate that increases with pH.

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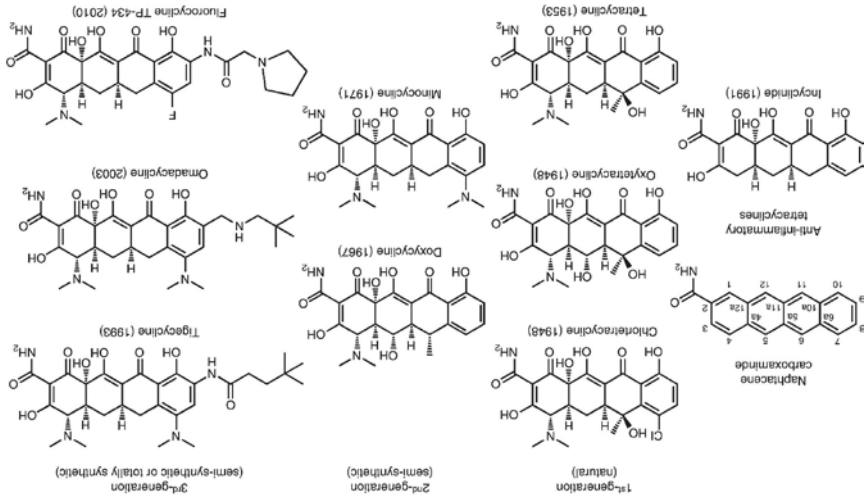

Figure 15.1. Structures of the tetracyclines scaffold (naphtacene carboxamide showing the carbon numbering) and an anti-inflammatory-only derivative, and of the most significant first-, second-, and third-generation tetracycline antibiotics.

Mechanism of Action

The tetracyclines are pleiotropic drugs that classically are used as protein synthesis inhibitors. Upon binding to the 16S RNA (rRNA) and S7 protein of the 30S bacterial ribosome, they allosterically inhibit the binding of aminoacylated transfer RNA (AA-tRNA) to their docking site (A-site) on the ribosome. This halts the process of peptide synthesis. Overall, they exert a bacteriostatic effect on susceptible bacterial pathogens, with time-dependent bactericidal activity that has been proven at least for tigecycline and doxycycline. They exert antiparasitic activity by inhibiting protein synthesis in endosymbionts or organelles that possess a genome and prokaryote-like ribosomal components. For instance, they alter the apicoplasts of *Plasmodium falciparum*, and likely of coccidia and *Babesia*. As a result, their progeny inherit defective organelles that shorten their lifespan. In filaria, they kill the endosymbiont *Wolbachia pipientis* that is essential to the growth and fertility of the nematode, and plays key role in its evasion from the host immune mechanisms (McHaffie et al., 2012).

The tetracyclines possess an adjunct anti-inflammatory activity that is valuable in controlling infectious disease. They inactivate the matrix metalloproteinases by interacting with the structural (not catalytic) Zn^{2+} and/or Ca^{2+} of these proteins, and they scavenge the reactive oxygen species. Finally, the tetracyclines have been shown to reduce the infectivity of pathogenic prions in animals and currently are subject to clinical trials against Creutzfeldt-Jakob disease.

Antimicrobial Activity

The tetracyclines are classic broad-spectrum antibiotics. They exhibit activity against a range of Gram-positive and Gram-negative bacteria, including the Mycoplasmataceae, *Coxiella* and Chlamydiales, and alpha-proteobacteria such as *Anaplasma* spp., *Ehrlichia* spp., *Neorickettsia* spp., *Rickettsia* spp., and *Wolbachia* spp. Their spectrum of activity also includes many protozoan parasites such as *Entamoeba histolytica*, *Giardia lamblia*, *Leishmania major*, *Plasmodium falciparum*, *Trichomonas* spp., and *Toxoplasma gondii*.

Tetracycline is the representative molecule in drug sensitivity testing because it is more stable in culture media than its congeners. However, the antibacterial potency of these drugs positively correlates with lipid solubility: the semisynthetic derivatives are most active, followed by the chlorinated tetracyclines, and lastly by oxytetracycline and tetracycline. It is noteworthy that the decay of chlortetracycline in culture media biases its estimation of antimicrobial potency, especially against slow-growing organisms (e.g., *Mycoplasma*). Table 15.1 lists the cumulative estimates of MIC for a number of pathogens: they must be considered with caution, as their associated MIC distributions must be examined for proper interpretation, since they are conservative potency estimates for molecules other than tetracycline, and the cumulative MIC estimates are always associated with exponential measurement error.

- *Good or moderate activity (MIC ≤ 4 μg/ml)*: The tetracyclines exhibit good to moderate activity against the following Gram-positive aerobes: *Bacillus* spp., *Corynebacterium* spp., *Erysipelothrix rhusiopathiae*, *Listeria monocytogenes*, some streptococci and against the following Gram-negative bacteria: *Actinobacillus* spp., *Bordetella* spp., *Borrelia* spp., *Brucella* spp., *Campylobacter fetus*, *Francisella tularensis*, *Haemophilus* spp., *Lawsonia intracellularis*, *Leptospira* spp., *Mannheimia* spp., *Pasteurell*a spp., including *P. multocida*, and *Yersinia* spp. (Table 15.1). They are also active against *Anaplasma* spp., *Chlamydia* and *Chlamydophila* spp., *Coxiella burnetii*, *Ehrlichia* spp., *Mycoplasma* spp., *Rickettsia* and *Neorickettsia*, and some anaerobes including *Actinomyces* spp. and *Fusobacterium* spp.
- *Variable susceptibility*: Because of acquired resistance, among Gram-positive bacteria, many isolates of enterococci, staphylococci and; streptococci may be resistant. Among Gram-negative bacteria many Enterobacteriaceae including *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp. and *Salmonella* spp. may be resistant. Anaerobes such as *Bacteroides* spp. and *Clostridium* spp. show variable susceptibility. Some isolates of *Mannheimia haemolytica* may also be resistant.
- *Resistant (MIC ≥ 16 μg/ml)*: Most *Mycobacterium* spp., some enterobacteria (*Proteus mirabilis*, *Serratia* spp.), *P. aeruginosa*, and some *Mycoplasma* spp. are resistant.

Resistance

To reach the ribosome, tetracyclines must first complex with Mg^{2+} to cross the Gram-negative outer cell wall via a porin. The periplasmic acidity dissociates the

Table 15.1. *In vitro* activity (MIC_{90}, μg/ml) of tetracycline against bacteria including *Mycoplasma*.

Organism	MIC_{90}	Organism	MIC_{90}
Gram-positive aerobes			
Arcanobacterium pyogenes	16	*Staphylococcus aureus*	> 64
Bacillus anthracis	4	*Streptococcus agalactiae*	0.25
Corynebacterium pseudotuberculosis	≤ 0.25	*S. dysgalactiae*	> 32
C. renale	4	*S. suis*	64
Erysipelothrix rhusiopathiae	0.25	*S. uberis*	0.5
Listeria monocytogenes	1	*S. equi (*ssp. *zooepidemicus* and *equi)*	> 16
Rhodococcus equi	8		
Gram-negative aerobes			
Actinobacillus spp.	≤ 0.25	*Klebsiella pneumoniae*	≥ 16
A. pleuropneumoniae	≥ 16	*Moraxella bovis*	1
Bordetella avium	≥ 16	*Manheinemia haemolytica*	≥ 16
B. bronchiseptica (pig)	≥ 16	*Pasteurella* spp. (horse)	≤ 2
Brucella canis	0.25	*P. multocida* (pig)	1
Campylobacter fetus	2	*Proteus* spp.	≥ 16
C. jejuni	≥ 64	*Pseudomonas* spp.	≥ 16
Escherichia coli	≥ 64	*Salmonella* spp.	≥ 16
Haemophilus parasuis	0.5	*Taylorella equigenitalis*	0.5
Histophilus somni	2		
Anaerobes			
Actinomyces spp.	1	*Clostridium* spp	8
Bacteroides fragilis	2	*C. perfringens*	32
Bacteorides spp.	25	*C. difficile*	16
Fusobacterium necrophorum	4	*Dichelobacter nodosus*	0.12
Mycoplasma			
Mycoplasma bovirhinis	0.5*	*M. hyorhinis*	2
M. bovis	4*	*M. hyosynoviae*	32
M. canis	16	*M. ovipneumoniae*	0.5
M. hyopneumoniae	0.03	*Ureaplasma* spp.	0.06
M. agalactiae	0.5		
Spirochetes			
Borrelia burgdorferi	1		
Leptospira spp.	4		

*Some reports show resistance.

drug-cation complex, and provides motor ions for carrier-mediated passage of drug molecules across the cytoplasmic membrane.

Resistance to tetracyclines can be mediated by different mechanisms: (1) energy-dependent efflux systems, most of which being antiporters that exchange an extracellular H^+ for a cytoplasmic drug-Mg^{2+} complex; (2) ribosomal protection proteins that dissociate the tetracyclines from their binding site near the ribosomal AA-tRNA docking site; (3) flavin-dependent enzymatic hydroxylation of carbon-11a, which disrupts the tetracyclines' β-keto-enol involved in the chelation of cations and ribosome binding; (4) ribosomal 16S RNA mutation at the primary binding site of tetracyclines; and (5) stress-induced down-regulation of the porins through which the drug crosses the outer Gram-negative wall. The first two mechanisms are by far the most common. Currently, almost 50 resistance genes have been reported, some of which are mosaic genes.

Acquired resistance to tetracyclines is widespread among enteric bacteria and mycobacteria, but they still are useful drugs against many pathogens of veterinary importance. Fortunately, resistance is extremely rare among obligate intracellular pathogens such as *Anaplasma*,

Chlamydia, and *Ehrlichia*. However, horizontal transmission of tetracycline resistance was recently found in a *Chlamydia suis* isolate. Tetracycline-resistant bacteria may carry more than one tetracycline resistance gene, which often are on different mobile elements (chapter 3).

Pharmacokinetic Properties

The absorption, distribution and elimination of the tetracyclines all depend on factors such as their molecular size, lipid/buffer partition behavior, plasma protein binding, the acidity of biological mediums, their exposure to multivalent cations (Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}), and the expression level of P-glycoprotein (P-gp) in the cell membranes they face.

To be absorbed, the tetracyclines administered as solid oral dosage or long-acting injectable formulations must undergo the process of drug release. Dissolution in the gastric fluids is the critical step to the absorption from solid oral tetracycline forms. Some excipients of the injectable products retain the tetracyclines at the injection site via different mechanisms that delay their absorption; for example, tissue irritation. The type of tetracycline salt influences its solubility and release, and therefore its extent of absorption (i.e., bioavailability). In dogs and cats, this parameter varies among oral tetracycline preparations. Water and feed acidifiers improve the release and absorption of tetracyclines from medicated feeds in pigs.

The bioavailability of these drugs after oral administration depends on their lipid/buffer partition, and is hampered by complexation with multivalent cations that precipitate with increasing pH, and by food particles (particularly of dairy products). For instance, the mean oral bioavailabilities of oxytetracycline and chlortetracycline respectively are 5% and 37% in non-fasting calves, and 5% and 28% in fed pigs. Bioavailability is further reduced when fed with milk or milk replacer but is much higher in fasted calves and pigs. The oral bioavailability of feed-administered doxycycline is approximately 22%. Hence, steady-state plasma drug concentrations reached with medicated feeds in food-animal species may not cover the whole MIC range of sensitive pathogens, but chlortetracycline and doxycycline are 2–3 doubling dilutions lower (i.e., more potent) than tetracycline. Oral doxycycline is of limited usefulness in horses due to the low systemic exposure it achieves, presumably as a result of poor oral bioavailability. In horses given 10 mg/kg doxycycline q 12 h for several days, the average peak serum concentration was 0.46 μg/ml. This is in contrast to peak serum concentrations of 3.5 μg/ml in dogs receiving a dose of 5 mg/kg.

The distribution of tetracyclines is highest in richly perfused organs: kidney > liver ≥ lungs > blood = synovia > muscle. Because they are substrates of P-gp, the tetracyclines cross the blood-brain barrier with difficulty, and at a rate that depends on their lipid solubility. The tetracyclines vary in their binding to serum albumin: doxycycline > minocycline = chlortetracycline > tetracycline > oxytetracycline. The limited evidence available suggests that minocycline has greater capacity than other tetracyclines to penetrate cellular barriers, as it attains higher concentrations in poorly accessible fluids such as tears and prostatic fluid. The tetracyclines are among a limited number of osteotropic drugs. Their multivalent cation-chelating properties cause their deposition in teeth and at sites of new bone formation. This feature has toxicological consequences that will be discussed further. The drugs cross the placenta to reach the fetus and are secreted in milk, where they reach concentrations approximating those of serum.

The tetracyclines are excreted primarily by glomerular filtration, by biliary secretion at an extent that depends on their lipid solubility (e.g., approximately 5% of total clearance for doxycycline in dogs), and by intestinal excretion via P-gp. Minocycline is also subject to oxido-reductive reactions, the main metabolites of which are 9-OH-minocycline and mono-N-demethylated molecules. As glomerular filtration is their mechanism of excretion, impaired renal function can increase their elimination half-life.

Tetracyclines undergo enterohepatic circulation, with much of the drug excreted in bile being reabsorbed from the intestine. This process contributes to the half-life of 6–10 hours, which is unusually long for drugs that are eliminated mainly by renal excretion.

Chlortetracycline has shorter mean residence time than other congeners because of its spontaneous degradation in neutral to basic mediums. Neighboring effects of the carbon-7 chlorine on the carbon-6 hydroxyl group result in the production of iso-chlortetracycline, a molecule devoid of antibiotic activity. Besides, all tetracyclines are subject to reversible epimerization of carbon-4 at pH values between 2 and 6, especially when exposed to substances such as phosphate, urea, and multivalent

cations. The 4-epitetracyclines are more pH-stable and water soluble, but their antimicrobial efficacy is much lower than the original molecules.

Drug Interactions

The absorption of tetracyclines is impaired by antacids containing Al^{3+} or other multivalent cations, by iron-containing preparations, and by bismuth subsalicylate. Synergism between tetracyclines and tylosin or tiamulin against respiratory pathogens including *Mycoplasma* and *Pasteurella* has been described and may occur with other macrolides and other bacteria. Combination with polymyxins may also give synergistic effects by enhancing bacterial uptake of the drugs. Doxycycline is synergistic with rifampin or streptomycin in the treatment of brucellosis. Doxycycline was synergistic with pyrimethamine in the effective treatment of toxoplasmosis in experimentally infected mice.

Toxicity and Adverse Effects

From a toxicologic perspective, the tetracyclines are relatively safe. They are irritants that may cause vomiting after oral dosing, and tissue damage at injection site. Similarly to other inhibitors of the bacterial protein synthesis, these antibiotics cause imbalances of the intestinal flora. Their ability to bind calcium is associated with acute cardiac toxicity. They also induce apoptosis in osteoclasts, which may cause chronic bone toxicity. Their most serious adverse effects are attributed to anhydrotetracyclines that damage the plasma membranes and bind to serum albumin. These tetracycline degradation products that are found in expired or poorly preserved drug products have been associated with renal toxicity, and likely in hepatic and cardiovascular toxicity.

Although not well documented in veterinary medicine, tetracyclines are associated with dose-related functional changes in renal tubules (Riond and Riviere, 1989). Tetracycline-induced renal toxicosis may be exacerbated by dehydration, hemoglobinuria, myoglobinuria, toxemia, or the presence of other nephrotoxic drugs (Riond and Riviere, 1989). Nephrotoxicosis has been reported especially in foals receiving high doses for the treatment of contracted tendons. In dogs, fatal nephrotoxicosis has been reported after the IV administration of tetracyclines at higher than recommended dosages.

Severe liver damage can follow overdosage of tetracyclines in animals with preexisting renal failure and may also be associated with late pregnancy. In cattle, high doses (33 mg/kg IV) have led to fatty infiltration of the liver and severe proximal renal tubule necrosis. Tetracyclines should be administered to cattle only in recommended doses to avoid problems of nephrotoxicosis (Lairmore et al., 1984). Transient hemoglobinuria with trembling and subnormal temperatures lasting 4 hours has been reported with long-acting formulations (Anderson, 1983). Rapid IV administration in cattle has been followed by collapse, probably the result of calcium binding and consequent cardiovascular depression (Gyrd-Hansen et al., 1981), although the propylene glycol vehicle may be responsible. Intravenous injections of all forms of tetracyclines should be given slowly to cattle over a period of not less than 5 minutes (Gyrd-Hansen et al., 1981).

Malabsorption because of moderate diarrhea may occur in calves after oral administration of therapeutic doses. In horses, the most feared side effect of tetracyclines is enterocolitis due to alteration of intestinal microflora and superinfection with resistant *Salmonella* or unidentified pathogens that may include *Clostridium difficile*. This occurs in only a small percentage of treated horses.

Oxytetracycline irritates tissues. Marked differences have been found in the different formulations of oxytetracyclines in this respect (Nouws et al., 1990). The more irritating the product, the lower the bioavailability and the greater the associated drug persistence at the injection site. The long-acting formulations containing glycerol formaldehyde or dimethylacetamide are particularly irritating.

Administration to growing puppies or pregnant bitches results in yellow discoloration of primary and, to a lesser extent, permanent teeth. But their chronic use in pig and rodent models induce apoptosis in the osteoclasts, which hinders the process of bone remodeling. This causes an increase in bone mineral density and conformation.

Tetracyclines have antianabolic effects that may produce azotemia. Such effects can be exacerbated by corticosteroids. The drugs may also cause metabolic acidosis and electrolyte imbalance.

Administration and Dosage

Recommended dosages are shown in Table 15.2. Tetracyclines are available both in capsular and tablet forms and are usually administered PO to dogs and cats. Milk, antacids, and ferrous sulfate interfere with absorption.

Table 15.2. Usual dosages of tetracyclines in selected domestic animal species.

Species	Drug	Route	Dose (mg/kg)	Interval (h)	Comments
Dogs and cats	Chlortetracycline, oxytetracycline	PO	20	8	
	Oxytetracycline	IV	10	12	slow IV
	Doxycycline, minocycline	PO, IV	5–10	12	slow IV
Horses	Oxytetracycline	IV	5	12	slow IV
	Doxycycline	PO	10	12	
	Minocycline	PO	4	12	
		IV	2.2	12	
Ruminants	Oxytetracycline, tetracycline	IM, IV	10	12–24	slow IV
	Long acting	IM	20	48	
Swine	Oxytetracycline, tetracycline	IM	10–20	12–24	
	Long acting	IM	20	48	
	Doxycycline	PO	10	12	

Because of poor water solubility, oxytetracycline dihydrate is subject to "flip-flop" absorption, and cannot reach similar plasma and tissue concentrations than the hydrochloride salt. Intramuscular injection of tetracyclines cannot be recommended for horses or companion animals because of local tissue damage and pain, and erratic absorption. The recommended dose in cattle is 10 mg/kg given IM or preferably IV, because of variability in absorption. The long-acting oxytetracycline parenteral preparation containing 2-pyrrolidone as excipient is approved for IM use in cattle and swine only. Owing to its "flip-flop" absorption kinetics, a single IM dose of 20 mg/kg provides serum concentrations of oxytetracycline above 0.5 μg/ml for 48 hours, but appears to offer no advantage over the same dose of the conventional drug IM (Nouws, 1986). Subcutaneous injection in cattle maintains similar serum concentrations to those following IM administration and appears to be better tolerated. To prevent adverse effects, it is important to differentiate between the conventional and the long-acting formulation in dosage decisions.

Clinical Applications

The primary indications for tetracyclines are in the treatment of bacterial pathogens involved in the bovine and porcine respiratory disease complexes, borreliosis, brucellosis, chlamydiosis, ehrlichiosis, *Lawsonia* proliferative enteropathy, leptospirosis, listeriosis, porcine mycoplasmosis, rickettsiosis, and tularemia. The older tetracyclines have been used for many years in managing infectious diseases in food animals because of their low cost, broad antimicrobial activity, ease of administration, and general effectiveness. However, their widespread use has undoubtedly contributed to very widespread resistance in Enterobacteriaceae and other important pathogenic bacteria.

The tetracyclines' capacity to attain effective concentrations in most tissues, together with their broad-spectrum of activity, makes them particularly useful in the treatment of mixed bacterial infections. The activity of the agents against obligate intracellular pathogens such as *Anaplasma*, *Chlamydia*, *Ehrlichia*, *Rickettsia*, and some *Mycoplasma* makes them the drugs of choice in treatment of infections caused by these microorganisms. Although recommended for the treatment of plague, results in the treatment of experimental infections in animals have sometimes been disappointing. The lipophilic character of the newer tetracyclines (minocycline, doxycycline) allows them to attain concentrations in sites such as the prostate, which are poorly accessible to older members of the group. One disadvantage of tetracyclines over a number of other antimicrobial drugs is their bacteriostatic action, so that treatment may need to be for longer than with bactericidal drugs.

Tetracyclines are commonly used in the treatment of brucellosis, usually in combination with rifampin or streptomycin. Doxycycline and minocycline are more effective than older tetracyclines because of better penetration into cells. Treatment with doxycycline should last 6 weeks and with streptomycin 7–14 days. Tetracyclines (particularly minocycline and doxycycline)

are also used in the treatment of infections caused by other intracellular bacteria, including *Coxiella* and *Ehrlichia*.

Cattle, Sheep, and Goats

Many of the microorganisms that cause bovine pneumonia are susceptible to tetracyclines at concentrations that can be achieved in lung tissue. The drugs are generally useful in the treatment of bovine pneumonias and also in their prophylaxis, especially in feedlots. Nevertheless, increasing resistance in *Mannheimia haemolytica* and variable susceptibility of *Mycoplasma bovis* limits their effectiveness. The long-acting parenteral formulation, which must be administered by IM injection (or in some formulations, SC), 20 mg/kg at 48-hour intervals on 2–4 occasions, may be adequate in treating lower respiratory disease in cattle, sheep, and goats.

If tetracyclines are administered orally to feedlot cattle in the prophylaxis of pneumonia, they should be given in feed and not water. Administration in water may increase mortality (Martin et al., 1982), possibly because of the difficulty of ensuring that even amounts are ingested. While prophylactic administration of drug in the ration appears often to reduce pneumonia and to improve growth and feed conversion efficiency, the cost-to-benefit ratio may not justify this approach. In addition, such a practice tends to promote resistance among *Mannheimia* organisms. In prophylaxis of feedlot pneumonia, parenteral administration gives better effects than oral administration because of higher bioavailability. An approach shown to be useful is to inject tetracyclines when animals enter feedlots or to inject a single dose of long-acting tetracycline to all animals as soon as some in the lots appear to be developing pneumonia.

Clostridial infections and listeriosis can be treated by tetracyclines. A recommended dosage in neural listeriosis is 10 mg/kg/day IV, but clinical trials are needed to determine whether the same dose given twice daily or the use of ampicillin or penicillin G might not be more effective. In listeriosis, IV administration of the conventional preparation (parenteral aqueous solution) is preferred. In human medicine, minocycline is a recognized alternative to ampicillin.

Oxytetracycline is the drug of choice in acute *Anaplasma marginale* infections. However, short-term therapy with oxytetracyclines fails to clear the *A. marginale* infections in carrier cattle (Coetzee et al., 2005). Long-acting tetracyclines are effective in preventing *Babesia bovis* and *B. bigemina* (redwater) in cattle. Tetracyclines are used in the treatment of, and prevention against, heartwater disease caused by *Ehrlichia ruminantium* (Mebus and Logan, 1988). The drugs are also used in the prophylaxis of East Coast fever caused by *Theileria parva* (Chumo et al., 1989) and tickborne fever caused by *Anaplasma phagocytophilum* (Cranwell, 1990).

For infectious keratoconjunctivitis in cattle, 2 doses of the long-acting preparation given 3 days apart can be recommended (George et al., 1988). Long-acting tetracyclines produced moderate cure rates in cattle with dermatophilosis. Long-acting tetracyclines (at 3- to 4-day intervals for 5 treatments) combined with streptomycin (IM daily for 7 days) successfully treated 14 of 18 cows with *B. abortus* infection (Nicoletti et al., 1985). Administration once daily as a topical spray (25 mg/ml) was effective in controlling bovine papillomatous digital dermatitis, the efficacy increasing with an increasing number of days of applications (Shearer and Elliott, 1998).

Tetracyclines achieve milk concentrations approximating those of blood, but because of poor bioavailability after IM injection, they are best given IV. They are second-choice parenteral antibiotics for serious infections of the udder caused by Gram-positive bacteria and possibly by coliforms, although susceptibility among the latter organisms is uncommon. Repeated intramammary administration of tetracycline in combination with tylosin cured experimentally induced *Mycoplasma californicum* mastitis in cows (Ball and Campbell, 1989).

In enzootic abortion in sheep caused by *Chlamydophila abortus*, experimental and field evidence suggests that 2 treatments of 20 mg/kg of the long-acting preparation at 2-week intervals, starting 6–8 weeks before lambing, will reduce the prevalence of abortions. The drug may be most useful at the start of outbreaks (Greig and Linklater, 1985). Tetracycline is the drug of choice in the prevention and treatment of Q fever (*Coxiella burnetii*). Lambs can be protected from the rickettsial agent of tickborne fever and associated infections by a single injection of long-acting tetracycline formulation (Brodie et al., 1986). Duration of the effect is between 2 and 3 weeks (Brodie et al., 1988). A single injection of long-acting tetracycline with topical tetracycline is an effective treatment of ovine keratoconjunctivitis caused by *Mycoplasma conjunctivae* (Hosie, 1988; Hosie and Greig, 1995). Long-acting oxytetracycline was highly successful

in preventing *M. haemolytica* pneumonia in sheep (Appleyard and Gilmour, 1990), and has been used successfully in the treatment of ovine footrot (Grogono-Thomas et al., 1994), and dermatophilosis (Jordan and Venning, 1995).

Long-acting tetracyclines combined with streptomycin were shown to successfully treat about 80% or more of rams with *Brucella ovis* infection (Marin et al., 1989; Dargatz et al., 1990). Daily intraperitoneal injections of 1000 mg oxytetracycline hydrochloride eliminated *Brucella melitensis* infection from sheep (Radwan et al., 1989).

Swine

Tetracyclines are commonly used in swine to prevent and treat atrophic rhinitis and bacteria associated with the porcine respiratory complex (*A. pleuropneumoniae*, M. hyopneumoniae, *P. multocida*). They also are effective against *L. intracellularis*. Field outbreaks of *Pasteurella* pneumonia have been controlled by feed medication (200–400 g/ton). Feed medication with chlortetracycline, 100 g/ton, has been used to control adenomatosis and at a much higher level, 800 g/ton, to eradicate *Leptospira* from the kidneys of swine (Stahlheim, 1967). Tetracyclines may be effective against erysipelas and *Haemophilus* infections, but these pathogens are better controlled with the beta-lactams. Enterotoxigenic *E. coli* and *S. suis* are usually resistant. Tetracyclines in feed or water have been used successfully to control streptococcal lymphadenitis and *M. hyopneumoniae* infection.

Orally administered oxytetracycline in pigs has an average bioavailability of 5% across studies, tetracycline is 18% bioavailable, chlortetracycline is between 18% and 28% bioavailable, and doxycycline is 22% bioavailable on average. The long-acting oxytetracycline formulations were more effective than the conventional formulations in preventing experimental *A. pleuropneumoniae* infections when administered 48 hours before challenge, but no more effective in treatment. An average dose of 11 mg doxycycline/kg bodyweight q 24 h in feed for 8 days was effective in controlling pneumonia due to *P. multocida* and *M. hyopneumoniae* in pigs (Bousquet et al., 1998).

Horses

The clinical use of oxytetracycline in horses has long been controversial because of early anecdotal reports of severe enterocolitis. While oxytetracycline may, like many other antimicrobial agents, cause enterocolitis, the vast majority of treated horses do not exhibit side effects. Nevertheless, the main factor limiting the use of tetracyclines in horses is their limited spectrum against common equine pathogens as well as the irritant nature of injectable products.

Oxytetracycline is active *in vitro* against most equine non-enteric Gram-negatives such as *Actinobacillus* spp. and *Pasteurella* spp., and approximately 70% of *Staphylococcus* spp. However, at clinically achievable concentrations, oxytetracycline is active against only 50–60% of Enterobacteriaceae and β-hemolytic streptococci. Doxycycline is generally safe when administered orally to horses, but it has poor bioavailability. Oxytetracycline or doxycycline is the treatment of choice for infections caused by *A. phagocytophilum*, *B. burgdorferi*, and *N. risticii* in horses. These microorganisms typically have a very low MIC (≤ 0.25 μg/ml). Oxytetracycline is also highly effective in the treatment of *A. phagocytophilum* and *N. risticii* infections in horses (Madigan and Grible, 1987; Palmer et al, 1992). Administration of oxytetracycline to ponies experimentally infected with *B. burgdorferi* by tick exposure resulted in elimination of persistent infection. In contrast, doxycycline or ceftiofur were inconsistent in eliminating persistent infection in this experimental model (Chang et al., 2005). Oxytetracycline or doxycycline has been used successfully in the treatment of infections by *Lawsonia* infection in foals (Sampier et al., 2006).

Dogs and Cats

Tetracyclines are drugs of choice for *A. phagocytophilum*, *Ehrlichia canis*, and *Rickettsia rickettsii* infections. Doxycycline administered orally to dogs infected with *R. rickettsii* is effective in preventing the disease or treating acute illness but may not remove the carrier state. In experimental *Brucella canis* infection, the most effective of several treatments, was minocycline (22 mg/kg every 12 hours for 14 days) co-administered with streptomycin (11 mg/kg every 12 hours for 7 days), but effectiveness must be monitored in the laboratory (Flores-Castro and Carmichael, 1978). Field efficacy of tetracycline and streptomycin was 74% in one study (Nicoletti and Chase, 1987). Tetracycline hydrochloride, 10 mg/kg PO every 8 hours, was successful for the treatment of *P. aeruginosa* urinary tract infections in dogs because of the high urine concentrations of the drug attained (Ling et al., 1980).

Other indications in dogs include treatment of Lyme borreliosis and leptospirosis. Minocycline delivered in a subgingival local delivery system improved the clinical and microbiologic response in dogs with periodontitis following root scaling and planing (Hayashi et al., 1998). Doxycycline administered orally for 3 weeks achieved complete remission of about half of canine patients with superficial pyoderma and partial remission in another 40%, but complete remission in only 14% of patients with deep pyoderma and partial remission in another 51% (Bettenay et al., 1998).

Cats suffering from *Chlamydophila felis* infection of the upper respiratory tract and conjunctiva should be treated with tetracyclines for 14 days to eliminate the organism and to remove the latent carrier state. Tetracyclines are drugs of choice for the treatment of *Mycoplasma haemofelis*. Prolonged oral treatment with doxycycline does not eliminate the carrier state in *Bartonella henselae* or *B. clarridgeae* infection (Kordick et al., 1997). Treatment by tetracyclines of a cat with *Yersinia pestis* infection was only temporarily effective (Culver, 1987).

Poultry

Tetracyclines are effective in the treatment of chlamydophilosis if administered for prolonged periods. Tetracycline or chlortetracycline can be administered in 1% medicated feed (45 days), and doxycycline has been administered at 100 mg/kg IM at 5-day intervals on 6 or 7 occasions (Gylsdorff, 1987) or orally twice daily for 20 days. Tetracyclines are also used in the treatment of chronic respiratory disease (*Mycoplasma gallisepticum*) and infectious synovitis (*Mycoplasma synoviae*), as well as of fowl cholera (*P. multocida*). Prolonged administration of oxytetracycline (250 ppm) in feed is required to control *M. gallisepticum* infection in birds. One report noted the surprising efficacy of tetracycline sorbate in the oral treatment of naturally occurring *Aspergillus fumigatus* infection (Roy et al., 1991).

Uses Unrelated to Their Antibacterial Activity

Tetracyclines have a number of non-antibiotic effects that are better documented for the second- and third-generation molecules. They include anti-inflammatory properties, immunosuppression, inhibition of lipase and collagenase, antinociceptive, antiosteoporotic, and wound-healing effects. Experimentally, tetracyclines have protected mice from endotoxin-induced shock by reducing inflammatory cytokine and nitric oxide production. Minocycline is neuroprotective in many experimental models of neurodegenerative diseases, central nervous system injury, and viral encephalitis owing to its antiapoptotic and reactive oxygen species-scavenging properties.

Its matrix-metalloproteinase inhibitory effects have been shown to be beneficial for various conditions such as rheumatoid arthritis, gingivitis, acute lung injury, myocardial disease, and cancer. This might be the main mechanism of action in the treatment of contracted tendons in foals. In one study, intravenous administration of oxytetracycline, at a dose of 44 mg/kg, resulted in a decrease in the angle of the metacarpophalangeal joint for approximately 96 hours. These high doses of oxytetracycline to foals with preexisting renal damage or hypovolemia, or to foals unable to nurse sufficiently, may result in acute renal failure.

Glycylcyclines

The glycylcyclines are the first approved members of the third-generation tetracyclines. They retain the mechanism of action of the tetracyclines and circumvent their main resistance mechanisms (i.e., efflux pump and ribosomal protection system), but they are substrates of the bacterial hydrolases.

Tigecycline is a minocycline holding a tert-butylglycylamino group on carbon-9 (Garrison et al., 2005). Tigecycline is available only as an injectable formulation, which restricts its use to hospital settings. It is unsuitable for oral administration due to its higher formula weight (584 Da), and lipid/buffer partition coefficient (log-P > 10).

Tigecycline binds 5 times more strongly to ribosomes than minocycline or tetracycline, which may lead to decreased sensitivity toward ribosomal protection resistance mechanisms. It is active against a broad range of Gram-positive, Gram-negative and anaerobic microorganisms including multidrug-resistant strains of *Staphylococcus* spp. and *Enterococcus* spp., but is not active against *Pseudomonas* spp. (Garrison et al., 2005). Several laboratory animal studies describing the efficacy of tigecycline have been published. In people, nausea and vomiting are the most important side effects. There are currently no published studies evaluating tigecycline in domestic animal species.

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16

Chloramphenicol, Thiamphenicol, and Florfenicol

Patricia M. Dowling

Chloramphenicol is a stable, lipid-soluble, neutral compound. It is a derivative of dichloracetic acid and contains a nitrobenzene moiety. This *p*-nitro group is associated with idiosyncratic (non-dose-dependent) aplastic anemia in humans (Figure 16.1). Thiamphenicol has a similar antibacterial spectrum to chloramphenicol but differs from the parent compound in that the *p*-nitro group attached to the benzene ring is replaced by a sulfomethyl group. Florfenicol is a structural analogue of thiamphenicol that also lacks the *p*-nitro group, and it is more active than thiamphenicol. Neither thiamphenicol nor florfenicol are associated with dose-independent aplastic anemia in humans or any other species, but both are associated with dose-dependent bone marrow suppression.

Chloramphenicol

Mechanism of Action

Chloramphenicol is a potent inhibitor of microbial protein synthesis. It binds irreversibly to a receptor site on the 50S subunit of the bacterial ribosome, inhibiting peptidyl transferase and preventing the amino acid transfer to growing peptide chains and subsequently inhibiting protein formation. Chloramphenicol also inhibits mitochondrial protein synthesis in mammalian bone marrow cells in a dose-dependent manner.

Antimicrobial Activity

Chloramphenicol is active against a wide range of Gram-positive and many Gram-negative bacteria (Table 16.1), against which it is usually bacteriostatic. Anaerobic bacteria are inhibited at usual therapeutic concentrations (5–15 μg/ml). Chloramphenicol suppresses rickettsial and chlamydial growth. While mycoplasma often show susceptibility *in vitro*, chloramphenicol therapy of mycoplasma pulmonary infections is often ineffective.

- *Susceptible organisms (MIC ≤ 8 μg/ml)* include among Gram-positive aerobic bacteria, including *Actinomyces* spp., *Trueperella pyogenes, Bacillus anthracis, Corynebacterium* spp., *Erysipelothrix rhusiopathiae, Listeria monocytogenes*, many *Enterococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudintermedius* (MRSP) have emerged as a significant pathogens in companion animals. Two major clonal MRSP lineages have disseminated in Europe and North America. Isolates originating from North America are often susceptible to chloramphenicol, whereas isolates from Europe are often resistant to chloramphenicol (Perreten et al., 2010). *Staphylococcus schleiferi* isolated from pyoderma dogs is typically susceptible (Vanni et al., 2009). Typically susceptible Gram-negative aerobic bacteria include *Actinobacillus* spp., *Bordetella*

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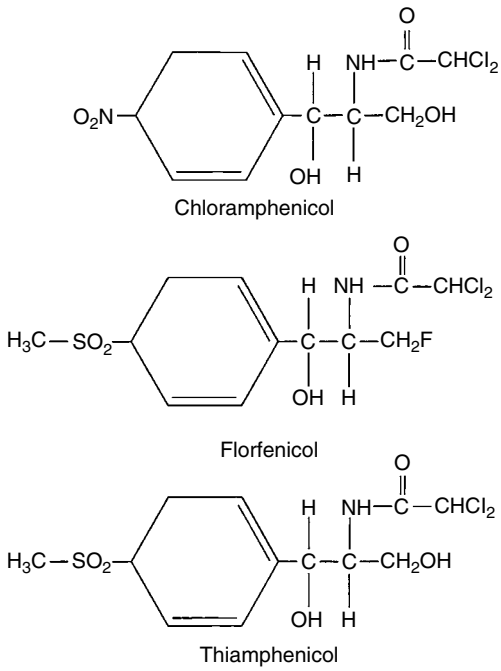

Figure 16.1. Chemical structure of chloramphenicol, florfenicol, and thiamphenicol.

bronchiseptica, Brucella canis, Enterobacteriaceae (including many *E. coli), Klebsiella* spp., *Proteus* spp., and *Salmonella* spp., *Haemophilus spp., Histophilus somni*, *Leptospira* spp., *Moraxella bovis*, *Mannheimia hemolytica*, and *Pasteurella* spp. Anaerobes (*Bacteroides* spp., *Clostridium* spp., *Prevotella* spp., *Porphyromonas* spp.) are commonly susceptible, including penicillin-resistant *Bacteroides fragilis*.

- *Intermediately susceptible organisms (MIC = 16 μg/ml)* include *Rhodococcus equi*.
- *Resistant organisms (MIC ≥ 32 μg/ml)* include *Mycobacterium* spp. and *Nocardia* spp. Resistance often emerges in Gram-negative enteric bacteria such as *E. coli*.

The most frequently encountered mechanism of bacterial resistance to chloramphenicol is enzymatic inactivation by acetylation of the drug by chloramphenicol acetyltransferases (CATs). Acetylation of the hydroxyl groups on chloramphenicol prevents drug binding to the 50S ribosomal subunit. There are also reports of other mechanisms of resistance, such as efflux systems, inactivation by phosphotransferases, and mutations of the target site or permeability barriers (Schwarz et al., 2004). The CAT genes are commonly found on plasmids in Enterobacteriaceae and Pasteurellaceae, and most of these plasmids carry one or more additional resistance genes. The efflux of chloramphenicol from bacteria can be mediated by either specific transporters or multidrug transporters. Specific transporters tend to have a substrate spectrum limited to a small number of structurally related compounds while the multidrug transporters often have a wide range of unrelated substances as substrates. Specific transporters commonly mediate higher levels of resistance compared to multidrug transporters. Many of the genes coding for the CAT genes or specific transporters are located on mobile genetic elements, such as plasmids, transposons or gene cassettes. When plasmids mediating resistance to chloramphenicol are transferred from one bacterium to another, they are not always able to replicate in the new host. Recombination between the new plasmid and the plasmids already resident in the new host effectively circumvents replication problems. Such recombination may lead to the formation of novel resistance plasmids that carry the resistance genes of both parental plasmids and are well adapted to replication in the new host.

Pharmacokinetic Properties

In monogastric animals and pre-ruminant calves, chloramphenicol is typically well absorbed from the gastrointestinal tract. The oral bioavailability of chloramphenicol in foals is 83%, but only 40% after a single administration in mares; declining to 20% after 5 doses (Brumbaugh et al., 1983; Gronwall et al., 1986). Chloramphenicol palmitate is poorly absorbed in cats. In ruminants, orally administered chloramphenicol is inactivated in the rumen. The apparent volume of distribution of chloramphenicol is large (> 1 L/kg) in all species. This can be attributed to widespread distribution, as partitioning of the drug is independent of pH and there is no evidence of selective tissue binding. Because of its lipid solubility and moderately low protein binding (30–46%), chloramphenicol attains effective concentrations in most tissues and body fluids, including cerebrospinal fluid (CSF) and the central nervous system. Chloramphenicol may achieve CSF concentrations up to 50% of plasma concentrations

Table 16.1. Activity (MIC_{90}) of chloramphenicol (μg/ml) against selected bacteria and mycoplasma.

Organism	MIC_{90}	Organism	MIC_{90}
Gram-positive aerobes			
A. pyogenes	1		
B. anthracis	2	*L. monocytogenes*	8
C. renale	4	*S. aureus*	8
Enterococcus spp.	>32	*S. dysgalactiae*	4
E.rhusiopathiae	2	*S. uberis*	2
Gram-negative aerobes			
Actinobacillus spp.	4	*Klebsiella* spp.	> 32
B. bronchiseptica	8	*Pasteurella* spp.	> 32
B. canis	4	*M. haemolytica*	2
Enterobacter spp.	> 32	*P. multocida*	2
E. coli	> 32	*Proteus* spp.	>32
H. somni	1	*P. aeruginosa*	>32
Anaerobes			
Bacteroides spp.	8	*D. nodosus*	0.25
B. fragilis	8	*Fusobacterium* spp.	1
C. difficile	4	*F. necrophorum*	2
C. perfringens	4	*S. hyodysenteriae*	4
Mycoplasma			
M. bovis	8	*M. hyopneumoniae*	4
M. bovirhinis	64	*M. ovipneumoniae*	16
M. canis	8		

when the meninges are normal and more when inflammation is present. Topical ophthalmic formulations achieve therapeutic concentrations in the aqueous humor. Chloramphenicol readily diffuses into milk, and pleural and ascitic fluids. It readily crosses the placenta, achieving concentrations 75% of those in maternal plasma. This may be of clinical significance, as the fetal liver is deficient in glucuronyl transferase activity. Penetration of the blood-prostate barrier is relatively poor unless inflammation is present.

The elimination half-life of chloramphenicol varies widely between species. Elimination is primarily by hepatic metabolism by conjugation with glucuronic acid. Its elimination is short in horses (1 hour; Sisodia et al., 1975) and long in cats (5–6 hours) because of feline deficiencies in glucuronide conjugation (Watson, 1991). A fraction of the dose is excreted unchanged by glomerular filtration in the urine of dogs (10%) and cats (20%), while a negligible amount is eliminated by renal excretion in herbivores. The metabolites, which are inactive, are excreted in the urine and to a much lesser extent in the bile. The glucuronide conjugate excreted in bile can be hydrolyzed by intestinal flora to liberate the parent drug.

In newborn animals the elimination half-life of chloramphenicol is considerably longer than in adult animals of the same species. This is due mainly to immature glucuronide conjugation mechanisms. Glucuronide conjugation develops most rapidly in foals, so that the half-life in the 1-week-old foal approaches that of the adult horse.

Drug Interactions

Chloramphenicol should not be used concurrently with bactericidal antimicrobials in treating infections where host defenses are poor. Concurrent chloramphenicol and penicillin G have been shown to be antagonistic in treating bacterial meningitis and endocarditis in humans. Chloramphenicol acts on the same ribosomal site as macrolides antibiotics. Chloramphenicol is antagonistic to the fluoroquinolones, as inhibition of protein synthesis by chloramphenicol interferes with the production of autolysins necessary for cell lysis after the fluoroquinolone interferes with bacterial DNA supercoiling.

Because chloramphenicol inhibits microsomal enzyme activity, hepatic metabolism (oxidative reactions and glucuronide conjugation) of drugs given concurrently is slowed, resulting in prolonged pharmacologic

effect. Thus chloramphenicol markedly prolongs the effect of barbiturates, and fatal effects have been observed in epileptic dogs treated concurrently with phenobarbital (Adams and Dixit, 1970).

Toxicity and Adverse Effects

The main toxic effects of chloramphenicol in humans are bone marrow depression, which can be either an idiosyncratic, non-dose-dependent aplastic anemia or a dose-dependent anemia from suppression of protein synthesis. Aplastic anemia appears to be a genetically determined idiosyncrasy of individual humans. The incidence of fatal aplastic anemia has been estimated as 1 in every 25,000–60,000 humans who use the drug. A few cases of aplastic anemia in humans have occurred following contact exposure (ophthalmic use, medicated sprays, handling), so that veterinarians and owners should wear protective gloves and face masks when handling chloramphenicol products (Wallerstein et al., 1969).

A "gray baby" syndrome occurs in newborn infants because their deficiency in glucuronic acid conjugation causes a dose-dependent anemia. In animals, chloramphenicol toxicity is related to both the dose and duration of treatment, and cats are more likely than dogs to develop toxicity. In cats, clinical signs of toxicity may be seen when the usual maintenance dosage of 25 mg/kg of base or palmitate ester is given twice daily for 21 days (Watson, 1991). Chloramphenicol causes changes in the peripheral blood and bone marrow due to reversible, dose-related disturbances in red cell maturation. Administration for less than 10 days using the maintenance dose is not likely to cause toxicity in either dogs or cats, unless the animals have depressed hepatic microsomal enzyme activity or severely impaired renal function. Use in dogs for MRSA and MRSP infections is associated with frequent adverse gastrointestinal effects (vomiting, diarrhea, weight loss, nausea, anorexia and decreased appetite), as well as lethargy, shaking, increased liver enzymes, and anemia (Bryan et al., 2012).

Administration and Dosage

Recommended drug dosages of chloramphenicol are given in Table 16.2.

Chloramphenicol is a broad-spectrum, time-dependent bacteriostatic drug that can attain effective concentrations at sites of infection that are relatively inaccessible to other antimicrobials. Therapeutic efficacy is maximized by maintaining an average steady-state plasma concentration of 5–10 μg/ml.

Chloramphenicol is available for either oral (free base or palmitate ester) or parenteral (sodium succinate) administration. For local treatment of eye or ear infections caused by susceptible organisms, topical preparations are available.

Because the drug is well absorbed from the gastrointestinal tract in small animals, it can be given orally as either the base or the palmitate ester. The ester is hydrolyzed prior to absorption of the free base. The intake of food does not influence bioavailability. Subcutaneous injection of chloramphenicol sodium succinate is an alternative to oral administration. While both routes may provide equivalent concentrations, the oral route is preferable as injection of the parenteral preparation is painful. The total length of treatment should not exceed 10 days, especially in cats. Do not administer chloramphenicol to patients with evidence of or suspected bone marrow suppression.

The short half-life of chloramphenicol in horses (1 hour), together with its generally bacteriostatic action, makes IV administration impractical. Oral tablets of the free base drug can be administered PO or the sodium succinate formulation can be given by IM injection. After absorption from injection sites, the inactive succinate ester is rapidly hydrolyzed to the active drug.

Because of the risks of idiosyncratic aplastic anemia in humans, chloramphenicol is banned for use in food animals in most countries. The drug should not be used in the early neonatal period unless plasma concentrations are monitored, and should be used with caution in pregnant animals because of the potential adverse effects on the fetus.

Clinical Applications

The potential for idiosyncratic fatal aplastic anemia in humans has led to prohibition of chloramphenicol use in food animals in many parts of the world. Florfenicol is the appropriate analogue to use in food animals. With the development of fluoroquinolone antimicrobials for companion animals, there were few primary indications for the use of chloramphenicol, but it was still considered for some anaerobic infections, serious ocular infections, prostatitis, otitis media/interna and salmonellosis in

Table 16.2. Usual systemic dosing rate (mg/kg) of chloramphenicol in animals.*

Species	Dosage Form	Route	Dose (mg/kg)	Interval (h)	Comments
Dogs, cats	Base, palmitate	Oral	50	12	Limit to 10 days of therapy
	Sodium succinate	IV, IM, SC	25–50	8–12	
Horses	Sodium succinate	IM	30–50	6	
	Base, palmitate	PO	25–50	6–8	

*Owners should be warned of the risks of their exposure to chloramphenicol.

horses, dogs and cats. Use in dogs and cats has been increasing in frequency due to the increase in MRSA and MRSP infections, but chloramphenicol is associated with more adverse effects (mainly gastrointestinal) than other treatment options such as doxycycline, clindamycin and amikacin (Bryan et al., 2012). Human toxicity from handling chloramphenicol should be discussed with the owner and appropriate precautions taken when prescribing chloramphenicol for use in dogs and cats. In addition, the zoonotic potential of animal-origin staphylococci should be discussed with owners (Guardabassi et al., 2004).

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Thiamphenicol

Thiamphenicol is a derivative of chloramphenicol, in which the *p*-nitro group been replaced by a sulfomethxyl group. Thiamphenicol is generally 1–2 times less active than chloramphenicol, although it has equal activity against *Haemophilus, B. fragilis*, and streptococci. Cross-resistance with chloramphenicol is complete in bacteria that possess CATs. Absorption and distribution are similar to chloramphenicol, and it is also equally well distributed into tissues. Oral bioavailability in pre-ruminant lambs and calves is 60% (Mengozzi et al., 2002). Thiamphenicol is not eliminated by hepatic gluronide conjugation but excreted unchanged in the urine. Unlike chloramphenicol, its elimination is unaffected by liver disease and by the use of other drugs metabolized in the liver. The pharmacokinetic parameters of thiamphenicol follow allometric scaling, in that values for elimination half-life and volume of distribution increase with body size from mice through rats, rabbits, dogs, pigs, sheep and calves (Castells et al., 2001). Therapeutic concentrations are achieved in milk of lactating cows (Abdennebi et al., 1994).

One reason for major interest in thiamphenicol is that, because it lacks the *p*-nitro group, it does not induce irreversible bone marrow aplasia in humans, although it may cause dose-dependent bone marrow suppression more frequently than chloramphenicol.

Thiamphenicol is used extensively in Europe and Japan but is not available in North America. Apart from

its bacteriostatic character and lower activity than chloramphenicol, thiamphenicol appears underutilized in the treatment of many infections caused by susceptible organisms. While detailed dosage information is not available because of the lack of pharmacokinetic and clinical studies, suitable dosage in animals would appear to be similar to that of chloramphenicol. Dosages for cattle and pigs are 10–30 mg/kg IM every 24 hours, 30 mg/kg PO every 12 hours for pre-ruminant lambs and every 24 hours for pre-ruminant calves, or 50–200 ppm in feed for pigs and 100–500 ppm in feed for chickens.

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Florfenicol

Florfenicol is a fluorinated derivative of thiamphenicol, in which the hydroxyl group at C-3 has been replaced with fluorine. Florfenicol is a potent inhibitor of microbial protein synthesis with the same mechanisms of action as chloramphenicol. Like thiamphenicol, florfenicol does not cause idiosyncratic aplastic anemia in humans but can cause dose-dependent bone marrow suppression in animals.

Antimicrobial Activity

Florfenicol is slightly more active than chloramphenicol in its range of antimicrobial activity (Table 16.3). Florfenicol remains highly active against the pathogens involved in bovine respiratory disease (Portis et al., 2012). It is bactericidal against *Histophilus somni* and *Pasteurella* spp. at concentrations only one dilution above those that are bacteriostatic. The MIC_{90} for *Actinobacillus pleuropneumoniae*, *Histophilus somni*, *Mannheimia haemolytica*, *Trueperella pyogenes*, *Pasteurella multocida* and *Streptococcus suis* is ≤ 2 μg/ml.

Table 16.3. Activity (MIC_{90}) of florfenicol (μg/ml) against selected bacteria and mycoplasma.

Organism	MIC_{90}
Porcine	
A. pleuropneumonia	0.5
P. multocida	0.5
B. bronchispetica	8
S. suis	2
Bovine	
M. haemolytica	2
P. multocida	0.5
H. somni	2
A. pyogenes	1.56
Salmonella dublin	32
M. bovis	4
L. monocytogenes	32.0
Fish	
Edwardsiella ictaluri	0.25
Aeromonas salmonicid	1.6
Vibrio anguillarum	0.5
Photobacterium damsela	0.6
Chryseobacterium spp.	32.0

The mutant prevention concentration for *Mannheimia haemolytica* is ≥ 32 μg/ml (Blondeau et al., 2012). *Fusobacterium necrophorum*, *Bacteroides melaninogenicus* and *Moraxella bovis* are highly susceptible. The MIC_{90} for Enterobacteriaceae, which are less susceptible, is higher; for example, for *Salmonella dublin* it is 32 μg/ml. Florfenicol is active against a number of important bacterial pathogens of fish including *Aeromonas salmonicida, Vibrio salmonicida, Vibrio anguillarum* and *Yersinia ruckeri* in salmon and trout and *Edwardsiella ictaluri* in catfish.

Because of the substitution of a hydroxyl group with a fluorine molecule, florfenicol is less susceptible to resistance from bacteria expressing CAT enzymes. But new mechanisms of bacterial resistance to chloramphenicol and florfenicol are being identified (Liu et al., 2012; Tao et al., 2012). Florfenicol resistance in Gram-negative bacteria is related to plasmid transfer of the *floR* gene. This gene codes for a membrane-associated exporter protein that promotes efflux of chloramphenicol and florfenicol (Schwarz et al., 2004). In cases of neonatal calf diarrhea from *E. coli*, if *floR* is present, the MIC range is 16 to ≥ 256 μg/ml (White et al., 2000).

The *floR* gene was identified in *Pasteurella multocida* isolated from a calf in 2005 (Kehrenberg and Schwarz, 2005) and has now been identified a bovine isolate of *Mannheimia haemolytica* (Katsuda et al., 2012). After a single dose of florfenicol, feedlot cattle show a shift in fecal flora to multiresistant *E. coli*, likely due to selection for plasmids containing the *floR* gene linked with other resistance genes. The antimicrobial resistance associated with florfenicol treatment declined over 4 weeks post-treatment but a higher proportion of fecal *E. coli* were resistant than when the cattle entered the feedlot (Berge et al., 2005).

Pharmacokinetic Properties

The oral bioavailability of florfenicol in horses is 83% (McKellar and Varma, 1996). It is 89% in 2- to 5-week-old calves, but decreases when administered with milk replacers (Varma et al., 1986). After intramuscular injection, bioavailability is 81% in horses and 38% in lactating dairy cattle but 54% after intramammary infusion (Soback et al., 1995). Ten hours after IM administration to dairy cows, milk concentrations peak at 1.6 μg/ml and it takes at least 5 days for milk concentrations to deplete to undetectable concentrations. Milk depletion is significantly prolonged with subcutaneous administration, so administration by this route should be avoided in dairy cows. While values of volume of distribution for florfenicol are slightly lower than for chloramphenicol, florfenicol is well distributed into many tissues including lungs, muscle, bile, kidney and urine. With IV administration, cerebrospinal fluid concentrations are 46% of plasma concentrations, achieving potentially therapeutic concentrations for *H. somni*, but not Gram-negative enteric bacteria (de Craene et al., 1997). With IM administration to beef calves, the serum concentration of florfenicol remains above 1 μg/ml for 22 hours (Lobell et al., 1994). The commercially available formulation of florfenicol is long-acting, so that "flip-flop" kinetics occurs, where elimination is prolonged due to slow absorption from the IM or SC injection site. In cattle, 64% of a dose is excreted as parent drug in the urine. Florfenicol amine is the slowest metabolite to deplete from the liver and is used as the marker residue for withdrawal times.

While not approved, florfenicol is used extra-label in a number of species. Pharmacokinetics have been described in sheep, goats, North American elk, rabbits, alpacas, and dogs (Alcorn et al., 2004; Ali et al., 2003; Atef et al., 2001; Holmes et al., 2012; Kim et al., 2011; Koc et al., 2009; Lane et al., 2004; Lane et al., 2008; Palma et al., 2011; Shen et al., 2004).

Drug Interactions

There are no published data on adverse drug interactions with florfenicol. Mechanistically, interactions should be similar to those seen with chloramphenicol.

Toxicity and Adverse Effects

Transient diarrhea or inappetance may occur in cattle treated with florfenicol, but resolves within a few days of discontinuing treatment. In swine, peri-anal inflammation and/or rectal eversion may occur in treated animals, but should resolve completely within 1 week. The injectable florfenicol formulations for cattle and swine are only labeled for a maximum of 2 doses, so bone marrow suppression has not been reported with clinical use in these species. Potentially fatal bone marrow suppression, from suppression of protein synthesis in erythroid cells, has been documented with over dose or prolonged florfenicol administration (Holmes, et al., 2012; Tuttle et al., 2006).

Administration and Dosage

Florfenicol is approved in numerous countries in beef cattle for the treatment of respiratory disease, pododermatitis and keratoconjunctivitis caused by highly susceptible bacteria (MIC ≤ 2 μg/ml) at 20 mg/kg IM twice at a 48-hour interval or 40 mg/kg SC once. Each injection site should not exceed 10 ml. The label dosage does not result in concentrations that would be effective against Gram-negative enteric pathogens. In some countries, florfenicol is approved for the treatment of swine respiratory disease from *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* at 15 mg/kg IM twice at a 48-hour interval. In swine it should be injected into the neck at no more than 5 ml per site.

In the United States, florfenicol is approved as a premix for swine for the control of swine respiratory disease associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Streptococcus suis*, and *Bordetella bronchiseptica*. In Canada, florfenicol is approved as a 2.3% concentrate solution for oral administration to swine for the treatment of swine respiratory disease associated with *Actinobacillus*

pleuropneumoniae and *Pasteurella multocida* and to broiler chickens for the treatment and control of air sacculitis associated with *E. coli* susceptible to florfenicol. As well in Canada, florfenicol is approved for the treatment of furunculosis caused by susceptible strains of *Aeromonas salmonicida* in salmon. In the United States, it is approved for control of catfish mortality due to enteric septicemia associated with *Edwardsiella ictaluri*. In Japan, florfenicol is labeled for the treatment of pseudotuberculosis and streptococcosis in *Perciformes* (yellowtail, amberjack, red sea bream, tilapia, etc.) and for the treatment of edwardsiellosis disease in eel. The fish formulation is mixed in unmedicated feed prior to pelleting or used to surface coat pelleted feed and fed to deliver 10 mg/kg per day for 10 consecutive days (Gaikowski et al., 2003).

Clinical Applications

Currently, florfenicol is used for metaphylaxis and for treatment of bovine respiratory disease caused by highly susceptible bacteria such as *Mannheimia, Pasteurella* and *Histophilus* (Hoar et al., 1998). The same dosage regimen will treat pododermatitis caused by *Fusobacterium necrophorum* and *Bacteroides melaninogenicus* and infectious bovine keratoconjunctivitis caused by *Morexella bovis*, but penicillin or oxytetracycline are less expensive and narrower in antimicrobial spectrum and should be used as first line treatments for these infections. When administered to lactating dairy cows, florfenicol readily crosses into milk, and residues are more prolonged after SC than IM administration. While it has high systemic bioavailability, intramammary administration of florfenicol for the treatment of bovine mastitis caused by a variety of pathogens had no advantage over cloxacillin (Wilson et al., 1996).

Florfenicol in feed or by injection reduces illness due to *Actinobacillus pleuropneumoniae* and *M. hyopneumoniae* in pigs.(Ciprian et al., 2012; Del Pozo Sacristan et al., 2012; Palacios-Arriaga et al., 2000). Oral florfenicol is effective in broiler chickens for the treatment and control of air sacculitis associated with *E. coli* susceptible to florfenicol.

Florfenicol is used in the treatment of susceptible bacterial diseases of fish, including furunculosis in salmon and vibriosis in salmon and cod, pseudotuberculosis in Japanese yellowtail, enteric septicemia in channel catfish, and enteric redmouth in trout.

The use of florfenicol in horses is not recommended. Despite a high oral bioavailability and good tissue distribution, florfenicol administration to horses altered fecal consistency with single doses administered IV, PO, or IM (McKellar and Varma, 1996). In a chronic dosing study using the cattle formulation at 20 mg/kg IM every 48 hours, all horses remained clinically normal but had significant alterations in fecal flora (Dowling, 2001).

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17

Sulfonamides, Diaminopyrimidines, and Their Combinations

John F. Prescott

The value of the sulfonamides as single antimicrobial agents has been greatly diminished both by widespread acquired resistance and by their relatively low potency compared to more modern antimicrobial drugs. However, when combined with antibacterial diaminopyrimidines such as trimethoprim, resistance occurs less frequently and thus their usefulness has been enhanced.

Sulfonamides

Chemistry

The sulfonamides are derivatives of sulfanilamide, which contains the structural prerequisites for antibacterial activity. The sulfonamides differ in the radical (R) attached to the amido ($-SO_2NHR$) group or occasionally in the substituent on the amino ($-NH_2$) group (Figure 17.1).

The various derivatives differ in physicochemical and pharmacokinetic properties and in degree of antimicrobial activity. As a group, sulfonamides are quite insoluble; they are more soluble at an alkaline pH than at an acid pH. In a mixture of sulfonamides, each component drug exhibits its own solubility. An example is the trisulfapyrimidine preparation, in which the antibacterial activity of the combined sulfonamides is additive, but the agents behave independently with respect to solubility. This mixture was developed to offset the precipitation of sulfonamide crystals in acidic fluid in the distal renal tubules and ureters.

The sodium salts of sulfonamides are readily soluble in water, and parenteral preparations are available for IV injection. These solutions are highly alkaline in reaction, with the notable exception of sodium sulfacetamide, which is nearly neutral and is available as an ophthalmic prepa ration.

Certain sulfonamide molecules are designed for low solubility (e.g., phthalylsulfathiazole), so they are slowly absorbed and are intended for use in treatment of enteric infections.

Mechanism of Action

Sulfonamides interfere with the biosynthesis of folic acid in bacterial cells by competitively preventing para-aminobenzoic acid (PABA) from incorporation into the folic (pteroylglutamic) acid molecule. Specifically, sulfonamides compete with PABA for the enzyme dihydropteroate synthetase. Their selective bacteriostatic action depends on the difference between bacterial and mammalian cells in the source of folic acid. Susceptible microorganisms must synthesize folic acid, whereas mammalian cells use preformed folic acid. The bacteriostatic action can be reversed by an excess of PABA, so that any tissue exudates and necrotic tissue should be removed if animals are to be treated with sulfonamides.

Antimicrobial Activity

Sulfonamides are broad-spectrum antimicrobial agents, inhibiting bacteria, toxoplasma, and other protozoal agents

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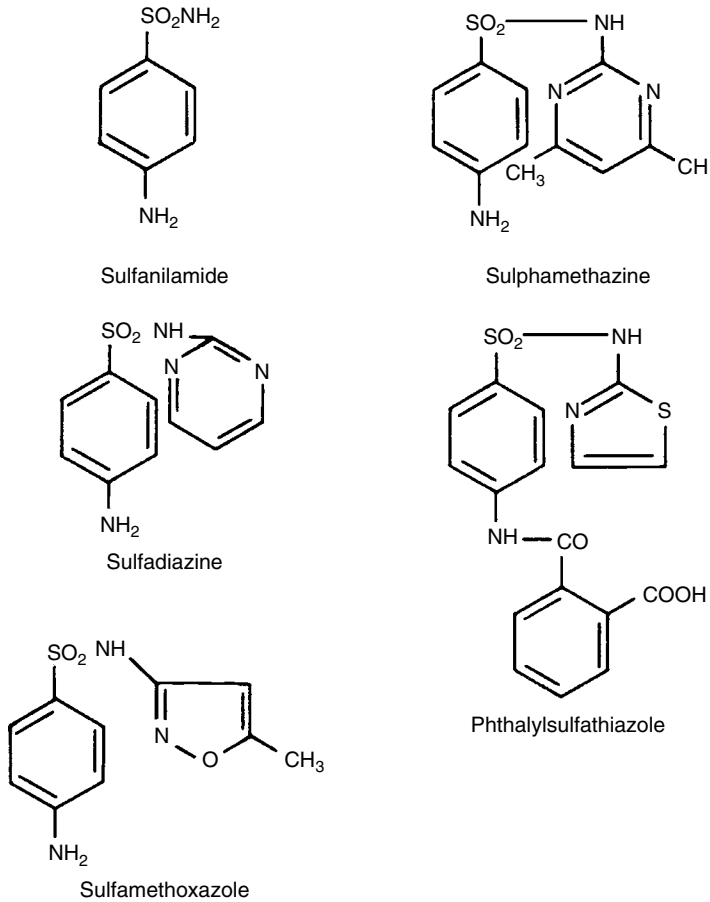

Figure 17.1. Structural formulas of some sulfonamides.

such as coccidia, but their antibacterial activity is significantly limited by the extensive resistance that has developed over 70 years. Different sulfonamides may show quantitative but not necessarily qualitative differences in activity.

The MIC of sulfonamides is markedly affected by the composition of the medium and the bacterial inoculum concentration. Because of this, *in vitro* tests may sometimes falsely report a bacterium to be resistant. This will not be the case if proper quality control with a thymidine-sensitive strain of *Enterococcus faecalis* is used. In agar diffusion tests, Mueller-Hinton agar containing lysed horse blood is the ideal medium because it contains thymidine phosphorylase that decreases the quantity of thymidine in the medium. The criteria of susceptibility for bacteria in systemic infections are not agreed because of difficulties in both determining MIC and variability in serum concentrations with different drugs and different doses. An MIC of 8–32 μg/ml is a reasonable definition of susceptibility for short-acting systemic sulfonamides; an MIC of ≥ 64–128 μg/ml can be interpreted as evidence of resistance.

Sulfonamide susceptibility testing in veterinary laboratories is usually done with high-potency triple-sulfonamide disks, designed to determine susceptibility to the high concentrations in the urinary tract (≥ 100 μg/ml); extrapolation of susceptibility to systemic infections is thus not appropriate. The CLSI criteria describe susceptibility in bacteria for urinary tract infections as those having an MIC of ≥ 256 μg/ml.

Table 17.1. Activity of sulfonamides, trimethoprim, and trimethoprim-sulfamethoxazole (μg/ml) against selected bacteria.

Organism	Sulfonamide[a] MIC_{90}	Trimethoprim MIC_{90}	Trimethoprim-Sulfamethoxazole MIC_{90}[b]
Gram-positive aerobes			
Arcanobacterium pyogenes	32	8	0.13
Corynebacterium pseudotuberculosis			≤ 0.5
C. renale	> 64		
Erysipelothrix rhusiopathiae	8	0.13	0.06
Listeria monocytogenes	8	0.06	0.03
Nocardia asteroides	128	128	8
Rhodococcus equi	> 128	64	32
Staphylococcus aureus	32	2	0.25
Streptococcus agalactiae	32	0.5	0.06
S. dysgalactiae	> 256	4	0.06
S. uberis	> 128	4	0.5
Beta-hemolytic streptococci	> 128	2	2
Gram-positive anaerobes			
Clostridium perfringens	16	64	
Gram-negative aerobes			
Actinobacillus spp.	64		≤ 0.06
A. pleuropneumoniae[c]	≥ 128	2	8
Bordetella bronchiseptica[c]	> 256		≤ 0.06
Brucella abortus	16	4	0.06
B. canis	2		
Campylobacter jejuni	≥ 256	≥ 512	≥ 512
Escherichia coli[c]	≥ 128	1	≤ 0.5
Histophilus somni	≥ 128		
Klebsiella pneumoniae[c]	≥ 128	4	≤ 0.5
Moraxella bovis	> 64	> 64	< 0.15
Pasteurella multocida	> 128	4	
Proteus spp.	> 256	8	≤ 0.5
Pseudomonas aeruginosa	> 515	512	128
Salmonella spp.[c]	128	4	0.5
Taylorella equigenitalis	> 128		
Yersinia enterocolitica	> 128	1	8

[a]Mainly sulfadimethoxine.
[b]Single figures refer to trimethoprim concentration; trimthoprim-sulfonamide ratio is 1:19.
[c]Many of these isolates are now reported as resistant to the combination; this table is partly designed to illustrate the synergism that can occur between sulfonamides and trimethoprim. Because of increasing resistance, susceptibility testing under properly controlled conditions is often required.

- *Good susceptibility: Bacillus* spp., *Brucella* spp., *E. rhusiopathiae, L. monocytogenes, Nocardia* spp., pyogenic *Streptococcus* spp., *Chlamydia* and *Chlamydophila* spp., coccidia, *Pneumocystis carinii*, and *Cryptosporidium* spp.
- *Moderate susceptibility, but often variable because of acquired resistance* (Table 17.1) includes among Gram-positive aerobes: staphylococci, some enterococci. Gram-negative aerobes: Enterobacteriaceae (including *Enterobacter* spp., *E. coli, Klebsiella* spp., *Proteus* spp.), *Actinobacillus* spp., *Haemophilus and Histophilus* spp., *Pasteurella* spp., *Pseudomonas* spp. Anaerobes such as *Bacteroides* spp. and *Fusobacterium* spp. are often susceptible *in vitro* if the medium is depleted of thymidine; this is, however, often not the case *in vivo*. *Clostridium* spp. (other than *C. perfringens*) and anaerobic cocci are often resistant.

- *Resistant: Mycobacterium* spp., *Mycoplasma* spp., most obligate intracellular pathogens (such as *C. burnetii* and *Rickettsia* spp.), *P. aeruginosa*, and spirochetes are resistant.

Resistance

Chromosomal mutation to resistance develops slowly and gradually and results from impairment of drug penetration, production of an insensitive dihydropteroate enzyme, or hyperproduction of PABA. Plasmid- and integron-mediated resistance, often encoded by *sul1, sul2* or *sul3* genes sometimes linked to other resistance genes including trimethoprim (*dfr*) resistance genes or streptomycin (*strA, strB*), is far more common and in enteric bacteria is the result of impaired drug penetration or the production of additional, sulfonamide-resistant, dihydropteroate synthetase enzymes (Maynard et al., 2003; Sheikh et al., 2012). Resistance to sulfonamides is extensively documented as widespread in bacteria isolated from animals, particularly farmed animals, reflecting extensive use of the drug over many years. A restriction of the *sul3* resistance gene to largely porcine *E. coli* has been noted (Kozak et al., 2009; Wu et al., 2010). There is complete cross-resistance between the sulfonamides.

Pharmacokinetic Properties

The sulfonamides constitute a series of weak organic acids with pK_a values ranging from 10.4 for sulfanilamides to 5.0 for sulfisoxazole. They exist predominantly in the non-ionized form in biologic fluids of pH lower than their pK_a. It is the non-ionized moiety that diffuses through cell membranes and penetrates cellular barriers.

Most sulfonamides are rapidly absorbed from the gastrointestinal tract and distribute widely to all tissues and body fluids, including synovial and cerebrospinal fluids. The sulfonamides are bound to plasma proteins to an extent varying from 15% to 90%. In addition, there is variation among species in binding of individual sulfonamides. Extensive (> 80%) protein binding increases half-life. In any one species, the extent of protein binding, apparent volume of distribution, and half-life vary widely among individual sulfonamides. This information, together with designating 100 μg/ml as the desired steady-state plasma sulfonamide concentration, facilitates calculation of dosages.

Sulfonamides are eliminated by a combination of renal excretion and biotransformation. This combination contributes to species variations in the half-lives of individual drugs. Sulfadimethoxine, for example, has half-lives of 12.5 hours in cattle, 8.6 hours in goats, 11.3 hours in horses, 15.5 hours in swine, 13.2 hours in dogs, and 10.2 hours in cats. These relatively long half-lives have been attributed to extensive binding to plasma albumin and pH-dependent passive reabsorption of the drug from acidic distal renal tubule fluid.

Sulfonamides undergo metabolic alterations to a variable extent in the tissues, especially the liver. Acetylation (which is the principal metabolic pathway for most sulfonamides), glucuronide conjugation, and aromatic hydroxylation take place in humans and in all domestic animals except dogs. It appears that dogs cannot acetylate aromatic amines. Acetylation takes place in the reticuloendothelial rather than the parenchymal cells of the liver and other tissues such as the lungs. This metabolic reaction has clinical significance, since the acetyl derivative of most sulfonamides (except sulfapyrimidines) has lower aqueous solubility than the parent compound. Acetylation therefore increases the risk of damage to the renal tubules due to precipitation. Aromatic hydroxylation, which may be the principal metabolic pathway for sulfonamides in ruminants, and glucuronide conjugation are microsomal-mediated metabolic reactions. The glucuronide conjugates are highly water-soluble and are rapidly excreted.

Renal excretion mechanisms include glomerular filtration of free (unbound) drug in the plasma, active carrier-mediated proximal tubular excretion of ionized unchanged drug and metabolites, and passive reabsorption of non-ionized drug from distal tubular fluid. The extent of reabsorption is determined by the pK_a of the sulfonamide and the pH of the fluid in the distal tubules. Urinary alkalinization increases both the fraction of the dose that is eliminated by renal excretion (unchanged in urine) and the solubility of sulfonamides in the urine.

Drug Interactions

The important synergistic interaction of sulfonamides with antibacterial diaminopyrimidines such as trimethoprim and baquiloprim is discussed below under diaminopyrimidines.

The agents appear not to antagonize the bactericidal effect of penicillins, but the procaine of procaine

penicillin is an analog of PABA that will antagonize sulfonamides. Combination with pyrimethamine is the treatment of choice for toxoplasmosis and some other protozoal infections.

Toxicity and Adverse Effects

The sulfonamides can produce a wide variety of usually reversible side effects, some of which may have an allergic basis and others are the result of direct toxicity. The more common adverse effects are urinary tract disturbances (crystalluria, hematuria, or even obstruction), hematopoietic disorders (thrombocytopenia, anemia, leukopenia), and dermatologic reactions. Significant reactions, however, are generally uncommon in animals treated with conventional doses of common sulfonamides (other than sulfaquinoxaline) for less than 2 weeks.

In a small proportion (approximately 0.25%) of humans or animals, sulfonamide therapy can produce idiosyncratic drug reactions, which are unpredictable and rare events occurring 10 days to 3 weeks after first exposure. The syndrome in dogs includes fever, arthropathy, blood dyscracias, epistaxis, hepatopathy, skin eruptions of various types, uveitis, and keratoconjunctivitis sicca (Trepanier, 2004). These reactions are sometimes described as hypersensitivity reactions (drug fever, urticaria) since they seem to involve immune reactions such as a T-cell-mediated response to proteins haptenated by sulfonamide metabolites (Trepanier, 2004) but may involve a limited capacity to detoxify metabolites of sulfonamides. Idiosyncratic reactions recur if individuals are retreated with sulfonamides. In dogs, serious but reversible sulfadiazine-induced reactions have been described in a number of reports on Doberman Pinschers, in which sulfonamides should probably be avoided.

Some adverse effects are associated with particular sulfonamides. Sulfadiazine and sulfasalazine given for long periods to dogs as a "geriatric stimulant" have caused keratoconjunctivitis sicca (KCS), which was not always fully reversible when the drug was discontinued. However, in one study KCS determined by decreased tear production occurred in 15% of 33 dogs treated with trimethoprim-sulfadiazine combination, within the first week of treatment (Berger et al., 1995). This effect occurred in dogs weighing less than 12 kg, suggesting that dosage must be particularly carefully calculated for small dogs. Trimethoprim-sulfamethoxazole has been used in the treatment of tear staining syndrome in dogs (YounSok et al., 2008).

Renal tubular damage can be minimized by ensuring that the patient is well hydrated throughout the course of treatment, by administering the most soluble sulfonamides, and by alkalinizing the urine. Prolonged dosage with sulfa-ethoxypiridine in dogs has produced cataracts. Sulfaquinoxaline has caused hypothrombinemia, hemorrhage, and death in puppies given the drug orally for control of coccidiosis; hemorrhagic diathesis was reported in other species because of the antagonistic effect of this drug on vitamin K.

Rare additional adverse effects reported include: hepatic necrosis leading to death or euthanasia, developing in some cases within days of treatment (Twedt et al., 1997) and hypothyroidism associated with prolonged treatment (Torres et al., 1996). An unusual goitrogenic effect in swine, which increased the number of stillborn or weak piglets born to sows fed sulfadimethoxine and ormetoprim in late gestation, was described by Blackwell et al. (1989). Goitrous hypothyroidism has also been described in a young dog treated with trimethoprim-sulfamethoxazole (Seelig et al., 2008). Congenital defects have been described in foals born to mares treated for equine protozoal myeloencephalitis during pregnancy (Toribio et al., 1998).

Administration and Dosage

In treating systemic diseases with sulfonamides, it is desirable to initiate therapy with a priming dose and to administer maintenance doses, each one-half the priming dose, at intervals approximately equal to the half-life of the drug (Table 17.2). When the drug is administered orally, the dose level must compensate for incomplete systemic availability from the oral preparation, that is, % bioavailability of oral preparations.

Although a large number of sulfonamide preparations are available for use in veterinary medicine, many of these are different dosage forms of sulfamethazine. This sulfonamide is most widely used in food-producing animals and can attain effective plasma concentrations when administered either orally or parenterally. Because of their alkalinity, most parenteral preparations should be administered only by IV injection. Rapid IV injection of high doses of sulfonamide preparations should be avoided. Sulfamethazine therapy should be initiated with an IV priming dose of 100 mg/kg, and effective

Table 17.2. Examples of usual dosages of sulfonamides in animals.

Drug	Route	Dose (mg/kg)	Dosing interval (h)	Comment
Short-acting sulfadiazine, sulfamethazine, trisulfapyramidine (triple sulfas)	IV, PO	50–60	12	Double first dose
Sulfamethoxazole	PO	50	12	Double first dose
Intermediate-acting sulfadimethoxine	PO, IV, IM, SC	27.5	24	Double first dose
(sustained release, cattle)	PO	137.5	96	
sulfadiazine	PO, IV	50	12	Double first dose
Sulfisoxazole	PO	50	8	Urinary tract infections
Gut-active phthalylsulfathiazole	PO	100	12	
Special-use salicylazosulfapyridine	PO	25	12	See text
silver sulfadiazine	Topical			

Table 17.3. Usual dosages of potentiated sulfonamide combinations in animals.

Drug (Species)	Route	Dose (mg/kg)	Dosing Interval (h)	Comment
Trimethoprim-sulfonamide	PO, IV, IM	(15–)30	12(–24)	Not IM in horses
Ormetoprim-sulfadimethoxine	PO	27.5	24	Double first dose
Baquiloprim-sulfadimethoxine				
Dogs	PO	30	48	
Cats	PO	30	24	
Cattle, swine	IM	10	24	

concentrations can then be maintained by administering maintenance doses of 50 mg/kg PO at 12-hour intervals. At least one prolonged-release oral preparation of sulfamethazine is available for use in calves and could be administered to sheep and goats. This is a convenient form of maintenance therapy in that a single dose provides an effective level for 36–48 hours. Different oral forms have different systemic availability (Table 17.3).

Sulfadimethoxine preparations are more widely used in companion animals. The parenteral preparation (40%), containing sulfadimethoxine sodium in solution, is suitable for IV administration to horses. Having initiated therapy with a priming dose of 50 mg/kg, effective concentrations can be maintained with maintenance dosage of 25 mg/kg administered IV at 12-hour intervals. In dogs and cats, sulfadimethoxine can be administered either as the parenteral solution IV or as the oral suspension. Therapy should be initiated with a priming dose (55 mg/kg, IV), and therapeutic concentrations can be maintained either by administering maintenance doses IV (27.5 mg/kg) or PO (55 mg/kg) at preferably 12-hour or 24-hour intervals. Selection of the dosing interval should be based on quantitative susceptibility of the pathogenic microorganisms and the site of infection.

Sulfisoxazole has higher aqueous solubility than most other members of the class. Its solubility in urine increases markedly with increase in urinary pH. It has a half-life in dogs of 4.5 hours, and because it is eliminated largely by renal excretion, sulfisoxazole is present in high concentrations unchanged in the urine. This makes sulfisoxazole an effective agent in the treatment of urinary tract infections caused by susceptible organisms. The usual oral dosage is 50 mg/kg administered at 8-hour intervals.

Unlike the sodium salts of other sulfonamides, sodium sulfacetamide is nearly neutral. It is the only sulfonamide available for topical ophthalmic use. When a 30% solution is applied to the conjunctivae, it penetrates well and attains high concentrations in ocular fluids and tissues.

Clinical Applications

Widespread resistance greatly limits the effectiveness of sulfonamides in treating bacterial diseases of animals, so that indications for primary use are few. Trimethoprim- or other antibacterial diaminopyrimidine-sulfonamide

combinations have largely replaced sulfonamides as therapeutic agents used in companion animals, although resistance also increasingly limits their use. Purulent material must always be removed, since free purines neutralizes the effect of sulfonamides. Primary uses include treatment of toxoplasmosis (when combined with pyrimethamine), of chlamydiosis, of *Pneumocystis carinii*, and possibly of nocardiosis (combined with minocycline), and the use of sulfasalazine in the treatment of chronic colitis.

Cattle, Sheep, and Goats

Widespread resistance limits the use of sulfonamides in these animals, and it is best to give these agents in combination with trimethoprim. Orally administered, long-acting, sustained-release dosage forms result in effective plasma concentrations for 3–5 days. Such a preparation has been effective in clinical trials assessing prevention and treatment of feedlot pneumonia, an unexpected result in view of the resistance reported in bovine *Mannheimia* and *Pasteurella*. Sulfonamides are used successfully to treat bovine interdigital necrobacillosis and coccidiosis. Sulfadimethoxine is the only sulfonamide approved for use in dairy cows over 20 months of age in the United States; extra-label use in dairy cows is prohibited. Sustained-release oral sulfamethazine and orally administered pyrimethamine, 0.5 mg/kg once daily, might be drugs of choice in preventing outbreaks of *Toxoplasma* abortion in sheep. Sulfonamides have been used with chlortetracyclines in feedlot lambs to improve performance and prevent clostridial enterotoxemias.

Swine

Sulfonamides have been used to promote growth and to control group E streptococcal infections and atrophic rhinitis in swine. The sulfonamides are often combined with chlortetracycline. In the United States, there have been moves to ban the use of sulfonamides for use in swine because of persistent problems of residues in carcasses in excess of legally permitted concentrations and evidence from chronic toxicity studies in mice that sulfamethazine was linked to the production of thyroid adenomas in rodents.

Horses

Sulfonamides are used in horses in combination with antibacterial diaminopyrimidines. For the treatment of equine protozoal myeloencephalitis, sulfadiazine (20 mg/kg PO SID or BID, for up to 12 weeks or longer) combined with pyrimethamine (1.0 mg/kg PO SID, for up to 120 days or longer; Dubey et al., 2001). Dapsone alone (3 mg/kg PO SID) has been used successfully in the treatment of *Pneumocystis carinii* pneumonia in a foal (Clark-Price et al., 2004).

Dogs and Cats

Use of sulfisoxazole to treat urinary tract infections in dogs has been largely replaced by antibiotics that are more effective because of their broader spectrum of activity or bactericidal action. Sulfonamides are one of the drugs of choice in the treatment of *Nocardia* infections; effectiveness may be increased by concurrent administration with minocycline. Silver sulfadiazine cream has been used as a treatment in chronic *otitis externa* caused by multiresistant *P. aeruginosa*, as the drug acts as a broad-spectrum antimicrobial antiseptic. This preparation has been effective in controlling bacteria that infect burn wounds in human patients; activity is almost certainly the result only of the silver component.

Sulfasalazine (salicylsulfapyridine) has been recommended as a drug of choice in the treatment of chronic colitis in dogs. It is hydrolyzed by colonic bacteria to yield sulfapyridine and 5-aminosalicylate; it is likely that the anti-inflammatory effect of the latter is responsible for the therapeutic effect. Comparably high concentrations of salicylate cannot be achieved in the colon by oral administration. The dosage of sulfasalazine for the dog is 25 mg/kg PO 3 times daily. The same dose in cats may induce salicylate poisoning. Some have suggested that a low dose of corticosteroid be administered simultaneously to reduce the overall duration of therapy, which is 3–4 weeks when the drug is administered alone. This dual dosage may decrease the frequency of keratoconjunctivitis sicca. In most cases of sulfasalazine treatment, cure is achieved within 4 weeks, and treatment should not be continued beyond this time without histologic confirmation of colonic inflammation.

Dapsone (diaminodiphenylsulphone) has been used in the treatment of dermatitis herpetiformis in dogs and in the treatment of leprosy in humans.

Poultry

Sulfonamides have been used in the prevention and treatment of coccidiosis, infectious coryza, pullorum disease, and fowl typhoid.

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Antibacterial Diaminopyrimidines: Aditoprim, Baquiloprim, Ormetoprim, and Trimethoprim

Diaminopyrimidines interfere with folic acid production by inhibition of dihydrofolate reductase. Some diaminopyrimidines have marked specificity for bacterial dihydrofloate reductases (aditoprim, baquiloprim, ormetoprim, trimethoprim), others for protozoal enzymes (pyrimethamine), and others for mammalian enzymes (methyltrexate). The earliest antibacterial diaminopyrimidine introduced for clinical use was trimethoprim (Figure 17.2), a synthetic drug that is widely used in combination with sulfonamides. It is a weak base with a pK_a of about 7.6 and is poorly soluble in water. Other antibacterial diaminopyrimidines have

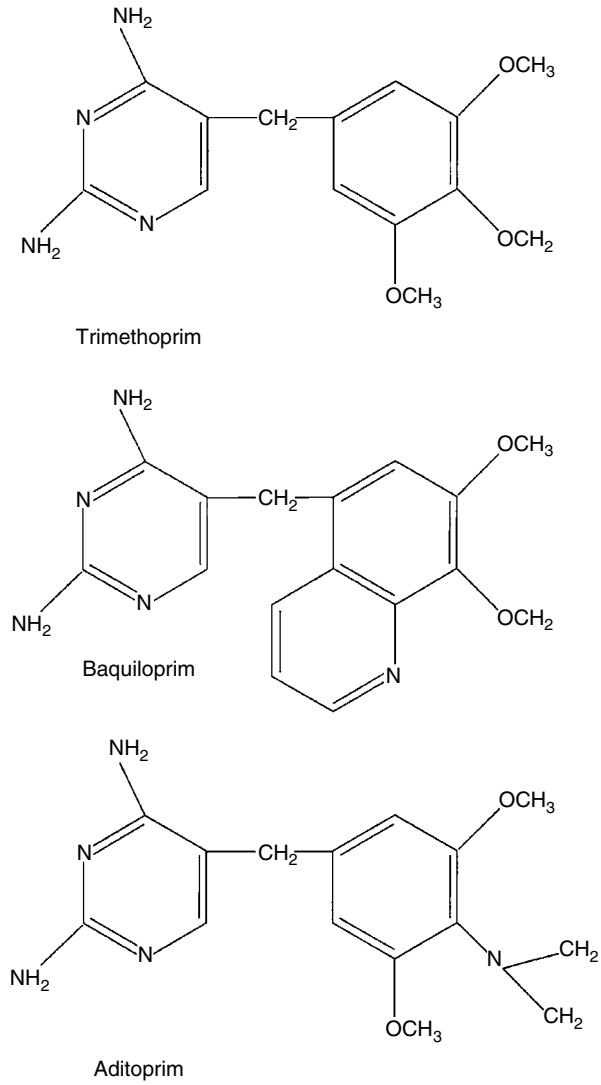

Figure 17.2. Structural formulas of some diaminopyrimidines.

similar antibacterial activities to trimethoprim but offer significant pharmacokinetic advantages, particularly those of greater half-lives and tissue distribution.

Mechanism of Action

Diaminopyrimidines interfere with the synthesis of tetrahydrofolic acid from dihydrofolate by combining with the enzyme dihydrofolate reductase. Selective antibacterial activity occurs because of greater affinity for the bacterial rather than the mammalian enzyme. Diaminopyrimidines thus inhibit the same metabolic sequence as the sulfonamides, preventing bacterial synthesis of purines and thus of DNA. A synergistic and bactericidal effect occurs when the diaminopyrimidines are combined with sulfonamides (see sulfonamide-diaminopyrimidine combinations), and for this reason these drugs are invariably used with a sulfonamide in veterinary medicine.

Interestingly, in the United Kingdom trimethoprim alone rather than the combination is now generally used in human medicine (Hughes, 1997). The reasons for the abandoned use of trimethoprim-sulfonamide combination in favor of trimethoprim alone are (1) bacteriostatic synergy is only demonstrable when the concentration of each drug is less than bacteriostatic, but the bacteriostatic effect of trimethoprim in urinary tract infections, for which the drug is most commonly used, is often detectable in urine for several days; (2) diaminopyrimidines are more widely distributed into tissues than sulfonamides, reaching sites, such as cells, which sulfonamides do not penetrate well; (3) most of the adverse effects of the combination are the result of the sulfonamide component; and (4) the original claim that the combination prevented the emergence of resistance is dubious because sulfonamide resistance is widespread and because plasmids conferring resistance to sulfonamides often also confer resistance to trimethoprim (Hughes, 1997). The licensed medical use in the United Kingdom of the combination is therefore restricted largely to the treatment of *Pneumocystis jirovecii* infection.

Antimicrobial Activity

Antibacterial diaminopyrimidines are generally bacteriostatic, broad-spectrum drugs active against Gram-positive and Gram-negative aerobic bacteria, but not usually against anaerobes (Table 17.1). Bacteria with an MIC $\leq 1\,\mu g/ml$ are usually regarded as susceptible. Activity against *Mycoplasma* spp., *Chlamydia* and *Chlamydophila* spp., *Mycobacterium* spp., and *P. aeruginosa* is negligible. Activity of aditoprim, baquiloprom, and ormetoprim is similar to or very slightly less than that of trimethoprim.

Resistance

High-level resistance to trimethoprim and other diaminopyrimidines is usually the result of transposon- or integron-encoded plasmid or chromosomal synthesis of a resistant dihydrofolate reductase enzyme (Skold, 2001). Changes that affect bacterial permeability or efflux pumps can result in moderate resistance. Resistance is increasingly reported, particularly among Enterobacteriaceae. Resistance to trimethoprim is extensively documented as widespread in bacteria isolated from animals, particularly in enteric bacteria isolated from farmed animals of all types exposed to trimethoprim. At least 30 phylogenetically different *dfr* resistance genes expressing dihydrofolate reductases have been characterized. Isolates with plasmid- or integron-mediated resistance commonly show multiple resistance, which includes sulfonamide resistance. Examples include multidrug-resistant *Salmonella* such as *S. typhimurium* DT104 and *S. Newport*. The apparent spread of a trimethoprim resistance gene from porcine to human *E. coli* has been described (Jansson et al., 1992).

Pharmacokinetic Properties

Diaminopyrimidines including trimethoprim are lipid-soluble organic bases that are approximately 60% bound to plasma proteins. They are rapidly absorbed from the intestine after oral administration. The drugs distribute widely, penetrating cellular barriers by non-ionic diffusion and attaining effective concentrations in most body tissues and fluids. The drug may concentrate in fluids, such as the prostate, that are acidic relative to plasma. The average milk-to-plasma equilibrium concentration ratio is 3:1. The dose, systemic availability from the dosage form, and route of administration determine the plasma concentration profile and tissue levels of the drug. Hepatic metabolism (oxidation followed by conjugation reactions) is the principal process for elimination. Because of this, the half-life and fraction of the dose that is excreted unchanged in the urine vary widely among species. In ruminants, the short half-life of trimethoprim is due to rapid demethylation to produce inactive compounds. Replacing the phenyl ring of trimethoprim with

the bicyclic ring of baquiloprim resulted in an increase in half-life from 1 hour (trimethoprim) to 10 hours (baquiloprim) in cattle and from about 2 to 5 hours in pigs, while replacement of a methyl group in trimethoprim by the dimethylamino group of aditoprim increased its half-life in cattle to 4–7 hours, in horses to 9–14 hours, and in pigs to 8–9 hours, or greater. Greater tissue distribution may be one factor responsible for prolonged half-life compared to trimethoprim.

Toxicity and Adverse Effects

The antibacterial diaminopyrimidines are relatively nontoxic drugs. Their main, though clinically unimportant, potential toxic effect is to induce folic acid deficiency at high doses, so care should be used in pregnant animals. Rarely, aseptic meningitis related to trimethoprim therapy has been reported in humans. Hyperkalemia may occur under unusual circumstances.

Clinical Applications

Antibacterial diaminopyrimidines are currently used only in combination with sulfonamides in animals, although there may be a need to reassess the benefits of the combination. Alone or in combination they may be a drug of choice for treating prostatic infections caused by Gram-negative bacteria, since prostatic concentrations may reach 10 times those of plasma, at which concentration the drug may be bactericidal. Nevertheless, clinical results in treating chronic prostatitis with trimethoprim may be disappointing, probably because of the nature of the disease process. Trimethoprim administered orally has been used to prevent relapse after treatment of *L. monocytogenes* meningitis in humans. Antibacterial diaminopyrimidines, including trimethoprim, combined with sulfonamides or dapsone may be the prophylactic drugs of choice for *Pneumocystis carinii* (*jirovecii*) pneumonia (Hughes, 1988).

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Antibacterial Diaminopyrimidine-Sulfonamide Combinations

Antibacterial diaminopyrimidines are combined with a variety of sulfonamides (sulfadiazine, sulfamethoxazole, and sulfadoxine) in a fixed (1:5) ratio, which in people produces a 1:20 ratio of drug concentrations in the plasma after oral or parenteral administration. This ratio is desirable since maximum synergy occurs when the drugs are present in the ratio of their MICs; diaminopyridines are 20–100 times more active than the sulfonamides, so that combinations are formulated to give a 1:20 ratio in human serum. This ratio occurs because diaminopyrimidines (lipid-soluble organic bases) are concentrated in tissues whereas sulfonamides (weak organic acids) remain largely in extracellular fluids. At these MICs and in this ratio, the combination produces a bactericidal effect against a wide range of bacteria, with some important exceptions, and also inhibits certain other microorganisms. Since the combinations of different diaminopyridines, with sulfonamides, give essentially similar antibacterial effects, comments will relate largely to trimethoprim-sulfonamide combinations but can be extrapolated to other combinations.

Veterinary preparations follow medical usage in that they contain diaminopyridines combined with a sulfonamide in the 1:5 ratio. For trimethoprim, the half-lives of the components (sulfadiazine, sulfadoxine, or sulfamethoxazole) do not coincide in any species (except humans) whereas they are more similar for baquiloprim (sulfadimidine, sulfadimethoxine) and ormetoprim (sulfadimethoxine). The dosage aims at maintaining bacteriostatic concentrations of the sulfonamide, which, for a time after each dose, is enhanced by the synergistic bactericidal action of the combination.

Mechanism of Action

The combination of a diaminopyrimidine with a sulfonamide inhibits sequential steps in the synthesis of

folic acid and thus of the purines required for DNA synthesis. The interference by the diaminopyrimidine methoprim with recycling of tetrahydrofolic or dihydrofolic acid is probably responsible for the synergistic interaction of the combination.

Antimicrobial Activity

Diaminopyrimidine-sulfonamide combinations have a generally broad and usually bactericidal action against many Gram-positive and Gram-negative aerobic bacteria, and protozoa such as *Toxoplasma*. They are not active against anaerobic bacteria *in vivo* because thymidine and PABA in the necrotic tissue antagonizes their antibacterial effect. Such an antagonistic effect is not limited to anaerobes so that this combination may not be fully effective in closed, non-draining, infections where there is significant tissue debris. *Pneumocystis carinii* (*jirovecii*)and some malarial parasites are susceptible; *Mycoplasma* spp. and *P. aeruginosa* are resistant.

Synergism occurs when the microorganisms are susceptible to both drugs in the combination. It may still be obtained, in up to 40% of cases, when bacteria are resistant to sulfonamides. Synergy often occurs if the organism is resistant to trimethoprim but sensitive to sulfonamides and in nearly 40% of cases in which the organism is resistant to each drug alone. Nevertheless, many organisms described as susceptible to the combination are susceptible to the diaminopyrimidine component only. Clinical response may sometimes be lower than expected from *in vitro* data, and better understanding of the use of MIC data in prediction of clinical outcome is required. One element of such disappointing responses may also be the presence of thymidine and PABA in infected tissue. Nevertheless, a more important element may be widespread resistance to sulfonamides and consequently the lack of synergism in many cases, so that only the diaminopyrimidine component is active. For trimethoprim, the short half-life in some species may exacerbate a lack of synergism.

Where synergistic interactions occur, a 10-fold increase in activity of the trimethoprim component and a 100-fold increase in activity of the sulfonamide component are common. Synergism occurs at different drug concentration ratios with different bacterial species. Because of differences between the diaminopyrimidine and sulfonamide in distribution and in the case of trimethoprim of elimination, the concentration ratios may differ considerably in tissues and urine from that in plasma. Such variation is said not to be important, as the synergistic interaction may occur over a wide range of concentration ratios of the drugs but clearly it would not occur in some tissues, since diaminopyrimidines are distributed more widely than sulfonamides. Because of these variations in the pharmacokinetics of diaminopyrimidines and sulfonamides, the length of effective action is difficult to assess based on serum concentrations alone. This has given rise to the suspicion that the manufacturer's recommended dosages are less than optimal, especially for trimethoprim combinations. A number of recent pharmacokinetic studies have resulted in recommendations to increase dosage (Ensink et al., 2003, 2005).

Errors in laboratory testing are common because of the presence of PABA or thymidine in media (Feary et al., 2005); in one study, half the strains reported as resistant in other laboratories were susceptible when tested in a reference laboratory. The use of lysed horse blood, which contains thymidine phosphorylase, will eliminate excess thymidine in the medium.

- *Good susceptibility (MIC ≥ 0.5/9.5 μg/ml)* is shown among the following Gram-positive aerobes: *S. aureus*, streptococci, *Arcanobacterium* spp., *Corynebacterium* spp., *E. rhusiopathiae, L. monocytogenes*. Gram-negative aerobes: *Acinetobacter* spp., *Actinobacillus* spp., *Bordetella* spp., *Burkholderia cepacia*, *Brucella* spp., *Dermatophilus congolense*, Enterobacteriaceae (*E. coli, Klebsiella* spp., *Proteus* spp., *Salmonella* spp., *Yersinia* spp.), *Haemophilus* spp., *Pasteurella* spp., *Stenotrophomonas maltophila*. Anaerobes: *Actinomyces* spp., *Bacteroides* spp., *Fusobacterium* spp., some *Clostridium* spp., and *Chlamydia* spp.
- *Moderate activity (MIC ≥ 2/38 μg/ml)* includes some *Mycobacterium* spp. and some *Nocardia* spp.
- *Resistance (MIC ≥ 4/76 μg/ml)* is shown by *Rickettsia*, *Leptospira* spp., *P. aeruginosa*, and *Mycoplasma* spp. (Table 17.2).

Resistance

Mechanisms of resistance were discussed under the individual components of the combination. Resistance to the combination has developed progressively with use. Multiple integron-associated resistance, which includes both sulfonamide and trimethoprim resistance,

has been described in some *Salmonella* serovars and in pathogenic *E. coli* isolated from animals.

Pharmacokinetic Properties

In humans the half-lives of trimethoprim and sulfamethoxazole are similar, and maintenance dosage provides continuous, therapeutic concentrations of both drugs in plasma. In animals the half-lives of the drugs are not similar, but the combination is often clinically effective because of the relatively broad range of drug ratio over which synergism occurs. The diaminopyrimidine component is concentrated in tissues whereas the sulfonamide component moves only slowly from plasma into tissues. The longer half-lives of newer diaminopyrimidines (baquiloprim, ormetoprim) give the advantage of better maintenance of the 1:20 ratio said to be desirable, and of less frequent dosing.

Following SC injection in cattle, trimethoprim seems to deposit in a slow-release form, so that serum concentrations remain below MIC. Because of this, the SC route cannot be recommended in cattle and perhaps in other species.

Drug Interactions

Trimethoprim-sulfonamide has sometimes been used in conjunction with ampicillin to provide "broad-spectrum bactericidal antimicrobial coverage" before microbiology data are available. However, one study showed that addition of ampicillin to trimethoprim-sulfonamide dosing regimens only marginally increased the spectrum of activity. There is no known mechanism to suggest that such a combination might be synergistic. Rather, such a combination may be effective in treating polymicrobial infections involving aerobic bacteria susceptible to the trimethoprim-sulfonamide combination and anaerobic bacteria susceptible to ampicillin.

Toxicity and Adverse Effects

The combination has a wide margin of safety, and adverse effects can mainly be attributed to the sulfonamide. These effects are discussed in the general description of the adverse effects of each drug class.

In horses, minor tissue damage and pain may occur after IM injection; transient pruritus has been reported to follow the first but not subsequent doses. In isolated incidents a fatal adverse reaction (possibly respiratory failure) followed IV injection of the combination preparation in horses, in some cases in anesthetized horses. A 7% incidence of diarrhea was observed in a study of the effect of twice-daily administration of oral 30 mg/kg trimethoprim-sulfadiazine in horses. The prevalence of diarrhea noted following trimethoprim-sulfonamide use in horses in another study was not significantly different from that observed in horses receiving other antibiotics, including penicillin (Wilson et al., 1996). Neurologic abnormalities in horses characterized by reversible hypermetric gait, agitation and by erratic behavior have been described as an unusual adverse reaction (Stack et al., 2011).

Administration and Dosage

Usual dosages are shown in Table 17.3. Dogs and cats can be given the oral form (tablets) at the same dosage. Twice-daily oral dosing of horses with 30 mg/kg trimethoprim-sulfadiazine combination paste, rather than once daily is recommended. Oral dosage with ormetoprim-sulfadimethoxine paste in mares recommended for susceptible organisms was a loading dose of 9.2 mg ormetoprim and 45.8 mg sulfadimethoxine/kg followed by half this dose every 24 hours (Brown et al., 1989).

Clinical Applications

Diaminopyrimidine-sulfonamide combinations have the advantage of good distribution into tissues, safety, a relatively broad-spectrum bactericidal activity, and oral administration. A disadvantage is antagonism of action by infected tissue debris.

The combination can be recommended in the treatment of urinary tract infections caused by common opportunist pathogens. The combination has a particular place in the treatment of bacterial prostatitis because of good tissue penetration. Other indications include the treatment of enteric infections (*E. coli, Salmonella, Y. enterocolitica*). The drug is of value in the treatment of brucellosis, often in combination with rifampin or doxycycline. The combination is a drug of choice in the treatment of *Nocardia* infections, but high oral dosage (3 mg trimethoprim equivalent/kg every 6 hours) must be used for prolonged periods.

Other indications include the treatment of *Pneumocystis carinii (jirovecii), Chlamydia* and *Chlamydophila* infections, of listeriosis, of certain fast-growing mycobacterial infections (*M. kansasii, M. marinum*), and of *Coxiella* infections. In human medicine, the combination is used

for the treatment of otherwise-resistant infections caused by *Acinetobacter*, *Burkholderia* and *Stenotrophomonas* species, as well as of methicillin-resistant *S. aureus* in humans (MRSA; Goldberg and Bishara, 2012). Livestock-associated MRSA have, however, been associated with multiple drug resistance, including a novel trimethoprim resistance gene (*dfrK;* Kadlec et al., 2012). The drug is also used in the treatment of acute upper and lower respiratory tract infections caused by susceptible organisms, as well as in infections in other sites.

Cattle, Sheep, and Goats

The drug combination is widely used in dairy and beef cattle and has been used successfully in the treatment of salmonellosis in calves, as well as in undifferentiated diarrhea, in bacterial pneumonia, in foot rot, and in septicemic colibacillosis. Baquiloprim-sulfadimidine was not as efficacious as danofloxacin in the treatment of experimentally induced *E. coli* diarrhea in calves (White et al., 1998), presumably because the organism is less susceptible to the combination drug. The potential for use in coliform septicemia and meningitis seems excellent but is increasingly limited by resistance. In meningitis the drug should be administered IV 3 or 4 times daily at the usual dosage. The potential for use in the treatment of *Listeria* meningoencephalitis appears excellent. The susceptibility of *Histophilus somni, Pasteurella multocida*, some *Mannheimia haemolytica*, and of *Arcanobacterium pyogenes* suggests a useful application in bovine respiratory disease that has been borne out by field studies. The drug combination should be administered parenterally (not orally). Clinical trials with undifferentiated bovine respiratory disease have failed to demonstrate improvement when dosage of trimethoprim-sulfadoxine was increased beyond that recommended or when the product was administered IV compared to IM, although pharmacokinetic studies suggest that manufacturer's once-daily recommended dosage of 17 mg/kg is too low. A preferred minimum dosage is 30 mg/kg SID or 15 mg/kg BID. Experimental studies have confirmed the antagonistic effect of infected tissue debris on the action of the combination (Greko et al., 2002).

When used to treat acute mastitis, the drug should be given IV at high dose because of poor bioavailability after IM injection and relatively poor udder penetration; a dosage of 48–50 mg/kg every 12 hours is appropriate for acute mastitis. A beneficial effect of trimethoprim-sulfonamide on the treatment of coliform mastitis has been noted, particularly when combined with non-steroidal anti-inflammatory drugs (Shpigel et al., 1998).

Other uses in cattle include the treatment of urinary tract infections and mixed aerobe-anaerobe infections such as those occurring in post-parturient metritis. The drug has potential but unproven use for the treatment of *L. monocytogenes* encephalitis in ruminants.

A special application in goats and sheep is in preventing *Toxoplasma* abortion; the drug is also potentially useful in preventing chlamydial abortion in sheep. In experimental *Toxoplasma* infections in mice, protection by trimethoprim-sulfonamide was inferior to pyrimethamine-sulfadiazine, but clinical results in naturally occurring infections in humans have been excellent.

Swine

Trimethoprim-sulfonamide combinations have been used successfully in controlling a wide variety of conditions in pigs, including neonatal and post-weaning colibacillosis, salmonellosis, atrophic rhinitis, greasy pig disease, streptococcal meningitis, and pneumonia. Atrophic rhinitis may be controlled by incorporating the drug in feed or water, or by injecting piglets at various times such as the third day of life and again in the third and sixth weeks. The mastitis-metritis-agalactia syndrome has been controlled by the prophylactic administration of 15 mg/kg PO for 3 days before and 2 days after parturition. The combination has been used in the eradication of *A. pleuropneumoniae* infection from herds by treating adults through the water for 3 weeks in combination with removal of serologically positive animals. Isolates of MRSA from clinical infections in Dutch swine were all found to be susceptible to the combination (Wolf et al., 2012), in marked contrast to nasal isolates from swine in Belgium (Crombé et al., 2012); the ST398 strain found in swine appears to be able readily to acquire multiple resistance genes (argudin et al., 011). Other diaminopyrimidine-sulfonamide combinations are available for swine for similar purposes to trimethoprim-sulfonamide combinations (Table 17.3). Susceptibility testing is required before instituting treatment in view of the variable reports of resistance of common swine pathogens to the combination, including bacteria such as *H. parasuis* that used to be highly susceptible.

Horses

The combination of trimethoprim-sulfadiazine is popular in horses because it can be administered as an oral antibiotic to horses with few adverse effects. It is painful when administered IM. It is, therefore, used orally to treat acute respiratory infections including strangles, acute urinary tract infections, and wounds and abscesses and is a drug of choice in salmonellosis. In recent years, however, resistance has apparently increased in *Streptococcus equi* subsp. *zooepidemicus*, so that in some studies less than 90% of isolates are susceptible *in vitro* (Peyrou et al., 2003), although Feary et al. (2005) have shown that reports of resistance may represent laboratory error. The combination is ineffective in eradicating *S. equi* subspecies *zooepidemicus* in a tissue chamber model of infection despite *in vitro* susceptibility of the isolate and high concentrations of the drugs in the tissue chamber fluid (Ensink et al., 2003). For these reasons, and because it can be partially antagonized by tissue debris, it is a less desirable choice than procaine penicillin G for treatment of streptococcal infections. In foals the combination is used in the treatment of *Actinobacillus* and coliform infections, although the latter use may be compromised by resistance. The drug may be used for coliform meningitis, in which high doses should be administered slowly IV 3 or 4 times daily. The drug may otherwise be administered orally but oral dosage recommended by the manufacturers may be low and there is apparent advantage to twice-daily dosage (30 mg/kg) of oral preparations (Van Duijkeren et al., 1994). The combination of sulfadiazine with pyrimethamine is a drug of choice in the treatment of protozoal encephalomyelitis (see antiprotozoal diaminopyrimidines). It is a drug of choice for *P. jiroveci* infections in foals. Direct infusion of the combination into the uterus may cause endometrial inflammation.

Dogs and Cats

Trimethoprim-sulfonamide or ormetoprim-sulfadimethoxine combinations have wide application in dogs and cats against specific and non-specific infections. The combination is highly effective against many opportunist bacteria present in canine urinary tract, skin and ear infections (*S. pseudintermedius*, streptococci, and Enterobacteriaceae including *E. coli* and *Proteus*). The drug has the potential for use in prophylaxis of urinary tract infections. Methicillin-resistant *S. pseudintermedius* are usually resistant to trimethoprim, as part of their common multidrug resistance (Perreten et al., 2010).

Consideration should be given to twice-daily dosing with trimethoprim-sulfadiazine. A blinded comparison of once versus twice-daily dosing with 30 mg/kg trimethoprim-sulfadiazine in the treatment of canine pyoderma showed an advantage of twice-daily dosing, although this was not statistically significant possibly because of small numbers of animals in the trial (Messinger and Beale, 1993). In one study, however, mean serum and skin concentrations using once-daily dosing were considered to achieve therapeutically effective concentrations (Pohlenz-Zertuche et al., 1992).

The combination drug is effective against *Bordetella bronchiseptica*, although relapses after treatment with trimethoprim-sulfadiazine for 5 days were common in experimental kennel cough. The drug should probably be administered for several weeks in the treatment of this infection. In one study, a significant number of isolates were found to be resistant to the combination drug (Speakman et al., 2000), so that doxycycline or amoxicillin-clavulanic acid may now be a better choice for treatment of kennel cough. The drug has been used successfully in the treatment of canine actinomycosis, often in conjunction with procaine penicillin; the combination may be particularly useful where *Nocardia* spp. and *A. viscosus* have not been distinguished properly. The combination has been effective in treating coccidiosis in dogs and cats.

The excellent penetration into the prostate makes the combination a treatment of choice in Gram-negative prostatic infections in dogs, equal to or better than minocycline, although now challenged by the fluoroquinolones. Similarly, the excellent penetration (50% of serum concentrations) of the aqueous and vitreous humors of the eyes by both drugs makes the combination suitable in the parenteral treatment of panophthalmitis caused by Gram-negative bacteria. The combination is used together with clindamycin and pyrimethamine in the initial treatment of *Hepatozoon* infections in dogs. The combination is also used with clindamycin in the treatment of *Neospora caninum* infection.

Poultry

Trimethoprim-sulfaquinoxaline and sulfamethoxazole-ormetoprim are used in the prophylaxis and treatment of *E. coli, Haemophilus*, and *Pasteurella* infections, as

well as of coccidiosis, and of *Reimerella anatipestifer* in ducks. The combination has been used successfully in the treatment of *Plasmodidium gallinaceum* malaria in chickens (Williams, 2005). Depending on the extent of use in different countries, which varies, resistance can be widespread among *E. coli* isolated from broilers,

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Antiprotozoal Diaminopyrimidines

Some diaminopyrimidines such as pyrimethamine have high activity against protozoa by inhibiting dihydrofolate reductase and thus preventing purine synthesis. These drugs are used in the treatment of systemic protozoal infections such as toxoplasmosis, neosporosis, and equine protozoal myelitis. They are also highly active against *Pneumocystis* spp.

Pyrimethamine and sulfadiazine are the most effective drugs in the treatment of toxoplasmosis in humans and are generally preferred over alternatives such as azithromycin and trimethoprim-sulfamethoxazole. The adult human dosage is 75 mg pyrimethamine and 4 g sulfadiazine PO/day in 4 divided doses, administered for up to 4 weeks. Dapsone combined with pyrimethamine has good activity experimentally against *Toxoplasma*.

Pyrimethamine combined with trimethoprim-sulfadiazine or with an oral sulfonamide alone (20 mg/kg q 24 h) has become a standard treatment for equine protozoal myeloencephalitis (EPM). Current maintenance dosage is 1 mg/kg daily given orally with trimethoprim-sulfadiazine or -sulfamethoxazole (20 mg/kg daily) for a minimum of 4 months (Fenger, 1997). The trimethoprim component is unnecessary. Anti-inflammatory drugs may also be administered. A small proportion of horses may develop anemia during treatment. Such animals can be treated with folic acid (40 mg daily). Alternate drugs for the treatment of EPM are required, since pyrimethamine is teratogenic for animals and may lead to myeloid, erythroid or lymphoid hypoplasia with epithelial dysplasia and renal hypoplasia or nephrosis in newborn foals. Such effects may be exacerbated by administering folic acid to mares being treated for EPM (Toribio et al., 1998). About 60% of horses with moderate to severe EPM will improve with any of the FDA-approved treatments (sulfadiazine/pyrimethamine, ponazuril or nitazoxanide), with about 10–20% recovering completely (MacKay et al., 2006).

Pyrimethamine and diaveridine are commonly combined with sulfaquinoxaline for their synergistic effect against coccidia. Pyrimethamine (1 mg/kg daily) combined with a sulfadoxine (20 mg/kg daily) or trimethoprim-sulfadiazine has been used successfully in the treatment of *Neospora caninum* infection in dogs (Thate and Laanen, 1998).

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18

Fluoroquinolones

Steeve Giguère and Patricia M. Dowling

Introduction

The fluoroquinolones, also known as quinolones, 4-quinolones, pyridine-β-carboxylic acids, and quinolone carboxylic acids, are a large and expanding group of synthetic antimicrobial agents. The first of these compounds, nalidixic acid, was initially described in 1962, introduced into clinical practice in 1963, and then approved for clinical use in 1965. Nalidixic acid had limited clinical application because of its poor absorption following oral administration, its moderate antibacterial activity (MICs of 4–16 μg/ml for Enterobacteriaceae), high protein binding (92–97%), and poor patient tolerance (Bryskier, 2005). Attempts to introduce an intravenous form of nalidixic acid administration were unsuccessful, primarily because of limited antibacterial activity and high protein binding. Between the mid-1960s and the early 1980s there were several other quinolones approved for clinical use, for example, oxolinic acid, pipemidic acid, piromidic acid, and flumaquine. These drugs exhibited increased antibacterial activity but still had limited absorption and distribution. In the 1980s, the addition of both a fluorine molecule at the 6 position of the basic quinolone structure and a piperazine substitution at the 7 position enhanced the antibacterial activity of these compounds, including activity against organisms such as *Pseudomonas aeruginosa* and staphylococci. These modifications also increased the oral absorption and tissue distribution (Ball, 2000). The quinolone nucleus possessing the fluorine molecule gave the group the name "fluoroquinolones." The first fluoroquinolone approved for use in clinical medicine was norfloxacin, followed shortly thereafter by ciprofloxacin. The first fluoroquinolone approved for use in animals was enrofloxacin, which was approved for use in the United States in companion animals in 1988. Since the approval of enrofloxacin, seven other fluoroquinolones have been approved for use in companion and/or food animals.

The fluoroquinolones that are marketed for use in veterinary medicine today are typically well absorbed orally, have a large volume of distribution, penetrate nearly every tissue and cell in the body, and have extended elimination half-lives, allowing for every 24- or 48-hour dosing. At appropriate drug concentration:MIC ratios, the fluoroquinolones are rapidly bactericidal, exhibit concentration-dependent killing, and may exhibit a prolonged *in vivo* post-antibiotic effect (PAE) on certain bacteria. However, the potential for fairly rapid selection of resistance in some pathogens is a disadvantage of this class of drugs. This can be minimized by appropriate dose selection directed against the right pathogen for the right infectious disease process.

The fluoroquinolones are classified into different groups based on their chemical structure or their biological activities. Classification by chemical structure is dependent on the number of rings associated with the pyridine-β-carboxylic acid nucleus (Bryskier, 2005). Group I is composed of monocyclic derivatives.

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Group II, which is the majority of fluoroquinolones on the market today, is composed of bicyclic derivatives. This group is divided into two subgroups based on substitutions at position 8 of the quinolone nucleus. Group III is composed of tricyclic derivatives and includes marbofloxacin. Group IV is comprised of those molecules that are quadricyclic, of which only a few have been synthesized and none are marketed for use in veterinary medicine. The biological classification places the 4-quinolones in three groups or generations. First-generation quinolones are those with antibacterial activity restricted to the Enterobacteriaceae (e.g., nalidixic acid and flumequine). Second-generation quinolones have an extended spectrum of antibacterial activity. Most fluoroquinolones approved for use in people (including ciprofloxacin, norfloxacin, and ofloxacin) and all but one of the fluoroquinolones approved for use in veterinary medicine are second-generation fluoroquinolones. Third-generation fluoroquinolones have considerably improved activity against streptococci and obligate anaerobes. Examples of third-generation fluoroquinolones approved for use in people include trovafloxacin, gatifloxacin, and moxifloxacin. Pradofloxacin is the only third-generation fluoroquinolone approved for use in animals. The fluoroquinolones can also be grouped according to their physiochemical properties (Bryskier, 2005). Newer compounds are being explored that optimize the various substitutions and allow for the fluorine atom at position 6 to be replaced, which may reduce side effects, decrease metabolism and decrease interactions with other drugs. The emergence of resistant bacterial strains, however, remains problematic.

To date there have been eight fluoroquinolones approved for use in veterinary medicine (danofloxacin, difloxacin, enrofloxacin, ibafloxacin [Europe only at this time], marbofloxacin, orbifloxacin, pradofloxacin, and sarafloxacin). These fluoroquinolones and their current clinical uses in veterinary medicine are listed in Table 18.1. Of these products, sarafloxacin has been voluntarily withdrawn from the market in the United States following a request by the Food and Drug Administration's Center for Veterinary Medicine. The use of enrofloxacin in poultry in the United States has been withdrawn following a Judicial Review (Federal Register, 2000). This chapter reviews chemical, microbiological, pharmacokinetic, pharmacodynamic, and clinical aspects of the fluoroquinolone antibacterial agents, with specific attention to those agents approved for use in animals (Table 18.1).

Table 18.1. Fluoroquinolones used in veterinary medicine.*

Fluoroquinolone	Comments
Enrofloxacin	Available as tablets and injectable formulation for dogs and cats and as an injectable solution for cattle. Only approved for treatment and control of respiratory disease in cattle in the United States and Canada.* Approved uses vary widely between countries, with some approvals for lactating dairy cows, swine, and poultry. Used extra-label in horses and exotic animals.
Ciprofloxacin	Only approved for humans, but used extra-label in small animals.
Danofloxacin	Only approved for treatment of respiratory disease in cattle in the United States and Canada, but approved for use in cattle, swine, and poultry in Europe.
Difloxacin	Only available as small animal oral formulations in the United States and Canada, but cattle and dog injectable formulations and poultry oral solution are available in Europe. Used extra-label in horses.
Ibafloxacin	Oral formulation available for small animals in Europe.
Marbofloxacin	Only available as small animal oral formulations in the United States and Canada, but large animal injectable formulations are available in Europe. Used extra-label in horses.
Pradofloxacin	Oral formulations for use in dogs and cats.
Orbifloxacin	Only available as small animal oral formulations. Used extra-label in horses.

*Off-label use of fluoroquinolones in food-producing animal species is illegal in the United States.

Chemistry

The fluoroquinolones, like sulfonamide and nitrofurans, are synthetic compounds (Grohe, 1998). The first clinically approved 4-quinolone-type compound was nalidixic acid. Nalidixic acid lacked several of the characteristics associated with the fluoroquinolones. For example, nalidixic acid has a nitrogen atom at position 8 instead of a carbon atom. With a nitrogen atom at position 1, nalidixic acid has

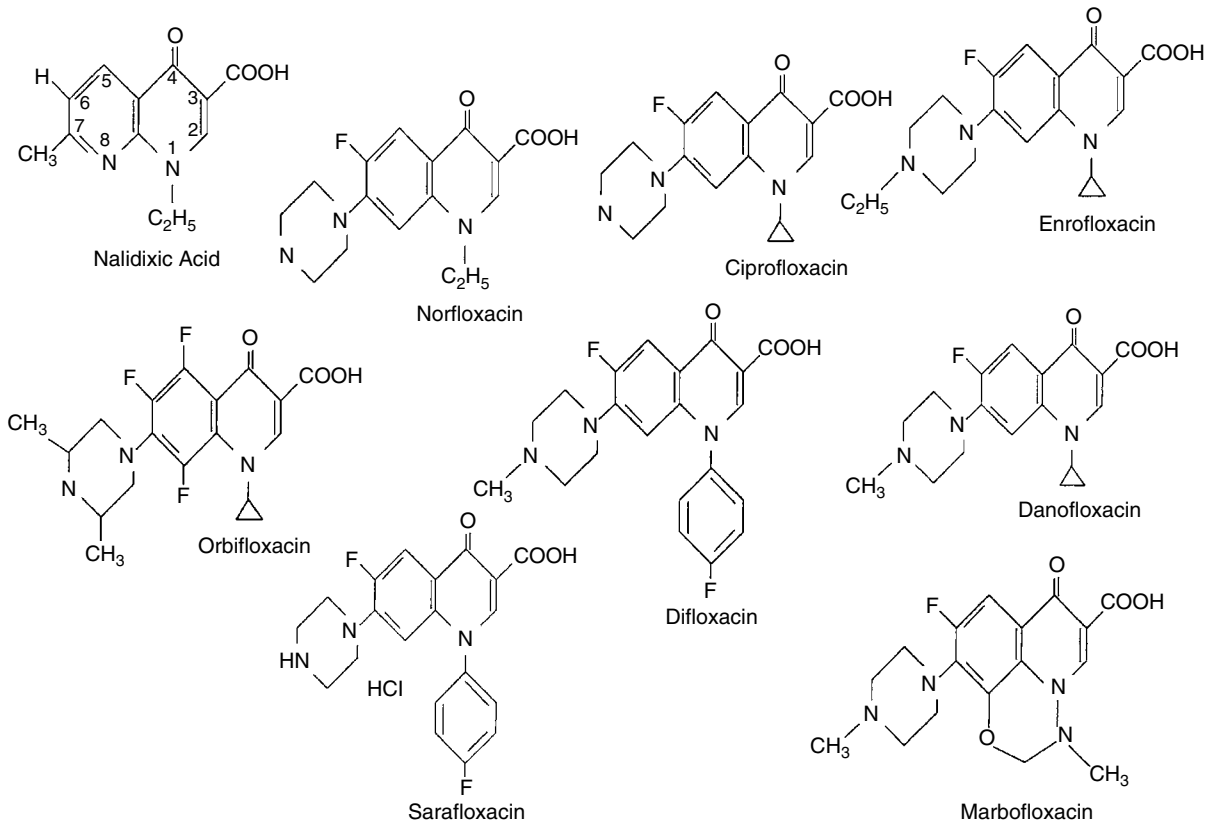

Figure 18.1. Structures of fluoroquinolones used in veterinary medicine.

two nitrogen atoms in its basic nucleus making it a naphthyridone molecule rather than a quinolone molecule. In addition, nalidixic acid is not halogenated like other quinolones. Since the discovery of nalidixic acid's antibacterial activities, more than 10,000 compounds have been designed from the parent bicyclic 4-quinolone molecule. Today the majority of fluoroquinolones marketed for clinical use in veterinary medicine are bicyclic derivatives. One exception is marbofloxacin, which is a tricyclic molecule (Figure 18.1).

Clinically, nalidixic acid has several limitations. These include a narrow spectrum of activity, poor pharmacokinetic properties, toxic effects, and a tendency to select for resistant organisms. Replacing the hydrogen atom at position 6 of the 4-quinolone molecule with a fluorine atom resulted in increased activity against both Gram-positive and Gram-negative bacteria. The increased activity is attributed to increased penetration of the bacterial cell membrane (Petersen and Schenke, 1998). Substituting a piperazinyl ring for the methyl group at position 7 increased Gram-negative activity including antipseudomonal activity. These modifications led to the development of the first broad-spectrum fluoroquinolone, norfloxacin, which was marketed in 1986. Additional studies demonstrated that substantial changes in potency could be obtained by variations at the N-1 and C-7 positions. For example, ciprofloxacin is similar in structure to norfloxacin but has a cyclopropyl group in place of the ethyl group at N-1. This substitution enhances ciprofloxacin's Gram-positive and Gram-negative activity. This cyclopropyl group is also found on enrofloxacin, danofloxacin, pradofloxacin and orbifloxacin. Difloxacin has a phenyl ring at position N-1 that reportedly gives it enhanced activity against Gram-positive bacteria, relative to enrofloxacin activity.

Difloxacin also has a second fluorine atom in its structure, whereas orbifloxacin has a total of three fluorine atoms. These additional fluorine atoms do not appear to influence the antibacterial activity of these compounds. Overall, there have been several chemical modifications at each of the eight positions in the 4-quinolone molecule. Some increase absorption, some increase antibacterial activity, and others increase toxicity. For example, ciprofloxacin and enrofloxacin are similar molecules except for the ethyl group on the piperazinyl ring of enrofloxacin. This ethyl group enhances the oral absorption of enrofloxacin over ciprofloxacin in the dog but decreases its antipseudomonal activity (Walker et al., 1990, 1992).

Mechanism of Action

The bacterial chromosome is a continuous, circular, double-stranded DNA molecule approximately 1,000 times longer than the bacteria in which it is contained. In order for such a long molecule to fit into the cell, it is densely packed in a negative supercoil, twisted in the opposite direction to the right-handed double helix of DNA. This supercoiled configuration is so highly strained that to improve function the chromosome is divided into approximately 50 topologically independent domains. Topoisomerase enzymes catalyze changes in coiling of the molecule. Topoisomerase I is characterized by reactions involving single-stranded DNA, whereas topoisomerase II is involved in reactions with double-stranded DNA. Topoisomerase II, also known as DNA gyrase, consists of two subunits, GyrA and GyrB. The *gyr*A gene encodes two α-subunits while the *gyr*B gene encodes two β-subunits; the active DNA gyrase is an A_2B_2 complex. DNA gyrase binds to DNA; a segment of approximately 130 nucleotide wraps around the DNA gyrase. This wrapped DNA is cleaved in both strands, forming a DNA-protein covalent bond between the GyrA subunit and the 5'-phosphates of the DNA molecule. Another segment of DNA is passed through this double-stranded break, which may then be resealed. The α-subunit of the DNA gyrase is important in the breakage and reunion that allow for this relaxation of the DNA molecule. In multiple species of bacteria it has been shown that the 4-quinolone molecule interrupts the DNA breakage-reunion step by binding to the DNA gyrase-DNA complex and thus leads to defects in the negative supercoiling.

Studies have also shown that the fluoroquinolones may have a second intracellular target, DNA topoisomerase IV (Topo IV; Kato et al., 1990, 1992). This is a bacterial type II DNA topoisomerase and is also a multimeric protein composed of two ParC sub-units and two ParE subunits, which exhibit sequence homology to GyrA and GyrB, respectively. This enzyme mediates relaxation of duplex DNA and the unlinking of daughter chromosomes following replication (Zechiedrich and Cozzarelli, 1995). However, unlike the DNA gyrase, Topo IV cannot supercoil DNA. Instead it is involved in the ATP-dependent relaxation of DNA. It is a more potent decatenase than DNA gyrase (Hoshino et al., 1994). Topo IV may be the primary target of fluoroquinolones in *S. aureus* and streptococci (Ferrero et al., 1994; Kaatz and Seo, 1998). This indicates that the primary target of fluoroquinolones varies in different bacteria.

The effect of fluoroquinolones on bacterial proliferation suggests three mechanisms of cell killing (Maxwell and Critchlow, 1998; Guthrie et al., 2004; Martinez et al., 2005):

1. Mechanism A: common to all quinolones. This requires RNA and protein synthesis and is only effective against dividing bacteria. Mechanism A appears to involve the blocking of replication by the gyrase-quinolone complex on DNA.
2. Mechanism B: does not require RNA and protein synthesis and can act on bacteria that are unable to multiple. Mechanism B (chloramphenicol insensitive) can be correlated with dislocation of the gyrase sub-units that constrain the ternary complex.
3. Mechanism C: requires RNA and protein synthesis, but does not require cell division. Mechanism C may correlate with trapping of topo IV complexes on DNA.

Antimicrobial Activity

Breakpoints for susceptibility to fluoroquinolones commonly used in veterinary medicine are listed in Table 18.2. The fluoroquinolones have excellent activity *in vitro* against a wide range of aerobic Gram-negative bacteria, including the Enterobacteriaceae, *Actinobacillus pleuropneumoniae*, *Histophilus somni*, *Mannheimia haemolytica*, and *Pasteurella* spp. including *P. multocida*. They are also active against *Bordetella bronchiseptica, Brucella* spp., *Chlamydia/Chlamydophila* spp., *Mycoplasma* spp., and *Ureaplasma*. Fluoroquinolones are active against rapidly growing mycobacteria isolated from dogs and cats (Govendir et al., 2011). In general, pradofloxacin tends to

Table 18.2. Minimal inhibitory concentration breakpoints for fluoroquinolones commonly used in veterinary medicine.

Drug	Species	Indications*	MIC breakpoint (μg/ml)	
			Susceptible	Resistant
Enrofloxacin	Cats, dogs	Dermal, respiratory, UTI	≤ 0.5	≥ 4
	Cattle	Respiratory	≤ 0.25	≥ 2
Ciprofloxacin	People	Various	≤ 1	≥ 4
Orbifloxacin	Cats, dogs	Dermal, UTI	≤ 1	≥ 8
Marbofloxacin	Cats, dogs	Dermal, UTI	≤ 1	≥ 4
Difloxacin	Dogs	Dermal, UTI	≤ 0.5	≥ 4
Danofloxacin	Cattle	Respiratory	≤ 0.25	≥ 2
Pradofloxacin	Cats, dogs	Dermal, respiratory, periodontal, UTI	≤ 1	≥ 2

*Indications may vary according to species and countries of approval.
UTI = urinary tract infection.

be more active (i.e., lower MICs) against most Gram-negative bacteria than other veterinary fluoroquinolones (Liu et al., 2012a; Schink et al., 2012). Activity against *Pseudomonas aeruginosa* is dependent on the fluoroquinolone, with ciprofloxacin being the most potent agent against this bacterium (Van Bambeke et al., 2005). For the most part, the first- and second-generation fluoroquinolones are less active against Gram-positive bacteria, especially enterococci, and have poor activity against anaerobic bacteria. Newer (third-generation) fluoroquinolones target this deficiency. For example, trovafloxacin, moxifloxacin, and gatifloxacin are newer fluoroquinolones with good *in vitro* activity against obligate anaerobes (Stein and Goldstein, 2006). Most fluoroquinolones approved for use in veterinary medicine should be considered to be ineffective against obligate anaerobes. The only exception is pradofloxacin, which is active against anaerobic bacteria from dogs in cats including *Clostridium* spp., *Bacteroides* spp., *Fusobacterium* spp., and *Prevotella* spp. (Silley et al., 2007).

The *in vitro* activities of fluoroquinolones used in veterinary medicine are listed in Tables 18.3, 18.4, and 18.5. Because the susceptibility of some bacterial isolates of animal origin to the fluoroquinolones decreases over time, the values listed in the table needs to be evaluated in relation to the isolation date of the microorganisms. In addition, the proportion of bacterial isolates resistant to various fluoroquinolones vary considerably between studies. In one study, approximately 20% of all Gram-negative isolates and 40% of *E. coli* isolates from dogs and cats were resistant to fluoroquinolones (Boothe et al., 2006).

Fluoroquinolones exhibit a biphasic dose response curve (paradoxical effect) in that they are less active at concentrations below, equal to or much higher than the MIC (Brown, 1996; Martinez et al., 2005). As the ratio of fluoroquinolone concentration to MIC increases from ≤ 1:1 to the optimal bactericidal concentration (usually shown to be approximately 10:1–12:1 but may be drug-bacterium dependent), bacterial killing increases and is usually very rapid (Maxwell and Critchlow, 1998; Preston et al., 1998). As illustrated in Figure 18.2, when a strain of *M. haemolytica* is exposed to a fluoroquinolone at concentrations that are 25% of its MIC, the drug exhibits a slight stationary effect but then the bacterium resumes growth at a rate similar to that of the untreated control. As the concentration of the drug is increased above the MIC there is a decrease in the number of viable organisms. For drug concentrations that are equivalent to the MIC, there is a slight decrease in the number of viable organisms but after 24 hours of exposure the number of viable organisms has increased to more than what was in the starting suspension. This is without an increase in MICs. This suggests that this fluoroquinolone, at concentrations that are equal to the MIC, has a static effect on *M. haemolytica*. When the concentration of this fluoroquinolone is increased to 4 times the MIC there is a nearly 4 $\log_{10}$ reduction in the number of viable organisms within 4 hours of exposure. However, this killing effect stabilizes and then the organisms begin to proliferate, again without an increase in MIC. This is in contrast to the growth

Table 18.3. Microbiological activity (MIC_{90}, μg/ml) of selected fluoroquinolones to common bacterial pathogens isolated from animals.

	Enrofloxacin[a]		Orbifloxacin[b]		Ibafloxacin[c]		Difloxacin[d]		Ciprofloxacin[e]		Pradofloxacin[f]	
Microorganism	MIC_{90}* Range	No. Isolates	MIC_{90} Range	No. Isolates	MIC_{90} Range	No. Isolates	MIC_{90} Range	No. Isolates	MIC^{90} Range	No. Isolates	MIC_{90} Range	No. Isolates
Aerobes												
Bordetella bronchiseptica	0.5–2.0	273			8	35	2–8	53	1	43	0.25	144
Staphylococcus intermedius	0.12–0.5	349	0.5–1.0	321	0.25	281	0.125–1.0	186	0.25	25	0.06	1606
S. pseudintermedius	0.25	177	0.5	177	0.12	177	0.5	177	0.25	177	0.12	178
S. aureus	0.12–0.25	202	0.5	15	0.25	86			0.5	50	0.5	269
Streptococcus canis			1.0–2.0	36	32	34	2	17				
Enterococcus spp.	1–2	59	16–32	35	4	31						
Escherichia coli	0.03– 0.125	529	0.5	78	0.5	150	0.125–0.25	81	≤0.015–0.06	95	0.25–2	1466
Klebsiella pneumoniae	0.06–0.12	104	0.25	12	0.5	24	0.5	20	0.06	37	0.25	38
Pasteurella multocida	0.03	48	0.03	48	0.03	48	0.03	48	0.015	48	0.015	57
Proteus spp.	0.12–0.5	147	1.0–2.0	24	0.5	43	1–4	48	0.03–0.06	58	4	185
Pseudomonas spp.	1–8	246	8–16	17	16	45	4	24	0.12	50	2–8	534
Anaerobes												
Clostridium spp.	8	32			4	32	8	32	8	23	0.5	32
Bacteroides spp.	8	28			32	28	8	28	> 32	108	1	28
Fusobacterium spp.	32	22			32	22	16	22	8–16	47	1	22
Prevotella spp.	8	20			16	20	16	20	8	74	1	20

[a]Sources are Carbone et al., 2001; Lautzenhiser et al., 2001; Speakman et al., 1997; Speakman et al., 2000; Walker 1998–1999 unpublished data; Watts, 1997; Schink et al., 2012; Silley et al., 2007.
[b]Ganiere et al., 2004; Schink et al., 2012; technical monograph, values adjusted to CLSI dilution schemes.
[c]Coulet et al., 2002; Schink et al., 2012; Silley et al., 2007.
[d]Carbone et al., 2001; van den Hoven et al., 2000; Schink et al., 2012; Silley et al., 2007.
[e]Carbone et al., 2001; Walker et al., 1990; Watts et al., 1997; Schink et al., 2012.
[f]deJong, 2004; Schink et al., 2012; Silley et al., 2007.
*MIC_{90} is presented for all organisms where more than 15 isolates were tested. MIC_{90} range is presented if the data was generated from more than one study and each study presented a different MIC_{90}.

Table 18.4. Susceptibility of bovine bacterial pathogens to marbofloxacin.[a]

Organism	Year Isolated	N	MIC µg/ml ≤ 0.06	0.12–1	≥ 2
Escherichia coli (enteric)	2000	151	93[b] (62)[c]	35 (85)	23 (100)
	2001	79	46 (58)	19 (82)	14 (100)
E. coli (mastitis)	2000	102	100 (98)	2 (100)	
	2001	96	93 (97)	2 (99)	1 (100)
Salmonella spp.	2000	57	50 (88)	7 (100)	
	2001	49	43 (88)	6 (100)	
Mannheimia haemolytica	2000	81	52 (64)	24 (94)	5 (100)
	2001	30	12 (40)	15 (90)	3 (100)
Pasteurella multocida	2000	109	94 (86)	14 (99)	1 (100)
	2001	67	56 (84)	11 (100)	
Staphylococcus aureus	2000	67	2 (3)	65 (100)	
	2001	45		45 (100)	
Streptococcus spp.[d]	2000	102	100 (98)	2 (100)	
	2001	96	93 (97)	2 (99)	1 (100)

[a]From Meunier et al., 2004.
[b]Number of isolates.
[c]Cumulative percentage.
[d]*Streptococcus* isolates tested included *S. agalactiae, S. dysgalactiae*, and *S. uberus*.

Table 18.5. Susceptibility of various canine and feline bacterial pathogens to marbofloxacin.[a]

Organism	Year Isolated	N	MIC µg/ml ≤ 0.06	0.12–1	≥ 2
Escherichia coli	1999	22	18[b] (82)[c]	1 (86)	3 (100)
	2000	34	27 (79)	3 (88)	4 (100)
	2001	20	17 (85)	1 (90)	2 (100)
Pseudomonas aeruginosa, skin	1999	33		30 (91)	3 (100)
	2000				
	2001	29[d]		27 (93)	2 (100)
P. aeruginosa, otitis	1999	21		18 (86)	3 (100)
	2000	16		16 (100)	
	2001	23		17 (74)	6 (100)
Staphylococcus intermedius	1999	33		32 (97)	1 (100)
	2000	33		33 (100)	
	2001	19		19 (100)	

[a]Meunier et al., 2004.
[b]Number of isolates.
[c]Cumulative percentage.
[d]Represents isolates from 2000 and 2001.

rate when the concentration of the fluoroquinolone is 8 times the MIC. Under this circumstance there is a very rapid bactericidal affect, 7 $\log_{10}$ reduction in viable organisms, and after a 24-hour exposure there was no detectable regrowth of the bacterium. This suggests that at this concentration to MIC ratio there was a 100% bactericidal effect. The concentration-dependent killing effect may plateau when the ratio of fluoroquinolone

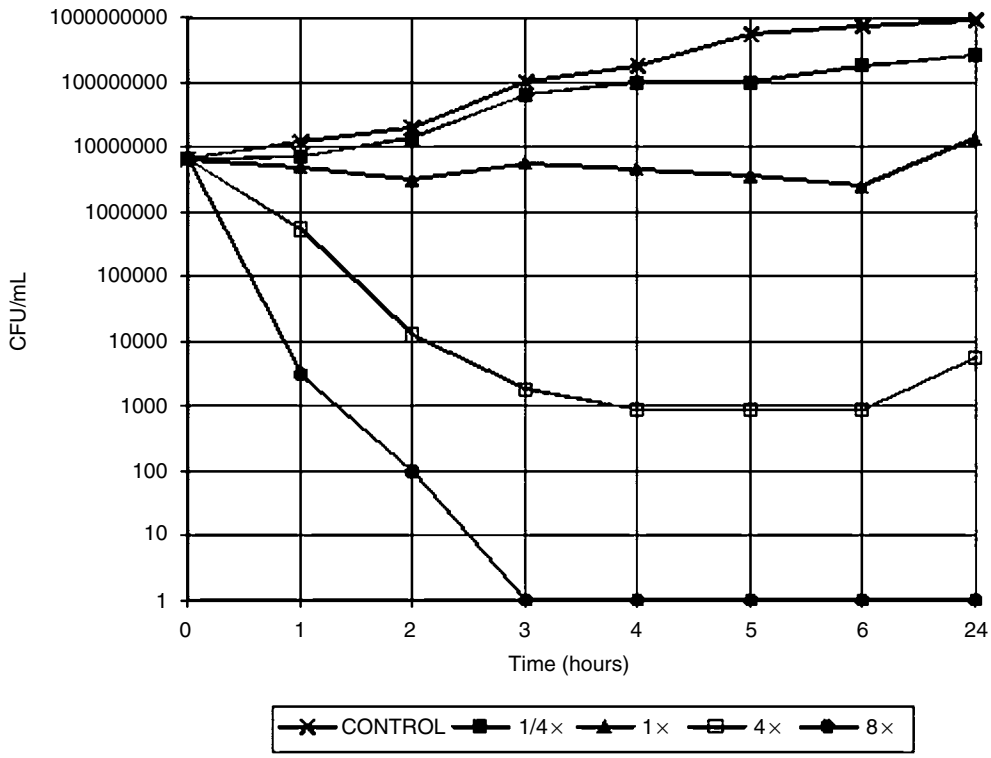

Figure 18.2. Concentration-dependent killing effect of a fluoroquinolone tested against *Mannheimia (Pasteurella) haemolytica*.

concentration to MIC reaches 15:1–20:1 and at ratios greater than 20:1 the fluoroquinolones may become bacteriostatic (Schentag and Scully, 1999). Others, however, have not observed this paradoxical effect, even at concentrations 200 times the MIC (Gould et al., 1990). The decrease in antibacterial activity at high drug concentrations is thought to be caused by the inhibition of RNA and protein. This implies that protein synthesis may be required for quinolone-mediated cell death. In this regard, it has been reported that protein synthesis inhibitors (such as chloramphenicol) and RNA synthesis inhibitors (such as rifampin) may reduce fluoroquinolone effectiveness in bacterial killing but this has not been demonstrated clinically (Guthrie et al., 2004; Maxwell and Critchlow, 1998).

While the antibacterial activity of the fluoroquinolones is dependent on the drug concentration relative to the MIC of the bacterium, the MIC is independent of the bacterial concentrations. As the bacterial concentration increases from 10^3 to 10^8 colony-forming units/ml (CFU/ml) the MIC remains constant. This is not the case with the minimal bactericidal concentration (MBC). As the bacterial concentration increases from 10^8 to 10^{10} CFU/ml, fluoroquinolone activity goes from decreased bactericidal activity to bacteriostatic (Bryskier, 2005). This phenomenon may be related to the lack of oxygen due to bacterial metabolism, as under anaerobic conditions ciprofloxacin becomes bacteriostatic.

The important feature of the antimicrobial activity fluoroquinolones is their general concentration dependent killing, which has the additional benefit of preventing the emergence of resistance. Targeting fluoroquinolone dosage to the MIC of the pathogen, as discussed below under pharmacodynamic properties, will not only increase clinical resolution but will reduce the emergence of resistance, which is the Achilles' heel of this group of antimicrobial drugs.

Bacterial Resistance

Resistance to the fluoroquinolone occurs by target modification, decreased permeability, efflux and/or target protection. Each of these fluoroquinolone resistance mechanisms can occur simultaneously within the same cell, thereby leading to very high resistance levels. To date, no mechanisms based on enzymatic inactivation/modification of fluoroquinolones have been discovered. Because fluoroquinolones are synthetic antimicrobials with no known natural analogues, it appears less likely that this type of mechanism will emerge. Selection of resistant mutants by decreased permeability or efflux mechanisms generally means a two- to eight-fold increase in MIC, whereas alteration of the DNA gyrase binding site or target protection may result in high-level resistance. Resistance to one fluoroquinolone frequently results in resistance to all. This is especially true for the older compounds and for high-level resistance. Fluoroquinolone resistance due to target mutations typically results in decreased susceptibility or resistance to other fluoroquinolones. Resistance due to alterations in permeability or activation of the efflux pump can confer resistance to other antimicrobial agents such as the cephalosporins, carbapenems, and tetracyclines (Everett et al., 1996; Piddock et al., 1998; Poole, 2000; Van Bambeke et al., 2005; Gibson et al., 2010; Liu et al., 2012b).

Because fluoroquinolones mediate DNA damage by binding to susceptible enzymes, fluoroquinolone-resistance mutations are recessive. For topoisomerase-mediated fluoroquinolone resistance to be transferred horizontally, an acquired mutated gene has to supplant the wild-type gene. The development of fluoroquinolone resistance via mutations in topoisomerases has been studied extensively. Resistance is mediated primarily by target mutations in DNA gyrase (topoisomerase II; Nakamura et al., 1989; Yoshida et al., 1990), with secondary mutations in topoisomerase IV contributing to higher levels of resistance (Vila et al., 1996). Amino acid substitutions that result in bacterial resistance have been localized to a specific topoisomerase subdomain termed the *quinolone resistance-determining region* (QRDR) within *gyrA* (Yoshida et al., 1988; 1990) and *parC* (Khodursky et al., 1995). In *E. coli*, most mutations associated with quinolone resistance occur in the QRDR at serine 83 (Ser83) and aspartate 87 of *gyrA*, and at serine 79 and aspartate 83 of *parC* and at analogous sites in other species (Takiff et al., 1994; Taylor and Chau, 1997; Bebear et al., 2003). DNA sequence analysis of *S. aureus* and *Streptococcus* genes shows that the situation can be reversed in Gram-positive bacteria, where topoisomerase IV (encoded by *grlA* and *grlB*) is the primary fluoroquinolone target (Munoz and De La Campa, 1996; Ng et al., 1996). In both cases, mutations decrease the quinolone affinity for the enzyme/DNA complex (Maxwell and Critchlow, 1998), and allow DNA replication to continue in the presence of fluoroquinolone concentrations that are inhibitory to wild-type cell growth.

In Gram-negative organisms quinolone resistance typically develops in a stepwise manner. A single QRDR mutation, usually at Ser83, confers resistance to nalidixic acid and decreases susceptibility to fluoroquinolones (ciprofloxacin MICs may go from a wild-type baseline of 0.015–0.03 μg/ml to 0.125–1 μg/ml). Secondary mutations in the *gyrA* QRDR lead to overt fluoroquinolone resistance (ciprofloxacin MICs ≥ 4 μg/ml). However, this does not hold true for all Gram-negative bacteria. In *Campylobacter* spp., which lack topoisomerase IV, a single mutation in *gyrA* is sufficient to impart high-level ciprofloxacin MICs (32 μg/ml; Wang et al., 1993). This feature helps explain the higher prevalence of resistance in C*ampylobacter*, compared to *E. coli*, from food animals exposed to fluoroquinolones (Van Boven et al., 2003).

As indicated above, fluoroquinolone resistance may also be mediated by decreased permeability of the bacterial cell wall through altered outer membrane porins (OmpF) and by the activity of energy-dependent efflux pumps. Most fluoroquinolones cross the Gram-negative outer membrane through protein channels called porins (Nikaido and Vaara, 1985), although some may diffuse directly across the lipid bilayer. Resistance due to decreased quinolone influx is generally reflected in low-level changes in susceptibility and may explain differences in potency among different fluoroquinolone derivatives. Porin deficiency has been associated with quinolone resistance in *E. coli* and *Pseudomonas*. For example, mutations of the *E. coli* porin OmpF produced about a two-fold increase in quinolone MICs (Alekshun and Levy, 1999).

However, it is difficult to experimentally assess the role of porins without also accounting for effects due to efflux. Permeability changes mediated by altered porins are often part of a coordinated cellular response to the presence of

numerous toxic agents, which includes simultaneous up regulation of efflux. In *E. coli*, de-repression in regulatory loci such as *marA* or *soxS* leads to decreased fluoroquinolone susceptibility via simultaneous up-regulation of the AcrAB-TolC efflux pump (Okusu et al., 1996) and down-regulation of the OmpF porin (Cohen et al., 1988). This mechanism confers decreased susceptibility to a large number of other antimicrobial agents in addition to fluoroquinolones. Analogous regulatory loci exist among other species of bacteria (Cohen et al., 1993).

In antimicrobial efflux systems, membrane-localized proteins actively pump drug from the cell before it can diffuse to its primary target within the active site of DNA gyrase. Because they are driven by the proton motive force, energy uncouplers can be used to study their role in resistance. The *E. coli* genome carries as many as 30 potential efflux pumps, many of which mediate antimicrobial efflux. Some are effective for specific agents, whereas others protect against a variety of structurally diverse compounds. In addition, a single bacterium may contain multiple efflux pumps (e.g., AcrAB and CmlA) that are capable of extruding the same antimicrobial agent. Constitutive and inducible efflux is a known mechanism of fluoroquinolone resistance in both Gram-negative and Gram-positive bacteria, and may be more important than secondary mutations in topoisomerase IV genes. For example, it has been shown that deletion of the gene encoding the inducible AcrAB efflux pump reduces ciprofloxacin MICs to near wild-type levels in cells carrying topoisomerase mutations (Oethinger et al., 2000). In *Campylobacter*, where efflux mediated by CmeAB is constitutive, fluoroquinolone MICs in wild-type cells are three- to four-fold higher than those typical of *E. coli*. Insertional inactivation of CmeAB in *C. jejuni* reduces ciprofloxacin MICs to levels near that of wild-type *E. coli* (0.003 μg/ml; Luo et al., 2003). These findings have led some drug developers to examine bacterial efflux systems as potential targets for compounded antimicrobial therapeutics.

Bacterial fluoroquinolone resistance was once thought to disseminate exclusively via clonal expansion under selective pressure. Recently, a plasmid-mediated quinolone resistance gene (*qnr*) was described, first in clinical isolates of *Klebsiella pneumoniae* (Martinez-Martinez et al., 1998) and later in *E. coli* (Jacoby et al., 2003; Wang et al., 2003; Kirchner et al., 2011). The *qnr* gene is located near sequences (*qacEA¨* 1, *sulI*) typically associated with class I integrons: the *qnr* gene encodes a 218 amino acid protein belonging to the pentapeptide repeat family (Tran and Jacoby, 2002). In a concentration-dependent manner, *qnr* functions by protecting *E. coli* DNA gyrase, but not topoisomerase IV, from inhibition by ciprofloxacin (Tran and Jacoby, 2002). The *qnr* gene confers a small decrease in quinolone susceptibility such that *qnr* + strains are still considered clinically susceptible. The presence of *qnr* permits selection of topoisomerase mutants at concentrations that normally would be toxic to the bacterium (Martinez-Martinez et al., 1998).

Pharmacokinetic Properties

The fluoroquinolones are rapidly and well absorbed from the gastrointestinal tract of monogastric animals and pre-ruminant calves. Enrofloxacin is more lipid soluble than ciprofloxacin and has a higher oral bioavailability than ciprofloxacin in horses and small animals. All of the oral veterinary products typically have high bioavailability in dogs and cats, but enrofloxacin bioavailability was poor in neonatal kittens (Seguin et al., 2004). The oral bioavailability of enrofloxacin is approximately 60% in adult horses and 42% in foals. While it is extremely low in adult cattle, it is surprisingly good in sheep (80%). The pharmacokinetic parameters of fluoroquinolones administered to dogs, cattle, horses, and pigs are given in Table 18.6. Ingestion with food may delay the time to peak serum concentrations without affecting total serum concentrations, unless the food is rich in magnesium or aluminum ions. Increases in oral dose usually produce linear increases in serum concentrations. Following absorption, fluoroquinolones exhibit rapid and extensive tissue distribution because of their hydrophilic nature and low (< 50%) protein binding. Their apparent volumes of distribution exceed total body water (> 1 L/kg). In general, fluoroquinolone concentrations in interstitial fluid, skin, and bones are 35–100% of those obtained in the serum, whereas bronchial secretions and prostatic concentrations may be 2–3 times the corresponding serum concentrations. Penetration into cerebrospinal fluid is approximately 25% of serum concentration. Therapeutic concentrations for Gram-negative bacteria may be achieved in the CSF and ocular fluids. High concentrations are found in the bile and organs of excretion (liver, intestine,

Table 18.6. Comparative pharmacokinetic parameters of selected fluoroquinolones administered orally to cats, dogs, horses, cattle, and pigs.

Fluoroquinolone	Animal Species	Route	Dose* (mg/kg)	C_{MAX} (µg/ml)	Vd (L/kg)	$T_{1/2\beta}$ (h)	$AUC_{0->24}$ (µg·hr/ml)	Bioavailability (%)
Ciprofloxacin	Cats	IV	10		3.9	4.5	17	
		PO	10	1.26		3.7	11	33
	Dogs	IV	10	3.1	2.2			
		PO	10	1.55		4.9		
	Ponies	IV	5		3.45	2.5		6
Enrofloxacin	Cats	IV	5		2.37	6.7	18.6	
	Kittens	IV	5		1.8	4.2	16.7	
		PO	5	0.5		4.8	5.7	33.7
	Dogs	IV	5		3.7	2.4		
		PO	5	1.41		4.1	8.74	83
	Horses	IV	5		2.3	4.4		
		PO	5	5.4		6.1	35.6	63
	Foals	IV	5		2.47	17.1	48.54	
		PO	10	2.12		18.4	58.47	42
	Cattle	IV	5		4.0	2.6	4.4	
		SC	8	0.81		7.3	7.51	
	Pigs	IV	5		6.11	10.5	11.2	
		PO	10	1.4				83
Danofloxacin	Cattle	SC	8	2.4		3.8	14.76	
Difloxacin	Dogs	PO	5	1.1	4.7	6.9	9.34	
Ibafloxacin	Cats	PO	15	6.86			37.14	
	Dogs	IV	15		1.14	5.2	29.13	
		PO	15	6.04		3.4	21.28	69.1
Marbofloxacin	Cats	IV	2		1.01	7.9	21.26	
		PO	2	2.34		7.8	24.73	100
	Dogs	IV	2		1.37	12.4		
		PO	2	1.47		9.1	13.07	94
	Cattle	IM	2	1.98		6.3	7.65	
Orbifloxacin	Cats	IV	2.5		1.3	4.5	10.6	
		PO	2.5	2.06		5.5	10.82	≈100
	Dogs	IV	2.5		1.2	5.4	14.3	
		PO	2.5	1.37		7.1	12.72	≈100
Pradofloxacin	Cats	PO	3	1.2	4	8	6	70
	Dogs	PO	3	1.6	2	7	13	≈100

and urinary tract). The fluoroquinolones are concentrated within phagocytic cells. Uptake occurs by simple diffusion, and intracellular concentrations may be several times greater than plasma concentrations. Intracellular drug is microbiologically active; *in vitro* studies indicate that ciprofloxacin reduces survival of intracellular pathogens such as *Brucella* spp., *Mycoplasma* spp., and *Mycobacterium* spp.

The fluoroquinolones are predominantly excreted as unchanged drug in the urine by glomerular filtration and active tubular secretion. The exception is difloxacin, where 80% is excreted in the feces. Metabolites and the parent compound may be excreted in an active form in the bile and urine. For example, the major metabolite of enrofloxacin is ciprofloxacin. The amount of ciprofloxacin produced varies with different species, with some producing ciprofloxacin concentrations that exceed the MIC of some pathogens (Kung et al., 1993). The elimination half-life of the fluoroquinolones is dependent on the

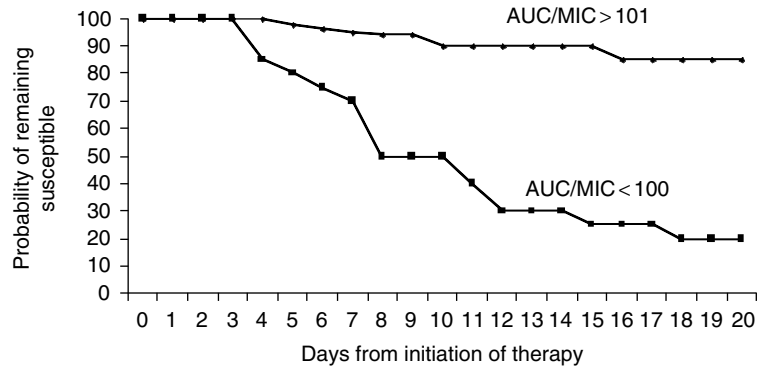

Figure 18.3. Relationship between $AUC_{0\text{-}24}$:MIC and the probability of selecting for resistant bacteria (in humans). Thomas et al., 1998; reproduced with permission.

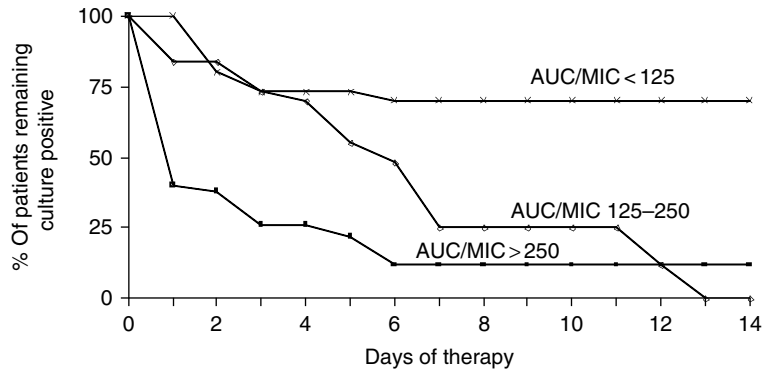

Figure 18.4. Time (days of therapy) to bacterial eradication (in humans) versus $AUC_{0\text{-}24}$:MIC ratio: a time-to-event (survival) plot. Forrest et al., 1993; reproduced with permission.

drug and the animal species, and may also be dose dependent. These long elimination half-lives make the fluoroquinolones ideal for every 24- or 48-hour dosing regimens.

Pharmacodynamic Properties

With ideal pharmacokinetic parameters but a potential to select for resistant bacteria, optimal therapeutic dosage regimens for fluoroquinolones requires integration of pharmacokinetics and pharmacodynamics (chapter 5). Pharmacodynamic indices describe the interaction of drug concentration, which is dependent on dose and pharmacokinetic properties, with the bacterial killing ability of the drug. The pharmacodynamic parameters best associated with fluoroquinolones efficacy are $AUC_{0\text{-}24}$/MIC or C_{max}/MIC ratios.

Studies with ciprofloxacin in critically ill people have shown that an AUC_{0-24}/MIC of ≥ 125 is linked with favorable clinical and microbiological outcomes, whereas an AUC_{0-24}/MIC of < 100 (or C_{max}/MIC of < 4) is associated with sub-optimal clinical and microbiological outcomes (Forrest et al., 1993; Van Bambeke, 2005). However, these ratios are dependent on the severity of the infection. For example, for less severe infections AUC_{0-24}/MIC values of 25–50 may be sufficient whereas AUC_{0-24}/MIC values exceeding 125 are required for severe infections or for immunosuppressed patients (Ambrose et al., 2007). Clinical data has shown that for severe infections, when the AUC_{0-24}/MIC ratio was ≥ 250 bacterial eradication was achieved at a faster rate than when the ratio was 125 (Schentag et al., 2003). In addition of maximizing clinical efficacy, these ratios have been shown to minimize selection of resistant organisms since a ratio of ≥ 125:1 is required for optimal bactericidal action (Figures 18.3 and 18.4; Thomas et al., 1998; Forrest et al., 1993).

The exact AUC/MIC ratio that would predict outcome of infection in domestic animals would likely vary according to animal species, infectious agent, site of infection, immune status of the host, and specific fluoroquinolones

Table 18.7. Usual dosages of fluoroquinolones in animals.[a,b,c]

Drug	Species	Route	Dose range (mg/kg)	Interval (h)	Comment
Enrofloxacin	DogsS	PO, IV	5.0–20	24	15- to 20-minute infusion
	Cats	PO, IV	5.0	24	
	Cattle	SC	2.5–5.0	24 for 3–5 days	
			7.5–12.5	Once	
	Horses[c]	IV	5.0	24	Slow IV bolus
		PO	7.5	24	
	Pigs[c]	IM	2.5–7.5	24	
Orbifloxacin	Dogs, cats	PO	2.5–7.5	24	
Difloxacin	Dogs	PO	5–10	24	
Ciprofloxacin	Dogs	PO	11–23	24	
Marbofloxacin	Dogs, cats	PO	2.75–5.5	24	
Danofloxacin	Cattle	SC	6.0	48 for 2 doses	
			8	Once	
Pradofloxacin	Dogs	PO	3–5	24	
	Cats	PO	5–10	24	

[a]Sources are from drug sponsors, package insert, or published data as indicated.
[b]Extra-label use of fluoroquinolones in food-producing animal species is illegal in the United States.
[c]Fluoroquinolones may cause arthropathies in juvenile animals.

selected. In one study predicting the efficacy of 5 fluoroquinolones used in dogs and cats based on pharmacodynamic and pharmacokinetic indices of efficacy, it was found that indices associated with a positive outcome (AUC/MIC > 125 and Cmax/MIC > 10) were more likely to be achieved with enrofloxacin, marbofloxacin, and ciprofloxacin (at the high dose listed in Table 18.7) than with orbifloxacin or difloxacin (Boothe et al., 2006).

Drug Interactions

The fluoroquinolones are synergistic when used with beta-lactams, aminoglycosides, and vancomycin against some bacterial pathogens. Some examples include *Staphylococcus aureus* (ciprofloxacin and azlocillin; levofloxacin and oxacillin), *Pseudomonas aeruginosa* (ciprofloxacin and imipenem, azlocillin, or amikacin) and enterococci (ciprofloxacin and ampicillin or vancomycin; Eliopoulos and Moellering, 1996). Antagonistic interactions have been demonstrated *in vitro* between ciprofloxacin and chloramphenicol and ciprofloxacin and rifampin (Eliopoulos and Moellering, 1996). Fluoroquinolones have been used with metronidazole to expand the antibacterial spectrum in the treatment of polymicrobial infections that involve obligate anaerobes. Oral administration of the fluoroquinolones with products containing divalent or trivalent cations, such as calcium, iron, magnesium, zinc or aluminum may reduce the absorption of the fluoroquinolones. Concurrent administration of fluoroquinolones can reduce elimination of drugs that depend on liver metabolism for excretion. For example, the fluoroquinolones decrease the hepatic clearance and thus increase the elimination half-life of theophylline and caffeine (Intorre et al., 1995). By inhibiting renal tubular secretion, probenecid has been shown to reduce the renal clearance of ciprofloxacin by 50% in humans (Stein, 1988).

Toxicity and Adverse Effects

Fluoroquinolones are relatively safe antimicrobial drugs. Administered at therapeutic doses, toxic effects are mild and generally limited to gastrointestinal disturbances such as nausea, vomiting, and diarrhea.

Chronic, high-dose fluoroquinolone therapy causes articular cartilage lesions in juvenile dogs, particularly in weight bearing joints (Burkhardt et al., 1992). Enrofloxacin inhibits cell proliferation, induces morphological changes, decreases total monosaccharide

content and alters small proteoglycan synthesis at the glycosylation level in equine tendon cell cultures (Yoon et al., 2004). These effects are more pronounced in juvenile tendon cells than in adult equine tendon cells. Arthropathies have been documented in 2-week-old foals after receiving 10 mg/kg of enrofloxacin orally (Vivrette et al., 2001). Damage was characterized by synovial joint effusion and lameness, erosion and cleft formation in articular cartilage. Arthropathies were not seen in adult horses that were given up to 25 mg/kg of enrofloxacin IV daily for 3 weeks or 15 mg/kg PO every 12 hours for 3 weeks (Bertone et al., 2000). The potential to induce arthropathy varies between different fluoroquinolones. While not recommended for use in pregnant humans or animals, the fluoroquinolones appear to have little effect on the developing fetus.

Retinal degeneration has been reported in cats treated with high doses (20 mg/kg every 24 hours) of enrofloxacin (Wiebe and Hamilton, 2002). Vision may or may not return after enrofloxacin therapy is discontinued. Although the exact mechanism retinal degeneration in cats is unknown, it appears that a similar retinal degeneration can be reproduced from either direct intravitreal injection of high concentrations of enrofloxacin or exposure to ultraviolet (UVA) light and enrofloxacin in laboratory animals. The fluoroquinolone molecular structure is similar structurally to other drugs known to directly induce retinal degeneration. Experimental evidence suggests that both enrofloxacin and its breakdown products induce retinal degeneration. Development of retinal degeneration also depends on the maximum concentration of enrofloxacin and/or its metabolites accumulating in the retina over time. Risk factors for cats appear to include (1) high doses resulting in high plasma concentrations of enrofloxacin; (2) rapid IV administration; (3) chronic treatment; and (4) advanced age. Other factors may include (1) prolonged exposure to UVA light while on enrofloxacin therapy; (2) drug interactions; and (3) altered metabolism or reduced elimination resulting in drug accumulation. Because of this it has been recommended that administration of high doses of all fluoroquinolones be avoided in the cat whenever possible. However, this toxicity may be fluoroquinolone-dependent, as limited manufacturer studies with marbofloxacin, and orbifloxacin did not demonstrate ocular toxicity in cats. Pradofloxacin at 6 and 10 times the recommended doses was shown to have no retinal toxic effects on rod or cone function in cats, as documented with electroretinography (Messias et al., 2008).

Neurotoxic effects causing central nervous system disturbances (seizures, dizziness, ataxia, insomnia, restlessness, somnolence, tremors) are common adverse effects of fluoroquinolones in humans and have been reported in horses, dogs and cats treated with enrofloxacin. Rapid IV administration of high doses of enrofloxacin to horses causes transient neurological signs, including excitability and seizure-like activity. The adverse CNS effects are due to GABA receptor antagonism, and are usually dose and specific fluoroquinolone dependent. Enrofloxacin has been associated with increased frequency and intensity of seizures in epileptic dogs (Van Cutsem et al., 1990). Because of greater penetration of the blood-brain barrier than ciprofloxacin, enrofloxacin causes hallucinations when administered to humans.

Photosensitivity and Achilles tendon rupture has been associated with the use of fluoroquinolones in humans but has not been reported in animals. Occasionally, mild interstitial inflammation of the kidney tubular walls has been associated with precipitation of fluoroquinolone complexes. Crystalluria, leading to obstructive uropathy, has been reported in human studies, but it is uncommon. Other renal toxicities may include acute renal failure, associated with interstitial nephritis. However, in human medicine, most cases of renal toxicity have been associated with overdoses.

The popularity of fluoroquinolones for use in dogs has been associated with the emergence of canine toxic shock syndrome and necrotizing fascitis caused by *Streptococcus canis* (Miller et al., 1996). Minor infections caused by *S. canis* have developed into very severe illness in dogs treated with fluoroquinolone monotherapy. Enrofloxacin can cause a bacteriophage-induced lysis of *S. canis* and superantigen expression (Ingrey et al., 2003). Superantigens are powerful inducers of T-cell proliferation, causing the release of massive amounts of host cytokines with potentially lethal effects. The toxic shock syndrome can be exacerbated by the concurrent use of corticosteroids or non-steroidal anti-inflammatory drugs.

In horses, the use of fluoroquinolones, like most antimicrobial agents has been associated with occasional cases of enterocolitis (Yamarik et al., 2010; Barr et al., 2012).

Administration and Dosage

Fluoroquinolones are usually administered orally or IV in small animals or horses and parenterally (typically SC) in ruminants. The usual dosages of some currently available fluoroquinolones are shown in Table 18.7.

Clinical Applications

Fluoroquinolones in veterinary use offer the advantages of oral administration in many species, high potency against many Gram-negative aerobic pathogens, moderate activity against Gram-positive aerobes, widespread distribution throughout the body, and low toxicity.

Their disadvantages include the tendency to select for resistant bacteria if dosed inappropriately and their only moderate activity against Gram-positive aerobes, such as pyogenic streptococci (e.g., *Streptococcus canis*). They are very effective in the treatment of urinary tract infections in animals and can be useful for serious infections such as septicemia and pneumonia caused by Gram-negative bacteria (*E. coli*, *Pasteurella* spp.), for the treatment of skin and many soft tissue infections caused by Gram-negative or some Gram-positive aerobic bacteria, and for intra-abdominal infections caused by Gram-negative aerobes. Human ophthalmic formulations are routinely used to treat Gram-negative infectious keratitis. Fluoroquinolones are the most effective antimicrobial agents for the treatment of chronic bacterial prostatitis caused by susceptible Gram-negative bacteria. They are effective in the treatment of *Mycoplasma* infections in some species. Because of their potency and ability to enter phagocytes, they have the potential to be valuable for the treatment of infections caused by atypical, intracellular, bacteria, including mycobacteria, *Brucella* spp., *Chlamydia*/*Chlamydophila* spp., *Coxiella* spp., *Ehrlichia* spp., and *Rickettsia* spp.

The introduction of fluoroquinolones for companion animals was associated with their promotion as drugs of choice for numerous infectious disease processes. One justification was that plasmid-mediated resistance was not likely to occur, or if it did, it would not be transferable. However, since the introduction of these drugs into clinical medicine, plasmid-mediated resistance has been described (Martinez-Martinez, 1998; Wang, 2003), and chromosomal resistance develops remarkably readily. Unless they are used with optimal dosing strategies, the fluoroquinolones may soon be ineffective in treating anything but the simplest infections, despite the promise they offered when they were first introduced into clinical medicine.

Cattle, Sheep, and Goats

Fluoroquinolones are quite active when tested against bacteria associated with acute respiratory disease in cattle, sheep and goats such as *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*. They also have the potential to be effective against several other species of bacteria known to cause disease in these animals, especially Gram-negative bacteria such as *E. coli* and *Salmonella*, although the MICs of these pathogens will most likely be higher than the MICs of the bacteria associated with acute respiratory disease thus requiring higher doses and longer withdrawal times. Other indications include mastitis, metritis, conjunctivitis, and infections caused by *Mycoplasma*, such as pneumonia and otitis media. The MICs for enrofloxacin and danofloxacin are within the same range as the respiratory pathogens for which these drugs are approved (Rosenbusch et al., 2005). There is some evidence of efficacy for otitis media in calves (Francoz et al., 2004), but prolonged therapy is required. Enrofloxacin is more effective than oxytetracycline for the treatment of experimentally induced bovine anaplasmosis (Facury-Filho et al., 2012).

Although fluoroquinolones should be effective in treating most of the indications noted above, in the United States enrofloxacin and danofloxacin are approved only for the treatment (enrofloxacin and danofloxacin) and control (enrofloxacin only) of pneumonia in beef cattle and veterinarians are legally prohibited from any extra-label use of fluoroquinolones in food animals. Extra-label use includes any alteration of dose, frequency of dosing, or dosing duration in any animal that may enter the human food chain. In Canada, both drugs are approved and there are cautions against extra-label drug use on product labeling, but there is no legal prohibition against extra-label drug use. In other countries, enrofloxacin, danofloxacin, and marbofloxacin have a variety of approvals for the treatment of bovine respiratory disease, colibacillosis and mastitis in lactating dairy cattle. Treatment regimens vary between products, but all should be dosed according to the ideal pharmacokinetic/pharmacodynamic methods described in this chapter. Injectable formulations tend to be irritating to muscle tissues, so most products are labeled for SC injection.

Swine

Fluoroquinolones have established value in the treatment of *Mycoplasma hyopneumoniae* infections and have the potential for the prevention or treatment of *Actinobacillus pleuropneumoniae*, *Escherichia coli*, and *Pasteurella multocida* infections. Their use should be optimized to the individual pathogen and infection. Fluoroquinolones should never be administered in feed because residues can contaminate the environment from the feed mill to the farm. Because of concern about resistance in zoonotic foodborne pathogens, fluoroquinolones are prohibited from use in pigs in the United States. Several fluoroquinolone products are approved for use in swine in other countries to treat respiratory disease and Metritis-Mastitis-Agalactia syndrome.

Horses

Because fluoroquinolones can be administered orally they are useful in horses for the treatment of a variety of Gram-negative infections caused by susceptible bacteria resistant to alternative, first-choice drugs. Fluoroquinolones are commonly used in combination with penicillin G to provide broad-spectrum antimicrobial coverage in adult horses, particularly when gentamicin is contraindicated because of compromised renal function. The main limitation of second-generation fluoroquinolones as stand-alone therapy in horses is the lack of activity against beta-hemolytic streptococci.

Kaartinen et al. (1997) found IM administration to be very irritating, resulting in swelling or tenderness at the injection site with elevated creatine kinase activity for up to 32 hours after injection. Cattle formulations can be administered slowly IV (Bertone et al., 2000) or formulated into a gel for oral administration (Epstein et al., 2004). Because of the potential of fluoroquinolones to cause cartilage erosion, their use is not recommended in young, growing horses.

Dogs and Cats

Fluoroquinolones have provided small-animal clinicians with a truly exciting new class of antimicrobials. Never before have they had products with such a broad spectrum of activity as the fluoroquinolones combined with the pharmacokinetic properties that allow for oral administration on a once-a-day basis. This has allowed clinicians to treat a larger number of patients as outpatients with more assurance of owner compliance. In most countries, only enrofloxacin is available as an injectable product. Intramuscular or SC injections are irritating but the product can be safely administered slowly IV. Enrofloxacin, difloxacin, ibafloxacin, marbofloxacin, pradofloxacin and orbifloxacin are available for oral use in small animals in many countries. Human formulations of ciprofloxacin can be used as long as the dose is corrected for bioavailability (33% in cats, 50% in dogs).

Because fluoroquinolones can penetrate nearly every tissue in the body, these drugs can be used to treat infections such as prostatitis and mastitis caused by susceptible bacteria; urinary tract infections; respiratory infections including rhinitis and pneumonia, including those caused by *Bordetella bronchiseptica*; deep and superficial pyoderma, otitis media and externa, and wound infections caused by susceptible organisms; peritonitis (used in combination with metronidazole if anaerobic bacteria are suspected); osteomyelitis caused by susceptible Gram-negative aerobes; and infections caused by mycoplasmas such as rhinitis/conjunctivitis and soft tissue infections. In one study, treatment of upper respiratory infection associated with *Chlamydophila felis* and *Mycoplasma* spp. in cats with pradofloxacin resulted in a marked improvement in clinical signs Hartmann et al., 2008). *Mycoplasma* was completely eliminated but *C. felis* DNA remained after treatment in some cats suggesting that infection might not have been eliminated (Hartmann et al., 2008). Pradofloxacin and enrofloxacin are also effective in the treatment of cats experimentally infected with *Mycoplasma hemofelis* (Tasker et al., 2004; Dowers et al., 2009).

At therapeutic doses the fluoroquinolones have proven to be relatively safe with few reported side effects. If adverse reactions do occur they are not as frequent as those reported in human medicine. The fluoroquinolones are not recommended for administration to animals less than 8 months of age or to large-breed dogs less than 18 months of age. However, since their approval in the 1980s they have been used to treat life-threatening infections in young dogs and cats without any published reports of arthropathic effects.

Poultry

In intensive poultry production, rapidly acting antimicrobial agents are needed in the face of explosive outbreaks of infectious disease. The most critical of such infections is *E. coli* septicemia and cellulitis (see chapter 35), but other important Gram-negative aerobic infections include salmonellosis and *Haemophilus (Avibacterium) paragallinarum* and *Pasteurella multocida* infections (Bauditz, 1987). Two fluoroquinolones, sarafloxacin and enrofloxacin, were developed for poultry use and approved as water medication. While studies have shown that the treatment of colibacillosis with enrofloxacin does not cause significant increases in resistant *E. coli* (van Boven et al., 2003), there is evidence that this treatment selects for ciprofloxacin-resistant *Campylobacter* in chickens (McDermott et al., 2002; Luo et al., 2003; Humphrey et al., 2005). In the United States, the approvals of both enrofloxacin and sarafloxacin have been withdrawn because of fears that fluoroquinolone-resistant *Campylobacter* from poultry contribute to human foodborne illness. In Canada, an egg dip solution for the treatment of salmonellosis in turkey eggs was once available but has been withdrawn from the market. Oral formulations of enrofloxacin and sarafloxacin have never been approved for Canadian poultry. Many of the veterinary fluoroquinolones are approved and continue to be administered orally to poultry in other countries.

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19

Miscellaneous Antimicrobials: Ionophores, Nitrofurans, Nitroimidazoles, Rifamycins, and Others

Patricia M. Dowling

This chapter discusses a variety of minor antimicrobial classes used in veterinary medicine—the ionophores, nitrofurans, nitrimidazoles, and rifamycins—in detail and briefly comments on other antimicrobials, including oxazolidinones, carbadox, fusidic acid, isoniazid, mupirocin, methenamine, and novobiocin.

Ionophore Antibiotics

Caboxylic ionophore polyether antibiotics are *Streptomyces* products used in agriculture primarily for feed efficiency and anticoccidial activity. The use of ionophores is ubiquitous; more animals have been medicated with ionophores than any other antimicrobial agents in the history of veterinary medicine. The prophylactic use of antimicrobials as growth promotants in food animals has fallen under greater scrutiny due to fears of the spread of antibiotic resistance. Reports of "tons of antimicrobials" used in food animals routinely include the ionophores. But because of the complexity and high degree of specificity of ionophore resistance, it appears that ionophores do not contribute to the development of antimicrobial resistance to important human drugs, do not affect fecal shedding of potential pathogens (e.g., *E. coli* O157:H7), and there is no need to eliminate them from use in animal feeds (Callaway et al., 2003; Lefebvre et al., 2006). These drugs behave as alkali metal ionophores to alter bacterial cell permeability; complexing with sodium in the cell membrane to cause passive extracellular transport of potassium ions and replacement by hydrogen ions, which kills the cell by lowering intracellular pH. By selectively affecting Gram-positive organisms, ionophore antibiotics cause rumen microflora to shift toward a more Gram-negative population. This increases propionic acid production while decreasing production of acetic and butyric acid. This shift in volatile fatty acids is related to increased feed efficiency. In the absence of ionophores, ruminal sugars are metabolized to acetic acid and butyric acid and lose some of their potential energy in the form of carbon dioxide and methane. However, when these sugars are converted to propionic acid, losses are reduced and energy content per unit of feed consumed is increased (Bergen and Bates, 1984). Ionophores reduce rumen methane production and ruminal protein-degradation, reduce the incidence of bloat from legume pastures, decrease rumen acidosis and help prevent tryptophane-induced atypical bovine pulmonary emphysema. Other effects of ionophores independent of ruminal effects include lowering of serum concentrations of potassium, magnesium and phosphorus and elevating serum glucose and volatile fatty acid concentrations.

Pharmacokinetic Properties

Monensin is rapidly absorbed following oral administration. Ruminants appear to absorb only about 50% of a dose, while monogastric species appear to absorb

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almost the entire administered dose. Oral bioavailability is 30% in broiler chickens (Henri et al., 2009). Ionophores do not accumulate in large amounts in tissues, even when toxic doses are administered orally (Donoho, 1984). Ionophores are rapidly and extensively metabolized by the liver into numerous metabolites, which are excreted in the bile and eliminated in the feces. Horses are not able to eliminate monensin from the blood as rapidly as cattle, which may explain why horses are the species most sensitive to monensin toxicity.

Toxicity and Adverse Effects

The relative toxicities of the ionophores from lowest to highest are salinomycin < lasalocid ≤ narasin < monensin < maduramicin (Oehme and Pickrell, 1999). Ionophore toxicity causes cellular electrolyte imbalances, elevating extracellular potassium and intracellular calcium, resulting in severe cellular damage and death. The dose necessary to cause toxicity is variable among species, with equine species being the most sensitive and turkeys being more sensitive than chickens (Table 19.1). Skeletal and cardiac muscle cells are generally the most severely affected; however, the specific tissues affected and resulting clinical signs vary from species to species. Skeletal muscle is primarily affected in dogs, ostriches, sheep and turkeys. Cardiac muscles are affected in cattle, and both myocardium and skeletal muscles are damaged in horses. Age-related differences in ionophore sensitivity occur in poultry, with adult birds more sensitive to the toxic effects of ionophores than young birds. In dogs, puppies are more sensitive to the toxic effects of narasin than adult dogs. In cattle, calves 5–8 months of age are much more susceptible to the toxic effects of maduramicin exposure than calves aged 9–16 months.

Table 19.1. Ionophore toxicity by drug and species.

Drug	Species	Toxicity
Lasalocid	Horses[a]	LD_{50} is 15 mg/kg
	Cattle	10–50 mg/kg causes depression, ataxia, paresis, inappetance, labored breathing, cardiomyopathy
		100–125 mg/kg is fatal
	Chickens	LD_{50} is 71.5 mg/kg
Maduramicin	Cattle	6 mg/kg of feed caused 50% mortality in calves
Monensin	Cattle	20–40 mg/kg caused cardiotoxicity in calves
	Chickens	LD_{50} is 200 mg/kg
	Deer	225 mg/kg of feed caused cardiomyopathy and death
	Dogs	LD_{50} is 20 mg/kg
		15 mg/kg daily for 3 months caused ataxia, cardiomyopathy, depression, diarrhea, muscle weakness, paresis, weight loss
	Goats	LD_{50} is 26 mg/kg
		50 mg/kg daily for 2 weeks caused death
	Horses	LD_{50} is 2–3 mg/kg
		125 mg/kg of feed for 28 days caused toxicity
		279 mg/kg of feed for 1–3 days caused death
	Pigs	LD_{50} is 17 mg/kg
	Ostriches	3–4 mg/kg daily for 13 days caused toxicity and death
	Sheep	12 mg/kg
	Turkeys	90 mg/kg of feed caused no adverse effects
		180–450 mg/kg of feed caused toxicity and death
Naracin	Dogs	LD_{50} is 3–10 mg/kg
		2 mg/kg daily results in mild toxicity in adults but more severe toxicity in puppies
	Rabbits	LD_{50} is 10.75 mg/kg
Salinomycin	Cattle	90 mg/kg of feed for 4–7 weeks caused toxicity and death
	Turkeys	13–18 mg/kg of feed caused toxicity and death
Semduramicin	Chickens	50–75 mg/kg of feed reduced feed intake and rate of weight gain and poor feathering

Ionophore toxicity occurs from dose errors in mixing with feed, accidental ingestion of treated feed by sensitive species, ingestion by ruminants of poultry litter from ionophore-treated flocks, concurrent administration with a medication that potentiates toxicosis, or accidental feed mill contamination of presumably untreated feed. Heat stress and water deprivation exacerbate toxicity in chickens when lasalocid is administered at 1–2 times the recommended dose. Cattle and sheep have manifested signs of ionophore toxicity following ingestion of poultry litter from chicken flocks treated with maduramicin (Bastianello et al., 1995); it is possible that toxicity might also occur if poultry litter from flocks treated with other ionophore antibiotics is fed to ruminants. Ionophore toxicosis is potentiated by medications that interfere with hepatic metabolism. Tiamulin administered concurrently with monensin caused signs of severe ionophore toxicity in chickens and pigs (Szucs et al., 2004).

Clinical Applications

Lasalocid

Lasalocid is approved in the United States for the control of coccidiosis in cattle, rabbits, chukar partridges, turkeys, broiler or fryer chickens, and sheep and for increased feed efficiency in cattle and sheep. In Canada, it is approved for improved feed efficiency and the control of coccidiosis in cattle and lambs, and to control coccidiosis in turkeys and broiler chickens. In both countries it is approved for growth promotion and improving feed efficiency in cattle. A total oral dose of 200 mg per animal per day initiated 6 days prior to tryptophan exposure is effective under experimental conditions for the prevention of acute bovine pulmonary edema and emphysema (fog fever) in cattle. It has been suggested that continuing lasalocid for 10 days following abrupt change in pasture will protect cattle during the critical period. This dose is within the labelled dose range for other indications. There is some evidence to suggest that the dose labelled for growth promotion is effective in preventing grain bloat in cattle (Bartley et al., 1983).

Laidlomycin

Laidlomycin is approved for use in feedlot cattle in the United States with similar growth-promoting effects as monensin.

Maduramicin

Maduramicin is approved as a premix for coccidiosis control in broiler chickens and turkeys in Canada. Reduced rate of growth and no improvement in feed efficiency occurs if feed concentrations of 6 parts per million are administered to chickens not suffering from coccidiosis.

Monensin

Monensin is a fermentation product of *Streptomyces cinnamonensis*. It is active against Gram-negative bacteria, some *Campylobacter* spp., and *Brachyspira (Serpulina) hyodysenteriae* (MIC 0.1 μg/ml), as well as against coccidia and *Toxoplasma*. Its antimicrobial effect in the rumen influences the production of volatile fatty acids, which promotes growth and feed efficiency, helps prevents bloat and aids in the prevention of ketosis in dairy cattle (Gallardo et al., 2005). Monensin prevents clinical signs of tryptophan-induced acute bovine pulmonary edema in cattle (Potchoiba et al., 1992) and appears to reduce the development of lactic acidosis in cattle suffering from grain overload (Burrin and Britton, 1986). Monensin may reduce abortion and control neonatal losses from toxoplasmosis in sheep (Buxton et al., 1988). Monensin supplementation decreased the duration of shedding in *E. coli* O157:H7 positive cows on a forage diet (Van Baale et al., 2004). Monensin is frequently used in poultry production for the control of coccidiosis (Chapman et al., 2010).

In the United States, monensin is available as a feed premix for use in beef cattle for improved feed efficiency and coccidiosis control, for lactating dairy cattle for improved milk production, for coccidiosis control in bobwhite quail, chickens, turkeys, and goats. In Canada, monensin premix is approved for improved feed efficiency in beef cattle and coccidiosis control in broiler chickens, turkeys and calves, increasing milk protein and reducing milk fat in lactating dairy cows and minimizing loss of body condition during lactation in dairy cows. Monensin is available in Canada as controlled-release capsules to prevent legume bloat control coccidiosis and reduce fecal shedding of *Mycobacterium avium* subsp. *paratuberculosis* in cattle and prevent subclinical ketosis in lactating dairy cattle. When monensin capsules are administered, the capsule's embossed number should be recorded with the corresponding animal identification number, and cattle should be observed for 1 hour following treatment. If the capsule is regurgi-

tated, the animal is identified and re-treated with an undamaged capsule. Cattle treated with monensin capsules should be checked for 4 days following treatment for bloat, coughing, drooling, and inappetence, which could indicate that the capsule is lodged in the esophagus. Regurgitated capsules must be disposed of properly as they can be lethal to dogs if chewed.

Narasin, Nicarbazin, and Semduramicin

Narasin is approved for use to control coccidiosis in broiler chickens and promote feed efficiency in swine in Canada, but it is only approved in broiler chickens in the United States. Narasin/nicarbazin is approved for coccidiosis control in broiler chickens in Canada and the United States. Semduramicin is approved for coccidiosis control in broiler chickens in the United States.

Salinomycin

Salinomycin is approved in the United States for coccidiosis control in broiler, roaster, replacement (breeder and layer) chickens and quail, while in Canada it is only approved for coccidiosis control in broiler chickens. In Canada, salinomycin is also approved for growth promotion and feed efficiency in cattle and swine. Salinomycin is toxic to turkeys and causes excessive mortality at the label dose for chickens (Van Assen, 2006).

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Nitrofurans

Nitrofurans (furazolidone, furaltadone, nitrofurantoin, and nitrofurazone) are a group of synthetic antimicrobials with broad-spectrum activity against Gram-positive and Gram-negative bacteria, but their toxicity limits their use. While effective for the treatment of intestinal and urinary tract infections in humans and animals, the carcinogenicity of the nitrofurans led to their ban in food animals in the United States, Canada, and the European Union. However, some nitrofurans, such as nitrofurantoin and nifuroxazide, are still used for antimicrobial therapy in humans. Because cross-resistance with other antimicrobial agents does not occur, nitrofurantoin is increasingly being used as first-line therapy for acute or recurrent urinary tract infections and nocosomial urinary tract infections caused by *E. coli* (including extended-spectrum beta-lactamase-producing strains; Tasbakan et al., 2012) and multidrug-resistant enterococci (Swaminathan and Alangaden, 2010). Nifuroxazide is available in Europe as oral therapy for acute bacterial diarrhea ("traveler's diarrhea"; Taylor, 2005). Nitrofurazone, once used orally as a veterinary antimicrobial drug, causes mammary and ovarian tumors in animals. Nitrofurazone stimulates the proliferation of estrogen-dependent cells, nitrofurazone metabolites are involved in tumor initiation through oxidative DNA damage, and nitrofurazone itself

enhances cell proliferation, leading to promotion and/or progression in carcinogenesis (Hiraku et al., 2004).

The only veterinary-approved products in the United States and Canada are topical wound formulations of nitrofurazone and furazolidone for use in non-food animals. But the use of oral human formulations of nitrofurantoin in the treatment of resistant urinary tract infections in dogs and cats is becoming more common, despite the lack of pharmacokinetic/pharmacodynamic studies and the risk of adverse effects (Maaland and Guardabassi, 2011). Nitrofurantion also shows promising activity against methicillin-resistant staphylococci (Rubin and Chirino-Trejo, 2011).

Because of carcinogenicity, the nitrofurans are of high regulatory concern. The nitrofurans are rapidly metabolized after administration, resulting in stable tissue-bound metabolites, which persist in muscle and liver for weeks to months. These metabolites, 3-amino-2-oxazolidinone (the metabolite of furazolidone), 1-aminohydantoin (the metabolite of nitrofurantoin), and semicarbazide (the metabolite of nitrofurazone), are the marker residues for their parent compounds in animal tissues. Nitrofuran metabolite residues in food animal products are resistant to degradation during storage or by cooking (Cooper and Kennedy, 2007).

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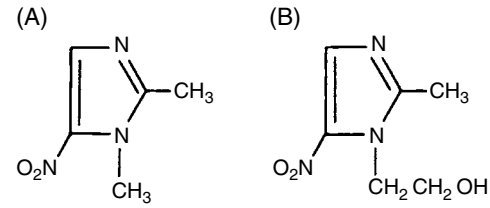

Figure 19.1. Structural formulas of nitroimidazole drugs: (A) metronidazole; (B) dimetridazole.

Nitroimidazoles

The nitroimidazoles include metronidazole, dimetridazole, ronidazole, tinidazole and ipronidazole. Like the nitrofurans, the nitroimidazoles were once widely used in veterinary medicine, but because of potential carcinogenicity, have now been banned for use in food animals in the United States, Canada, and the European Union. Metronidazole is still used human medicine and in companion animals for its excellent activity against anaerobes and protozoa. Ronidazole is used in cats for the treatment of *Tritrichomonas foetus*.

Chemistry

Nitroimidazoles are heterocyclic compounds based on a 5-membered nucleus similar to that of the nitrofurans (Figure 19.1).

Mechanism of Action

After entry into the cell, nitroimidazoles undergo reduction of the nitro group to produce a variety of unstable intermediates, including antibacterial products. Reduction occurs under anaerobic conditions, but unlike the nitrofurans, it is not enzymatically controlled. The reduction system of aerobic bacteria is insufficiently low for reduction to occur, but there is the suggestion that these agents or their metabolites, produced by anaerobic bacteria, may have some activity against aerobic bacteria under anaerobic conditions. Nitroimidazoles cause extensive breakage of DNA strands and inhibition of the DNA repair enzyme DNAase 1.

Antimicrobial Activity

The antimicrobial activity of the clinically useful nitroimidazoles is similar. They are bactericidal to most Gram-negative and many Gram-positive anaerobic

Table 19.2. *In vitro activity* (MIC_{90}, μg/ml) of metronidazole against selected anaerobic bacteria.

Organism	MIC_{90}	Organism	MIC_{90}
Gram-positive anaerobes			
Clostridium spp.	4	*Actinomyces* spp.	> 128
C. perfringens	2	*Eubacterium* spp.	4
C. difficile	0.5	*Peptostreptococcus* spp.	≥ 64
C. septicum	2	*Peptococcus* spp.	1
Gram-negative anaerobes			
All anaerobes	2	*Porphyromonas asaccharolytica*	2
Bacteroides fragilis	2	*Fusobacterium* spp.	0.5
Bacteroides spp.	2	*Brachyspira hyodysenteriae*	0.5

bacteria (Table 19.2). They are highly active against *Brachyspira* (*Serpulina*) *hyodysenteriae* and a variety of protozoa (*Tritrichomonas foetus, Giardia lamblia, Histomonas meleagridis*). *Campylobacter* spp. are moderately susceptible. *Heliocobacter pylori* of human origin are commonly susceptible, but the susceptibility of animal-origin *Helicobacter* species has not been sufficiently investigated and treatment does not clear infection in dogs and cats (Happonen et al., 2000). Trichomonads, such as *Tritrichomonas foetus* are susceptible to nitroimidazoles drugs because they utilize reductive metabolic pathways.

Resistance

Resistance to metronidazole is rare among usually susceptible bacteria (Lofmark et al., 2010). Resistance involves reduced intracellular drug activation. Cross-resistance between nitroimidazoles is complete. Equine and canine isolates of *Clostridium difficile* and *Clostridium perfringens* resistant to metronidazole have been described, so susceptibility testing is warranted in patients with clostridial diarrhea (Gobeli et al., 2012; Magdesian et al., 2006; Marks and Kather, 2003). *Bacteroides fragilis* resistant to metronidazole therapy has been reported in a horse with pleuropneumonia (Dechant, 1997). Administration of metronidazole has been identified as a risk factor for dogs becoming rectal carriers of multidrug-resistant *E. coli* during hospitalization (Gibson et al., 2011). *Tritrichomonas foetus* isolates from cats may demonstrate resistance to metronidazole and ronidazole when cultured under aerobic conditions (Gookin et al., 2010). These resistant isolates exploit oxygen in the environment to out-compete the nitroimidazoles for ferredoxin-bound electrons by decreasing the activity of their own oxygen scavenging pathways.

Pharmacokinetic Properties

Metronidazole is a weak base that is moderately lipophilic with a low molecular weight, which facilitates penetration of cell membranes and allows almost complete systemic absorption. Metronidazole is rapidly but variably absorbed after oral administration, with an oral bioavailability of 75–85% in horses, 59–100% in dogs, and 28–90% in cats (Neff-Davis et al., 1981; Sekis et al., 2009; Steinman et al., 2000). In horses with gastrointestinal ileus, metronidazole may be administered per rectum and is rapidly absorbed; however, the bioavailability is only 30%. Metronidazole is lipophilic and widely distributed in tissues. It penetrates bone, abscesses and the central nervous system. The volume of distribution is 0.7–1.7 L/kg in mares, 0.95 L/kg in dogs, and 0.6 L/kg in cats. It crosses the placenta and is distributed into milk in concentrations similar to those in plasma. Metronidazole is primarily hepatically metabolized by oxidation and conjugation. Both metabolites and unchanged drug are eliminated in urine and feces. The plasma elimination half-life is 3–4 hours in horses, 8 hours in dogs, and 5 hours in cats.

Ronidazole is rapidly and completely absorbed after oral administration to cats (LeVine et al., 2011). The volume of distribution is 0.7 L/kg and the elimination half-life is prolonged, at 10 hours. Therefore, twice-daily dosing resulting accumulation may explain the neurotoxicity associated with ronidazole administration in cats.

Drug Interactions

Interference with the susceptibility of anaerobic bacteria has not been reported *in vitro* when metronidazole is combined with a variety of other anaerobe-active drugs, such as clindamycin, erythromycin, penicillin G, amoxicilin-clavulanic acid, cefoxitin, and rifampin. Combined with a beta-lactam and gentamicin or enrofloxacin, metronidazole is commonly used for therapy of bacterial pleuropneumonia in horses (Mair and Yeo, 1987). The hepatic metabolism of metronidazole may be decreased when administered concurrently with cimetidine, possibly resulting in delayed elimination and increased

serum concentrations of metronidazole. Phenobarbital may induce microsomal liver enzymes, increasing the metabolism of metronidazole and decreasing serum concentrations.

Toxicity and Adverse Effects

Nitroimidazoles have been shown to be carcinogenic in some laboratory animals and mutagenic in some *in vitro* assays. These drugs are banned for use in food animals in the United States, Canada, and the European Union, but metronidazole is still directly used in people, without reports of cancer-associated morbidity. The adverse effects of metronidazole in humans include seizures, ataxia, peripheral neuropathy, and hematuria. Oral use in horses is associated with anorexia. Adverse effects of metronidazole in the dog and cat have been reported and include vomiting, hepatotoxicity, neutropenia, and neurologic signs such as seizures, head tilt, falling, paresis, ataxia, vertical nystagmus, tremors, and rigidity (Caylor and Cassimatis, 2001; Dow et al., 1989; Olson et al., 2005). Neurologic toxicity from metronidazole has been reported in dogs receiving 60 mg/kg/day for an average of 3–14 days, but there are reports of toxicity at lower dosages. The mechanism of the neurotoxic effects of metronidazole is thought to be a vasculitic neuropathy. Initially, the recommended therapy for metronidazole toxicosis was discontinuation of the drug and supportive therapy. With supportive therapy, the reported recovery times of dogs with neurologic manifestations of metronidazole toxicosis are 1–2 weeks. The recovery time can be significantly shorted by the administration of diazepam, with an initial IV bolus of 0.5 mg/kg and then PO every 8 hours for 3 days (Evans et al., 2003). Recovery time is markedly shorter for diazepam-treated dogs (40 hours) compared to untreated dogs (11 days). While the mechanism of this effect is unknown, it is likely that diazepam at therapeutic concentrations competitively reverses the binding of metronidazole to the benzodiazepine site on the GABA receptor.

Ronidazole is also associated with neurotoxicity in dogs and cats, particularly with dosages > 60 mg/kg/day (Rosado et al., 2007). Clinical signs include altered mentation, trembling, weakness, ataxia, and hyperesthesia. The rapid absorption and low elimination of ronidazole in cats may increase the risk of neurotoxicity with high doses and/or frequent dosing.

Administration and Dosage

Since antibacterial effect is concentration-dependent, twice a day therapy is now recommended over 3 times a day therapy. All nitroimidazoles are now banned for use in food animals in the United States, Canada, and the European Union. There are no veterinary formulations of metronidazole, so human formulations are used. Metronidazole USP induces salivation and inappetence when administered orally to some cats. Products containing metronidazole benzoate are commercially available in some countries and the drug is available for formulation in the United States and Canada (Groman, 2000). Metronidazole benzoate is very well tolerated by cats. The recommended dose for treatment of giardia in dogs and cats is 25 mg/kg every 12 hours for 5–7 days. Lower doses (10–20 mg/kg every 12 hours) are used chronically for the treatment of inflammatory bowel diseases. High doses (25–50 mg/kg every 12 hours) are sometimes used in the treatment of serious anaerobic infections (peritonitis, meningitis), but there is an increased risk of neurotoxicity. Doses of 10–25 mg/kg PO every 12 hours are used in horses; withholding feed for 2 hours after administration may improve bioavailability.

Ronidazole is not approved as a drug by the US Food and Drug Administration or the Canadian Veterinary Drugs Directorate, so it must be compounded from active pharmaceutical ingredient by a compounding pharmacist for use in cats. It is usually compounded into tablets or capsules and dosed at 30 mg/kg orally once a day for 14 days. Higher doses or more frequent dosing increases the risk of neurotoxicity.

Clinical Applications

Metronidazole is used to treat anaerobic infections, especially pleuropneumonia and lung abscesses caused by penicillin-resistant *Bacteroides fragilis* and clostridial enterocolitis in horses (Baverud et al., 2003; Mair and Yeo, 1987). It is typically administered orally along with a parenteral beta-lactam and aminoglycoside or enrofloxacin to achieve Gram-positive, Gram-negative and anaerobic coverage. Although rectal absorption is inferior to oral absorption, it is a viable option for treatment when oral administration is not feasible.

In small animals, metronidazole is used in the therapy of anaerobic infections, including bacterial stomatitis,

osteomyelitis, hepatitis, pneumonia and lung abscessation, clostridial enteritis, and peritonitis (Jang et al., 1997; Sarkiala and Harvey, 1993; Weese and Armstrong, 2003). It is also used in the treatment of giardiasis and other protozoal infections (*Trichomonas, Balantidium coli*). Metronidazole appears efficacious for the treatment of *Giardia* in cats, but fenbendazole may be more efficacious against giardia in dogs, with fewer side effects (Barr et al., 1994; Scorza and Lappin, 2004). Metronidazole is sometimes effective in the treatment of inflammatory bowel diseases by inhibiting leukocyte-endothelial cell adhesion in post-capillary venules (Craven et al., 2004) and may be useful in the presurgical management of perianal fistulas (Tisdall et al., 1999). Oral administration of metronidazole decreased the number of aerobic bacteria and altered indigenous flora in the small bowel of cats (Johnston et al., 2000). The alteration in bacterial flora appeared to have an impact on nutrients, because serum albumin and cobalamin concentrations increased during administration and returned to preadministration concentrations after therapy was discontinued. Metronidazole is used as part of combination therapy in the treatment of *Helicobacter*-associated gastritis in dogs and cats. While clinical improvement is seen, such therapy does not eradicate infection (Khoshnegah et al., 2011; Leib et al., 2007).

Ronidazole is the only known effective treatment for *T. foetus* infection in cats (Gookin et al., 2006; Lim et al., 2012). Treatment with other drugs such as fenbendazole, paromomycin, tinidazole, metronidazole, and furazolidone improves fecal consistency during treatment, but *T. foetus* is not eradicated and diarrhea returns after the drugs are discontinued.

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Rifamycins

Rifampin (Figure 19.2) is the most important synthetically modified member of the rifamycins, antibiotic products of *Amycolaptopsis mediterranei*. Rifampin is a highly active first-line oral drug for the treatment of tuberculosis in humans. Because of the ready development of resistance, rifampin is always combined with other antimicrobials. Care must be taken however, as there are numerous interactions with other drugs. In addition to antibacterial activity, rifampin also has some antiviral and antifungal activity. Rifabutin and rifapentine are other semisynthethetic derivatives of rifamycin that are used in human medicine and have the advantage of causing less hepatic enzyme induction than rifampin.

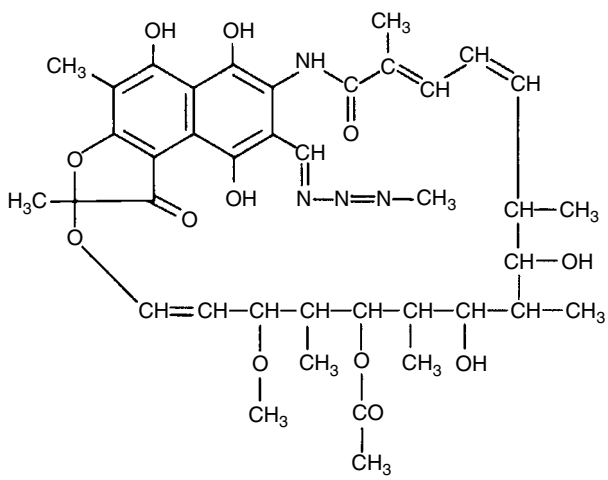

Figure 19.2. Structural formula of rifampin.

Chemistry

Rifampin is an ansamycin, with an aromatic ring system spanned by an aliphatic bridge. It is soluble in organic solvents and in water at an acid pH.

Mechanism of Action

Rifampin inhibits DNA-dependent RNA polymerase in bacteria. At therapeutic doses, it does not affect mammalian polymerase. Due to its high degree of lipid solubility, rifampin is effective against intracellular pathogens as well as against extracellular pathogens. Rifampin enters neutrophils and macrophages to kill intracellular bacteria, without interfering with phagocytosis. Rifampin penetrates the outer membrane of Gram-positive bacteria more easily than that of Gram-negative bacteria (Frank, 1990).

Antimicrobial Activity

Rifampin is a broad-spectrum antibiotic, with activity against many Gram-positive and some Gram-negative aerobic bacteria as well as facultative anaerobic organisms. Rifampin is bacteriostatic, shows time-dependent activity and has a long post-antibiotic effect. Rifampin is active against most strains of *Staphylococcus aureus* and *Staphylococcus pseudintermedius*, even methicillin-resistant strains (Rubin et al., 2011; Rubin and Chirino-Trejo, 2011). Because of unpredictable susceptibilities, Gram-negative bacteria should be considered resistant unless indicated by susceptibility testing. Because of the rapid development of resistance, rifampin is typically administered with other antimicrobial agents. The ability of rifampin to reach intracellular bacteria makes it difficult to predict clinical results from *in vitro* susceptibility tests. Rifampin is active against equine *Corynebacterium pseudotuberculosis*, *Rhodococcus equi*, *Staphylococcus* species, *Streptococcus equi*, *S. equisimilis*, and *S. zooepidemicus* isolates. Rifampin is synergistic with erthyromycin, clarithromycin, and azithromycin against *R. equi* (Giguère, et al., 2012). Susceptibility is variable for equine Gram-negative non-enteric bacteria. Rifampin has moderate activity against *Actinobacillus suis*, *A. equuli*, and *Pasteurella* spp. isolates. Equine isolates of *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus* spp., and *Salmonella* spp. are resistant (Wilson et al., 1988). Porcine isolates of *Actinobacillus pleuropneumoniae* and

Table 19.3. *In vitro* activity (MIC_{90}, μg/ml) of rifampin against selected bacteria.

Organism	MIC_{90}	Organism	MIC_{90}
Gram-positive aerobes			
Bacillus anthracis	0.03	*Staphylococcus aureus*	0.03
Corynebacterium pseudotuberculosis	≤ 0.25	*Rhodococcus equi*	0.06
Enterococcus spp.	≥ 4	*Nocardia* spp.	> 256
Listeria monocytogenes	0.25	Beta-hemolytic streptococci	≤ 0.5
Mycobacterium avium complex	4		
M. fortuitum	> 64		
M. tuberculosis	< 0.03		
Gram-negative aerobes			
Actinobacillus pleuropneumoniae	0.5	*Escherichia coli*	16
Bordetella bronchiseptica	≥ 128	*Klebsiella pneumoniae*	32
Brucella canis	1	*Pasteurella* spp.	1
B. abortus	2	*Proteus* spp.	32
Campylobacter jejuni	> 128	*Pseudomonas aeruginosa*	64
Gram-positive anaerobes			
Actinomyces spp.	0.06	*Clostridium* spp.	1
Clostridium perfringens	0.13	*C. septicum*	≤ 0.13
C. difficile	≤ 0.25	*Peptostreptococcus* spp.	32
Gram-negative anaerobes			
Bacteroides fragilis	1	*Porphyromonas asaccharolytica*	0.25
Fusobacterium spp.	2		

Pasteurella multocida are susceptible to rifampin, but *Bordatella bronchiseptic* can be resistant. Human and animal strains of *Mycobacterium avium* subsp. *paratuberculosis* are susceptible (Chiodini, 1990). Anaerobes found to be susceptible *in vitro* include *Bacteroides fragilis* and *Fusobacterium* spp. (Bach and Thadepalli, 1980). Bacteria with MIC ≤ 2 μg/ml are regarded as susceptible and those with MIC 2–4 μg/ml as moderately susceptible (Table 19.3).

Resistance

The antimicrobial activity of rifampin is inhibition of bacterial RNA polymerase, by binding to conserved amino acids in the active centre of the enzyme and blocking initiation of transcription. Most of the bacterial resistance to rifampin is due to mutations of these amino acids. These mutations often occur with high frequency; therefore, rifampin is administered in combination with other antimicrobials. Other reported mechanisms of resistance include duplication of the target, action of RNA polymerase-binding proteins, modification of rifampin and modification of cell permeability (Tupin et al., 2010). Resistance may occur as a single-step mutation of the DNA-dependent RNA polymerase at a high rate (1 in 10^7 or 10^8 bacteria). Initial susceptibility can rapidly diminish as small populations of resistant cells soon outnumber susceptible cells. This effect is diminished when rifampin is administered in combination with other antimicrobials. Rifampin resistance in *Rhodococcus equi* isolates from foals has been documented (Boyen et al., 2011; Kenney et al., 1994). The use of rifampin monotherapy in dogs with methicillin-resistant *Staphylococcus pseudintermedius* infection results in rapid emergence of rifampin resistance (Kadlec et al., 2011). Cross-resistance among the different rifamycin derivatives occurs, and cross-resistance to drugs unrelated to rifampin has been documented (Xu et al., 2005).

Pharmacokinetic Properties

Although parenteral pharmacokinetic studies have been performed in horses, rifampin is generally administered by the oral route in animals. Rifampin is rapidly absorbed after oral administration to people, calves, dogs, and horses, although bioavailability is low in horses (Frank, 1990; Wilson, et al., 1988). Oral dosing

for horses is adjusted for poor bioavailability. Administration with food prolongs the time to maximum serum concentration in adult horses and people.

Rifampin is very lipophilic and penetrates most tissues including milk, bone, abscesses and the central nervous system. Rifampin is well distributed into milk, with a milk to serum concentration ratio of 0.9:1.28 in sheep. Rifampin penetrates phagocytic cells to kill susceptible intracellular bacteria. Rifampin crosses the placenta and is teratogenic in rodents. Feces, saliva, sweat, tears, and urine are discoloured red-orange by rifampin and its metabolites. The volume of distribution of rifampin in horses is 0.6–0.9 L/kg. Rifampin is highly bound to plasma proteins in humans and horses. In horses, serum concentrations > 2 μg/mL are reached 45 minutes after intragastric administration of 20 mg/kg and serum concentrations are maintained at > 3 μg/mL for at least 24 hours. In dogs, serum concentrations are 9–10 μg/mL 24 hours after a single oral dose of 10 mg/kg.

Induction of hepatic enzymes occurs in response to administration of rifampin in many species. Rifampin induces hepatic CYP3A12 and intestinal CYP3A in dogs (Kyokawa et al., 2001). The biotransformation and elimination of rifampin in animals is not well known, and the major metabolites of the parent drug in most animals have not been traced. Desacetylrifampin was not detected in equine serum samples after IV or oral dosing. It was detected in urine, but the parent compound was much more predominant; however, only 6.82% of the total dose was recovered in the urine as either compound. It is not known if the unrecovered rifampin is sequestered in tissues or excreted in bile as desacetylrifampin, a more polar and more easily excreted metabolite (Kohn et al., 1993).

The elimination half-life of rifampin in horses is 6–8 hours after IV administration and 12–13 after oral administration. Due to immature hepatic metabolism, elimination of rifampin is delayed in very young foals and the elimination half-life is 17.5 hours. In dogs, the elimination half-life is 8 hours. As a hepatic enzyme inducer, rifampin induces its own metabolism, so that multiple oral dosing significantly decreases the elimination half-life. Enzyme induction is typically not been seen with less than 5 days of therapy, but once induction occurs, the increase in enzyme activity may last for more than 2 weeks after discontinuation of treatment.

Drug Interactions

Rifampin increases intestinal expression of P-glycoprotein transporters, reducing the oral bioavailability of drugs that are P-glycoprotein substrates. Concurrent induction of hepatic and intestinal cytochrome P450 enzymes results in lower plasma drug concentrations and increased clearance of prednisolone in dogs (Van der Heyden et al., 2012). Microsomal enzyme induction from rifampin may shorten the elimination half-life and decrease plasma drug concentrations of chloramphenicol, corticosteroids, theophylline, trimethoprim, itraconazole, ketoconazole, warfarin, and barbiturates.

Rifampin has *in vitro* synergistic activity with erythromycin, clarithromycin and azithromycin against *R. equi*, but is antagonistic with gentamicin or amikacin (Giguère et al., 2012). Rifampin may be synergistic in the treatment of staphylococcal infections in combination with vancomycin, linezolid and quinupristin-dalfopristin.

Toxicity and Adverse Effects

Rifampin is well tolerated by horses. There is little published information regarding the effects of rifampin in small animals; however, there is anecdotal information that a significant percentage of dogs receiving 5–10 mg/kg a day develop increases in hepatic enzymes and may develop hepatitis. Monotherapy should be avoided due to the rapid emergence of resistance.

Administration and Dosage

Rifampin is available as human labelled capsules or suspension for oral administration or as a diluted solution for IV use. Most horses object to the taste of rifampin, so care must be taken to deposit a dose well back on the tongue and rinse the horse's mouth afterward. Oral dosing of rifampin in horses is adjusted for poor bioavailability with a suggested dose of 10 mg/kg every 12 hours. Parenteral rifampin should be administered only by the intravenous route, not intramuscularly or subcutaneously.

Clinical Indications

The use of rifampin in food-producing animals is not approved in the United States or Canada, therefore there are no tolerances or established withdrawal times and the global FARAD centers have no data upon which to base withdrawal recommendations. The issue of whether rifampin should be used in food animals is further

complicated by its link to hepatic tumors in mice. The significance of this link is not known, but any residue of a known carcinogen in animal products for human consumption is a violation of the Food, Drug, and Cosmetic Act of the United States. The United States Pharmacopoeia Veterinary Medicine Advisory Panel has concluded that rifampin should not be administered to food-producing animals.

Rifampin is primarily used in foals for the treatment of *Rhodococcus equi*. Originally, it was combined with erythromycin, but because of adverse side effects from the erythromycin, combinations with new human labelled macrolides have been investigated. The combination of clarithromycin and rifampin appears superior to erythromycin/rifampin or azithromycin/rifampin (Giguère et al., 2004).

Because of hepatoxicity, rifampin is cautiously used in dogs. As methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* usually are susceptible to rifampin, its use is now more frequently considered (Kadlec et al., 2011).

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Oxazolidinones

The oxazolidinones are a novel chemical class of synthetic antibacterial agents. They exhibit a unique mechanism of protein synthesis inhibition and are active against many important human pathogens, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* (Diekema and Jones, 2000). In 2000, linezolid became the first oxazolidinone approved for human use and many analogs are currently under development.

Mechanism of Action

Oxazolidinones reversibly block protein synthesis by binding to the 23s ribosomal RNA (rRNA) of the 50s ribosomal subunit, near the interface formed with the 30s ribosomal subunit. Linezolid binds near the chloramphenicol and lincomycin binding sites and competes with these agents for binding. Although they share binding sites, their mechanism of action is different, with chloramphenicol inhibiting peptide bond formation and linezolid inhibiting initiation complex formation. As a result, there is only rare cross-resistance between linezolid and chloramphenicol or lincomycin (Zhanel et al., 2001).

Antimicrobial Activity

In vitro, linezolid is active against many Gram-positive bacteria. It is bacteriostatic against staphylococci and enterococci and often bactericidal against streptococci. *Staphylococcus* spp. with an MIC ≤ 4 μg/ml, as well as *Enterococcus* spp. and *Streptococcus* spp. with an MIC of ≤ 2 μg/ml are considered susceptible to linezolid. Isolates with an MIC of ≥ 8 μg/ml are considered resistant. Linezolid is active against staphylococci including methicillin-resistant *S. aureus* and *S. epidermidis*. It is also active against *S. aureus* isolates with intermediate susceptibility to vancomycin. Linezolid is active against *Enterococcus faecium* and *E. faecalis*, including isolates resistant to vancomycin, and against *Listeria monocytogenes* and *Rhodococcus equi*. Linezolid does not have clinically useful activity against aerobic Gram-negative bacteria. It is active against most anaerobes including *Clostridium perfringens*, *C. difficile*, *Peptostreptococcus* spp., and *Fusobacterium* spp. *Bacteroides fragilis* isolates are resistant or are intermediate in susceptibility.

Resistance

Linezolid is typically active against Gram-positive cocci that are resistant to other antimicrobials. In addition, it is difficult to induce *in vitro* resistance to linezolid because it has a very low spontaneous resistance mutation rate. Linezolid is active against > 98% of human staphylococci, with resistance identified in 0.05% of *Staphylococcus aureus* and 1.4% of coagulase-negative *Staphylococcus* spp. The most common mechanisms for linezolid resistance are mutation to the 23S rRNA or the presence of a transmissible ribosomal methyltransferase. The emergence of linezolid resistance in staphylococci and enterococci poses significant challenges to the clinical treatment of infections caused by these organisms (Gu et al., 2012; Herrero et al., 2002).

Pharmacokinetics

Linezolid is available in oral and parenteral forms. Rapid and extensive absorption occurs after oral administration in people and dogs with a bioavailability greater than 95% and maximum serum concentrations achieved less than 2 hours following administration (Slatter et al., 2002). The plasma elimination half-life of linezolid in dogs is approximately 4 hours and the volume of distribution is 0.63 L/kg. Linezolid is only 30% protein bound and is well distributed in all body tissues including the CSF. Elimination is equally distributed between renal and hepatic elimination in the dog.

Toxicity and Adverse Effects

Clinical reports of adverse effects in humans are typically associated with long-term use, and most effects are reversible upon discontinuing the drug (Ager and Gould, 2012). Some of the adverse effects appear to be due to direct inhibition of mitochondrial ribosomes (Barnhill et al., 2012). The most commonly reported adverse reactions are diarrhea, headache, nausea and vomiting. Myelosuppression, lactic acidosis and hepatic dysfunction have been reported. The safety of linezolid at clinically relevant dosages has not been established in domestic animal species.

Clinical Indications

Linezolid is indicated in humans for the treatment of infections due to vancomycin-resistant enterococci, nosocomial and community-acquired pneumonia due to *S. aureus* or multidrug-resistant *Streptococcus pneumoniae*, and skin infections including those caused by methicillin-resistant *Staphylococcus* spp. Linezolid therapy was required in the treatment of methicillin-resistant *S. pseudintermedius* sinusitis acquired from the owner's pet dog (Kempker et al., 2009). There are no published reports of the clinical use of linezolid in domestic animals, but the increasing rates of methicillin-resistant *Staphylococcus pseudintermedius* in dogs with pyoderma or orthopaedic infections and limited alternative treatment options will surely lead to veterinary use of linezolid (Weese et al., 2012). The decision to use linezolid to treat a highly resistant pathogen in a veterinary patient should only be made when all other alternative therapies have been attempted and with consideration of the health risks to in-contact humans and other animals (Frank and Loeffler, 2012; Papich, 2012).

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Carbadox

Carbadox is a quinoxaline *NN* dioxide derivative used to promote growth and for prevention and control of dysentery and bacterial enteritis in pigs. In many areas of the world it is used in animals up to 4 months of age with a 4-week withdrawal period prior to slaughter for human consumption. Other quinoxalines used as growth promoters in animals in some countries include olaquindox and cyadox. Carbadox inhibits bacterial DNA synthesis and denatures preexisting DNA. It is more active under anaerobic than aerobic conditions and its effect on DNA, like that of the nitrofurans, is believed to be caused by an unstable quindoxin-reduction product.

Carbadox is highly active against clostridia (MIC ≤ 0.25 μg/ml), *Brachyspira hyodysenteriae* (MIC < 0.005 μg/ml), and aerobic bacteria under anaerobic conditions. Quinoxalines have some activity against *Chlamydia/Chlamydophila* spp. and protozoa. Resistance in field cases of swine dysentery has been described, but the mechanism has not been elucidated.

Carbadox is used for growth promotion and feed efficiency in swine at 55 ppm in feed. Doses of carbadox as low as 50 ppm induce hypoaldosteronism from dose- and time-dependent damage to the zona glomerulosa of the adrenal cortex (van der Molen, 1988). Also at 50 ppm, mild effects of increased fecal dryness, urine drinking, growth retardation and poor condition are seen. At 300 ppm, posterior paresis and death may occur (Power et al., 1989). Olaquindox causes similar toxicity in pigs, but cyadox is less toxic (Nabuurs et al., 1990).

Carbadox remains approved for use in swine in the United States, but is banned in Canada, Australia, and the European Union because of carcinogenicity and genotoxicity concerns. In 2003, Health Canada requested that the Joint Expert Committee on Food Additives (JECFA) review the safety of carbadox residues and the analytical methodology used to assess these products. Carbadox and some of its metabolites (desoxycarbadox and hydrazine) were found to be genotoxic and carcinogenic in rodents. The final metabolite, quinoxaline-2-carboxylic acid (QCA), was not found to be carcinogenic or mutagenic in animals. Initial studies of residues showed rapid depletion of carbaox and its genotoxic metabolites in liver and muscle to concentrations of < 2 μg/kg, within the limit of detection of the analytical method available at that time (MacIntosh et al., 1985). QCA was the most persistent metabolite and was the only residue detectable in edible tissues of pigs 72 hours after dosing. After a 28-day withdrawal period, its concentration was < 30 μg/kg in liver and 5 μg/kg in muscle, representing the limits of quantification of the analytical method used at that time. Carbadox was reviewed by JECFA primarily on the basis of new information on residue concentrations, which indicated that the metabolite desoxycarbadox was present in edible tissues even at the end of a 15-day experimental withdrawal period. Reports of misuse and cross-contamination of swine finishing rations, combined with a better analytical capacity to detect desoxycarbadox, raised human safety concerns over the use of carbadox. The Committee confirmed that both carbadox and desoxycarbadox should be regarded as carcinogens that act by a genotoxic mechanism. The Committee concluded that it was not possible to identify a dose of carbadox in swine that posed an acceptable risk to consumers. The Committee therefore did not establish an acceptable daily intake (ADI) for carbadox. The US FDA also considers carbadox and its metabolites to be carcinogens, but allows its use in swine with a 70-day withdrawal time. Olaquindox has been withdrawn from use in Europe because of cases of a photoallergic "phototoxic contact dermatitis" that developed in pig farmers.

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Fusidic Acid

Fusidic acid is a lipophilic steroid antibiotic, a fusidane (like cephalosporin P1 and helvolic acid). It is a product of *Fusidium coccineum* and available as a readily soluble sodium salt. It prevents protein synthesis by inhibiting the binding of aminoacyl tRNA to the ribosomal A site. Sodium fusidate is active mainly against Gram-positive bacteria. Initially, there is excellent bactericidal activity against *Staphylococcus aureus* and *Staphylococcus pseudintermedius* (MIC ≤ 0.03 μg/ml) but resistance emerges rapidly due to acquired resistance genes. Gram-negative rods are inherently resistant.

Fusidic acid is used orally in humans for the treatment of serious staphylococcal infections (Wang et al., 2012) but is not approved in North America for such use (Fernandes and Pereira, 2011). It is used topically in dogs for local treatment of staphylococcal infections (Guardabassi et al., 2004; Saijonmaa-Koulumies et al., 1998; Valentine et al., 2012), but resistance in *Staphylococcus pseudintermedius* has been documented (Loeffler et al., 2008; Pedersen et al., 2007). It is available in some countries as an ophthalmic ointment for the treatment of Gram-positive bacterial keratitis in dogs and cats.

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Isoniazid

Isoniazid is the hydrazide of isonicotinic acid, a low molecular weight, water-soluble drug. It is the most potent antituberculosis drug used in humans, and is bactericidal to *Mycobacterium tuberculosis* at concentrations of 0.05–0.2 μg/ml. *M. bovis* is similarly susceptible, but *M.avium-intracellulare* and other atypical mycobacteria are resistant. Many *M. kansasii* are susceptible. *Actinomyces bovis* is susceptible. Isoniazid is always administered in combination with other antimicrobials, because bacteria readily develop resistance. The antibacterial mechanism of action of isoniazid is still being investigated but it appears to inhibit mycolic acid cyclopropane synthase (Banerjee and Bhattacharyya, 2012).

Isoniazid is well absorbed from the intestine and distributes well into tissues, including cerebrospinal fluid. Toxic effects occur in people who are genetically slow acetylators of isoniazid (Kinzig-Schippers et al., 2005). Isoniazid has been used in cattle for the treatment of actinomycosis (Watts et al., 1973) and in the treatment of Johne's disease (*Mycobacterium avium* subsp. *paratuberculosis*) in cattle (Fecteau and Whitlock, 2011). Isoniazid in conjunction with a vaccine from *Mycobacterium tuberculosis* cell wall fragments was efficacious in *Mycobacterium caprae*-infected goats (Domingo et al., 2009). Isoniazid is not approved for use in food animals and there is no information available on pharmacokinetics or residue depletion.

A single dose of 300 mg isoniazid in dogs causes life-threatening central nervous system toxicity (Doherty, 1982; Haburjak and Spangler, 2002). As with metronidazole, diazepam is antidotal by improving GABAergic transmission in the central nervous system, and has proved effective in protecting animals from further convulsions and death (Villar et al., 1995).

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Mupirocin

Mupirocin (pseudomonic acid) is a novel antibiotic, isolated from *Pseudomonas fluorescens*. By preventing the incorporation of isoleucine into protein chains, this powerful inhibitor of bacterial isoleucyl transfer RN synthetase (IleS) stops protein synthesis. It is active against a variety of Gram-positive bacteria, but it is most valuable in the topical treatment of staphylococcal infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates from dogs and cats are typically susceptible to mupirocin (MIC ≤ 2 μg/ml; Loeffler et al., 2008). Mupirocin is bacteriostatic but appears to be bactericidal at a lower pH approximating that of many parts of the skin. It is rapidly metabolized after systemic administration, so it is only used topically.

Mupirocin was introduced into clinical practice in the United Kingdom in 1985, and has been extremely effective for treatment of human staphylococcal skin infections and for the clearance of nasal colonization with MRSA. The skin ointment (with polyethylene glycol) and nasal cream (with soft paraffin) are currently registered for use in more than 90 countries worldwide. Bacterial resistance soon emerges with clinical use, and is seen in staphylococci isolates from humans and dogs (Fulham et al., 2011; Rubin and Chirino-Trejo, 2011). Low-level resistance is due to mutations in a chromosomally encoded IleS, is stable and non-transferable. High-level mupirocin resistance (MIC of ≥ 512 μg/ml) is mediated by the expression of mupA (ileS2), which encodes an alternate isoleucyl-tRNA synthetase. Cross resistance with other antimicrobials does not occur, due to mupirocin's novel mechanism of action (Cookson, 1998), but the MupA gene may co-transfer with other antibacterial resistance genes. This has been observed already with resistance genes for triclosan, tetracycline, and trimethoprim. MupB, a new high-level mupirocin resistance mechanism has been identified in *Staphylococcus aureus* (Seah et al., 2012). A dog served as a reservoir for mupirocin-resistant MRSA colonization in its owners. The MRSA infection and nasal-colonization in the couple was resolved only after successful eradication of MRSA from the family dog's nares with a vancomycin ointment (Manian, 2003). While remaining susceptible, mupirocin was not effective in decolonizing all personnel who were MRSA carriers in an equine hospital (Sieber et al., 2011). Failure to eradicate MRSA may be due to slime production that limits drug penetration (Ogura et al., 2012).

Mupirocin is available as a veterinary product in the United States for topical treatment of pyoderma in dogs; however, in Canada, it is only available as the human labelled product. The mupirocin ointment penetrates well into granulomatous lesions such as interdigital abscesses. However, given the value of mupirocin for treatment of human staphylococcal infections and the rising rates of resistance, veterinary use of this drug for routine use of skin infections is not prudent.

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Methenamine

Methenamine (hexamine) is a highly soluble, basic substance of the chemical formula (CH2)6 N4, which decomposes under acidic urine conditions to release formaldehyde. It is available as a salt of mandelic acid or hippuric acid. After oral administration, methenamine is well absorbed and excreted unchanged in urine by glomerular filtration and tubular secretion. If the urine is strongly acidic (pH < 5.5), methenamine releases formaldehyde, which acts as a non-specific urinary antiseptic. The minimal inhibitory concentrations of uropathogens are significantly lowered in more acidic urine, so ensure urine acidity by concurrent administration of ascorbic acid or ammonium chloride.

Methenamine is used for long-term prophylaxis of recurrent urinary tract infections in dogs and cats at 0.25 mg/15 kg every 6 hours. Urease-producing bacteria such as staphylococci and *Proteus* that make urine strongly alkaline through the release of ammonia from urea are not susceptible to methenamine. Methenamine should not be used in patients with preexisting hepatic insufficiency, the small amounts of ammonia and formaldehyde that are produced may cause further hepatic damage.

Novobiocin

Novobiocin (Figure 19.3) is an antibiotic product of *Streptomyces* that is used in the local treatment of *Staphylococcus aureus* infections, including mastitis in dairy cows and in an oral product for dogs for the treatment respiratory tract infections (in combination with tetracycline). Novobiocin is a coumarin antibiotic, formulated as a dibasic acid available as the poorly water soluble calcium salt or as the more soluble monosodium salt.

Novobiocin inactivates the beta subunit of DNA gyrase, inhibiting supercoiling, DNA-dependent adenosine triphosphatase, and catenation/uncatenation. Novobiocin is very active against *S. aureus*, less active against streptococci and the more fastidious Gram-negative bacteria (*Histophilus*, *Brucella*), and least active against Enterobacteriaceae and *Pseudomonas* (Table 19.4). In a study of bovine mastitis isolates, 95% of *S. aureus*, 60% of *Streptococcus dysgalactiae*, and 40% of *S. agalactiae* were susceptible to the drug. There is renewed interest in novobiocin as a treatment for methicillin-resistant and methicillin-susceptible staphylococcal infections in dogs as the majority of isolates are susceptible (Fulham et al., 2011; Owens et al., 2001). *S. xylosus* and *S. sciuri* isolated from animals are novobiocin-resistant. Many mycoplasma species are moderately susceptible. Bacteria with MIC ≤ 4 μg/ml are regarded as susceptible, MIC = 8 μg/ml as intermediate, and MIC ≥ 16 μg/ml as resistant. Chromosomal resistance develops fairly readily *in vitro* and has been reported during treatment of *S. aureus* infections.

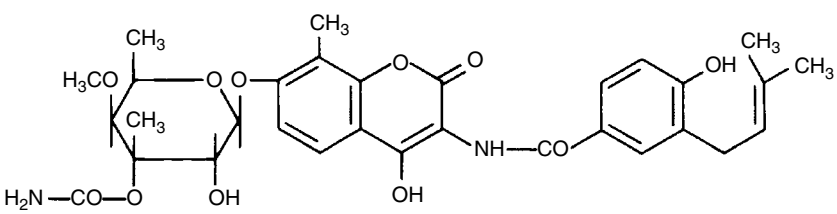

Figure 19.3. Structural formula of novobiocin.

Table 19.4. *In vitro* activity (MIC_{90}, μg/ml) of novobiocin against selected bacteria.

Organism	MIC_{90}	Organism	MIC_{90}
Gram-positive aerobes			
Arcanobacterium pyogenes	64	*Rhodococcus equi*	> 64
Corynebacterium bovis	1	*Staphylococcus aureus*	2[a]
Enterococcus faecalis	64	*Streptococcus agalactiae*	16
Erysipelothrix rhusiopathiae	> 64	*S. pyogenes*	4
Listeria monocytogenes	2	*S. uberis*	2[a]
Gram-positive anaerobes			
Actinomyces spp.	16	*Clostridium perfringens*	1
Gram-negative aerobes			
Brucella canis	2	*Pasteurella multocida*	16
Escherichia coli	> 64	*Proteus* spp.	64
Histophilus somni	≤ 0.13	*Pseudomonas aeruginosa*	> 64
Mannheimia haemolytica	64	*Taylorella equigenitalis*	2
Mycoplasma			
M. ovipneumoniae	8		

[a]Some reports are far higher because of resistance.

Moderate synergism with penicillin G against bovine *S. aureus* and streptococci has been described. The claim that novobiocin is synergistic with tetracycline may be a laboratory artifact associated with magnesium chelation by tetracycline.

Novobiocin is well absorbed from the gastrointestinal tract in humans and has an elimination half-life of 2–4 hours. Penetration into tissues is relatively modest. The drug is mainly excreted in the bile, and enterohepatic recirculation occurs. Skin eruptions in humans are common. Novobiocin is an inhibitor of hepatic metabolism. Eosinophilia, thrombocytopenia, and leukopenia are occasional seen. Skin rashes may occur in cows treated with intramammary infusions containing novobiocin.

The main use of novobiocin in veterinary medicine is in the local treatment of *S. aureus* mastitis in dairy cows. The drug is combined with procaine penicillin G in dry cow therapy, with reasonable clinical efficacy (Owens, et al., 2001). Prepartum therapy of heifer mammary glands with penicillin-novobiocin significantly reduced the percentage of heifers and quarters infected with mastitis pathogens during early lactation (Oliver et al., 2004). Novobiocin is available in the United States for the treatment of respiratory tract infections in dogs in two combinations: one with tetracycline and one with tetracycline and prednisolone. Use of the combination product appears effective in the treatment of infectious tracheobronchitis ("kennel cough"; Maxey, 1980). Use may increase in small animals due to its activity against staphylococci, including methicillin-resistant strains.

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20

Antifungal Chemotherapy

Steeve Giguère

An increased incidence of fungal infections has been documented over the last 2 decades. Advances in patient management technologies and therapies such as bone marrow and solid organ transplant, new and more effective chemotherapeutic agents, more aggressive use of chemotherapy, and the rise of the numbers of patients with HIV infection are all factors contributing to the considerable increase in infections with various fungi in people. Some of these factors are likely contributing to an increase in the incidence of fungal infections in domestic animals as well. Fungi are also emerging as important nosocomial pathogens causing considerable morbidity and mortality in hospitalized patients. In addition to immunosuppression, risk factors common to many hospitalized veterinary patients include malnutrition, indwelling catheters, and disruption of host normal microbial flora by potent broad-spectrum antibacterial drugs.

Until relatively recently, the range of antifungal drugs available for systemic use was limited to a few agents, the most effective of which was amphotericin B, which is highly toxic. As fungal infections became an important public health issue, newer agents with broader spectrums of activity, different targets of action, or fewer side effects have been developed. However, despite continued efforts, the number of antifungal agents for systemic use remains limited (Table 20.1). This is because mammals and their fungal pathogens have many common cellular characteristics and potential drug targets that are unique and important to the fungus, but not the host, are few. The main sites of action of antifungal drugs are the (1) cytoplasmic membrane (polyenes, azoles); (2) cell wall (ecchinocandins, nikkomycins); and (3) DNA and protein synthesis machinery (flucytosin, sordarins; Figure 20.1).

Antifungal Susceptibility Testing

In vitro antifungal susceptibility tests differ from the susceptibility tests performed against bacteria in that a given fungal species may be in the form of yeast or of filamentous fungi. The Clinical and Laboratory Standards Institute (CLSI) has described standardized testing methods for both of these forms of fungi, M27 and M38, respectively. The M27 document is intended for the susceptibility testing of yeasts that cause invasive infections and include organisms such as *Candida* spp. and *Cryptococcus neoformans*. The M38 document describes testing methods for common filamentous fungi that cause invasive infections such as *Aspergillus*, *Fusarium*, *Rhizopus*, *Pseudallescheria* and the mycelial form of *Sporothrix schenckii*. The methods described in these CLSI documents have not been standardized for testing the yeast forms of the dimorphic fungi such as *Blastomyces*, *Coccidioides* and *Histoplasma*. For specific details on how to perform antifungal susceptibility tests the reader is referred to these documents.

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Table 20.1. Systemic and topical antifungal agents in use.

Class	Agent	Formulations	Spectrum
Allylamine	Terbinafine	O, T	Broad spectrum[a]
	Naftidine	T	Broad spectrum
Pyrimidine synthesis inhibitors	Flucytosine	O, IV*	Yeasts,[b] some *Aspergillus*
Azole (Imidazole)	Ketoconazole	O, T	Dermatophytes, yeasts, dimorphic fungi[c]
	Miconazole	T	Broad spectrum
	Enilconazole	T	Broad spectrum
	Clotrimazole	T	Broad spectrum
	Others[d]	T	
Azole (Triazole)	Fluconazole	O, IV, T	Yeasts, dimorphic fungi
	Itraconazole	O, IV	Broad spectrum
	Voriconazole	O, IV	Broad spectrum
	Posaconazole	O	Broad spectrum
Echinocandin	Caspofungin	IV	*Candida* spp., *Aspergillus*
	Anidulafungin	IV	*Candida* spp., *Aspergillus*
	Micafungin	IV	*Candida* spp., *Aspergillus*
Polyene	Amphotericin B	IV, T	Broad spectrum
	Nystatin	T	Yeasts
	Natamycin	T	Broad spectrum
Other	Griseofulvin	O	Dermatophytes
	Amorolfine	T	Dermatophytes, *Candida* spp.
	Butenafine	T	Dermatophytes
	Ciclopirox	T	Dermatophytes, yeasts
	Haloprogin	T	Dermatophytes, *Candida* spp.
	Tolnaftate	T	Dermatophytes
	Undecylenic acid	T	Dermatophytes

O: oral; IV: intravenous; T: topical.
[a]Broad spectrum: dermatophytes, yeasts,[b] *Aspergillus*, dimorphic fungi.[c]
[b]Yeasts: *Candida* spp., *Cryptococcus neoformans*, *Malassezia pachydermatis*.
[c]Dimorphic fungi: *Blastomyces dermatitidis, Histoplasma capsulatum*, *Coccidioides immitis*, and *Sporothrix schenckii*.
[d]Many other imidazoles such as bifonazole, butoconazole, oxiconazole, sulconazole, terconazole, and tioconazole are available for topical use.
*The parenteral formulation is not available in the United States.

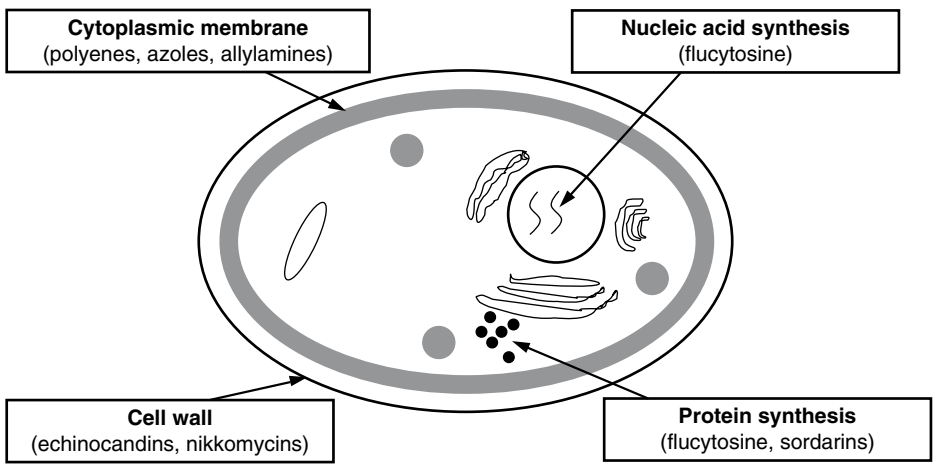

Figure 20.1. Action of antifungal agents on the fungal cell.

Since antifungal susceptibility testing is not routinely performed in veterinary clinical microbiology laboratories, referral of isolates to laboratories that specialize in antifungal testing is recommended in most instances. This can result in a considerable increase in costs and an additional delay in obtaining the results. To compensate for this, the clinician should be familiar with the types of pathogenic fungi most likely to be encountered and the susceptibility of those fungi to the antifungal agents they have at their disposal. Such knowledge will facilitate the initiation of the appropriate empirical therapy. One should keep in mind, however, that the susceptibility of fungi, as with bacteria, is not always predictable. Both acquired and intrinsic resistance have been described.

In order to be useful clinically, *in vitro* susceptibility testing needs to reliably predict clinical outcome of therapy. Many factors may affect this outcome, including drug pharmacokinetics, drug interactions, host immune response, patient management, and virulence of the infecting microorganism. Because so many factors can affect the outcome of antifungal therapy, a low MIC does not necessarily predict clinical success. Similarly, a report that indicates that a fungus is resistant to an antifungal agent does not always mean that the use of that antifungal agent will result in an unfavorable clinical outcome. Nevertheless, many recent studies have provided evidence that *in vitro* antifungal susceptibility tests correlate with the outcome of therapy in human medicine (Rex and Pfaller, 2002). In the absence of specific veterinary criteria, the standards developed in human medicine may be useful.

Antifungal Drug Resistance

Antifungal drug resistance can be intrinsic or acquired. Intrinsic resistance is an inherited characteristic of a species or strain. In contrast, acquired resistance occurs when a previously susceptible isolate develops a resistant phenotype, usually as a result of prolonged treatment with antifungals. The precise mechanism associated with acquired resistance depends on the mode of action of the class of antifungal drug, and includes reduced drug uptake, drug export through efflux pumps, or reduced affinity of target enzymes. Unlike bacterial cells, intact fungal cells do not readily take up exogenous DNA. As a result, transferable drug resistance has not been described among widely divergent fungal taxa, and the spread of resistance has been considerably slower than that observed in bacteria. Prevention of emergence and spread of resistant fungi depends on taking maximal advantage of the pharmacodynamic properties of the particular drug class, on the use of local rather than systemic treatment (thus reducing general exposure of the animal's fungal flora to antifungal agents), and on hygienic precautions. Additionally, combination antifungal therapy is a well-recognized strategy to prevent emergence of flucytosine resistance.

Pharmacodynamics of Antifungal Agents

In vitro and laboratory animal model studies have begun to define the pharmacodynamic characteristics of antifungal agents. Analysis of clinical data in humans also suggests that pharmacodynamic targets identified in animal models are predictive of outcome in humans (Andes, 2004). The activity of antifungal agents may be concentration dependent, time dependent, or exhibit both. The polyenes and echinocandins exert a long post-antifungal effect and are concentration dependent. The best predictor of efficacy for these drugs is a maximum serum concentration (Cmax) to MIC ratio of 3–10/1, with high ratios conferring better activity. In contrast, flucytosine has a short post-antifungal effect and the best predictor of efficacy is the time period for which serum concentrations exceed the MIC of a given pathogen. The triazoles exert characteristics of both time and concentration dependent activity. The best predictor of efficacy for these drugs is a 24-hour area under the serum concentration versus time curve (AUC)/MIC ratio of 25/1.

Antifungal Drugs for Systemic Administration

Allylamines: Naftifine, Terbinafine

Naftifine is used topically to treat dermatophyte infections, while terbinafine is available for both oral and topical use in human medicine. Terbinafine is used in the treatment of dermatophytic, *Malassezia*, and *Sporothrix schenckii* infections, and there is interest in it for its activity against *Candida*, dimorphic, and filamentous fungi.

It is used in people in the systemic treatment of persistent or intractable dermatophyte infections, in which it is more effective than ketoconazole, itraconazole, or griseofulvin.

Mechanism of Action. Allylamines are synthetic drugs that inhibit squalene epoxidase, a critical enzyme in biosynthesis of ergosterol, the principal sterol in the cell membrane of susceptible fungi. This causes fungal cell death primarily due to the increase in membrane permeability mediated by the accumulation of high concentrations of squalene.

Antimicrobial Activity. Isolates with an MIC ≤1 μg/ml are considered susceptible, 2–4 μg/ml represent intermediate susceptibility, and isolates with an MIC ≥ 8 μg/ml are resistant. The MIC of terbinafine is low *in vitro* against dermatophytes species and a broad spectrum of non-dermatophyte organisms including *Aspergillus* spp., *Blastomyces dermatidis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Malassezia* spp., *Scopulariopsis brevicaulis*, *Sporothrix schenckii*, and certain *Candida* spp. The fungicidal activity of terbinafine offers a considerable advantage over many other antifungal agents.

Resistance. Acquired resistance to terbinafine has not been reported for dermatophytes even after prolonged exposure.

Pharmacokinetic Properties. Terbinafine is a lipophilic allylamine compound that is well absorbed (> 70% in people) after oral administration and binds strongly and non-specifically to plasma proteins. The absorption characteristics are not altered when terbinafine is taken with food. The drug is rapidly absorbed after oral administration in dogs (Sakai et al., 2012). In horses, relative oral bioavailability of terbinafine is less than 20% of that observed in dogs (Williams et al., 2011). The excretion of terbinafine in the urine and feces is 80% and 20%, respectively in people. Terbinafine penetrates keratinized tissues, and enters the stratum corneum and sebum by direct diffusion through the dermis and living epidermis. Plasma terbinafine concentrations are not particularly good indicators of concentrations in the target organs since the drug persists in the skin for prolonged periods of time. In a study in cats, there was no difference in plasma concentrations between low- (10–20 mg/kg q 24 h) versus high-dose (30–40 mg/kg q 24 h) terbinafine but concentrations in hair were significantly greater with the high dose (Kotnik et al., 2001). Therapeutic concentrations of terbinafine in cat hair persist for over 5 weeks after 14 days of oral therapy (Foust et al., 2007).

Drug Interactions. *In vivo* studies have shown that terbinafine is an inhibitor of the CYP450. Co-administration of terbinafine with drugs predominantly metabolized by the CYP450 2D6 isozyme should be done with careful monitoring and may require a reduction in dose of the 2D6-metabolized drug. Terbinafine clearance is increased 100% by rifampin, a CYP450 enzyme inducer, and decreased 33% by cimetidine, a CYP450 enzyme inhibitor. Terbinafine clearance is unaffected by cyclosporine.

From a theoretical point of view, combinations of azoles and terbinafine should exhibit synergy since they are acting at different points of the same pathway. This has been corroborated in several studies *in vitro*. Combinations of terbinafine with fluconazole, itraconazole, or voriconazole have shown synergy *in vitro* against species of *Aspergillus*, *Candida*, *Mucor* and even against fluconazole-resistant *Candida* isolates and itraconazole-resistant *Aspergillus* strains (Cuenta-Estrella, 2004). The combination of terbinafine with caspofungin or fluconazole is synergistic *in vitro* against many strains of *Pythium insidiosum* (Cavalheiro et al., 2009).

The interaction of terbinafine with amphotericin B or flucytosine has also been assessed. *In vitro* studies have indicated that these combinations exhibit no interactions or are antagonistic against *Aspergillus* and other fungi.

Toxicity and Adverse Effects. Terbinafine is well tolerated with a low incidence of adverse reactions in dogs and cats. Adverse effects involve the gastrointestinal system and the skin. Abnormalities in liver enzymes and hematologic parameters are rarely observed in people.

Administration and Dosage. Dosage is summarized in Table 20.2.

Clinical Applications. Due to its high rate of efficacy, low incidence of adverse reactions, and ability to

Table 20.2. Usual dosages of selected systemic antifungal agents in domestic animals.

Species	Drug	Dosage (mg/kg)	Route	Interval (h)
Dog/cat	Terbinafine	30	PO	24
	Amphotericin B (conventional)	0.5(dog); 0.25(cat)	IV*	3 x/week
	Amphotericin B (lipid)	1–3 (dog); 1 (cat)	IV*	3 x/week
	Flucytosine	50–75	PO	6–8
	Ketokonazole	10	PO	12
	Itraconazole	5	PO[b]	12–24
	Voriconazole	4 (dogs only)	PO	12
	Fluconazole	5–10	PO	12–24
	Griseofulvin (micro size)	50	PO	24
	Griseofulvin (ultramicro size)	10	PO	24
Horses	Amphotericin B (conventional)	0.3–0.9 mg/kg	IV*	24–48
	Ketoconazole	30 (in 0.2 N HCl)	NGT[a]	12
	Fluconazole	5[c]	PO	24
	Itraconazole	5	PO[b]	12–24
	Voriconazole	4	PO	24

*Diluted to 1 mg/ml in 5% dextrose and administered over 1–2 hours.
[a]Nasogastric intubation is required to avoid the irritant effect of HCl on the oral cavity and throat.
[b]The bioavailability of the oral suspension is superior to that of the capsules.
[c]A loading dose of 14 mg/kg is recommended.

achieve clinical success after a relatively short course of therapy compared to other agents, terbinafine is often the treatment of choice for various dermatomycoses in people. Terbinafine therapy has also been efficacious in some patients with sporotrichosis, aspergillosis, chromoblastomycosis, and leishmaniasis. There is also evidence that resistant *Candida* infections may respond to a combination of terbinafine and a triazole.

Terbinafine is more active *in vitro* than griseofulvin against *Microsporum canis*, *M. gypseum*, and *Trichophyton mentagrophytes* (Hofbauer et al., 2002). In dogs and cats, terbinafine has been shown to be effective for the treatment of both experimental and naturally acquired dermatophytosis. The length of therapy for mycological cure has ranged between 33 and 63 days (Kotnik et al., 2002; Moriello, 2004). Terbinafine has also been shown to be at least as effective as ketokonazole in reducing yeast counts in dogs with *Malassezia* dermatitis (Rosales et al., 2005). There are isolated reports of successful treatment of canine pythiosis using a combination of terbinafine and itraconazole.

Polyenes: Amphotericin B

The polyene group of antifungal agent includes amphotericin B, nystatin, and natamycin. Amphotericin B is typically administered systemically whereas nystatin and natamycin are used topically. Amphotericin B was the mainstay of systemic antifungal treatment for many years. Although its place in the systemic treatment of yeast or dimorphic fungal infections is now challenged by the azole antifungal drugs, Amphotericin B is still the mainstay for systemic treatment of filamentous fungal infections. A major advantage of this drug is its fungicidal nature, so that it is often used in treatment of life-threatening yeast or dimorphic fungal infections. Its toxicity has been circumvented in recent years by development of lipid formulations that, though expensive, are coming into clinical use in veterinary medicine.

Chemistry. Amphotericin is a heptaene product of *Streptomyces nodosus* (Figure 20.2). It is an amphoteric polyene macrolide that is poorly soluble in water and unstable at 37°C. The antifungal effects of the antibiotic are maximal between pH 6.0 and 7.5 and decrease at lower pHs. The amphotericin B sodium deoxycholate

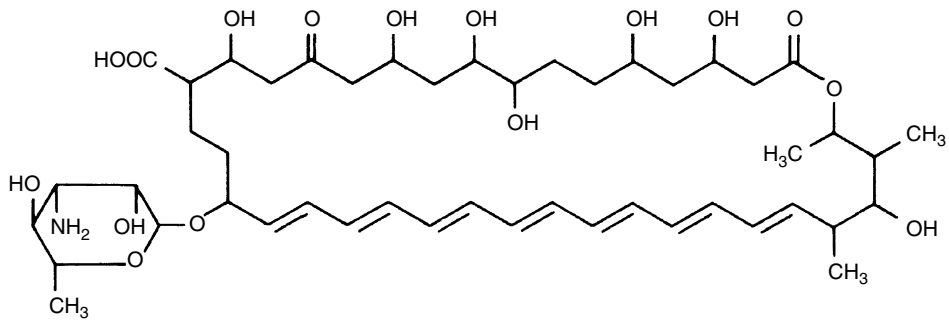

Figure 20.2. Structural formula of amphotericin B.

Table 20.3. *In vitro* activity (MIC_{90}, μg/ml) of selected systemic antifungal agents against common fungal pathogens.

	Activity of antifungal agents						
Organisms	Amphotericin B	Flucytosine	Ketokonazole	Fluconazole	Itraconazole	Voriconazole	Caspofungin
Filamentous fungi							
Aspergillus fumigatus	1	> 64	16	> 64	0.5	0.25	0.06
Mucor spp.	0.25	> 64	> 64	> 64	> 8	8	> 16
Rhizopus spp.	0.25	> 64	> 64	> 64	4	> 8	> 16
Yeasts							
Candida albicans	0.5	0.125	0.125	0.25	0.03	0.03	1
C. glabrata	1	0.125	32	32	4	1	1
C. tropicalis	1	0.25	0.5	0.5	0.25	0.12	1
Cryptococcus neoformans	0.5	8	0.25	2	0.25	0.12	> 16
Malassezia pachydermatis	4		< 0.03	8	< 0.03	2	> 256
Dimorphic fungi							
Blastomyces dermatidis	0.5		0.5	32	0.25	0.25	8
Coccidioides immitis	0.25	> 64	0.5	4	0.5	0.25	32
Histoplasma capsulatum	0.25	> 64	0.25	2	0.25	0.25	4
Sporothrix schenckii	4	> 64	4	> 64	4	4	> 16

compound with phosphate buffer is more water-soluble and is used for IV administration. Lipid-based formulations (liposomal [AmBisome], colloidal [Amphocil or Amphotec], or lipid complex [Abelcet]) are less toxic than the micellar suspension, which is the conventional formulation (Fungizone).

Mechanism of Action. Amphotericin B binds to ergosterol, the principal sterol of the fungal cell membrane, causing leakage of cell contents. The drug binds cholesterol in mammalian cell membranes less avidly, but its ability to bind to mammalian cells makes this the most toxic of the clinically useful systemic antifungal drugs. In addition to its effects on the cell membrane, amphotericin B can cause oxidative damage to fungal cells.

Antimicrobial Activity. Amphotericin B is a broad-spectrum, antifungal agent with the advantage of fungicidal activity against most pathogenic fungi. Isolates with an MIC ≤ 1 μg/ml are considered susceptible. *Blastomyces dermatitidis, Candida* spp., *Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum*, and *Sporothrix schenckii* are typically susceptible, in decreasing order (Table 20.3). Most *Aspergillus* spp. are susceptible, with the exception of *A. terreus* and *A. lentulus*. Dermatophytes and strains of *Pseudoallescheria boydii* are often intrinsically resistant to amphotericin B. *Prototheca*, an algae associated

with cutaneous, subcutaneous and systemic infections in several animals species and bovine mastitis, is also susceptible.

Resistance. Resistant isolates of *Candida* spp., *C. immitis*, and *Mucor* spp. have been described. Although rare, development of resistance during treatment of susceptible fungi such as *Candida* spp. and *C. neoformans* has been documented.

Pharmacokinetic Properties. Amphotericin B is poorly absorbed orally (< 5%), and parenteral (IV) administration is required. The half-life in dogs after IV injection of conventional amphotericin B is about 26 hours (Kukui et al., 2003). The drug is thought to bind to plasma or cellular lipoproteins and to be released slowly from these sites. Although only about 5% of the injected dose is excreted by the kidneys, the agent continues to be excreted in the urine of humans for several weeks after cessation of therapy. Penetration into cerebrospinal fluid (CSF) is poor (5%) but increases in meningitis. Systemic absorption from the lungs following aerosol administration is poor; therefore, this route has been used successfully in the treatment of pulmonary aspergillosis. The pharmacokinetics of lipid-based formulations of amphotericin B are quite diverse in people. Peak plasma concentrations after administration of the liposomal formulation are much higher than those achieved with conventional amphotericin B. In contrast, peak plasma concentrations after administration of the lipid complex or colloidal formulations are lower due to more rapid distribution of the drug to tissues. Lipid-based formulations appear to be taken up extensively by the reticulo-endothelial system, which may give them considerable therapeutic advantage. The lipid complex, but not the liposomal or conventional amphotericin B, are concentrated and accumulate in lung tissue (Matot and Pizov, 2000). This affinity for the lung may have implications in the treatment of fungal pneumonia.

Drug Interactions. Due to both the serious nature of systemic fungal infections and the toxicity of amphotericin B, considerable effort has been expended to find synergistic combinations of drugs that will enable reduction of dosage and expedite clinical cure.

Flucytosine and amphotericin B show additive or synergistic effects *in vitro* against *Candida, Cryptococcus*, and *Aspergillus*. The combination is synergistic in cryptococcal meningitis in humans, producing faster cure, fewer relapses, more rapid sterilization of CSF, and less nephrotoxicity.

There is a theoretical concern that co-administration of amphotericin B and azole agents will lead to antagonism because of azole inhibition of ergosterol synthesis, resulting in less ergosterol in the cell membrane available for the polyene to bind to. Amphotericin B can also interfere with the influx of the azole agents by damaging the membrane structure. Combination therapy with various imidazoles or triazoles against *Candida* spp. and *C. neoformans*, and *Aspergillus* spp. has produced complex interactions *in vitro* that are hard to interpret. Results of animal models of candidiasis have given contradictory results with most studies showing either indifference or antagonism. In contrast, a clinical trial in people revealed a significant advantage of the combination fluconazole-amphotericin B over fluconazole monotherapy in invasive candidiasis (Rex et al., 2003). Results in animal models of invasive aspergillosis and cryptococcal infection have given equivocal results with some studies showing synergism, some studies showing antagonism and most studies showing indifference (Cuenca-Estrella, 2004). Combination of amphotericin B with ketoconazole has been used successfully to treat systemic mycoses in dogs (Richardson et al., 1983), but such use may be premature until it is shown that such a combination produces optimal effects.

Toxicity and Adverse Effects. Renal toxicity inevitably accompanies treatment with micellar (conventional) amphotericin B. In humans, the damage is reversible when the total dose is below 4 g. Monitoring of blood urea nitrogen (BUN) or creatinine shows the extent of renal damage, which can be reversed either by temporarily stopping treatment or by decreasing the dosage. Dosing every other day reduces nephrotoxic effects compared to administering the same dose daily. Other side effects include thrombophlebitis at the injection site and hypokalemia with resulting cardiac arrhythmias, sweating, nausea, malaise, and depression. In dogs and cats, signs of nephrotoxicity develop within 3 or 4 weeks of starting treatment, associated with BUN levels of 60–70 mg/dl. The effect is reversible and the drug should be discontinued until BUN falls below 40 mg/dl. Blood urea nitrogen should be monitored twice weekly during

treatment. In addition, serum potassium should be monitored and hypokalemia corrected by oral supplementation. Hypokalemia does not seem to be as common in dogs and cats as in humans. Concurrent use of flucytosine decreases the dosage of amphotericin B required to treat cryptococcal infection.

Lipid-based formulations of amphotericin B lessen the infusion-related toxicities (nausea, fever, chills) and markedly reduce nephrotoxicity. Because of this reduced toxicity, higher daily doses of the lipid-based formulations may be used ranging in humans up to 3–5 mg/kg daily compared to 0.5–1 mg/kg q 48 h for the conventional form. In a meta-analysis of the human literature, lipid formulations conferred a significant advantage over conventional amphotericin B in terms of reduced risk of mortality and renal toxicity (Barrett et al., 2003).

Doses > 5 mg/kg of conventional amphotericin B in dogs resulted in death as a result of cardiac abnormalities. Doses of 2–5 mg/kg occasionally caused cardiac arrhythmias in dogs, but doses < 1 mg/kg had no effect on the heart. Administration of the liposomal amphotericin B formulation to dogs at daily dosages of 8 and 16 mg/kg resulted in weight loss, vomiting and tubular necrosis. A daily dose of 4 mg/kg for 30 days was associated with occasional vomiting, a moderate increase in BUN and creatinine concentrations, and histopathologic changes consistent with moderate tubular nephrosis. In contrast, a daily dose of 1 mg/kg was well tolerated and only associated with an increased urine volume and lower specific gravity (Bekerski et al., 1999). Renal and clinicopathologic changes observed with administration of the liposomal formulation at a daily dose of 4 mg/kg were similar to those reported after administration of the colloidal formulation at a dose of 5 mg/kg, or to the conventional amphotericin B formulation administered at a daily dose of 0.6 mg/kg.

Administration and Dosage. Dosage is summarized in Table 20.2. There is no general agreement as to the optimum dosage, total dose, or duration of treatment required for amphotericin B in veterinary medicine. The dosage used for the conventional formulation has ranged between 0.25 and 1.0 mg/kg/day.

For otherwise healthy dogs, an initial IV dosage of 0.5 mg/kg q 48 h is often used; BUN is monitored for evidence of kidney damage. If BUN exceeds 60 mg/dl, the dose is discontinued or reduced by 25–50% until BUN falls below 40 mg/dl. Administration by slow IV infusion is preferable because it reduces the severity of systemic toxicity (vomiting, diarrhea, weight loss) and results in less renal damage (Legendre et al., 1984; Rubin et al., 1989). In severely debilitated dogs an initial dosage of 0.2 mg/kg IV has been proposed, increasing by 0.1 mg/kg daily until day 4 (0.5 mg/kg), then using this maintenance dosage. In cats with cryptococcal infection, combining amphotericin B with flucytosine reduces the length of treatment course required for successful therapy.

Subcutaneous administration of amphotericin in 0.45% saline with 2.5% dextrose in dogs (0.5–0.8 mg/kg in 500 ml) and cats (same dose, in 400 ml) 2–3 times weekly was described as a way of administering large quantities of amphotericin without producing the marked azotemia associated with IV injection (Malik et al., 1996a). These amounts were given subcutaneously 2 or 3 times weekly over several months, to a total cumulative dose of 8–26 mg/kg body weight. In the aforementioned study, the drug was administered in combination with a triazole drug for the treatment of cryptococcal infection. Treatment duration with conventional amphotericin B varies with clinical response but may be up to 12 weeks. For blastomycosis the total cumulative dose used is about 12 mg/kg.

Clinical experience with lipid-based formulations in animals is limited but dosages of 1–3 mg/kg 3 times weekly for a total of 9–12 treatments (cumulative dose of 24–27 mg/kg) have been used in dogs. In cats, a lower dose of 1 mg/kg 3 times weekly for a total of 12 treatments (cumulative dose of 12 mg/kg) has been recommended (Grooters et al., 2003). In veterinary medicine, the advantages of lipid-based formulations may be offset by their high cost.

Clinical Applications. Amphotericin B is the most toxic antimicrobial in clinical use, but its fungicidal action makes it the drug of choice for most systemic fungal infections (*Candida*, *Blastomyces, Coccidioides, Histoplasma*) in immunocompromised hosts. In non-compromised hosts, the less toxic, though fungistatic, triazole drugs may be equally valuable for yeast infections. Comparative clinical trials in veterinary species are required to support this statement. For systemic infections caused by dimorphic fungi in non-immunocompromised hosts, amphotericin B may be combined

with, preceded by, or replaced with ketokonazole or itraconazole treatment. In a retrospective study of 115 dogs with blastomycosis, treatment with itraconazole was as effective as treatment with a combination of amphotericin B and ketoconazole (Arceneaux et al., 1998). Amphotericin B lipid complex (Abelcet) has been used to treat dogs with blastomycosis at 1 mg/kg q 48 h, for a total cumulative dose of 8–12 mg/kg. Most dogs given a cumulative dose of 12 mg/kg became clinically free of blastomycosis; the two dogs in the study receiving a total dose of 8 mg/kg had a relapse of blastomycosis. No dogs developed evidence of renal damage (Krawiec et al., 1996).

Historically, amphotericin B was the only reliable antifungal drug for systemic aspergillosis and zygomycosis (*Mucor, Rhizopus*), however, the newer triazoles itraconazole and voriconazole are now challenging amphotericin's use for this purpose. Aerosol treatment of pulmonary aspergillosis may be one way to assure high lung levels and low toxicity due to low systemic absorption from the lungs. Amphotericin B has not always been effective in nasal or disseminated *Aspergillus* infections in animals, possibly because of the lack of susceptibility of the causative fungal species to the drug. Amphotericin B may be a drug of choice in the treatment of *Prototheca* infections alone or in combination with itraconazole (Stenner et al., 2007). In a recent study, intralesional amphotericin B and oral itraconazole resulted in clinical remission in 22 of 26 cats with sporotrichosis (Gremiãno et al., 2011). Lipid formulations of amphotericin B have been successfully used for the treatment canine leishmaniasis but relapses have been reported (Lamothe, 2001; Cortadellas, 2003).

In horses, amphotericin B is not suitable for the local treatment of mycotic keratitis because of its poor activity against some filamentous fungi and its locally irritating nature. There are several reports of intralesional or systemic use of amphotericin B in horses. A wide range of dosages and administration protocols have been used for systemic administration (Table 20.2). In one report, successful treatment of pulmonary cryptococcosis was reported with daily infusions of amphotericin B at 0.5 mg/kg for a month. Recently, administration of amphotericin B by intravenous regional limb perfusion was effective for treating pythiosis of the distal limbs in horses (Dória et al., 2012).

Pyrimidine Synthesis Inhibitors: Flucytosine

Flucytosine (or 5-fluorocytosine) is a fluorinated pyrimidine, a low-molecular-weight compound slightly soluble in water but readily soluble in alcohol. It is the only antifungal agent in use that acts as an antimetabolite. In the United States, flucytosine is only available for oral administration; in most other countries, it is also available for parenteral administration.

Mechanism of Action. After permease-mediated entry into the fungal cell, flucytosine is deaminated to 5-fluorouracil, which is incorporated into messenger RNA. This disrupted mRNA functions poorly, garbling codon sequences and producing faulty proteins. Conversion of 5- fluorouracil to 5-fluorodeoxyuridine monophosphate, on the other hand causes inhibition of thymidylate synthase, which functions in fungal DNA synthesis and nuclear division.

Antifungal Activity. Flucytosine has a narrow spectrum of antifungal activity, being active against most *C. neoformans*, 80–90% of *Candida*, and most *Torulopsis*. The majority of yeast isolates from bovine mastitis are resistant. While a few *Aspergillus* strains are susceptible, dermatophytes, other filamentous fungi, and dimorphic fungi are resistant. An MIC ≤ 4 μg/ml is considered susceptible, 8–16 μg/ml intermediate, and ≥ 32 μg/ml is resistant. The drug becomes fungicidal at concentrations 5 times the MIC.

Resistance. About 10–20% of *Candida* spp. but only 1–2% of *C. neoformans* show resistance to flucytosine. However, resistance develops readily *in vitro* and *in vivo*, thus flucytosine should never be used as a single agent but rather, always in combination with other antifungal agents.

Pharmacokinetic Properties. Flucytosine is well absorbed from the intestine after oral administration in humans, giving peak plasma concentrations of 70–80 μg/ml 1–2 hours after a dose of 37.5 mg/kg. Half-life in humans is about 4 hours; in the presence of renal impairment the half-life is increased. Penetration into tissues, including CSF, is excellent. The drug is largely excreted unchanged in the urine via glomerular filtration.

Drug Interactions. Combination with amphotericin B is commonly synergistic, because amphotericin B increases fungal permeability to flucytosine. Combination of flucytosin with amphotericin B or with an azole is superior to amphotericin B or azole monotherapy for the treatment of cryptococcosis.

Toxicity and Adverse Effects. Flucytosine is generally well tolerated. Occasional side effects reported are reversible anorexia, nausea, vomiting, diarrhea, mild elevations of liver enzymes, and bone marrow depression resulting in leucopenia. Skin eruptions characterized by depigmentation, followed by ulceration, exudation and crust formation have been reported in dogs (Malik et al., 1996b). Lesions resolve following discontinuation of therapy.

Administration and Dosage. Dosage is summarized in Table 20.2. The drug is given in capsule form at a dosage of 150–225 mg/kg daily in 3 or 4 divided doses. Flucytosine should always be used in conjunction with amphotericin B or an azole to prevent the emergence of resistant mutants.

Clinical Applications. The major clinical application of flucytosine is in the treatment of cryptococcal infection in cats. However, its use for this purpose has now largely been replaced by triazole drugs (Trivedi et al., 2011). The drug should be combined with amphotericin B or an azole to prevent rapid development of resistance. The usual dose of amphotericin B can be reduced by half or less, or administered for a shorter period when used with flucytosine. Ketoconazole can substitute for amphotericin B and significantly reduces the length of treatment required with either drug alone (Shaw, 1988). The use of other azoles drugs has shown the same effect, experimentally. There are reports that describe the successful treatment of cryptococcosis in cats treated with flucytosine as a single agent. However, because of the likelihood of resistance, this is not recommended.

Azoles: Imidazoles and Triazoles

The azoles constitute a large group of synthetic agents containing many compounds that are effective in the topical treatment of dermatophyte infections and superficial forms of candidiasis. A number of these agents are suitable for systemic administration.

Azole drugs were first extensively evaluated in the early 1970s for their antifungal activity. Two imidazoles, clotrimazole and miconazole are effective topical antifungal agents but neither can be used parenterally as clotrimazole rapidly induces hepatic-inactivating enzymes and the toxicity of the solubilizing agent required for IV administration of miconazole limits its use. Another imidazole, ketoconazole, was developed in the late 1970s and became a major addition in antifungal therapy with the advantages of a broad antifungal spectrum, the option of oral administration, and relatively low toxicity. Further development of the azoles, for example, substitution of the imidazole ring by a triazole ring, produced compounds such as fluconazole, itraconazole, voriconazole, and posaconazole (Figure 20.3). These products all have greatly increased half-lives, increased bioavailability following oral administration, lower toxicity, and enhanced antifungal activity compared to many of the imidazole drugs.

Mechanism of Action. Imidazole and triazole drugs have the common antifungal action of inhibiting 14α-demethylase, a cytochrome P_{450}-dependent enzyme responsible for the demethylation of lanosterol to ergosterol. Ergosterol is the principal sterol in fungal cell membranes just like cholesterol is the principal sterol in mammalian cells. This results in in the accumulation of various methylated sterols and the depletion of ergosterol with subsequent disruption of cell membrane structure and function. The synthesis of cholesterol in mammalian cells is also affected but the dose of azoles required to obtain this inhibition is much higher than inhibitory concentrations for fungi. Most azoles are considered fungistatic drugs although some of the newer triazoles can exert fungicidal effects again some mold species.

Resistance. The emergence of strains resistant to azoles has occurred in parallel with their use. As all members of the azole family act on the same target, cross-resistance with multiple azoles is common. Three resistance mechanisms have been postulated. The first is genetic alteration of the target of action (ERG11 gene coding for 14α-demethylase). The second is over production of the target of action by overexpression of the ERG11 gene. The third is upregulation of multidrug efflux transporter genes.

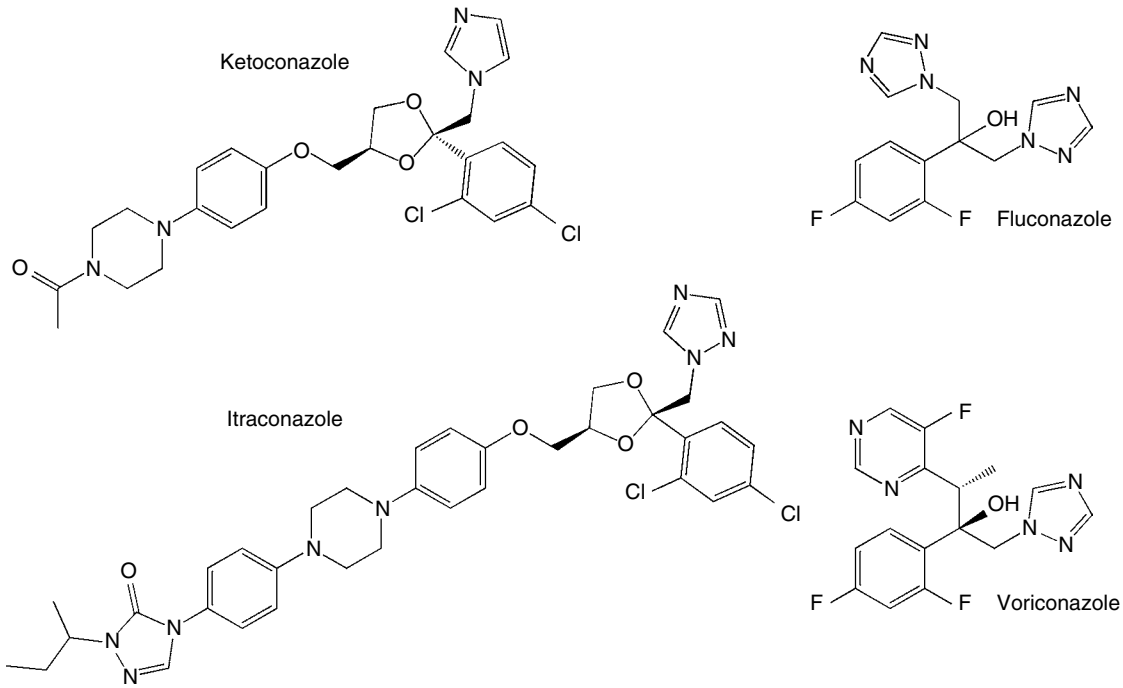

Figure 20.3. Structural formulas of representative azole compounds. Courtesy of Jérôme del Castillo.

Drug Interactions. The azoles may be associated with three types of drug interactions. Firstly, because azoles are important inducers of the CYP3A4 enzyme system, they may slow the metabolism and increase plasma concentrations of drugs that are metabolized by the CYP pathway. Secondly, drugs that are CYP inducers can speed the metabolism of the azoles, thereby lowering their plasma concentrations. Thirdly, when an azole is given concurrently with some drugs, there may be two-way interactions in which the azole can raise the serum level of a concomitant drug and in turn, the concomitant drug can lower the concentration of azole. Administration of azoles with drugs that are potent inhibitors of the cytochrome $P\text{-}_{450}$ enzyme system, such as rifampin, results in marked reduction in plasma concentrations, especially with itraconazole and ketoconazole.

Imidazoles: Ketoconazole

Chemistry. Ketoconazole is a poorly water-soluble, highly lipophilic, weak dibasic compound that requires an acid pH for dissolution, which precedes absorption from the stomach. There are conflicting reports on the effect of feeding on the absorption of ketokonazole.

Antimicrobial Activity. Ketoconazole is generally fungistatic against a wide range of fungi including dermatophytes, yeasts, and dimorphic fungi (Table 20.3). Isolates with an MIC ≤ 0.125 μg/ml are considered susceptible, 0.25–0.5 μg/ml represent intermediate susceptibility, and isolates with an MIC ≥ 1 μg/ml are resistant. Most isolates of *C. albicans* are susceptible but *C. tropicalis* and *C. glabrata* are resistant. *Malassezia pachydermatis* isolates are susceptible. The drug has favorable *in vitro* activity against *H. capsulatum*, *C. immitis*, and *B. dermatidis*. However, *Aspergillus* spp. *Fusarium* spp., and the *Zygomycetes* group of fungi (e.g., *Rhizopus* spp., *Mucor* spp.) are usually resistant. Ketoconazole is active against some Gram-positive bacteria, and the drug has activity against *Leishmania, Plasmodium*, and other protozoa. The *in vitro* resistance of *Prototheca* is apparently contradicted by *in vivo* response to ketoconazole treatment.

Pharmacokinetic Properties. Ketoconazole is well absorbed after oral administration. In dogs, plasma concentration after an oral dose of 10 mg/kg peaks at 8.9 μg/ml within 1–2 hours. The drug requires an acid environment for full dissolution and absorption and should be given with food. Ketoconazole is extensively metabolized in the liver to inactive compounds, which are excreted in the bile. The distribution of ketoconazole is limited, and its penetration into CSF is minimal. The drug does, however, enter milk. Little active drug is excreted in urine. Administration of ketokonazole orally to adult horses at a dose of 30 mg/kg does not result in detectable serum concentrations. Administration of the same dose in 0.2 N HCl resulted in peak serum concentrations of 3.7 μg/mL and a bioavailability of only 23% (Prades et al., 1989).

Drug Interactions. Combination of amphotericin B with ketoconazole gives additive effects in the treatment of cryptococcal infection. Experimentally, however, ketoconazole antagonizes the activity of amphotericin against *Aspergillus*. Combination with flucytosine in the treatment of cryptococcal infections may prevent the emergence of resistance to flucytosine and reduce the length of time required for treatment. The azoles may be associated with three types of drug interactions as indicated above.

Toxicity and Adverse Effects. Nausea, vomiting, dizziness, itching, and increases in liver enzyme levels are adverse effects in humans. In a retrospective study of 632 dogs treated with ketoconazole (2.6–33.4 mg/kg), adverse effects occurred in 14.6% and included vomiting (7.1%), anorexia (4.9%), lethargy (1.9%), diarrhea (1.1%), pruritus (0.6%), and erythema (0.3%; Mayer et al., 2008). In the same study, adverse effects were significantly more often recorded in dogs that were concurrently treated with cyclosporine or ivermectin. Other adverse effects reported include ataxia, alopecia, and reversible lightening of the hair (Mayer et al., 2008; Moriello, 1986). Long-term treatment of dogs (mean 13.6 months, range 3.5–37) has been associated with the development of cataracts (da Costa et al., 1996). The mean time from the initiation of treatment to development of cataracts was 15 months. Cats appear to be more susceptible to the toxic effects of ketoconazole and may develop anorexia, depression, weight loss, diarrhea, and fever. In a few human patients (1 in 15,000) severe hepatitis may develop. This reaction does not appear to be dependent on dose. High doses in dogs (greater than 80 mg/kg/day) for prolonged periods have produced severe hepatitis. Cats treated concurrently with flucytosine have shown evidence of liver damage and developed leukopenia, possibly because of additive or synergistic toxicity. Significant inhibition occurs of mammalian P_{450} systems that are responsible for cholesterol, cortisol, and testosterone synthesis. Gynecomastia, decreased libido, and azoospermia have been reported in a small percentage of men but not in dogs or cats. Ketoconazole at therapeutic dosage suppressed plasma cortisol and testosterone but increased progesterone concentrations in dogs (Willard et al., 1986a); therefore, care should be taken when using the drug in male breeding dogs. Similar effects were not observed in cats (Willard et al., 1986b). Ketoconazole may be embryotoxic and teratogenic, and should not be given to pregnant animals.

Administration and Dosage. Dosage is summarized in Table 20.2. Absorption from the gastrointestinal tract may be erratic. The oral dosage of ketoconazole in dogs and cats for the treatment of ringworm was extrapolated from human clinical studies and varies from 5 to 10 mg/kg daily for 4–6 weeks. Recommended dosage of ketoconazole for systemic fungal infections in dogs and cats is 10 mg/kg q 12 h.

Clinical Applications. Ketoconazole used to be the most widely used antifungal drug in veterinary medicine, because of its efficacy, safety relative to amphotericin B, oral dosing route, and cost. However, ketoconazole is now being eclipsed by fluconazole and itraconazole, because of their greater activity, lower toxicity, and improved pharmacokinetic properties. Ketoconazole is now a second line drug for the treatment of dimorphic fungi (candidiasis, cryptococcosis), as well systemic mycosis (coccidioidomycosis, histoplasmosis, blastomycosis) in dogs and cats.

Legendre et al. (1984) suggested that amphotericin B was better than ketoconazole (10 mg/kg daily) for the treatment of canine blastomycosis, but that a course of amphotericin (total 4 mg/kg) followed by ketoconazole (10 mg/kg daily for 2 months) was as effective as more prolonged treatment with amphotericin (total 8–9 mg/kg), and produced less kidney damage.

Ketoconazole is not useful in zygomycosis and its efficacy against *Aspergillus* infections is questionable in that only about 50% of dogs treated for nasal aspergillosis were cured by the use of ketoconazole alone (5 mg/kg, q 12 h; Sharp and Sullivan, 1989). The combination ketoconazole and 5-flucytosine reduced the dose and duration of treatment required for feline cryptococcosis compared to either drug alone (Shaw, 1988).

Ringworm in dogs and cats has been treated successfully with 10 mg/kg of ketoconazole daily for 10–20 days. Because of the adverse effects of ketoconazole (especially in cats), its lower cost, and its greater activity against dermatophytes *in vitro*, griseofulvin is preferred for the treatment of ringworm. Animals with lesions may require 6 weeks (range 4–10) for complete resolution (Medleau and Chalmers, 1992). Ketoconazole is effective in the oral treatment of soft tissue sporotrichosis in humans, but high doses are required and relapses may occur. Ketoconazole has been the systemic treatment of choice for *Malassezia* infections, although topical treatment with miconazole is more usual. In a recent study, oral fluconazole was at least as effective as ketoconazole for the treatment of dogs with *Malassezia* dermatitis (Sickafoose et al., 2010).

Triazoles: Itraconazole

Chemistry. Like ketoconazole, itraconazole is a poorly water-soluble, highly lipophilic, weakly dibasic, compound that also requires an acid pH for absorption from the stomach. It is now available as both IV and oral preparations.

Antimicrobial Activity. Itraconazole is a potent inhibitor of most fungal pathogens of animals, because of its greater selectivity for the fungal cytochrome system compared with ketoconazole (Table 20.3). The spectrum includes dimorphic fungi, *Cryptococcus, Sporothrix, Alternaria*, most *Aspergillus, Candida* spp. and the dermatophytes. While itraconazole is considered to be a fungistatic agent it has been shown to be fungicidal, at low concentrations, against some fungi. Isolates with MIC ≤ 0.125 μg/ml are regarded as susceptible, with MIC 0.25–0.5 μg/ml as intermediate, and ≥ 1 μg/ml as resistant.

Pharmacokinetic Properties. A lipophilic drug, itraconazole is well absorbed following oral administration and widely distributed to tissues (except the CSF), where it achieves concentrations several times those found in plasma. Skin concentrations exceed plasma concentrations and marked keratin binding occurs; this is significant in the treatment of dermatophyte infections. Administration with food and an acidic environment significantly enhance absorption. It is cleared mainly by intrahepatic metabolism and detectable concentrations of the do not appear in the urine or CSF, even though the drug has been successfully used in the treatment of cryptococcal meningitis. A steady-state in serum concentration was achieved in cats after 2–3 weeks of administration of 10 mg/kg q 24 h (Boothe et al., 1997). In horses and in cats, the oral suspension is better absorbed than the capsules. The half-life in horses is 6.5 hours (Davis et al., 2005). As with other azoles, concurrent administration of rifampin will increase hepatic metabolism of itraconazole.

Toxicity and Adverse Effects. Toxicity reported in humans is minimal and limited to nausea in a small proportion of patients and to rare, transient increases in hepatic enzymes. Blockage of adrenal steroid or testosterone synthesis has not been described. There were no adverse effects reported in cats treated with itraconazole at 10 mg/kg/day for 3 months compared to anorexia and weight loss in cats treated with the same dosage of ketoconazole (Medleau et al., 1990). Adverse effects reported in dogs and cats have, apart from occasional anorexia and vomiting, been minimal. Dosage can be progressively decreased in animals that vomit or become anorectic until these effects are no longer observed. Fatal hepatoxicity was reported in one cat treated with over 20 mg/kg (Medleau et al., 1995). Cutaneous lesions suggestive of drug eruption have been described in a dog (Plotnick et al., 1997). Itraconazole is contraindicated in pregnancy.

Administration and Dosage. Recommended dosage is summarized in Table 20.2. Itraconazole is available in oral capsule, oral suspension, and intravenous formulations. The oral suspension is preferred to capsules in domestic animals because of enhanced bioavailability. Itraconazole, administered orally, preferably with food at a dose of 5 mg/kg q 12–24 h is recommended for dogs, cats, horses and other monogastric animals. Duration of treatment should be tailored to clinical and mycological response. For example, a dose of 5 mg/kg q 24 h for

60 days was as effective as 10 mg/kg q 24 h for the treatment of canine blastomycosis and was associated with fewer adverse effects (Legendre et al., 1996); however, about 20% of treated dogs relapsed. A dosage of 5 mg/kg q 12 h for 60 days or more was used to treat histoplasmosis in cats; recurrence of disease occurred in two of eight treated cats, which required further treatment (Hodges et al., 1994). The dosage of 5 mg/kg q 12 h in cats can safely be increased to 10 mg/kg q 12 h (Boothe et al., 1997). Dosage of 1.5–3 mg/kg q 24 h, usually for 15 days (but sometimes for longer), was effective in controlling dermatophytosis in cats (Mancianti et al., 1998). Dosage in humans is ≤ 400 mg/day but higher doses (600 mg) have been used in infections that have not responded to the lower dose, although toxicity was observed in long-term use of high dosage (Sharkey et al., 1991).

Clinical Applications. Because of its potency, pharmacokinetic advantages, clinical efficacy and safety, itraconazole has become the systemic treatment of choice for aspergillosis, blastomycosis, coccidioidomycosis, histoplasmosis, and sporotrichosis. It has similar application to ketoconazole but its broader spectrum includes *Aspergillus* and the agents of phaeohypomycosis. It has greater activity than ketoconazole against *Sporothrix*. It is as effective and less toxic than ketoconazole in the treatment of cryptococcosis and dermatophyte infections. It is as effective as griseofulvin in the treatment of dermatophyte infection in cats (Moriello and DeBoer, 1995). Treatment of serious infections with this generally fungistatic drug needs to be prolonged (3+ months), and relapses anticipated. The effectiveness of treatment may be monitored by serology, for cryptococcal (Jacobs et al., 1997) and possibly for other systemic mycoses. In the treatment of serious systemic mycoses, combination with amphotericin B in the initial stages of treatment is recommended.

Although itraconazole has been used successfully to treat disseminated *Aspergillus* infections in dogs (Kelly et al., 1995), its oral administration has been found to be ineffective in treatment of canine nasal aspergillosis. In high doses, it has been used to successfully treat cerebral aspergillosis in humans (Verweij et al., 1997). The drug has a particularly useful place in the systemic treatment of aspergillosis in pet birds; pharmacokinetic studies in Blue-fronted Amazon Parrots suggested that a dosage of 10 mg/kg q 24 h was appropriate (Orosz et al., 1996).

In horses, topically applied 1% itraconazole with 30% DMSO ointment gave considerably higher corneal concentrations than drug without DMSO (Ball et al., 1997a); applied every 4 hours for a median of 35 days, it was effective in resolving keratomycosis in the majority of cases (Ball et al., 1997b). Administered orally for 3.5–5 months, itraconazole was effective in the treatment of mycotic rhinitis in horses (Korenek et al., 1994). Oral administration of 5 mg/kg q 24 h was combined with locally applied enilconazole in the successful treatment of guttural pouch mycosis (Davis and Legendre, 1994).

Triazoles: Fluconazole

Fluconazole is a specific inhibitor of the fungal enzyme lanosterol 14α-demthylase. This inhibition prevents the conversion of fungal cell lanosterol to the membrane lipid ergosterol. It is highly selective for fungal cytochrome P_{450} enzymes.

Chemistry. Fluconazole is a water-soluble bis-triazole compound with marked pharmacokinetic differences from ketoconazole and itraconazole.

Antimicrobial Activity. Fluconazole possesses the narrowest spectrum of all the azoles antifungals available for systemic use. It is active against most *Candida* spp. However, *C. kruzei* is intrinsically resistant. Fluconazole is also active against dimorphic fungi including *Cryptococcus neoformans*, *Coccidioides immitis*, and *Histoplasma capsulatum* (Table 20.3). Fluconazole has limited activity against *Blastomyces dermatitidis*. It is ineffective against *Aspergillus and Fusarium* spp. Organisms with MIC ≤ 8 μg/ml are regarded as susceptible, with MIC 16–32 μg/ml as intermediate, and ≥ 64 μg/ml as resistant.

Resistance. *Candida krusei* is intrinsically resistant to fluconazole and as many as 15% of *C. glabrata* isolates may exhibit resistance. Progressive development of acquired resistance in *C. albicans* has been reported during long-term treatment, particularly in immunosuppressed patients. Resistance may also develop when fluconazole is used to treat histoplasmosis.

Pharmacokinetic Properties. In contrast to other azoles, fluconazole is a water-soluble, weakly protein-bound drug, and oral absorption is unaffected by gastric pH. It is well

absorbed after oral administration and, because of its low molecular weight, water-solubility, and lack of protein binding, it distributes widely to tissues. Its ability to reach high concentrations (50–90% of serum) in CSF is a particular advantage in treating yeast (e.g., *Cryptococcus* infections) in the brain. Food does not affect absorption. It is excreted unchanged in the urine. Half-life in humans is 25–30 hours, so that single oral dosing is used for some types of infections. Half-life in cats has been reported as 14 (Malik et al., 1992) or 25 hours (Vaden et al., 1997). Half-life in horses is 40 hours (Latimer et al., 2001). Oral bioavailability is 100% in both cats and horses. In contrast to ketoconazole, fluconazole can be administered IV.

Toxicity and Adverse Effects. Fluconazole is well tolerated after oral or IV administration, with minimal side effects other than nausea, skin rash and headaches in some human patients. There is no evidence of interference with steroid biosynthesis but elevations in hepatic enzymes, which are usually mild, have been reported. It can interfere with the metabolism of drugs whose metabolism is dependent on hepatic P_{450} enzymes.

Administration and Dosage. Fluconazole is available in both oral and IV formulations although it is used almost exclusively orally in veterinary medicine. Dosage recommendations for fluconazole in animals are presented in Table 20.2. Cryptococcal infections in cats were successfully treated with 50 mg/cat q 12 h (Malik et al., 1992); in one animal 100 mg q 12 h was required. Pharmacokinetic considerations led Vaden et al. (1997) to suggest a dose in cats of 50 mg/cat q 24 h. A dose of 11 mg/kg q 24 h was used in the effective treatment of cryptococcosis in a dog. This dose was reduced to 4.2 mg/kg several weeks later, when anorexia developed (Tiches et al., 1998).

Clinical Applications. Fluconazole has had excellent success in oral treatment of local or systemic candidiasis in humans, and is a drug of choice for this purpose. In severe candidiasis, it may be combined with amphotericin B. Fluconazole is also the treatment of choice for cryptococcal meningitis in AIDS patients. It is as effective as amphotericin B in treatment of acute cryptococcal meningitis in all patients and is more effective in maintenance therapy in AIDS patients. Initial concurrent treatment with amphotericin B is recommended. It is a drug of choice for candidal cystitis. In animals, fluconazole is probably the drug of choice for cryptococcal infections, for the systemic treatment of candidal infections, and for the treatment of coccidioidosis.

Efficacy against blastomycosis, histoplasmosis and sporotrichosis in humans has been moderate at the dosages assessed. Fluconazole is not as effective as itraconazole for these infections. In a recent retrospective study of 144 dogs with blastomycosis, remission was achieved in 90% of dogs treated with itraconazole compared to 75% of dogs treated with fluconazole (Mazepas et al., 2011). Despite treatment duration being significantly longer in dogs treated with fluconazole, total treatment cost was significantly lower in the fluconazole group (Mazepas et al., 2011). Fluconazole has little *in vitro* activity against *Aspergillus* spp. and it thus, not recommended for the treatment of aspergillosis. Paradoxically, fluconazole was administered PO at 2.5–5 mg/kg/day to dogs in a divided dose in the successful treatment of nasal aspergillosis or penicilliosis in six of ten dogs (Sharp et al., 1991).

Triazoles: Voriconazole

Voriconazole is the first licensed member of the second generation of triazoles, which includes posaconazole and ravuconazole (see below). It is structurally related to fluconazole rather than to itraconazole.

Antimicrobial Activity. Voriconazole is active against a wide spectrum of medically important fungi, including dermatophytes, opportunistic yeasts (*Candida* spp, *Cryptococcus neoformans*), opportunistic filamentous fungi (*Aspergillus* spp., *Fusarium* spp.) and dimorphic fungi (*Histoplasma*, *Coccidioides*, *Blastomyces*, *Sporothrix*). Isolates with an MIC ≤ 1 μg/ml are considered susceptible. In contrast to fluconazole, voriconazole is active against most *C. krusei* and most *C. glabrata* isolates. However, cross-resistance of resistant *C. albicans* and *C. glabrata* strains has been reported. Voriconazole exerts time-dependent fungicidal activity against *Aspergillus in vitro*. This process is slightly better than with itraconazole but slower than with amphotericin B, as would be expected from their respective mechanism of action.

Pharmacokinetic Properties. Voriconazole is available in oral and IV formulations. It is extensively metabolized

by the liver and, unlike fluconazole and amphotericin B, does not depend on renal function for excretion. However, the IV formulation contains sulfobutyl ether ß-cyclodextrin sodium, which is excreted by the kidneys and tends to accumulate in patients with renal failure. Unlike itraconazole, voriconazole is not dependent on gastric acid for absorption and the drug is entirely absorbed in dogs and horses after oral administration (Roffey et al., 2003; Davis et al., 2006; Colitz et al., 2007). Voriconazole has excellent tissue penetration and distributes widely into body fluids (Passler et al., 2010). In a guinea pig model CSF concentrations were about half that of plasma whereas brain tissue concentrations were two-fold higher (Lutsar et al., 2003).

Toxicity and Adverse Effects. Voriconazole is generally well tolerated in humans. Transient visual disturbance is the most common adverse effect occurring in 20–40% of human patients. The effect is dose related and it is seldom necessary to stop therapy. This effect has not been described with other triazoles. Other adverse effects and drug interactions are similar to that reported with other triazoles. In a recent report, three cats treated with voriconazole (approximately 10 mg/kg/day) developed ataxia that, in two cats, progressed to paraplegia of the rear limbs (Quimby et al., 2010). Additionally, two of the cats had visual abnormalities including mydriasis, decreased to absent pupillary light responses, and decreased menace response. Arrhythmia and hypokalemia were noted in two cats (Quimby et al., 2010).

Clinical Applications. Voriconazole is used in people for the treatment of invasive aspergillosis and serious infections caused by *Scedosporium* spp., *Fusarium* spp., or invasive fluconazole-resistant *Candida* spp. In one study, voriconazole was more effective than amphotericin B in humans with invasive aspergillosis, regardless of the site of infection, the neutrophil count, and the underlying disease (Herbrecht et al., 2002). Experience with the use of voriconazole in domestic animal species is limited. The drug has been used topically (1% solution) for the treatment of fungal keratitis in horses and dogs (Grundon et al., 2010). In one study, fungal isolates from equine ulcerative keratomycosis were significantly more susceptible to voriconazole than to natamycin, itraconazole, fluconazole, and ketoconazole (Pearce et al., 2009). There are isolated reports of oral administration of voriconazole to dogs with various fungal infections. Systemic administration to cats is not recommended because of the adverse effects described above.

Triazoles: Posaconazole. Posaconazole is similar in structure to itraconazole and has potent broad-spectrum antifungal activity. It is available only as an oral suspension. As opposed to voriconazole, posaconazole is active *in vitro* and *in vivo* against zygomycetes, a group of fungi for which there are limited treatment options. The drug has a good oral bioavailability and a half-life of 15 hours in dogs (Nomeir et al., 2000). Information on the use of posaconazole in domestic animal species is limited to isolated case reports in cats.

Echinocandins: Caspofungin, Micafungin, and Anidulafungin

Echinocandins are novel lipopeptide antifungal agents that are 1,3-β-D-glucan synthase inhibitors, preventing production of an essential polysaccharide in the cell wall of many fungi. Three echinocandins have been approved for systemic use in humans: caspofungin, micafungin, and anidulafungin. Caspofungin is approved for the treatment of candidiasis and invasive aspergillosis whereas micafungin and anidulafungin are currently only approved for the treatment of candidiasis.

Antimicrobial Activity. These drugs are active against yeast of the genus *Candida* including isolates resistant to azoles and amphotericin B. Echinocandins are also highly active against *Aspergillus* spp. These drugs are not active or have limited activity against *C. neoformans*, *B. dermatitidis*, *C. neoformans*, *C. immitis*, or *Fusarium* spp. Echinocandins are active against *Pneumocystis jiroveci*. Isolates with an MIC ≤ 2 μg/ml are considered susceptible.

Acquired Resistance. Acquired resistance to echinocandins is rare at present, but resistant strains of *Candida* spp. have been recovered from patients failing to respond to therapy. Resistance has been associated with mutations in the glucan synthase subunit *FKS1*.

Pharmacokinetic Properties. Echinocandins have limited oral bioavailability and only IV formulations are available. These drugs are extensively distributed to tissues, but concentrations in the CSF are negligible.

Caspofungins and micafungin are metabolized by the liver and eliminated as inactive metabolites in the urine and the feces. Anidulafungin is not metabolized by the liver but undergoes non-enzymatic degradation to an inactive peptide in the blood.

Drug Interactions. Echinocandins have few drug interactions because their metabolism is independent of the cytochrome P_{450} system. Because of their distinct targeting of the fungal cell wall, echinocandins have been proposed to be ideal agents for use in combination with drugs that act on the cytoplasmic membrane (polyene or azoles). *In vivo*, the combination of caspofungin with fluconazole improved clearance of *C. albicans* in a murine model of disseminated candidiasis. Similarly, animal models of invasive aspergillosis have shown improved survival and enhanced clearance of the pathogen when an echinocandin is combined with amphotericin B or a triazole.

Toxicity and Adverse Effects. Caspofungin is well tolerated. The most common adverse effects are fever, nausea, and phlebitis at the infusion site. Transient elevations in liver enzymes have been reported in a few human patients

Clinical Applications. Echinocandins are indicated for the treatment of invasive aspergillosis or candidiasis unresponsive to amphotericin B or triazoles, or in patients intolerant of these drugs. There is no information regarding the use of these drugs in domestic animal species.

Other Antifungal Agents for Systemic Use

Griseofulvin

Chemistry. Griseofulvin (Figure 20.4) is a benzofuran cyclohexene antibiotic, a product of *Penicillium griseofulvum*. It is poorly soluble in water.

Mechanism of Action. Griseofulvin is a fungistatic antibiotic that inhibits mitosis, probably by disorganizing the spindle microtubules. It may also interfere with cytoplasmic microtubules.

Antimicrobial Activity. Virtually all dermatophytes of animal origin are inhibited by griseofulvin concentrations of 0.2–0.5 μg/ml. Other hyphal fungi, yeasts, dimorphic fungi, and bacteria are unaffected by

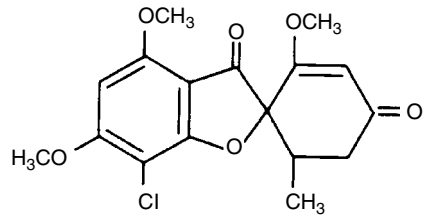

Figure 20.4. Structural formula of griseofulvin.

griseofulvin. Resistance (MIC ≥ 3 μg/ml) to griseofulvin has been described occasionally in dermatophytes of human origin.

Pharmacokinetic Properties. Absorption, after oral administration, depends greatly on particle size. It is enhanced in humans after a high-fat meal. Half-life in humans is about 20 hours but is considerably shorter (less than 6 hours) in dogs. Most of the drug is excreted in feces. Griseofulvin appears to be metabolized in the liver and as such, concurrently administration of drugs that are liver enzyme inducers (e.g., rifampin) may increase griseofulvin metabolism. Griseofulvin is selectively deposited in the newly formed keratin of hair, nails, and skin, and gradually moves from these deep layers to the site of infection in the superficial keratinized epithelium, where keratinized cells mature and are progressively desquamated. Actively growing fungi may be killed, but dormant cells are only inhibited, so that cure occurs when infected keratinized cells are shed. For this reason, treatment is prolonged.

Toxicity and Side Effects. Prolonged medication in humans has occasionally been associated with mild and transient adverse effects such as headaches, dizziness, fatigue, photosensitivity, and gastrointestinal disturbances (nausea, vomiting, diarrhea).

Griseofulvin is teratogenic in cats, particularly in the first weeks of gestation. Congenital defects reported include brain malformations, skeletal abnormalities, spina bifida, anophthalmia, and atresia ani. High doses in cats have also been associated with anemia, a possibly idiosyncratic reaction (Kunkle and Meyer, 1987). This may relate to feline immunodeficiency virus (FIV) infection (Shelton et al., 1990). Cats that are FIV-positive should probably be treated with another drug, such as itraconazole. All cats may exhibit signs of toxicosis

including anorexia, vomiting, ataxia, anemia, leukopenia, anorexia, depression, jaundice, pruritus, and pyrexia (Helton et al., 1986; Wack et al., 1992). These signs are usually, but not always, reversible. Because of the teratogenic effect for all species (Schutte and van den Ingh, 1997), griseofulvin should not be given to any pregnant animal. Dogs and cats may vomit if given griseofulvin on an empty stomach.

Administration and Dosage. The drug should be given for 1 or 2 weeks beyond clinical or mycologic cure. A single daily dose of 50 mg/kg can be reduced to 25 mg/kg once clinical response occurs. The optimal dose in cats has not been firmly established but toxicity appears to be idiosyncratic rather than dose-related (Levy, 1991).

Clinical Applications. Griseofulvin is effective only against dermatophytic infections and effective against ringworm only if administered orally. In recent years, griseofulvin has been largely replaced by itraconazole or terbinafine therapy but it is still considered an effective antifungal agent for dermatophytosis in dogs and cats. The drug reaches the superficial, dead, parasitized epithelium only through progressive maturation of basal cells. Prolonged therapy is necessary, typically 3–6 weeks in dogs and cats.

Iodides

Iodides have been used for many years to treat mycotic infections. Their mechanism of action is poorly understood, but action may result from enhancement of the immune response of the host or by spurring on the halide-peroxide killing system of phagocytic cells. Historically, sodium iodide has been the treatment of choice in sporotrichosis but itraconazole is now becoming the preferred treatment. Ketoconazole and sodium iodide administered together appear to have additive effects. The dose of iodide is 20 mg/kg in cats and 40 mg/kg in dogs. The drug is administered orally once or twice daily and a response occurs in 1–4 weeks; treatment should be continued for several weeks past clinical cure. Treatment should be temporarily stopped if signs of iodism (e.g., severe coryza, weakness, salivation) occur. Sodium iodide has been used as an adjunct in the treatment of nasal aspergillosis in dogs. Sodium iodide has been administered IV, 1 g/15 kg in a 10% solution, in the treatment of ringworm in cattle. The use of iodine preparations in animals that will enter the human food chain is discouraged because of prolonged tissue residues.

Lufenuron

Lufenuron is a benzoylphenyl urea-derived insecticide used as an oral product for flea control in dogs and cats. The drug interferes with chitin synthesis and the deposition of chitin in the cuticle of insects. Chitin is also an important component of the outer cell wall of fungi suggesting that the drug may have antifungal activity as well. In a retrospective study of 297 dogs and cats with dermatophytosis or superficial dermatomycosis, time to resolution of gross lesions was significantly shorter in lufenuron-treated animals than in untreated controls (Ben-Ziony and Arzi, 2000). In contrast, oral lufenuron did not prevent dermatophytosis following experimental infection of cats with *Microsporum canis* (Moriello et al., 2004). There are anecdotal reports of the use of lufenuron for the treatment of fungal endometritis in mares and cutaneous mycosis in chimpanzees (Hess et al., 2002; Dubuis and Lucas, 2003). Lufenuron demonstrates no *in vitro* activity against *Aspergillus* spp., *Fusarium* spp. and *Coccidioides immitis* (Hector et al., 2005; Scotty et al., 2005). Further therapeutic use of lufenuron as an antifungal agent should be based on proven *in vitro* activity against specific species of clinically relevant fungi with pharmacokinetic data demonstrating sufficient drug concentration at the site of infection.

Antifungal Drugs for Topical Application

An extensive range of antifungal drugs, some described in Table 20.1, is available for topical application. These preparations include creams, lotions, sprays, ointments, powders, solutions and nail lacquers for the treatment of onychomycosis. Clotrimazole, itraconazole, miconazole, enilconazole and natamycin are drugs of choice for topical treatment of fungal infections in veterinary medicine. Many other chemicals have antifungal properties, including phenolic antiseptics such as thymol and hexachlorphene; iodides; 8-hydroxyquinoline; quaternary ammonium and bisquaternary antiseptics; salicylamide; propionic, salicylic, and undecanoic acids; silver sulfadiazine; and chlorphenesin. All these compounds and

Table 20.4. *In vitro* activity (MIC_{90}, μg/ml) of selected topical antifungal agents against common fungi.

Organisms	Natamycin	Clotrimazole	Nystatin
Filamentous fungi			
Alternaria spp.	2	–	32
Aspergillus fumigatus	8	8	≤ 64
Fusarium spp.	1	8	≤ 64
Mucor spp.	1	1	8
Microsporum canis	8	2	4
Trichophyton spp.	8	8	16
Yeasts			
Candida spp.	8	0.5	4
Cryptococcus neoformans	8	4	2
Malassezia pachydermatis	8	2	0.25

others have been used for the topical treatment of fungal infections of the skin and sometimes of mucosal surfaces. The topical antifungal drugs discussed here are of interest for their potency or their broad-spectrum activity.

Natamycin

Natamycin is a fungicidal polyene antibiotic derived from *Streptomyces natalensis* with action against the fungal cell membrane. It is effective against a wide range of filamentous and dimorphic fungi and yeasts (Table 20.4). Natamycin is used for local application against ringworm, in the udder for yeast mastitis, and on the eyes for mycotic keratitis. After topical ocular administration, natamycin penetrates superficially into the cornea, as demonstrated in rabbits. However, the drug is water insoluble and penetration into internal ocular structures is poor. Therefore, natamycin is not effective against deep mycotic infections of the eyes. In one study examining *in vitro* activity against fungal isolates from the eyes of horses with ulcerative keratomycosis in the southeastern United States, natamycin and miconazole had the broadest spectrum followed by itraconazole and then ketoconazole (Brooks et al., 1998). In contrast, natamycin was more active than miconazole active against isolates from equine keratomycosis in the northeastern United States (Ledbetter et al., 2007). In a more recent study voriconazole was found to be more active than natamycin against *Aspergillus* spp. from horses with keratomycosis. In contrast, natamycin was more active against *Fusarium* spp. (Pearce et al., 2009).

Natamycin has been used successfully to treat cows with *Candida* mastitis (20 ml of a 2.5% solution, or 10 ml of a 5% solution, infused into the affected udder quarter once daily for 3 days). Total-body spraying or sponging with natamycin suspension is effective in the treatment of ringworm in cattle and horses. It is important that all grooming utensils be thoroughly cleansed or immersed in the natamycin suspension, which should be prepared in plastic or galvanized containers. Natamycin is used successfully to treat filamentous fungal keratitis in horses, and is the drug of choice for this purpose. A recommended treatment is one drop of a 5% suspension every 1 or 2 hours, decreasing to 6 or 8 times daily after a few days. Some clinicians have found natamycin to be locally irritating. *In vitro*, natamycin causes more damage to equine keratinocytes than miconazole or itraconazole (Mathes et al., 2010). Topical application in the treatment of nasal aspergillosis in horses has been clinically effective in some cases but controlled studies are lacking.

Nystatin

Nystatin is a polyene antibiotic that disorganizes the membrane of fungi, occupying ergosterol-binding sites and altering membrane permeability, so that intracellular ions leak from the cell. The drug is effective against *Candida, Malassezia, Cryptococcus*, and some dermatophytes. Several *Candida* species other than *C. albicans* are resistant. Nystatin is fungicidal at concentrations about 4 times MIC. *Prototheca* are reported to be susceptible. Nystatin is used clinically as a topical, broad-spectrum antifungal drug although the azole compound clotrimazole has a broader spectrum and is more active. In the treatment of bovine yeast mastitis, the recommended dose is 300,000 units/quarter on 3 occasions as a single daily dose; the drug can be diluted in saline to 5,000 units/ml and 50 ml administered. However, in one study, about one-fifth of yeasts isolated from bovine mastitis were resistant. Nystatin has been used in dogs to treat *Malassezia* infections of the outer ear and in horses to treat *Candida* metritis.

Azole Antibiotics: Clotrimazole, Enilconazole, Itraconazole, Ketoconazole, and Miconazole

Clotrimazole is an azole with chemical structure and mechanism of action described under the systemic azoles. It is inhibitory *in vitro* to a wide range of filamentous fungi, including *Aspergillus* spp. and dermatophytes, yeasts such

as *Candida*, and dimorphic fungi. Concentrations above 10 μg/ml are fungicidal. At present, few naturally occurring strains of fungi are resistant.

Clotrimazole is a broad-spectrum antifungal agent reserved for topical administration. Local application in mycotic keratitis in horses is well tolerated; the 1% solution is used for *Aspergillus* infections of the cornea. In dogs with nasal aspergillosis, administration of 100 ml of a 1% clotrimazole solution over 1 hour under general anesthesia is effective Pomrantz et al., 2010). In one study, a single topical application by either surgically implanted catheters or catheters placed non-surgically in the nose resulted in cure in 65% of dogs; a second treatment increased cure rate to 87% (Mathews et al., 1998). Prolonged recovery in a dog after barbiturate anesthesia and intranasal treatment with clotrimazole for nasal aspergillosis was attributed to hepatic microsomal enzyme induction by clotrimazole (Caulkett et al., 1997).

Clotrimazole is used in humans to treat *Candida* vaginitis. In the local treatment of mycotic endometritis in cows or horses, infusions of 400–600 mg clotrimazole every other day for 12 days has been recommended, using sufficient volume of saline diluent to gently fill the uterus. It may be the drug of choice for yeast mastitis in cows. Intramammary administration of 100–200 mg/quarter/day of 1% solution or cream, on 1–4 occasions as a single daily dose, has given good clinical results in the treatment of mycotic mastitis in cows.

Miconazole has similar activity to clotrimazole and has also proven useful for topical treatment of dermatophyte, candidal, *Aspergillus* spp., and *Malassezia* infections. It is commonly used topically for the treatment of keratomycosis in horses. Miconazole combined with chlorhexidine was more effective as a shampoo than selenium sulfide for treatment of seborrheic dermatitis in dogs caused by *M. pachydermatis* (Bond et al., 1995).

Enilconazole has become the treatment of choice for nasal aspergillosis in dogs. Administered via intranasal infusion, the drug is effective following both surgical removal of necrotic and foreign material, and on its own. In one study, rhinoscopic debridement followed by intranasal infusion of 1% or 2% enilconazole was successful in 24 of 26 treated dogs (Zonderland et al., 2002). In cases in which the microorganism is so invasive that complete surgical debridement is difficult, topical enilconazole combined with oral itraconazole has been effective (Claeys et al., 2006). Itraconazole should be an effective alternative to clotrimazole and enilconazole for local treatment of nasal aspergillosis. Topical enilconazole has also been used successfully for the treatment of dermatophytosis in small animals. Local infusion of enilconazole has been used successfully in the treatment of guttural pouch mycosis and fungal rhinitis in a small number of horses. Enilconazole has been used successfully in environmental decontamination of poultry houses to prevent aspergillosis.

Ketoconazole is also available for topical antifungal therapy, though it is less active *in vitro* than clotrimazole, itraconazole, or miconazole. Like other topical azoles, it is used in the treatment of *Malassezia pachydermatis* ear and skin infections.

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Section III

Special Considerations

21

Prophylactic Use of Antimicrobial Agents, and Antimicrobial Chemotherapy for the Neutropenic Patient

Steeve Giguère, Anthony C.G. Abrams-Ogg, and Stephen A. Kruth

Infectious diseases of bacterial etiology occur because the host has been exposed to a sufficient number of organisms that have the capability of causing disease (e.g., salmonellosis), or because there has been an assault on the host's specific and non-specific defense mechanisms (e.g., traumatic injury, surgical procedure, dramatic change in environment, or neutropenia). These assaults on physical barriers or defense mechanisms may render the host susceptible to infection from its normal flora or from other organisms with which it might come in contact. It is not uncommon for a clinician, recognizing the assault on the host's defenses, to initiate antimicrobial chemotherapy in an effort to ward off the pending infection or to assist the host in combating the infection until its natural defenses have recovered. When such therapy is initiated in an animal that is about to undergo a surgical procedure or has experienced a traumatic injury and the clinician wants to protect against infection, such therapy is referred to as prophylaxis. When an antimicrobial agent is administered to a herd or flock of animals that are at risk of a disease outbreak due to transport, crowding, or some other exposure to infectious agents, the therapy is referred to as metaphylaxis. When therapy is initiated in a neutropenic animal, with or without an ongoing infection, the use of antimicrobial agents may be considerably different from that in animals with intact defense mechanisms. This chapter discusses the prophylactic use of antimicrobial agents in a herd situation, prior to a surgical procedure, and in neutropenic animals.

Prophylactic or Metaphylactic Use of Antibiotics in Livestock

Steeve Giguère

The prophylactic or metaphylactic use of antimicrobial agents has had a tremendous impact on the prevention and control of infectious diseases in veterinary medicine particularly in farm animals. However, it has not been without its drawbacks, the most obvious being the risk of selection for resistant organisms. To minimize the risk of selecting for resistant organisms there are a few guidelines that should be followed when using antimicrobial agents prophylactically. These include:

1. Knowledge of the pathogen(s) putting the patient at risk.
2. Knowledge of the antimicrobial agents to which the suspected pathogen(s) are susceptible.
3. Initiation of therapy before the onset of infection to ensure there are adequate drug concentrations at the site of concern before the bacterial pathogen reaches sufficient concentration to cause disease. For herds or flocks, this should be at the time of exposure or at

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the first signs of a disease outbreak before it has fully manifested itself.
4. The duration of prophylaxis should be as short as possible, consistent with efficacy, and should be used only where its efficacy is clearly established.
5. The dosage must be the same as that used therapeutically.

Antimicrobial agents are often administered prophylactically when young animals (pigs, calves) are moved from breeding to growing areas, because disturbances in microbial flora and physiology and the sudden exposure to pathogens can spark outbreaks of infectious disease. Because of the disadvantages, the use of antimicrobial drugs for such purposes should be replaced, wherever possible, by adequate preventive husbandry practices. Addressing the immune status of the animals, the stress to which the animals are exposed and the pathogen load in the animal's environment may all contribute to decreasing the incidence of infection. For example, Berge et al. (2005) investigated the influence of prophylactic antibiotics on health and performance in 120 preweaned dairy calves. The most important factor associated with morbidity and mortality was inadequate transfer of passive immunity through colostrum. In-feed antibiotics delayed the onset of morbidity, decreased overall morbidity, and increased weight gain. However, rearing the calves that did not receive adequate transfer of passive immunity was more difficult and labor intensive than raising calves with adequate immunoglobulin concentrations, despite the use of prophylactic antibiotics. Many antimicrobial agents used as growth promoters also have an impact on infectious disease prevention. The use of antimicrobial agents as growth promoters and their effects on disease prophylaxis is discussed in chapter 24.

Metaphylaxis is employed extensively in veterinary medicine where herd health is at risk. Examples of metaphylaxis include preemptive medication in a dairy herd in the form of dry-cow therapy (chapter 30). Such drug use is based on knowledge that disease is present in the population and will continue to affect susceptible individuals. Preemptive medication of the herd or individual reduces shedding of pathogens. The concept of herd medication is to treat the whole group at risk rather than individuals. Typical examples are (1) giving drugs at prophylactic concentrations to prevent swine dysentery (chapter 33); (2) using "blitz" therapy with intramammary penicillin G to eradicate *Streptococcus agalactiae* infection from a cow herd; (3) ensuring specified disease-free pigs by the medicated early weaning system; and (4) mass medication on arrival at the feedlot to decrease the incidence of bovine respiratory disease (chapter 29).

Administration of parenteral products to calves that are at high risk for bovine respiratory disease (metaphylaxis) has consistently been found to reduce morbidity whereas the benefits of oral medications are less certain with some trials showing a negative effect of oral antimicrobial agents (Taylor et al., 2010). A meta-analysis of 107 field trials in cattle indicated that mass medication with oxytetracycline or tilmicosin on arrival at the feedlot consistently reduced morbidity but effects on mortality and performance were inconsistent (Van Donkersgoed, 1992). Since then, many antimicrobial agents have been approved for the control of bovine respiratory disease in cattle at risk of developing respiratory disease. These agents include ceftiofur crystalline free acid, enrofloxacin, florfenicol, gamithromycin, tildipirosin, tilmicosin, and tulathromycin.

In one study, medication with tulathromycin was more effective in preventing natural outbreaks of bovine respiratory disease than tilmicosin (Godinho et al., 2005). The relative efficacy of many of the available products has not been clearly established and may vary from farm to farm. However, the selection of an antimicrobial agent for prophylaxis or metaphylaxis depends not only on efficacy, but also on overall cost/benefit analysis. For example, one study comparing the prophylactic efficacy of tilmicosin and oxytetracycline found that there was a net economic advantage of using oxytetracycline because of lower cost even though tilmicosin was significantly more effective in preventing undifferentiated fever (Schunich et al., 2002).

The use of prophylactic antibiotics in veterinary medicine has also been shown to have adverse affects on some animals. For example, the routine use of neomycin intrauterine infusions to prevent post-parturition metritis in cows has been shown to have an adverse affect on subsequent fertility and concurrent intrauterine infusions of gentamicin in inseminated mares adversely affects their ability to conceive. Tetracyclines administered via drinking water to feedlot calves have been associated with increased mortality (Martin et al., 1982). Examples of well-established prophylactic or metaphylactic use of antimicrobial drugs are shown in Table 21.1.

Table 21.1. Selected examples of antimicrobial prophylaxis or metaphylaxis in large animals.

Species	Disease/Purpose	Drugs	Duration	Comment
Cattle	Pneumonia of feedlot cattle	CCFA, enrofloxacin, florfenicol, gamithromycin, tilmicosin, tildipirosin, tulathromycin,	Single dose	Treat upon arrival at feedlot
	Dry-cow therapy	Many	Single dose	Intramammary infusion
	Leptospirosis	Oxytetracycline, tilmicosin	Single dose	Eradicates urinary shedding
Swine	Swine erysipelas	Penicillin, long acting	Single dose	Treat pigs at risk
	Atrophic rhinitis in pigs	Oxytetracycline	First weeks of life	
	Swine dysentery	Tiamulin, valnemulin, lincomycin	Varies with drug	
	Proliferative enteropathy	Tylosin, lincomycin, tiamulin, valnemulin	Varies with drug	
	Clostridial enteritis	Salinomycin[a]	Prolonged	Administered in feed
Horse	Strangles	Penicillin	Depends on duration of exposure	Treat horses at risk before development of lymphadenopathy

[a]No longer approved for use in the United States as of January 2006.
CCFA = ceftiofur crystalline free acid.

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Antimicrobial Prophylaxis for Surgery

Steeve Giguère

The implementation of prophylactic antimicrobial use to reduce the incidence of surgical site infection was a major milestone in the field of surgery. However, antimicrobial use does not replace aseptic techniques and adherence to proper surgical principles such as minimizing trauma and hemorrhage, using adequate instrumentation, careful choice of suture material and implants, debriding devitalized tissues, and minimizing dead space. Although the benefit of proper prophylactic antimicrobial use prior to surgery is indisputable, unrestricted prophylactic use of antimicrobial agents may result in an increase risk of superinfection, development of resistant microorganisms, increased cost of hospitalization, and increased incidence of adverse effects for the host. Therefore, strict adherence to simple principles must be followed for optimal perioperative antimicrobial use.

The principles upon which drugs are used prophylactically to prevent surgical infections in animals are for the most part based on studies in human medicine because of the paucity of randomized veterinary trials. The selection and duration of antimicrobial prophylaxis should have the smallest impact possible on the normal bacterial flora of the patient and the microbiologic ecology of the hospital. This section summarizes the current state of knowledge on prophylactic use of antimicrobial agents for the prevention of surgical site infections as it relates to veterinary species.

Risk Factors for the Development of Surgical Site Infections

All surgical wounds are contaminated at some point. Fortunately, infection at the site of surgery is the exception rather than the rule. Incisional site infections

usually develop within 30 days of the procedure or within 1 year if an implant was left in place. The development of infection results from interactions between the nature and extent of microbial contamination, the virulence of microorganisms, the integrity of host innate and adaptive defense mechanisms, and factors that relate to the surgery itself.

A few studies have attempted to identify risk factors that influence infection rate in veterinary medicine. Epidemiologic evaluation of post-operative infections in 239 dogs and cats showed that intact males and animals with concurrent endocrinopathy are at higher risk of development of post-operative wound infection (Nicholson et al., 2002). Total surgery time and total anesthesia time are also well established risk factors in dogs in cats (Brown et al., 1997; Beal et al., 2000; Nicholson et al., 2002). One epidemiologic study of 1255 dogs and cats found that the risk of infection for animals undergoing a 90-minute procedure is twice as high as that of animals undergoing a 60-minute procedure, and the risk doubles for each additional hour of surgery (Brown et al., 1997). Similarly, equine orthopedic surgeries longer than 90 minutes are 3.6 times more likely to develop a surgical site infection than shorter procedures (MacDonald et al., 1994). Complications at the site of ventral celiotomy in horses are significantly associated with duration of surgery (Wilson et al., 1995; Freeman et al., 1012). In addition, the use of staples for skin closure was significantly associated with incisional complications after exploratory celiotomy in horses (Torfs et al., 2010). Preparation of the surgical site is also important. For example, surgical sites clipped before anesthetic induction in dogs and cats are 3 times more likely to become infected than sites clipped after induction (Brown et al., 1997).

Additional risk factors for surgical site infections recognized in humans include, among others, advancing age, obesity, corticosteroid therapy, chronic inflammation, the use of electrocautery, the use of braided/multifilament suture material, and severe concurrent illnesses. Some of these risk factors may also be valid in veterinary medicine. For example, the incidence of incisional complications for horses undergoing emergency surgery for acute abdominal disease (39%) is significantly higher that that of horses undergoing elective abdominal surgeries (7%; Wilson et al., 1995).

Patient Selection

Recommendations for antimicrobial prophylaxis for surgery in veterinary medicine are based on the extent of operative contamination as predicted by the National Research Council wound classification system (Table 21.2). This classification, developed in people, may not be totally accurate in veterinary surgery and its accuracy may vary according to the type of procedure. For example, in equine abdominal surgery, performing an enterotomy or intestinal resection does not influence the incidence of surgical site infection (Kobluk et al., 1989; Phillips and Walmsley, 1993). In contrast, there is a strong association between wound classification and the risk of surgical site infection for equine orthopedic procedures, where a clean-contaminated procedure is approximately 24 times more likely to develop a post-operative infection than a clean procedure (MacDonald et al., 1994).

Antimicrobial drugs are highly effective and necessary in preventing certain post-operative infections and should be used in surgical procedures where infection rates associated with a particular procedure exceed 5%. These typically include patients undergoing clean-contaminated or contaminated procedures. Prophylactic antimicrobials are not warranted for most clean surgical procedures because the risk of contamination is low. However, the use of prophylactic antimicrobials in clean procedures is recommended for procedures in which an implant is placed, or when an infection would be catastrophic to the outcome (e.g., total hip replacement; Dunning, 2003). Prophylactic antimicrobials may also be indicated for clean surgical procedures in patients with concurrent debilitating diseases and in animals receiving immunosuppressive doses of corticosteroids.

Although these principles were originally borrowed from studies in people, there are now several studies in dogs, cats, horses, and cattle indicating that prophylactic antimicrobials provide no benefit for clean surgical procedures (Holmberg, 1985; Vasseur et al., 1985; Klein and Firth, 1988a; MacDonald et al., 1994; Brown et al., 1997). On the other hand, studies in animals have demonstrated the benefit of prophylactic antimicrobials in clean-contaminated or contaminated procedures (Haven et al., 1992; Brown et al., 1997). By definition, dirty surgical procedures require therapeutic rather than prophylactic administration of antimicrobial

Table 21.2. Classification of operative wounds based on the likeliness of bacterial contamination and associated risk of surgical site infection.

Classification	Criteria	Approximate Risk (%)
Clean	Elective Non-traumatic Primarily closed No inflammation encountered No break in aseptic technique Respiratory, alimentary, biliary, and genitourinary tracts not entered	< 5
Clean-contaminated	Urgent or emergency case that is otherwise clean Elective opening of respiratory, gastrointestinal, biliary, or genitourinary tract with minimal contamination and no encounter with infected urine or bile Minor break in technique	5–10
Contaminated	Non-purulent inflammation Gross spillage from gastrointestinal tract Entry into biliary or genitourinary tract in the presence of infected bile or urine Major break in technique Penetrating trauma < 4 hours old Chronic open wounds to be grafted or covered	10–20
Dirty	Purulent inflammation encountered during the procedure (e.g., abscess) Preoperative perforation of respiratory, gastrointestinal, biliary, or genitourinary tract Penetrating trauma > 4 hours old	> 20

Adapted from Cruise and Ford, 1980.

agents and the guidelines of antimicrobial prophylaxis for surgery do not apply. Unfortunately, there are often considerable discrepancies between antimicrobial use practices in small animals and horses and actual perioperative antimicrobial use guidelines (Weese et al., 2009, Knights et al., 2012).

Antimicrobial Drug Choice

The selection of a prophylactic antibacterial drug must be based on the microorganisms most likely to contaminate the surgical site, known activity of the drug against those microorganisms, low incidence of adverse effects, cost, pharmacokinetics of the drug in the species of interest, and pharmacodynamic indices associated with a favorable clinical and/or microbiological outcome. The use of newer broad-spectrum drugs should be avoided in surgical prophylaxis to decrease emergence of bacterial isolates that are resistant to these frontline therapeutic agents (Bratzler et al., 2005).

In dogs and cats, as in people, cefazolin is the prophylactic antimicrobial of choice for most procedures owing to its activity against most surgical wound pathogens, affordable cost, and minimal adverse effects (Dunning, 2003; Nichols et al., 2005). The microorganisms most commonly associated with orthopedic and abdominal surgical site infections in horses are Enterobacteriaceae (Moore et al., 1992). Therefore, it is common practice to administer gentamicin, in addition to either penicillin or cefazolin, to broaden the Gram-negative spectrum when antimicrobial prophylaxis is indicated in equine patients. In ruminants, penicillin or ceftiofur have been used historically for perioperative antimicrobial prophylaxis. Both drugs have distinct advantages and disadvantages. Penicillin offers the advantage of being more active than ceftiofur against *Arcanobacterium pyogenes* and many anaerobic pathogens commonly isolated from ruminants. Unfortunately, the duration of withdrawal time for milk and meat is a major disadvantage and penicillin is not active against most Gram-negative bacterial isolates, e.g., Enterobacteriaceae. Conversely, ceftiofur has good activity against most Gram-negative pathogens isolates from ruminants. When used as labeled, ceftiofur sodium has no withdrawal time and ceftiofur hydrochloride has only a 2-day withdrawal time for meat and no withdrawal time for milk. As of 2012, the use of perioperative ceftiofur or any other cephalosporin in major

food-producing species (cattle, cattle, swine, chickens, and turkeys) would be illegal in the United States.

Although the antimicrobial agents mentioned above often represent the default choice for prophylaxis in each species, clinicians must continue to evaluate current literature and carefully examine *in vitro* susceptibility patterns of bacterial isolates within their own institution or animal population. Emergence of resistance in bacterial pathogens associated with nosocomial surgical site infections have been reported in both large and small animal veterinary hospitals.

Timing and Duration of Antimicrobial Prophylaxis

The goal of antimicrobial prophylaxis is to achieve serum and tissue drug concentrations > MIC for microorganisms likely to be encountered for the entire duration of the surgery. Prophylactic antimicrobials should be administered at least 30 minutes but no greater than 60 minutes before a surgical incision so that they are in adequate concentrations in tissues at the time of potential contamination. As early as 1961, it was demonstrated that incisions contaminated with *Staphylococcus aureus* could not be distinguished from uncontaminated controls when antimicrobial agents were administered before the incision (Burke, 1961). In the same study, antimicrobial agents were effective in minimizing severity of infection when administered no later than 3 hours after bacterial contamination. Since then, multiple studies in human medicine have shown that administration of the first antimicrobial dose after surgery results in surgical site infection rates almost identical to those of patients who did not receive prophylactic antimicrobials (Stone et al., 1976; McDonald et al., 1998). Administration of antimicrobial agents should be repeated intraoperatively if the surgical procedure is continuing for a time equivalent to two half-lives after the first dose, to ensure adequate drug concentrations until wound closure (Bratzler et al., 2005). The half-life of cefazolin is slightly less than 1 hour in dogs and horses. The half-life of IV potassium or sodium penicillin and gentamicin in horses is approximately 3 hours, whereas procaine penicillin administered IM has a half-life of approximately 12 hours.

The optimal duration of antimicrobial prophylaxis in veterinary medicine is unknown. The vast majority of published evidence in human medicine demonstrates that antimicrobial prophylaxis after wound closure is unnecessary (Aber and Thore, 1991; Meijer et al., 1990). Prolonged use of prophylactic antimicrobial agents is associated with emergence of resistant bacteria and is more likely to result in adverse effects (Harbarth et al., 2000). Based on published data, current recommendation from the National Surgical Infection Prevention Project is that prophylactic antimicrobial agents should be discontinued within 24 hours of the end of surgery (Bratzler et al., 2005). These guidelines should be followed in veterinary medicine as well. Consistent with findings in people, a single preoperative dose of penicillin prior to rumenotomy in cattle is as effective in preventing post-surgical complications as a 7-day course of the same antibiotic (Haven et al., 1992). It must be emphasized, however, that principles of perioperative surgical prophylaxis do not apply to dirty surgical procedures. Antimicrobial administration in these procedures is therapeutic rather than prophylactic and a longer course of therapy may be indicated. For example, the surgical infection rate in calves with complicated umbilical hernia is significantly lower after a 4-day course of antimicrobials compared to calves treated for only 1 day (Klein and Firth 1988b).

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Management of Infections Associated with Neutropenia in the Dog and Cat

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Neutropenic animals are at increased risk of developing bacterial and fungal infections, and established infections in neutropenic patients are more difficult to eradicate with appropriate antimicrobial therapy. Such infections may be due either to organisms that are normally considered to be pathogenic or to opportunistic pathogens, caused by organisms that rarely cause disease in animals with normal defense mechanisms. This section on the management of infection in the neutropenic dog and cat focuses on neutropenia resulting principally from impaired granulopoiesis and the attendant risk of opportunistic bacterial and fungal infection. Considerable attention has been given to the use of granulocyte and granulocyte-macrophage colony-stimulating factors to increase neutrophil production, but antimicrobial therapy remains the cornerstone of managing neutropenia, especially in dogs and cats. A number of factors influence the risk and outcome of infection during neutropenia, but in most cases prompt therapy with appropriate antimicrobial agents will result in successful patient outcome (Vail, 2009). In cases of prolonged, severe neutropenia, patient management strategies must be extrapolated from the therapy of human neutropenic patients.

Causes of Neutropenia

Neutropenia may occur as a primary or secondary disorder, and as an isolated hematologic abnormality or as a feature of pancytopenia (Brown and Rogers, 2001; Schnelle and Barger, 2012). Inherited disorders with clinically relevant neutropenia include cyclic haematopoiesis of Grey Collies, trapped neutrophil syndrome in Border Collies, and cobalamin deficiency (reported in a Border Collie and in a family of Giant Schnauzers). Some Belgian Tervuren and Greyhounds may have a physiologic neutropenia, where neutrophil counts are lower than the normal canine reference interval, but there is no associated illness. Idiopathic neutropenia is occasionally seen in both dogs and cats. In some cases this is a result of immune-mediated mechanisms; granulocyte colony-stimulating factor deficiency has also been reported (Lanevschi et al., 1999).

Neutropenia may also occur secondary to infectious diseases. Canine parvovirus-2 (CPV-2) and *Ehrlichia canis* (and potentially other members of Anaplasmataceae) are the principal infectious causes of neutropenia in the dog. Neutropenia may also be seen with *Babesia* spp. and *Leishmania chagasi* infections. Feline parvovirus (FPV), feline leukemia virus and feline immunodeficiency virus are the principal infectious causes of neutropenia in the cat. *Histoplasma capsulatum* may cause neutropenia in both dogs and cats secondary to bone marrow invasion. Neutropenia is also occasionally seen in association with other systemic mycoses and protozoal infections. Overwhelming bacterial infection may cause neutropenia in animals with normal granulopoiesis, by exhausting marrow granulocyte reserve. Neutrophil consumption exacerbates neutropenia in animals with impaired granulopoiesis.

Neutropenia may result from primary bone marrow neoplasia or from bone marrow involvement in metastatic disease. In either case there is likely to be concurrent anemia and thrombocytopenia. Sertoli cell tumours in dogs may cause pancytopenia due to paraneoplastic estrogen toxicosis.

Cytotoxic chemotherapeutic agents, radiation therapy, and tyrosine kinase inhibitors used for neoplastic and immune-mediated diseases predictably cause myelosuppression. The degree of resulting neutropenia may vary with the agent, the dose administered, the species, and the breed (Collies and other breeds with MDR1 mutations are at increased risk for some drug-induced neutropenias). Other drugs with a known but unpredictable risk for causing neutropenia include estrogen and phenylbutazone in dogs, and chloramphenicol, griseofulvin, propylthiouracil, methimazole, carbimazole and lithium in cats. Theoretically, any drug may be associated with an idiosyncratic reaction resulting in neutropenia. Such reactions have been reported with cephalosporins in dogs and cats, and with sulfonamides, captopril, quinidine, phenobarbital, primidone, trimeprazine, fenbendazole, and albendazole in dogs.

Other causes of neutropenia include Autumn crocus poisoning (the toxic principle is colchicine), myelofibrosis (concurrent anemia is common), bone marrow necrosis secondary to a variety of causes, and disseminated intravascular coagulation in dogs. Mild asymptomatic neutropenia may occur with hypoadrenocorticism in dogs.

Infectious Complications of Neutropenia

Risk Factors

Factors important in determining the probability of acquiring, and the severity and outcome of an established infection during neutropenia include the severity and duration of neutropenia, disruption of natural barriers, defects in specific defenses, organisms involved, site of infection, type of tumor and its biological stage, and age, performance status, and species of the host (Crawford et al., 2004; Freifeld et al., 2011; Sipsas et al., 2005; Van der Meer and Kullberg, 2002).

The risk of infection is related to the degree of neutropenia, and neutropenia is graded to assist in predicting such risk (Veterinary Co-operative Oncology Group, 2011). The risk of opportunistic infection occurs when the neutrophil count falls below 2.0×10^9/L. From 1.5×10^9/L to $< 2.0 \times 10^9$/L (grade 1 neutropenia), there is a marginal risk of infection. From 1.0×10^9/L to $< 1.5 \times 10^9$/L (grade 2 neutropenia), the risk is mild; and from 0.5×10^9/L to $< 1.0 \times 10^9$/L (grade 3 neutropenia), the risk is moderate. Animals with neutrophil counts $< 0.5 \times 10^9$/L (grade 4 neutropenia) have a high risk of infection; below 0.2×10^9/L the risk of infection is very high. Below 0.2×10^9/L there is still a relationship between worsening myelosuppression and adverse clinical consequences, but this is not reflected in the peripheral blood since any neutrophils released from the bone marrow immediately migrate into tissues. For a given degree of neutropenia, a higher risk of infection is associated with a falling, rather than a stable, neutrophil count. These figures are based upon a classic study of humans with leukemia (Bodey et al., 1966). No such studies have been conducted with dogs or cats, but, based upon experimental studies with total body irradiation and clinical experience with veterinary cancer patients, these figures appear to be applicable to the dog and cat (Couto, 1990; Abrams-Ogg et al., 1993; Veterinary Co-operative Oncology Group, 2011).

The outcome of infection is related to the duration of neutropenia. Humans with neutropenia of short duration (< 7 days) are unlikely to have severe infections that cannot be controlled with appropriate antimicrobial therapy. Infections accompanying neutropenia of moderate duration (7–14 days) are more difficult to manage. Infections in patients with prolonged neutropenia

(> 14 days) are even more difficult to manage, especially if the neutrophil count is < 0.2×10^9/L (Feld, 1989). This difficulty is because antimicrobial agents act in concert with host defenses in eradicating infections.

The risk of infection during neutropenia is increased by disruption of natural physical barriers, and suppression of humoral and cell-mediated immunity. Natural barriers are disrupted, for example, with gastrointestinal damage during parvoviral infections and with anticancer chemotherapy, facilitating translocation of enteric bacteria. Intravenous catheterization and percutaneous biopsy procedures increase the risk of infection with skin organisms. Immunosuppression may accompany myelosuppression, because of the primary disease, anticancer therapy, and malnutrition. The risk of infection in neutropenic humans is greater if there is concurrent lymphopenia and monocytopenia. The effects of chemotherapy on immune responses in dogs with cancer have not been studied extensively. In one study evaluating immune function in dogs with lymphoma and osteosarcoma, doxorubicin treatment did not cause a significant decrease in T- or B-cell numbers, whereas treatment with combination chemotherapy caused a significant and persistent decrease in B-cell numbers (Walter et al., 2006). However, antibody titers after vaccination were not significantly different between control and chemotherapy-treated dogs (Walter et al., 2006).

The severity of infection is affected by the type of organism. Infections with Gram-positive organisms tend to be more easily managed than infections with Gram-negative organisms. The site of infection is also important in determining outcome. Bacteremia and pneumonia are more difficult to treat than soft tissue, gastrointestinal or urinary tract infections. The type of tumor and its stage are important factors in humans. Infections are more likely to be severe in patients with acute compared to chronic hematologic malignancies, hematologic malignancies in relapse compared to those in remission, and hematologic malignancies compared to solid tumors. In a case-control study to evaluate risk factors for the development of neutropenia (< 2.5×10^9/L) and fever (> 39.2°C or 102.5°F) in dogs receiving chemotherapy, dogs with lymphoma were at greater risk compared to dogs with solid tumors, although stage of the disease, and remission versus relapse, did not affect risk (Sorenmo et al., 2010). In this study, patient age did not affect risk (Sorenmo et al., 2010).

Microbiology

Infections in neutropenic animals may occur with exogenous or endogenous organisms. Exogenous organisms are acquired from the environment. Nosocomial organisms are an important source of exogenous infections in neutropenic patients in human hospitals (Wade, 1994; Ellis, 2004), and probably represent a risk to neutropenic animals in veterinary hospitals (Warren et al., 2001). Endogenous infections occur with organisms from the host's own flora. The most important source is the intestinal tract. Other sources of endogenous infections include the oral cavity, skin, upper respiratory tract and lower urogenital tract. Exogenous and endogenous pathogens do not represent two entirely distinct groups of organisms, and the same organism may act as both an endogenous and exogenous pathogen for different individual animals.

Organisms causing infections in humans with neutropenia due to cytotoxic therapy have been extensively characterized (Sipsas et al., 2005). Gram-negative organisms, especially *E. coli*, *Klebsiella* spp. and *Pseudomonas aeruginosa* were initially the most common causes of infections. Gram-positive organisms, especially *Staphylococcus* spp., now account for up to 69% of infections. This change reflects the use of fluoroquinolones for antimicrobial prophylaxis treatment and the increasing use of long-term central venous lines (Picazo, 2004).

Infections in dogs and cats with neutropenia secondary to cytotoxic therapy have not been as well characterized. The majority of data have been anecdotally reported for myelosuppression in the dog. Similar to humans, the most frequent sites of infection appear to be the bloodstream (bacteremia) and the lung. Local cellulitis may occur, manifested as edema of one or more limbs. Other possible sites of infection include the oral cavity, gastrointestinal tract, genitourinary tract, heart, and central nervous system.

Similar to the initial pattern of infection seen in humans, bacteremia is probably most often of intestinal origin and corresponds to the pattern of bacterial translocation seen in healthy dogs (Dahlinger et al., 1997). Members of the Enterobacteriaceae, especially *E. coli* and *Klebsiella* spp., are most commonly isolated (Couto, 1990). *Pseudomonas* spp. are less frequently isolated, but have historically been associated with the most severe infections, because antibiotics effective against this organism were not initially available. Although the

majority of bacteria in the intestinal tract are obligate anaerobes, they are not commonly the first invaders in opportunistic infection during neutropenia. *Clostridium difficile*–associated diarrhea may occur in neutropenic humans and dogs (Gorschlüter et al., 2001; Weese and Armstrong, 2003). It is not known if neutropenia is a risk factor in addition to hospitalization, cytotoxic therapy and antimicrobial therapy; bacteremia is rare. Gram-positive bacteremia, usually with *Staphylococcus* spp. and *Streptococcus* spp., is less common than Gram-negative bacteremia, but more common than anaerobic bacteremia. Gram-positive bacteremia can arise from the skin, the intestinal tract or the oral cavity. Urinary tract infections are a possible source of bacteremia.

Pneumonia may occur as an opportunistic infection with upper respiratory flora or from translocation of intestinal bacteria. The same organisms are implicated as in bacteremia. Neutropenic dogs should probably also be considered at risk for *Bordetella bronchiseptica* pneumonia. Cats are likely at risk for pneumonia with *B. bronchiseptica* and *Pasteurella multocida*.

There is better documentation of bacterial infections secondary to parvoviral infections. Gram-negative organisms are believed to be the principal cause of sepsis; bacteremia and pneumonia may occur. *E. coli* was isolated from post-mortem tissues of 88 of 98 dogs with CPV-2 infection (Turk et al., 1990). *E. coli* is also the most common isolate from post-mortem tissues of cats dying from FPV (Scott, 1987). In a report of bacterial colonization of IV catheters in 100 dogs with CPV-2 infection, 22 catheters became colonized with one or more organisms (Lobetti et al., 2002). *E. coli* and other enteric organisms were isolated from 13 catheters, there was one isolate each of *Staphylococcus* spp. and *Streptococcus* spp., and 18 isolates were of environmental origin. In another study of 43 dogs with CPV-2 infection, 11 dogs had asymptomatic bacteruria, 10 of which had infections with *E. coli* and 2 with *Staphylococcus* spp. (Koutinas et al., 1998). In one study of experimental FPV infection, 10 of 30 blood cultures were positive (Hammon and Enders, 1939). Isolates included *Pasteurella* spp., Gram-negative bacilli, *Streptococcus* spp. and *Staphylococcus* spp. A *Bacillus* species was isolated in one culture along with a *Staphylococcus* spp. It is widely assumed, but not proven, that anaerobic bacteria contribute to bacteremia during parvoviral infections. It has been documented that *C. perfringens* proliferates in the intestinal tract of dogs with CPV-2 infection (Turk et al., 1992), but the role of the organism in sepsis is not known.

Local and systemic infections with *Aspergillus* spp., *Candida* spp., and less frequently organisms of the order *Mucorales* (zygomycosis), are an important cause of disease in neutropenic humans (Brown, 2005; Freifeld et al., 2011; Sipsas et al., 2005; Van der Meer and Kullberg, 2002). Risk factors for fungal infections are the same as those for bacterial infections. In addition, the risk of fungal infection increases with the duration of antibacterial therapy and concurrent immunosuppressive therapy (e.g., with cyclosporine). Invasive fungal infections are not as common in neutropenic dogs and cats. This may be due in part to the use of less aggressive cytotoxic therapy for cancer. However, the risk for fungal infection is comparatively low even in experimental dogs with prolonged, severe neutropenia (Ehrensaft et al., 1979). Systemic candidiasis has been reported in a pup with CPV-2 infection (Rodriguez et al., 1998). Pneumonia due to *Aspergillus* spp. has been reported in a dog following autologous bone marrow transplantation for treatment of lymphoma (Rosenthal, 1988) and in cats with FPV infection (Fox et al., 1978; Holzworth, 1987). Intestinal candidiasis associated with intensive antibiotic therapy occurred in three of six dogs with severe neutropenia induced by cytotoxic therapy (Abrams-Ogg et al., 1993). Intestinal candidiasis has also been reported as a complication of CPV-2 infection (Ochiai et al., 2000), and intestinal candidiasis, aspergillosis and zygomycosis have been reported as complications of FPV infection (Fox et al., 1978; Holzworth, 1987).

Patient Management

The majority of neutropenia that is managed in small animal medicine is of short duration (< 7 days) and/or of mild to moderate severity. Animals with prolonged neutropenia usually have only mildly depressed counts. This reflects a tendency on the part of veterinarians to reduce or discontinue cytotoxic therapy when neutropenia develops, and to euthanize animals with severe pancytopenia that have a poor prognosis for prompt recovery. As veterinarians continue to employ more aggressive cytotoxic protocols and manage dogs and cats with complex hematologic problems, the management of severe and prolonged neutropenia may be more frequently required.

The risk of acquiring an exogenous infection is reduced by isolation. Neutropenic animals that do not require critical supportive care should be maintained at home. Cats should be kept indoors and dogs confined to the house and yard. In the hospital, contact with the general hospital population should be avoided. Hands should be thoroughly washed and laboratory coats changed before handling a neutropenic animal, and barrier nursing procedures, such as wearing gloves, gowns and isolation boots, should be considered for severe cases. The thermometer used for the neutropenic animal should not be used for other patients. A "low microbial diet" may be recommended for human patients with severe neutropenia, although the benefits are not clear (van Dalen et al., 2012). The role of dietary pathogens has not been evaluated in neutropenic pet animals, but it is reasonable to recommend that only canned and well-cooked foods be offered to dogs and cats with neutropenia.

Antimicrobial therapy for neutropenic animals may be divided into three categories: (1) prophylactic therapy; (2) empirical treatment during febrile episodes; and (3) treatment of documented infection. The Infectious Disease Society of America has recently updated its clinical practice guidelines for the use of antimicrobial agents in neutropenic human patient with cancer (Freifeld et al., 2011). Optimal protocols have not been completely defined for the management of infections in people with neutropenia from other causes. The protocols recommended for dogs and cats in this chapter are adapted from recommendations in people, from clinical experience in dogs and cats, and when available, from studies performed in dogs and cats.

Prophylaxis

Prophylactic therapy is directed at the intestinal flora. The principal objective is "selective decontamination of the digestive tract" (Van der Waaij, 1988; Ellis, 2004). This refers to reduction of the aerobic Gram-negative organisms most often responsible for severe infections. The anaerobic population is left relatively undisturbed since it contributes to resistance to fungal overgrowth and colonisation by exogenous organisms. A second objective of prophylactic therapy is to provide sufficient blood and tissue antimicrobial concentrations to contain an incipient bacterial infection.

Choices for prophylactic therapy are presented in Table 21.3. Neomycin and polymyxin B were first used but have been replaced by trimethoprim-sulfonamide combinations and fluoroquinolones in humans and dogs (Klastersky, 1989; Ellis, 2004). Amoxicillin and amoxicillin-clavulanate are not ideal choices because of activity against intestinal anaerobes, but are readily available practical choices for cats, which often do not tolerate other choices and where prolonged use of fluoroquinolones is not recommended because of the risk of retinopathy. Cephalexin has also been used in dogs because of its activity against *E. coli* and *Klebsiella* spp., while causing less disturbance of the anaerobic population than amoxicillin. Amoxicillin and cephalexin also have good activity against susceptible Gram-positive organisms, which may be beneficial if surgical wounds are present.

Prophylactic therapy for human neutropenic patients has been reviewed (Freifeld et al., 2011; van de Wetering et al., 2005). Its use is controversial. The benefits are not clear, both with respect to reducing infection rates and with respect to reducing mortality rates. In general, prophylactic therapy appears to be more beneficial in reducing infection rates in humans with neutropenia of greater severity and duration than in humans with mild to moderate neutropenia (Freifeld et al., 2011). In a study of veterinary cancer patients receiving vincristine-doxorubicin-cyclophosphamide chemothe zrapy, which resulted in neutropenic episodes of short-duration with a median neutrophil count of 0.8×10^9/L, trimethoprim-sulfonamide prophylaxis reduced the number of antibiotic-responsive febrile episodes, presumably of infectious etiology, from 40% to 20% (Couto, 1990). In a recent double-blinded, placebo-controlled study in dogs with lymphoma or osteosarcoma, administration of trimethoprim-sulfonamide (30 mg/kg PO q 12 h) for the first 14 days after the dogs' first doxorubicin chemotherapy resulted in modest but significantly reduced hospitalization rate, non-hematologic toxicity, and gastrointestinal toxicity (Chretin et al., 2007). The potential advantages of prophylactic therapy include a reduction in infection rate, a reduction in the time to onset of infection, and a reduction in the speed in which an incipient infection develops into overwhelming sepsis. These benefits may facilitate home management of neutropenic animals and improve quality of life. Potential disadvantages include shifts in the host's flora, development of resistant organisms, adverse drug reactions, and expense (Williamson et al., 2002; Trepanier,

Table 21.3. Prophylactic oral antimicrobial therapy for the neutropenic dog and cat.

Antimicrobial	Doses	Comment
Diaminopyrimidine sulfonamides		
Trimethoprim-Sulfamethoxazole (dogs)	15 mg/kg (combined dose) q 12 h 30 mg/kg (combined dose) q 12–24 h	Relatively inexpensive No prophylaxis against *Pseudomonas* spp.
Trimethoprim-Sulfadiazine (dogs)		Risk for keratoconjunctivitis sicca, cutaneous, hematologic, and other immune-mediated abnormalities (Trepenier, 2004; Williamson et al., 2002) May retard marrow recovery following severe myelosuppression
Fluoroquinolones		Relatively expensive
Enrofloxacin (dogs)	**5–20 mg/kg q 24h**	Lower dose effective for selective decontamination of the digestive tract > 10 mg/kg needed to achieve tissue levels effective against *Pseudomonas* spp.
Ciprofloxacin (dogs)	**10–30 mg/kg q 24 h**	As for enrofloxacin
Orbifloxacin (dogs)	2.5–7.5 mg/kg q 24h	Relatively expensive Less well evaluated in neutropenia than enrofloxacin or ciprofloxacin
Marbofloxaxin (dogs, cats)	**2.5–5 mg/kg q 24 h**	As for orbifloxacin Retinopathy has not been observed but prolonged therapy with high doses of fluoroquinolones is not recommended in cats
Difloxacin (dogs)	5–10 mg/kg q 24 h	As for orbifloxacin
Beta-lactams		Relatively expensive
Cephalexin (dogs)	**30 mg/kg q 12 h**	No prophylaxis against *Pseudomonas* spp.
Amoxicillin (dogs, cats)	10–20 mg/kg q 12 h	Relatively inexpensive No prophylaxis against *Pseudomonas* spp. Ampicillin causes more intestinal disturbance than amoxicillin
Amoxicillin-Clavulanate (dogs, **cats**)	**12.5–25 mg/kg q 12 h**	As for amoxicillin but more expensive Increased activity against *Staphylococcus* spp., *Klebsiella* spp., *Escherichia coli*, *Bacteroides* spp. compared to amoxicillin
Combinations		
Fluroquinolone + beta-lactam	As above	Reserved for animals with severe prolonged neutropenia

Notes: Doses adapted from Greene and Calpin, 2012; Plumb, 2011. Drugs and dosages presented in boldface text are those most commonly used in the authors' practice.

Use of certain drugs for prophylaxis during neutropenia may be extra-label usage. Flexible labelling may specify once- to twice-daily use of certain drugs in dogs and cats depending upon the clinical situation. Once-daily use at the lower dose in the dose range probably results in selective decontamination of the digestive tract, although this has not been established with all drugs. Flexible dosing may specify twice-daily use when treating systemic infections and may be more appropriate than once-daily use if the goal of antimicrobial prophylaxis is also to provide more consistent tissue drug levels to treat incipient bacterial infections.

2004; van de Wetering et al., 2005), although preventing sepsis is less expensive than treating it.

Antimicrobial prophylaxis in the asymptomatic patient should be considered whenever a neutrophil count of ≤ 0.5–1.0 × 10^9/L is present or anticipated. Routine prophylactic therapy during anticancer chemotherapy is not recommended if the owner can closely observe the animal for signs of infection and if the anticipated neutropenia is of short duration, such as occurs with many commonly used protocols. Prophylactic therapy is specifically discouraged in cats because they have a better tolerance of neutropenia than dogs, but are more susceptible to antibiotic-induced gastrointestinal disorders (Kunkle et al., 1995). Prophylaxis is, however, initiated in the asymptomatic animal when a neutrophil count < 0.5–1.0 × 10^9/L is noted or anticipated during pretreatment evaluation. Under these circumstances the chemotherapy treatment is discontinued, and antimicrobial prophylaxis is continued until the animal is returned for its next chemotherapy treatment 4–7 days later, at which point the neutrophil

count has usually recovered. If the neutrophil count has not recovered sufficiently to administer the next chemotherapy treatment, antimicrobial prophylaxis is discontinued if the neutrophil count is > 1.0–2.0 × 10^9/L.

If an animal has had a previous episode of chemotherapy-induced sepsis, then antimicrobial prophylaxis is often given following the next treatment with the offending agent, but prophylaxis may be restricted to the period of 5–10 days post-treatment, that is, the period when most post-chemotherapy neutropenias occur.

Antimicrobial prophylaxis is also recommended if severe and prolonged neutropenia is anticipated, such as with pancytopenia caused by estrogen toxicosis. Prolonged neutropenia may also occur during the chronic phase of ehrlichiosis in dogs. Ehrlichiosis is usually treated with tetracyclines. Doxycycline is less likely to disturb colonization-resistance than tetracycline and may be a superior choice in dogs with chronic neutropenia due to ehrlichiosis.

Antifungal prophylaxis, using topical decontamination with amphotericin B, nystatin and clotrimazole, had been practiced widely for many years in neutropenic humans. Despite these measures the incidence of invasive fungal infections increased as anticancer therapy became more aggressive. This led to the use of fluconazole and then itraconazole and newer antifungal drugs for systemic antifungal prophylaxis (Freifeld et al., 2011; Glasmacher et al., 1996; de Pauw, 2004). Routine antifungal prophylaxis is not recommended in veterinary medicine, but may be considered in hematopoietic stem cell transplantation.

Empirical Treatment of Febrile Neutropenic Patients

Neutropenia itself does not cause clinical signs; these result from the underlying disease and infection. Most septic neutropenic animals will develop a fever, because macrophages, rather than neutrophils, are largely responsible for the production of interleukin-1 and other endogenous pyrogens. Occasionally, inactivity, inappetence, and tachycardia are the only signs of sepsis. This occurs mostly in older animals and in animals receiving corticosteroids, which may have blunted febrile responses. Septic animals may also present with vomiting, diarrhea, or in septic shock. Local signs of inflammation are subtle or absent if granulopoiesis is impaired, and the site of infection may be difficult to determine. Coagulation disorders, hypoglycemia and/or hypocalcemia, if present, support a diagnosis of sepsis (Holowaychuk et al., 2012), and several biomarkers of sepsis are under investigation (Ivády et al., 2011). In many cases it is not possible to document a suspected infection and fevers many remain unexplained (Freifeld et al., 2011).

Body temperature should be monitored in the asymptomatic neutropenic animal and in the animal at risk for neutropenia. Depending upon perceived risk, this may vary from recording temperature when the animal shows signs of lethargy or inappetence to regular temperature recordings 2–4 times a day. Axillary temperature measurements facilitate home monitoring and minimize rectal trauma, and are considered to measure 0.5–1°C lower than rectal temperature measurements in normothermic dogs. In a recent study, axillary temperature had a sensitivity of 67% for detecting hyperthermia (Goic et al., 2012), emphasizing the importance of determining rectal temperature in a sick animal. The definition of pyrexia depends to some extent on baseline body temperatures obtained for an individual animal. In general, a rectal temperature > 39°C in dogs and 39.2°C in cats should be regarded with suspicion and the animal either treated for sepsis or the temperature rechecked in several hours to detect progressive elevation. A temperature above 39.5°C in most cases represents true fever.

A febrile episode or unexplained depression or inappetence in a neutropenic animal should be considered bacterial in origin until proven otherwise and antimicrobial therapy should be initiated promptly. The animal should be closely examined for any signs of inflammation, and an appropriate specimen collected for culture. If there is no obvious site of infection, blood cultures should be considered. Our protocol is to obtain two simultaneous samples for culture from different veins (Reller, 1994). Blood cultures are expensive, results take 2–7 days to report, and they are often negative or do not alter initial therapy. For these reasons blood cultures are not always performed during anticancer chemotherapy when the anticipated duration of neutropenia and fever is short, nor are they routinely performed in animals with parvoviral infections. Blood cultures are always recommended if the cause of neutropenia is not known or if the animal is very sick. Broad range real-time PCR assays for bacterial 16s rRNA genes have been reported to yield rapid diagnoses in septic humans, however, these assays are not yet validated for veterinary patients (Tsalik et al., 2010; Avolio et al., 2010).

Additional tests may be performed in an effort to localize infection and determine the severity of illness. Recommended baseline measurements in hospitalized animals include serum glucose, urea, and electrolyte levels, and urine specific gravity. Activated clotting time and/or a coagulogram should be considered. Thoracic radiographs may be considered as part of the minimum data base, and should always be obtained if the animal is coughing, is dyspneic, or has nasal discharge. Culture of airway (transtracheal or bronchoalveolar) lavage samples should be performed if there are radiographic signs of pneumonia. Normal thoracic radiographs, however, do not rule out pneumonia, and airway lavage cultures should be considered if the animal has signs of respiratory tract disease, is severely ill without localizing signs, or does not respond to antimicrobial therapy.

Urinalysis and urine culture are recommended if there are any signs of urinary tract disease (and may be routinely considered), but therapy should not be delayed more than 1–2 hours, or less depending upon the clinical status of the animal, while awaiting adequate urine production for collection. This recommendation applies to obtaining other cultures as well. Catheterization should be avoided because of the risk of introducing infection. If cystocentesis cannot be performed because of thrombocytopenia ($< 20–50 \times 10^9$/L), a properly collected free catch sample submitted for quantitative culture will suffice. A serum chemistry profile, abdominal radiographs and/or abdominal ultrasound examination are recommended if the animal is vomiting or has abdominal pain. All the preceding tests may be needed to characterize the illness if the cause of neutropenia is not known, if the animal is severely ill, or if there is no response to antimicrobial therapy.

Because the likelihood is that pyrexia is due to infection, untreated infection may be rapidly fatal, and because neutropenic animals have died of sepsis with negative ante-mortem cultures, the recommendation is to initiate empirical antimicrobial therapy while awaiting culture results, and, in most cases, to continue therapy in spite of negative results (Freifeld et al., 2011; Rolston, 2004). Antimicrobial selection may be assisted by previous culture results (e.g., a dog with a history of recurrent urinary tract infection), localization and nature of the infection, clinical signs, Gram stain of body fluid (e.g., airway wash), and the antimicrobial susceptibility pattern of a suspected pathogen. If there is a history of prophylactic therapy with a fluoroquinolone, a febrile episode is most likely due to a Gram-positive organism. Cultures of feces, the oral cavity and the skin of an animal without clinical signs prior to the induction of neutropenia are not likely to yield useful information.

In many cases the choice of antimicrobial agents must be empirical. Numerous trials with various antibiotic combinations have been conducted in humans (Freifeld et al., 2011; Picazo, 2004; Sipsas et al., 2005). Veterinary reports are limited. The antibiotics chosen should be bactericidal, should have limited toxicity to the bone marrow, should be given parenterally, and should be active against Enterobacteriaceae, *Pseudomonas* spp., and Gram-positive cocci. Standard recommended drug doses should be employed. A representative selection of antibiotics is presented in Table 21.4. These protocols provide some activity against anaerobic organisms (except for imipenem-cilastatin and meropenem, which have broad-spectrum antianaerobe activity). More complete therapy against anaerobic organisms is not recommended for initial therapy since anaerobic infections are not common under conditions of neutropenia and such therapy may alter colonisation of mucosal surfaces. Until recently (Freifeld et al., 2011), combination therapy has been preferred over therapy with a single agent in order to increase the antibacterial spectrum, take advantage of additive and synergistic effects while minimizing toxicity, and possibly to reduce the development of antimicrobial resistance. Most approaches have combined an aminoglycoside antibiotic with a beta-lactam antibiotic. Combination therapy with beta-lactam antibiotics has been used as well in order to avoid aminoglycoside nephrotoxicity. This may also be accomplished by substituting a fluoroquinolone for an aminoglycoside. Although fluoroquinolones are considered broad-spectrum antimicrobial agents, in neutropenic patients they have limited activity against Gram-positive organisms. Fluoroquinolones are similar in spectrum to aminoglycosides, with excellent activity against Enterobacteriaceae and *Pseudomonas* spp. and limited activity against anaerobes. Single-agent therapy with an anti-pseudomonal beta-lactam agent or carbapenem is another option that has recently superseded combination therapy in humans (Freifeld et al., 2011; Klastersky, 1997; Rolston, 2004). Cefoxitin has not been used as a single-agent in humans presumably because of its lack of activity against *Pseudomonas* spp., but is has been used

Table 21.4. Initial parenteral empirical antimicrobial therapy for the febrile neutropenic dog or cat.

Drug(s)	Comments
Combinations	
Aminoglycoside + cefazolin or cephalothin (first-generation cephalosporins)	Once commonly used in veterinary medicine for cancer patients Once commonly used in human medicine Relatively inexpensive Spectrum may not cover *Pseudomonas* spp. Cephalosporin may increase risk of nephrotoxicity
Aminoglycoside + ampicillin	Commonly used in veterinary medicine for patients with parvoviral infections (use decreasing) Relatively inexpensive Spectrum may not cover *Pseudomonas* or *Staphylococcus* Increased activity against anaerobes over aminoglycoside + first-generation cephalosporin More likely to disturb colonization resistance Can inhibit beta-lactamase activity by using ampicillin-sulbactam (parenteral substitute for amoxicillin-clavulanate)
Aminoglycoside + antipseudomonal penicillin or ceftazidime (third-generation cephalosporin)	Once commonly used in human medicine for cancer patients More expensive than above combinations Synergy against *Pseudomonas* and Enterobacteriaceae Less activity against Gram-positive organisms Can inhibit beta-lactamase activity by using ticarcillin-clavulanate or piperacillin-tazobactam
Fluoroquinolone substituted for aminoglycoside in above combinations	Currently commonly used in dogs More expensive than aminoglycoside Combinations more likely to be additive than synergistic Avoids aminoglycoside nephrotoxicity
Combination of two beta-lactam antibiotics[a]	Avoids aminoglycoside nephrotoxicity Potential antagonism Resistance more likely to develop? Prolongation of neutropenia?
Single agents	
Cefoxitin (second-generation cephalosorin [cefamycin])	Substitute for aminoglycoside + ampicillin No activity against *Pseudomonas* spp. Activity against anaerobes More likely to disturb mucosal colonization
Ceftazidime (third-generation cephalosporin)	Less well evaluated in veterinary medicine Once commonly used in human medicine for cancer patients Relatively expensive Less activity against gram-positive organisms than combination therapy
Ceftiofur (third-generation cephalosporin)	Veterinary drug Less well evaluated than other treatments Has been used for CPV-2 infection (Macintire, 1999)
Imipenem-cilastatin (carbapenem)	Commonly used in human medicine, and to a lesser extent in veterinary medicine, for cancer patients Relatively expensive Has a broad antimicrobial spectrum
Meropenem (carbapenem)	As per imipenem-cilastatin

Notes: Doses are adapted from Greene and Calpin, 2012; Plumb, 2011; and current use in the authors' practice; optimal doses in recommended dose ranges are not known. IV routes of administration are preferred, and all intravenous injections are given over 15–20 minutes unless indicated otherwise. *Aminoglycosides*: amikacin 15–20 mg/kg, 24 h, IV, IM, SC; gentamicin 5–6 mg/kg q 24 h, IV, IM, SC; netilmycin 6 mg/kg q 24 h, IV; tobramycin 6 mg/kg q 24 h, IV, IM, SC. Recommendations to reduce the risks of nephrotoxicity due to aminoglycoside antibiotics are (1) once-daily administration; (2) avoid use in dehydrated animals; and (3) avoid use in animals receiving furosemide. *Fluoroquinolones*: ciprofloxacin 5–10 mg/kg q 12–24 h, IV (1-hour infusion; dogs only); enrofloxacin 5–10 mg/kg q 12–24 h, IV, IM (dogs only). The initial dose in the authors' practice is usually 5 mg/kg q 12 h, IV. Higher doses are reserved for those cases where bacteria with higher MICs are suspected or isolated (e.g., *Pseudomonas* spp.). *These drugs are NOT recommended in cats*. Enrofloxacin is approved for IM use only, but the solution is irritating to tissues and IV administration is preferred. For IV injection, the solution should be injected over 20–60 minutes; some recommend dilution of 1 part parenteral solution with 9 parts sterile water for injection. The parenteral solution should not be given SC. Reduction in the frequency of administration and/or dose may be necessary in animals at risk for seizure activity (see text). *Aminobenzyl penicillins*: ampicillin 20–40 mg/kg q 6–8 h, IV, IM, SC; ampicillin-sulbactam 50 mg/kg q 6–8 h, IV, IM. *Antipseudomonal penicillins*: piperacillin 25–50 mg/kg q 6–8 h, IV, IM; piperacillin-tazobactam 25–50 mg/kg q 6–8 h, IV, IM; ticarcillin 40–75 mg/kg q 6–8 h, IV, IM; ticarcillin-clavulanate 30–50 mg/kg q 6–8 h, IV, IM. *Cephalosporins*: cefazolin 20–30 mg/kg q 6–8 h, IV, IM, SC; cephalothin 25–40 mg/kg q 6–8 h, IV, IM, SC; cefoxitin 20–30 mg/kg q 6–8h, IV, IM, SC; ceftazidime 25–30 mg/kg IV, IM, SC q 8 h—these cephalosporins are typically dosed at 30 mg/kg q 8 h, IV; ceftiofur (dogs only) 2.2–4.4 mg/kg q 12 h, SC. *Carbapenems*: imipenem-cilastatin 2–10 mg/kg q 6–8, IV (1-hour infusion); 5 mg/kg q 8 h, IV is the typical dose in the authors' practice. Meropenem 12 mg/kg q 8 h IV.

[a]E.g., First-generation cephalosporin + antipseudomonal penicillin; first-generation cephalosporin + third-generation cephalosporin; third-generation cephalosporin + antipseudomonal penicillin.

in animals, especially in cats and immature animals where fluoroquinolone therapy may not be appropriate. For infections complicating the mild to moderate episodes of neutropenia usually encountered by veterinarians, the various protocols are probably of near equivalent efficacy. In the authors' practice, enrofloxacin plus cefazolin or ampicillin is the most frequent choice for canine cancer patients. This combination may also be used for initial therapy in animals with sepsis associated with neutropenia of unknown cause, although some clinicians prefer imipenem-cilastatin or meropenem.

Intravenous administration is preferred, to ensure rapid drug distribution, minimize tissue trauma and patient discomfort, and minimize bleeding in thrombocytopenic animals. IV catheterization is preferred to repetitive venipuncture, and is necessary for fluid therapy. However, there must be strict adherence to aseptic procedure during catheter placement. A sterile adhesive strip or plaster (e.g., Band-Aid) should be placed over the skin entry site and the site bandaged. Injection ports should be cleansed with alcohol and allowed to dry before each injection. The catheter should be removed promptly and cultured if signs of phlebitis occur.

Drug toxicity should be considered during therapy. Animals receiving aminoglycosides should be monitored for evidence of nephrotoxicity (e.g., urinary casts, glucosuria, azotemia), especially when the duration of therapy is greater than 5 days. The order of aminoglycosides with respect to increasing nephrotoxicity (and decreasing cost) is netilmicin, amikacin, tobramycin, and gentamicin. Fluoroquinolones should be avoided in animals less than 6 months old because of the possibility of inducing cartilage defects. However, the risks for such defects following 3- to 5-day courses of treatment at standard doses is not known and its use in treating severe CPV-2 infection in pups has been recommended (Macintire, 1999). Fluoroquinolones may cause seizures and other neurologic signs at higher doses, especially with repetitive administration. Geriatric animals, animals with hypoalbuminemia, and animals with a history of seizures are at increased risk. Antibiotics may inhibit platelet function; this effect is most pronounced with penicillins in humans. Any such effects do not appear to be important in dogs (Wilkens et al., 1995; Webb et al., 2005) and are unlikely to be in cats.

Reduction of fever is expected within 72 hours after starting antimicrobial therapy, and the animal should be more alert. Increasing depression coinciding with a falling temperature may be a sign of septic shock. In many cases improvement is noted after the first dose. The duration of antibacterial therapy, once pyrexia has resolved, is controversial. Prolonged therapy increases expense, hospitalization, side effects, risk of selecting for resistant bacteria, and risk of a fungal infection. Therapy should be continued for 1–7 days beyond achievement of a neutrophil count of $0.5–1.0 \times 10^9$/L. Changing from IV therapy to oral therapy (Table 21.4) during this period facilitates discharge from the hospital and reduces expense. For cancer patients without a documented site of infection, it is recommended to stop IV antimicrobial drugs the day after recovery of the neutrophil count to 1.0×10^9/L and resolution of pyrexia. Oral antimicrobial therapy is continued in those patients that were receiving it prophylactically, and initiated for 7 days in those that were not. Following up with oral antimicrobials is not recommended in patients recovering from parvoviral infections. In animals with pancytopenia with prolonged neutropenia, IV or oral antimicrobial therapy is continued for a minimum of 10 days beyond resolution of fever. At this time withdrawal of antimicrobial therapy may be attempted.

Pyrexia may not resolve if (1) it is not bacterial in origin (and this should be reconsidered); (2) the organism is not susceptible to the antimicrobial drug(s); (3) drug doses are too low; and (4) there is such a severe compromise of host defenses that the infection and associated fever will not respond to any antimicrobial agent. The latter occurs with prolonged, severe neutropenia. This is infrequently encountered in veterinary medicine, but has been observed during hematopoietic stem cell transplantation. Initial culture results may assist therapeutic decision making with unresponsive fever. If a resistant organism is documented, antimicrobial therapy may be changed based upon susceptibility testing. For animals with a bacterial pathogen that is susceptible to the drugs chosen empirically but that has not responded to empirical therapy, increasing the dose may result in clinical improvement. Once the animal is clinically stable, the medication may be continued until resolution of fever and achievement of a neutrophil count of 1.0×10^9/L. If there is a need to change the therapeutic regime the choice of additional drugs depends on which antibiotics were used for initial therapy. Traditionally, failure of response to empirical therapy with cefoxitin or an

aminoglycoside and first-generation cephalosporin, would prompt additional therapy against *Pseudomonas* spp. with an antipseudomonal penicillin. Ceftazidime, imipenem-cilastatin, and meropenem could also be used to intensify activity against *Pseudomonas*. If a resistant Gram-negative organism is suspected (e.g., if there are signs of intestinal damage or respiratory signs), choices for additional therapy may include an aminoglycoside, fluoroquinolone, cefoxitin, ceftazidime and other third-generation cephalosporins, and imipenem-cilastatin or meropenem. Aztreonam may also be used in humans to intensify therapy against Gram-negative organisms and *Pseudomonas* spp., but there is limited veterinary experience with this drug. Resistant Gram-positive organisms are increasingly responsible for infections in neutropenic humans, for which vancomycin and teicoplanin are the drugs of choice for empirical treatment. Veterinary experience with these drugs in neutropenia is limited. If a resistant Gram-positive organism is suspected (e.g., if there are signs of phlebitis, injury to the skin or oral cavity, or respiratory signs), the drug of choice in animals is clindamycin, 10 mg/kg q 12 h, IV, SC, although it is bacteriostatic. Imipenem-cilastatin and meropenem could also be used, although activity against *Streptococcus* spp. may not be complete.

A non-responding fever may also be due to a resistant anaerobic infection. Additional therapy could include metronidazole (15 mg/kg IV [1-hour infusion] q 12 h), clindamycin, cefoxitin, ampicillin-sulbactam, imipenem-cilastatin and meropenem. The latter two are suitable for increasing broad-spectrum antibacterial activity. Although imipenem-cilastatin and meropenem are expensive, they are less expensive than combined administration of an aminoglycoside or fluoroquinolone, first-generation cephalosporin, and metronidazole, and in some cases are substituted for such combinations. If multiple antimicrobial agents are being used, then selective withdrawal of agents may be considered once there is clinical improvement.

The preceding recommendations are appropriate for most cases, but may not be feasible due to cost restrictions, and inability or unwillingness of the owner to return the animal to the hospital. In such cases, initial use of oral antimicrobial agents may be used if the animal is clinically stable. In addition, oral antimicrobial agents may be sufficient for initial treatment of neutropenic animals that have been febrile and clinically stable for several days. There is an increasing trend in the use of oral antimicrobial therapy for the treatment of humans with low-risk febrile neutropenia (the MASSC Risk Index may be used to stratify humans as having a low or high risk for serious complications of febrile neutropenia; Klatersky et al., 2000), where the drugs of choice are ciprofloxacin plus amoxicillin-clavulanate (Freifeld et al., 2011; Rolston, 2004). For animals with mild neutropenia and mild pyrexia, therapy with trimethoprim-sulfonamide, a fluoroquinolone, amoxicillin, or amoxicillin-clavulanate is recommended. For animals with moderate to severe neutropenia or pyrexia, a fluoroquinolone plus cephalexin, amoxicillin or amoxicillin-clavulanate is recommended. Doses may be increased within standard recommendations (Greene and Calpin, 2012; Plumb, 2011) above those given in Table 21.4. Therapy with tetracyclines or doxycycline for ehrlichiosis may also control secondary infections. In all cases, the animal should be closely observed for clinical deterioration and arrangements made to initiate parenteral therapy. Oral antimicrobial therapy should not be used when the animal is hypovolemic, hypotensive, vomiting or there is disruption of the intestinal mucosa.

With high-risk human neutropenic patients, if there is no response to multiple antibacterial agents after 4–7 days of therapy, then empirical antifungal therapy may be initiated (Freifeld et al., 2011). Such patients are often already receiving antifungal prophylaxis, and intensification may be with amphotericin B, voriconazole, or an echinocandin (caspofungin or micafungin), depending on the prophylactic drug. This situation is rarely encountered in veterinary medicine, and antifungal therapy is not recommended in the dog or cat unless a fungal infection is documented. If neutropenia and antibacterial therapy persist beyond 10 days, then stools should be monitored by culture or cytologic studies for candidal overgrowth and prophylaxis with nystatin, ketoconazole, fluconazole, or itraconazole considered, especially if antibacterial agents are being used that disturb the mucosal bacterial flora (e.g., ampicillin, cefoxitin, metronidazole, imipenem-cilastatin, and meropenem).

Therapy of Documented Infections

An infection is considered documented in strict terms when the site of infection and infecting organism are both known. In broader terms an infection is also considered documented if only the site of infection is known

(e.g., radiographic evidence of pneumonia). Therapy of documented bacterial infections should consist of bactericidal antibiotics, with the choice based upon susceptibility testing. The guidelines for choosing parenteral or oral routes of administration are the same as those previously discussed. In most situations, by the time culture results are obtained, empirical therapy will have already been started. The guidelines for duration of therapy with documented bacteremia but no localization into other organs are also as previously discussed. Treatment for documented pneumonia, and urinary tract and soft tissue infections should be continued to a minimum of 7 days beyond recovery of the neutrophil count to 1.0×10^9/L *and* resolution of clinical and radiographic signs. The infection may transiently appear to become worse as neutrophil recovery occurs, due to increased inflammation. However, fever should be decreasing if the antimicrobial therapy is appropriate. The guidelines for intensifying therapy if fever and clinical signs are progressing are similar to those previously discussed, with drug selection aided by susceptibility test results.

Documented fungal infections should be treated with antimycotic drugs used at standard recommended doses (Greene and Calpin, 2012; Plumb, 2011). Amphotericin B is the current therapy of choice for infections caused by *Aspergillus* spp. Nephrotoxicity may be reduced by using the newer lipid-complex formulations, but the drugs are considerably more expensive. Some cases of topical and systemic aspergillosis can also be treated successfully with itraconazole. Amphotericin B is also indicated for treatment of systemic candidiasis, but therapy with ketoconazole or itraconazole may suffice (Weber et al., 1985). Intestinal candidiasis can be treated with nystatin, ketoconazole, itraconazole or fluconazole. Fluconazole is the drug of choice for urinary candidiasis. There is limited veterinary experience with the newer antifungal drugs voriconazole (except for topical ophthalmic therapy), posaconazole, and the echinocandins. In a recent report, 3 cats treated with voriconazole (approximately 10 mg/kg/day) developed ataxia that, in 2 cats, progressed to paraplegia of the rear limbs (Quimby et al., 2010). Neurologic abnormalities appeared to be reversible.

Role of Hematopoietic Growth Factors (G-CSF or GM-CSF) in Managing Neutropenic Patients

In humans, the prophylactic use of hematopoietic growth factors is recommended for patients in whom the anticipated risk of fever and neutropenia are greater than 20% (Friefeld et al., 2011), which is not likely with most veterinary chemotherapy protocols (Vail, 2009). These agents are not generally recommended for treatment of established fever and neutropenia (Friefeld et al., 2011). Controlled studies in dogs and cats are limited. In a study of normal dogs, recombinant human (rh) G-CSF improved neutrophil counts and survival following radiation-induced myelosuppression (Yu et al., 2011). In a study of dogs with lymphoma receiving high-dose chemotherapy and autologous bone marrow transplantation, rhG-CSF improved neutrophil counts (Lane et al., 2012). Administration of recombinant canine (rc)G-CSF after treatment with mitoxantrone or cyclophosphamide decreased the severity of neutropenia and accelerated recovery (Ogilvie et al., 1992; Yamamoto et al., 2011). Only rhG-CSF is commercially available. At this point, there are no accepted guidelines or consensus for the use of ru- or rc-G-CSF in veterinary cancer patients (Vail, 2009).

Treatment of young dogs with parvovirus-induced neutropenia with rhG-CSF has provided equivocal results with one study documenting significantly increased neutrophil counts compared to untreated controls (Kraft and Kuffer, 1995) whereas 2 other studies failed to detect an improvement in neutrophils counts, duration of hospitalization, or survival compared to untreated animals (Rewerts et al., 1998; Mischke et al., 2001). More recently, administration of rcG-CSF to dogs with parvovirus infection resulted in significantly higher neutrophil counts and shorter hospitalization compared to untreated dogs (Duffy et al., 2010). However, mortality was significantly higher in dogs treated with rcG-CSF (Duffy et al., 2010). The use of rhG-CSF was not beneficial in one study of cats (Kraft and Kuffer, 1995) At this point, treatment of neutropenia caused by parvovirus infection with rhG-CSF or rcG-CSF is not widely recommended.

The use of rhG-CSF has been reportedly or anecdotally beneficial in cases of estrogen or phenobarbital-induced neutropenia in dogs, and griseofulvin and retroviral-induced neutropenia in cats. Although recombinant canine and feline GM-CSF are available commercially as laboratory reagents, their use is not recommended as GM-CSF appears to be less effective than G-CSF in stimulating granulopoiesis and have a greater risk for side effects.

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22

Performance Uses of Antimicrobial Agents and Non-antimicrobial Alternatives

Thomas R. Shryock and Stephen W. Page

Antimicrobial agents administered with the primary intention of improving physiological performance (i.e., "growth promotion") have been used since the 1950s by food animal producers. They have been available without a prescription and decisions on their use have often been based on economic, nutritional, or animal performance considerations. Concerns regarding the possibility of adverse public health impacts arising from the selection and dissemination of foodborne antimicrobial-resistant bacteria from such uses in livestock and poultry were raised by Anderson in the UK in 1968 following the emergence of *Salmonella* Typhimurium DT29 in calves (Anderson, 1968).

As a consequence, veterinarians, public health officials, regulatory authorities and other stakeholders have become increasingly involved in exploring the risks, implementing risk management measures and searching for alternative products. The literature associated with the benefits and risks of antimicrobial agents used for performance is vast and has been accumulating for more than half a century. This chapter provides an introduction to this area, highlighting key historical findings and issues, as well as exploring future options.

History

The 1940s was a fertile time for nutritional and biochemical investigations. The role of many essential dietary factors, including many vitamins, was discovered. During this decade, increased growth rates in chickens consuming diets supplemented with arsenicals, sulfonamides, streptomycin or chlortetracycline were also observed. However, the era of the antimicrobial growth promoters began with an announcement at the American Chemical Society meeting in Philadelphia on 9 April 1950 by Stokstad and Jukes, both pioneers in vitamin research (Stokstad and Jukes, 1950). They described their observations that the addition of the crude mycelial mass produced by the fermentation of *Streptomyces aureofaciens* to the feed of poultry and pigs resulted in spectacular increases in rates of growth. Rather than a simple response to the supply of vitamin B_{12}, as they had initially hypothesized, much of the improved performance was directly attributable to the presence of low concentrations of chlortetracycline.

This serendipitous discovery of antimicrobial growth promotion coincided with a revolution in animal husbandry as pasture production was being replaced by more intensive housing. Much was still to be learned about the nutritional requirements and disease control interventions needed under these new environmental conditions. The advent of antimicrobial growth promotion, however, permitted improved food production at a time of fundamental change and increasing demand.

While much of the initial study of antimicrobial growth promotion concentrated on the tetracyclines and penicillin, other agents were progressively discovered and developed, in many cases displacing their predecessors.

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Table 22.1. Timeline of discovery of antimicrobial drugs with growth-promoting activity and other events.

Decade	Compound	Discovery	Other Events
1940s	Penicillin	1940	← 1940 Chain & Florey isolate and characterize penicillin
	Roxarsone	1941	
	Bacitracin	1945	← 1946 Moore & others*
	Chlortetracycline	1948	← 1949–1950 Stokstad & others*
	Oxytetracycline	1950	
	Lasalocid	1951	
1950s	Kitasamycin	1953	
	Virginiamycin	1955	
	Oleandomycin	1956	← 1959 Transferable resistance first described
	Avilamycin	1961	
	Tylosin	1961	
	Lincomycin	1963	← 1962 Netherthorpe report*
1960s	Carbadox	1964	← 1963 *Salmonella typhimurium* PT29 in UK
	Bambermycins	1965	
	Monensin	1967	
	Avoparcin	1967	← 1969 Swann report*
	Olaquindox	1970	← 1970 FDA task force
	Salinomycin	1972	
1970s	Tiamulin	1973	
	Laidlomycin	1974	
	Narasin	1975	
	Efrotomycin	1975	
1980s			← 1980 NAS study
			← 1988 IOM review
	Alexomycin	1989	← 1988 human VRE infection described
	LL-E19020	1989	
1990s			← 1997 WHO consultation
			← 1998 NRC report
			← 1999 GAO reports
2000			← 2000 WHO principles of resistance containment
			← 2006 final EU use of antibiotics for performance indications
2010s			← 2012 U.S. FDA CVM issues draft Guidance for Industry #213 that will eliminate performance indications for medically important antibiotics

*1946—Moore and colleagues first described growth responses to antibiotics; 1949—Stokstad and others announced growth responses to chlortetracycline fermentation mash (mycelium), findings soon to be published on front page of global press; 1962—Lord Netherthorpe chairs committee evaluating whether feeding antibiotics to farm animals constitutes any danger to human health. Found no danger and recommended extension of use to calves.
1969 Professor Swann chairs committee formed to assess likelihood and impact of transferable resistance on human health. Finds risks and significant benefits and presents criteria for selection of feed antibiotics.

Table 22.1 provides an insight into the age of many of the agents still in use in some countries. Notably, there are no antimicrobial agents with performance uses that have been approved in the last 4 decades.

Among the numerous observations made in the early decades of antimicrobial growth promoter research, it was noted that unsupplemented control groups of animals had improved weight gains and reduced mortality when raised in the vicinity of groups receiving antimicrobial growth promoters. This finding was attributed to a reduction in the total environmental load of pathogenic bacteria. Other observations during this period included the retention of

the effectiveness of antimicrobial growth promotion after prolonged use (even after decades of use), greater responses in young animals, significant reductions in enteric diseases in supplemented animals, reduction in vitamin and protein requirements, and (not unexpectedly) reduced responses as animals approached their genetic potential for growth. A summary of the diverse array of physiological, metabolic, nutritional, and disease control effects that have been documented is presented in Table 22.2.

In a statement that remains valid today, having completed a comprehensive review of antimicrobial growth promotion in pigs and poultry, Hays (1979) concluded that:

> the magnitude of the response to antibacterial agents varies with stage of life cycle, stage of production, and the environmental conditions to which animals are exposed. The response is greater in young animals than in more mature animals. The response is greater during critical stages of production such as weaning, breeding, farrowing or immediately post hatching in chicks and turkeys. Environmental stresses such as inadequate nutrition, crowding, moving and mixing of animals, poor sanitation and high or low temperatures also contribute to increased responses. Such stresses are ordinary and to a large degree unavoidable.

Table 22.2. Some physiological, nutritional, and metabolic effects ascribed to antibiotic feed additives.

Effect	Change	Effect	Change
Adverse bacteria	↓	Gut urease	↓
Alpha-toxin production	↓	Gut wall diameter	↓
Ammonia production	↓	Gut wall length	↓
Beneficial bacteria	↑	Gut wall weight	↓
Beneficial *E. coli*	↑	Limiting amino acid supply	↑
Beneficial lactobacilli	↑	Liver protein synthesis	↑
Calcium absorption	↑	Methane emission	↓
Clostridium perfringens	↓	Mucosal cell turnover	↓
Competition for nutrients by gut flora	↓	Nitrogen excretion	↓
Debilitation of pathogens	↑	Nitrogen retention	↑
Energy retention	↑	Nutrient synthesis by gut flora	↑
Fatty acid absorption	↑	Pathogenic *E. coli*	↓
Fatty acid oxidation	↓	Pathogenic streptococci	↓
Fecal fat excretion	↓	Phosphorus excretion	↓
Fecal moisture	↓	Plasma nutrients	↑
Feed intake	↕	Stress	↓
Glucose absorption	↑	Toxic amine production	↓
Gut absorptive capacity	↑	Trace element absorption	↑
Gut alkaline phosphatase	↑	Transferable resistance	↕
Gut energy loss	↓	Vitamin absorption	↑
Gut food transit time	↑	Vitamin synthesis	↑

Source: Adapted from Rosen, 1995, and Page, 2003.

Mechanism of Action

It was recognized very early in the history of antimicrobial growth promotion that the action of antimicrobial agents in increasing growth, feed efficiency and animal health was largely confined to effects on the bacteria within the gastrointestinal tract. This observation rests primarily on the following findings: (1) antimicrobials of widely varying chemical structure are effective, precluding the possibility of incorporation into any growth factor essential for the animal; (2) antimicrobials do not promote growth in germ-free animals; (3) antimicrobials are ineffective in increasing growth in the developing chick embryo; (4) sanitation affects the magnitude of the antimicrobial growth response; (5) the growth-promoting effect is observed with orally administered unabsorbed agents such as bacitracin; and finally, (6) the growth-promoting effects of certain parenteral antimicrobials may be explained by their excretion into the intestine.

Many hypotheses have been proposed to explain the mode of action of antimicrobial growth promoters. There remains no unifying principle or single mode of action, and it is likely that different mechanisms predominate in different situations. The magnitude and characteristics of bacterial metabolism in the intestine are dependent on the animal species, age of the host, diet, and segment of the intestinal tract investigated. Interactions between the enteric flora and the host have been described as either competitive or cooperative. Competitive interactions are typical of carnivores, in whom physiological mechanisms (such as low gastric pH and rapid gut transit) have evolved to limit the interaction of flora and nutrients. By contrast, cooperative interactions have evolved in herbivores, notably ruminants, where the host provides optimal conditions for bacterial fermentation. The mode of action of antimicrobial growth promoters must be consistent with this diversity of situations.

Among the hypotheses already proposed and tested in monogastric species (poultry, pigs, and pre-ruminant calves) are the following:

1. Stimulation of intestinal synthesis of vitamins by bacteria. The addition of vitamins at high levels to the diet reduces the response to antimicrobial agents. It has been reported that oral chlortetracycline may increase vitamin availability by increasing fecal elimination of vitamin $B_{12,}$ and that streptomycin has been observed to increase the population of vitamin B_{12}-producing *Bacillus megaterium*.
2. Reduction in total numbers of bacteria in the intestinal tract with a lowering of competition between microorganisms and host animal for nutrients.
3. Inhibition of harmful bacteria that may be mildly pathogenic or toxin-producing. A number of antimicrobial agents have been shown to prevent the growth of *Clostridium perfringens* in the intestinal tract of broilers, turkeys, and pigs. Other researchers have suggested or demonstrated that growth depression is associated with the presence of *Enterococcus faecalis* or *Enterococcus faecium*. Tsinas et al. (1998) observed that in pigs the ability to control *Lawsonia intracellularis* was directly related to growth enhancement. Animals raised in pristine environments benefit less from antimicrobial supplementation, while those growing in well established facilities respond sometimes dramatically to the inclusion of antimicrobial agents in their diet, consistent with the presence of growth depressing agents. Bacterial deamination and decarboxylation of amino acids can lead to the production of toxic degradation products. For example, decarboxylation of lysine yields cadaverine, whereas tyrosine and tryptophan are converted to a number of volatile phenolic and aromatic metabolites (including 4-methylphenol and 3-methylindole or skatole), which are both malodorous and potentially toxic. Various antimicrobial growth promoters have been shown to decrease the production of these metabolites.
4. Inhibition of bacterial urease. It has been suggested that ammonia produced by bacterial urease damages the intestinal mucosa, impairing nutrient absorption and impeding growth. However, caprylohydroxamic acid, a synthetic urease inhibitor, has been shown to have no effect on growth rate and feed efficiency in chicks.
5. Improved energy efficiency of the gut. The gut attracts a high proportion of cardiac output and contributes a commensurate rate of heat production, parameters that are influenced by nutritional status. Antimicrobial administration has been shown to improve nutrient digestibility and to enhance energy utilization mediated by intestinal microbes. The gut mucosa is the most metabolically active tissue in the body, and it has been demonstrated that antimicrobial supplementation reduces cell turnover in the small intestine and increases the rate of glucose uptake by isolated brush border vesicles.
6. Inhibition of bacterial cholyltaurine hydrolase activity. Conjugated bile acids are secreted via the bile into the small intestine where they aid digestion, emulsification and absorption of fats, lipids and fat soluble compounds such as α-tocopherol. Bacteria, principally Gram-positive genera, hydrolyse conjugated bile acids, reducing their function and also increasing the concentration of the hydrolysis product lithocholic acid, which is hepatotoxic and causes inflammation of the small intestine. Feighner and Dashkevicz (1987) found an inverse relationship between the growth performance of antimicrobials and cholyltaurine hydrolase activity, raising the possibility of a discrete mode of action. This hypothesis is supported by recent studies in broilers that have shown high levels of bile salt hydrolase activity expressed by *C. perfringens*. Enzyme activity, unconjugated bile acids, and *C. perfringens* numbers are reduced and ileal absorption of fatty acids was improved by supplementation with avilamycin and salinomycin (Knarreborg et al., 2004).
7. Nutrient sparing. Studies in the early 1950s found that efficient utilization of protein by pigs was obtained only when the diet contained the mycelial Lederle APF (animal protein factor) supplement and observed that a diet containing APF and 18% protein led to equivalent growth rates to pigs consuming a diet with 19.6% protein. It was suggested that the accepted values for the protein requirements of pigs may need to be re-evaluated by using adequate amounts of vitamin B_{12} plus other factors present in the Lederle APF supplement in the ration. Many subsequent studies have corroborated this early observation on protein sparing and established that energy, vitamins and minerals can also be spared, with particular significance for

reduced inputs and outputs of environmentally important greenhouse gases and nutrients such as nitrogen and phosphorus.

8. Improved nutrient absorption from morphological changes to small intestinal epithelium. A notable feature of germ-free animals and those whose diets are supplemented with antimicrobial growth promoters is a reduction in mass, manifested as shortening and thinning of the intestinal wall. It has been suggested that these changes may allow improved nutrient absorption.
9. Modification of intestinal enzyme activity. The characteristics of intestinal enzyme activity are significantly influenced by the presence of the microflora and factors modifying this ecosystem such as the antimicrobial growth promoters could favorably influence enzyme activity and the availability of nutrients.
10. Reduced immune stimulation. Microbial challenges, while infrequently resulting in clinical disease, do provoke immune responses that are metabolically expensive and lead to increased basal metabolic rate, changes in nutrient absorption, and partitioning of dietary nutrients away from skeletal muscle accretion. It has been demonstrated that dietary antimicrobial supplementation results in improved performance coupled with a reduction in several indicators of immune system activation.
11. Anti-inflammatory effects on intestinal cells. A case is made that the physiologic response of "growth permittants" is due to the anti-inflammatory effect of low concentrations of antimicrobials on host immune cells (Niewold, 2007). Although it was thought in the 1950s that oral antimicrobial administration was detrimental to ruminants, when dose rates were lowered and when novel agents such as the ionophores were introduced in the 1970s significant benefits in performance were realized. An additional mode of action specific to ruminants includes:
12. Modification of rumen microbial metabolism. Fermentative digestion is advantageous for substrates that cannot be digested by host enzymes. However, fermentation results in losses of energy and protein and is therefore disadvantageous for nutrients such as protein, amino acids and sugars readily digested by host enzymes. Optimal productivity in ruminants depends on an appropriate balance of fermentative and host digestion. The principal mode of action of most antimicrobial growth promoters in ruminants is to manipulate the ruminal ecosystem. Energetic efficiency is improved by manipulating carbohydrate fermentation in favor of propionate with simultaneous decreased methane production and loss. In addition, starch utilization is improved if the microbiota are shifted away from net lactic acid production. Nitrogen metabolism can be enhanced by reducing bacterial proteolysis and increasing ammonia assimilation. Ruminal lipid metabolism can be favorably manipulated if lipolysis is inhibited, allowing reduced biohydrogenation and increased flow of unsaturated fatty acids to the small intestine.

Advanced molecular biology techniques have allowed fundamental improvements in the understanding of the complex microbial ecology of the gut (Backhed et al., 2005). Bacterial and archaeal genera and species have been studied using specific 16S rRNA-targeted oligonucleotide hybridization probes and denaturing-gradient gel electrophoresis. Such studies have allowed researchers to identify and enumerate the culturable and non-culturable flora of cattle (Stahl et al., 1988; Shanks et al., 2011), sheep (Edwards et al., 2005), pigs (Collier et al., 2003; Lamendella et al., 2011) and poultry (Knarreborg et al., 2002; La-ongkhum et al., 2011) and the impact of antimicrobial exposure.

Regulatory Oversight

In common with many other veterinary medicines, the use of antimicrobials to improve food animal productivity has been highly regulated over the years. Thorough demonstration of manufacturing quality, efficacy, and safety (including tissue residues, toxicology, target animal safety, occupational safety and environmental safety) are required. Manufacturers of feed additives submit to regulatory agencies comprehensive studies on environmental toxicology and fate that describe the soil half-life of the antimicrobial and related metabolites, as well as effects on soil-associated micro- and macroorganisms, fish, wildlife, and plants. Specific U.S. approval guidance is available at the Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM; U.S. FDA CVM, 2012a). Similar requirements apply in other countries, facilitated by the development

and adoption of common guidelines by Japan, the United States, and Europe under the auspices of the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products (VICH, 2012). In 2012, the U.S. FDA CVM published final Guidance for Industry #209, which states: "FDA believes the use of medically important antimicrobial drugs in food-producing animals for production purposes (e.g., to promote growth or improve feed efficiency) represents an injudicious use" (U.S. FDA CVM, 2012b). A companion document, draft Guidance for Industry #213 (U.S. FDA CVM, 2012c), states: "FDA will be working with affected drug sponsors who notify us of their intent to voluntarily withdraw approved production uses of their medically important antimicrobial new animal drugs and combination new animal drug products." Thus, an orderly transition to therapeutic indications (i.e., judicious use that includes prevention, control, and treatment indications under the supervision of a veterinarian using a Veterinary Feed Directive [VFD] to "prescribe" an in-feed antimicrobial) is coupled to the discontinuation of performance indications.

Regulatory Oversight of Medicated Feeds

In the United States, medicated feed products are classified as Category I or II, and A, B, or C (irrespective of the intention of use), depending on the withdrawal time, concentration, and mixing status (Feed Additive Compendium, 2012). A Type A premix contains the highest drug concentration and can be manufactured only under FDA approval and in compliance with Current Good Manufacturing Practices (cGMP). A Type B premix contains a lower concentration of drug than a Type A premix, and can be further mixed. A Type C premix is the final product for incorporation into the feed that cannot be further mixed. Mixing of Category II, Type A premix into a Type B or C feed is done only by FDA-licensed feed mills, which requires establishment registration, full cGMP, and mandatory 2-year inspections as conditions for a Feed Mill License. All mixed feeds must display specific labeling information clearly listing ingredients, feeding instructions, cautions or warnings, feed withdrawal information (for unsafe residue avoidance), and other relevant information. The final medicated feedstuff is manufactured at the feed mill to conform to tight inclusion range specifications for potency, then bagged or delivered in bulk to the farm where it is to be used.

The concentration in feed of most antimicrobials for performance indications is in the order of 5–125 ppm (or mg/kg of feed), which equates to a much lesser mg/kg body weight for the individual animal based on a daily feed intake. For example, every kg of a product containing 10 ppm of an antimicrobial feed additive contains 10 mg of the antimicrobial. If the animal consuming this feed weighs 100 kg, then the intake of antimicrobial is 0.1 mg antimicrobial per kg body weight for every kg of the feed consumed. Analytical assays have been developed for all drug ingredients in order to be able to confirm proper mixing, prevention of cross-contamination, and for other quality-related purposes.

Antimicrobials approved as feed additives for performance indications in the United States are listed in Table 22.3. Products formerly used in the European Union for productivity enhancement are listed in Table 22.4. In 1996, avoparcin was suspended from the list of European Union approved products pending a re-evaluation of the potential medical impact associated with the selection of glycopeptide-resistant enterococci associated with its use. Following this precedent, in late 1998, the European Agriculture Council and Commission voted to invoke the "precautionary principle" for drugs in classes also used in human medicine, which included bacitracin, spiramycin, tylosin and

Table 22.3. Antibacterial feed additives approved for performance uses in cattle, swine, and poultry in the United States.

Drug	Antibiotic Class	Cattle	Swine	Poultry
Arsenicals	Arsenical		+	+
Bacitracin	Polypeptide		+	+
Bambermycins	Glycophospholipid		+	+
Carbadox	Quinoxaline		+	
Tetracyclines	Tetracycline	+	+	+
Chlortetracycline, sulfamethazine, penicillin	Combination		+ (< 75 lbs)	
Lasalocid	Ionophore	+		
Lincomycin	Lincosamide		+ (> 75 lbs)	+
Monensin	Ionophore	+		
Penicillin	B-lactam		+	+
Tylosin	Macrolide	+	+	+
Virginiamycin	Streptogramin	+	+	+

Source: Feed Additive Compendium, 2012.

Table 22.4. Antibacterial feed additives formerly approved for growth promotion in cattle, swine, and poultry in the European Union.

Drug	Antibiotic Class	Cattle	Swine	Poultry
Avoparcin[1]	Glycopeptide	+	+	+
Bacitracin[2]	Polypeptide	+ (calves)	+	+
Flavomycin[3]	Glycophospholipid	+	+	+
Monensin[3]	Ionophore	+		
Salinomycin[3]	Ionophore		+	
Spiramycin[2]	Macrolide	+ (calves)	+	+
Tylosin[2]	Macrolide		+	
Virginiamycin[2]	Streptogramin	+ (calves)	+	+
Avilamycin[3]	Orthosomycin		+	+
Carbadox[4]	Quinoxaline		+ (< 4 months)	
Olaquindox[4]	Quinoxaline		+ (< 4 months)	

[1]Suspended, then withdrawn in 1998.
[2]Authorization withdrawn under EU Council Regulation (EC) 2821/98; effective July 1999.
[3]Authorization withdrawn effective January 2006.
[4]Authorization withdrawn under EU Commission Regulation (EC) No. 2788/98; effective January 1999.
Source: Lawrence, 1998; Corpet, 1996; Anon., 1997.

virginiamycin, thereby removing their claims to improve productivity, effective July 1999, resulting in discontinuation of the use of these products for growth promotion. The remaining antimicrobials, avilamycin, bambermycin, monensin and salinomycin, although not used in human medicine, had their productivity claims removed effective January 2006. It is important to note that antimicrobial ionophores (when used as anticoccidial agents) remain unaffected by the EU directives. Other countries use some of the same products for performance responses. Some countries, such as Japan, also have unique performance enhancing products such as bicozamycin, nosiheptide, and enramycin.

Usage Practices and Benefits

Over the past 6 decades, numerous changes in the food animal production systems have taken place, most notably the consolidation of operations to large, company-operated farms that raise the vast majority of livestock and poultry in groups indoors, or outdoors in the case of beef cattle feedlot enterprises. Improvements in animal genetics, herd/flock management, medicinal usage practices, feedstuffs, biosecurity, and infection control have allowed increased production of meat and other foods of animal origin in a safe, cost-efficient manner to meet the ever-increasing consumer demands for animal protein. The use of antimicrobials to improve performance changed considerably during this period, so that today a wide variety of application strategies have been developed targeting product choice, age of animal medicated, duration of medication, and utilization of professional consultation (MacDonald and McBride, 2009).

There is a general public misperception that the only benefit to the use of antimicrobials for performance indications is a positive economic return to the producer, while risks to human health and the environment are ignored. However, the administration of antimicrobials for performance purposes in modern food animal production programs actually offer a number of significant benefits. It should be noted that not all of the benefits summarized below have been approved by all regulatory authorities as label indications for the antimicrobials listed, and not all antimicrobials that are used in food animal production are discussed.

Six of the benefits associated with the use of antimicrobials for performance benefits are summarized below and other benefits are listed in Table 22.5. First, enhancing the efficiency of nutrient utilization by animals allows additional lean muscle gain to be added per pound of feed consumed, resulting in an overall reduction in feed consumption. Logically, reduced feed intake means less cropland, water, and energy are needed for feed production. Second, less feed intake results in reduced fecal output, lessening the environmental burden from excess nutrients such as nitrogen and phosphorus. Third, maintaining a stable fermentation process within the rumen, small intestine, and hindgut of ruminants not only decreases the likelihood of metabolic disorders such as ketosis, but can reduce emissions of methane, an important greenhouse gas. Fourth, by reducing or shifting the populations of certain bacteria in the gut, there is a reduced need for the animal's immune system to respond, thus contributing to a healthier animal and improvement in animal welfare. Fifth, suppression of potential pathogens that may be present in low numbers can prevent important enteric diseases, which in a group setting, benefits overall flock

Table 22.5. Summary of performance benefits of antimicrobials.

Benefit	Avilamycin	Bacitracin	Bambermycin	Lasalocid	Monensin	Narasin	Salinomycin	Kitasamycin	Oleandomycin	Tylosin	Virginiamycin
Environmental Benefits											
Reduced methane emission (primarily ruminants)			+	+	+	+	+			+	+
Reduced nitrogen excretion (all species)	+	+	+	+	+	+	+			+	+
Reduced phosphorus output (all species)				+	+	+	+				+
Performance Improvements											
Increased rate of bodyweight gain	+	+	+	+	+	+	+	+	+	+	+
Lower feed requirements for each unit of gain	+	+	+	+	+	+	+	+	+	+	+
Improved carcass yield	+		+								
Improved sow performance							+				+
Improved piglet survival and growth							+				+
Increased dairy cow milk production				+	+						+
Increased wool growth			+								+
Disease Control											
Necrotic enteritis in poultry	+	+		+	+	+	+	+			+
Clostridial enteritis in pigs							+				+
Porcine proliferative enteropathy	+	+			+		+			+	+
Swine dysentery							+				+
Acute pneumonia in cattle				+	+	+	+				
Coccidiosis in calves and sheep				+	+	+	+				
Toxoplasmosis in ewes					+						
Prevention of Metabolic and Fermentative Disorders											
Decreased lactic acidosis				+	+	+	+				+
Decreased laminitis				+	+	+	+				+
Decreased ketosis					+						
Decreased ruminal bloat				+	+						
Other Benefits											
Protein sparing	+	+	+	+	+	+	+			+	+
Energy sparing	+	+	+	+	+	+	+			+	+
Improved mineral absorption				+	+						+
Improved heat tolerance	+			+	+	+	+				+
Decreased boar taint		+									+
Reduction in antibiotic resistance and its transfer		+	+								
Improved immune status		+								+	
Drier litter and reduced foot problems in broilers	+										+
Decreased fly survival in cattle feces				+	+						

Source: Page, 2003.

or herd health and welfare. Sixth, recent information suggests that a reduction in the variation in size of slaughter animals simplifies carcass processing and improves the quality of the meat product.

Economic Aspects of Performance Uses

The percentage improvement in performance of U.S. pigs fed an antimicrobial for performance is summarized in Table 22.6. Daily gain refers to the units of

Table 22.6. Percentage improvement in performance of pigs fed antimicrobials for specific years.

		Improvement, %	
Year	Periods[a]	Daily Gain	Feed/Gain
1950–1977	Starter	16.1	6.9
	Grower-finisher	4.0	2.1
1978–1985	Starter	15.0	6.5
	Grower-finisher	3.6	2.4

[a]Starter period from about 8 to 26 kg and grower-finisher period from 27 to 92 kg body weight.
Source: Zimmerman, 1986.

weight added per day, and feed efficiency is a measure of the amount of body weight gain per unit of feed consumed. The percentage of response was constant over the two periods compared, and for the two categories of pigs reported. The growth response (measured as percent gain) is greater in the starter than the grower-finisher; an observation consistent with actual use practices (Zimmerman, 1986; McBride et al., 2008).

The economic benefits of using antimicrobials in production animals have been described from various perspectives in the United States, including the consequences of their discontinued use (Zimmerman, 1986; U.S. General Accounting Office, 2004; McBride et al., 2008). Whereas the financial return per animal (in terms of gain accrued from the performance of an antimicrobial) is small, the cumulative effect on an industry that produces millions of cattle, sheep, and pigs and billions of chickens each year is economically significant and varies with the input feed costs. For an individual producer, the profit margin attributed to the use of a growth promoter can make the difference between profit and loss.

A US General Accounting Office report (2004) summarizes several studies that assess the economic effects from discontinuation of antimicrobial growth promotion in major food animal species. Several of the studies projected that to maintain animal production in the absence of growth promoters, an increase in the total number animals to produce the same amount of meat would be required and would actually increase the need for environmental resources. In general, the various reports describe a loss to the producer, with minimal food price increases of products at the retail counter.

Environmental Benefits

The environmental benefits of using antimicrobials in production animals arise from more efficient production by reducing the time needed to reach market weight, thereby lowering the quantity of feed and water required, hence less nitrogen and phosphorous excretion via urine and feces (Lawrence, 1998; Page, 2003). This improved feed efficiency means less land (and associated herbicides, fertilizers, agricultural equipment, etc.) required for crop production, as well as reductions in transportation costs for feed, etc. A major benefit for cattle raised with an ionophore such as monensin results from the reduction in the production and emission of methane, an important greenhouse gas (Tedeschi et al., 2003). In four European countries, an annual reduction of approximately 140–190 million cubic meters of methane from cattle was ascribed to the use of monensin (CEAS, 1991).

Prevention of Metabolic and Fermentative Disorders

In cattle, the use of ionophores in particular reduces ketosis and bloat, while virginiamycin reduces the risk of lactic acidosis in sheep and cattle (Page, 2003).

Disease Prevention

The intended use of antimicrobials for performance, and not disease prevention, does in fact, eliminate subclinical disease associated with bacterial, or in some cases, protozoal pathogens. The rationale is that food animals may be exposed to low numbers of pathogens that occasionally colonize the gut. In spite of the low antimicrobial concentrations present, there is sufficient activity to inhibit the small number of susceptible bacteria before they can multiply to achieve a "quorum" that results in clinical disease. Diseases such as necrotic enteritis in poultry, ileitis and clostridial enteritis in swine, and liver abscesses and coccidiosis in cattle may be prevented (Tsinas, 1998; Page, 2003).

Other Benefits

A diversity of other beneficial effects specific to individual antimicrobials include improved heat tolerance, increased mineral absorption, and enhanced immune status (Page, 2003).

Public Health Issues of Antibiotic Resistance

The critical public health issue associated with the use of antimicrobials for performance enhancement is that of antimicrobial resistance selection and the potential for resistant foodborne bacteria, or their resistance determinants, to cause a foodborne disease in humans that is subsequently less responsive to treatment. This is also discussed in chapter 3.

A brief chronology of reviews and major public health actions specific to antimicrobial use in animal feeds, mainly in the United States, is presented in Table 22.1 (U.S. Congress OTA, 1995; Institute of Food Technology, 2006). In the UK, two separate reports of the Netherthorpe Committee (1962, 1966), which had been formed to specifically "examine the possible consequences of the feeding of antimicrobials to farm animals," reviewed the potential public health impacts arising from the use of antimicrobials for growth promotion and concluded, perhaps surprisingly when viewed through the prism of today, there was no "reason to discontinue the permitted usage of feed additives," and indeed recommended that "the use of feed additives could be extended to young calves" (Swann, 1969). However, because of the emergence of transmissible resistance in the form of *Salmonella* Typhimurium phage type 29 in calves in the UK in 1963 (Anderson, 1968), the Netherthorpe committee recommended a new committee be formed to specifically review "the phenomenon of infective drug resistance, to consider the implications for animal husbandry and also for human and animal health."

The Swann Committee was established in 1968 with this objective. The final report of the Swann committee made a number of important recommendations about the use of therapeutic and feed additive antimicrobials including a recommendation that non-prescription antimicrobials used in feed should be restricted to those that "have little or no application as therapeutic agents in man or animals and will not impair the efficacy of a prescribed therapeutic antimicrobial … through the development of resistant strains of organisms" (Swann, 1969).

Following the recommendations of the Swann Committee in 1969, most growth promoters in Europe were non-therapeutic antimicrobials, ionophores, or synthetic compounds, hence the absence of tetracyclines and penicillins as growth promoters. During this period, public health officials were mainly concerned with Gram-negative zoonotic bacteria, especially *Salmonella, Campylobacter* and *E. coli*. Those antimicrobial growth promoters with primarily a Gram-positive spectrum of activity were thought to have the potential to provide Gram-negative bacteria with a competitive advantage if the protective Gram-positive flora were reduced (i.e., competitive exclusion barrier disruption).

In the United States during the 1970s, as a consequence of the UK actions, the FDA conducted several reviews of antimicrobial use in animal feeds. A new requirement in the Code of Federal Regulations (21 CFR 558.15) made it necessary for drug sponsors to conduct *Salmonella* shedding studies and *E. coli* resistance selection studies for all feed additive products. In 1977, the CVM issued a Notice of Opportunity for Hearing (NOOH) for subtherapeutic uses of penicillin and tetracycline. In 1978, a Congressional request to the National Academy of Sciences (NAS) was made and the National Research Council (NRC) undertook a review of the effects of subtherapeutic uses of antimicrobials.

In its 1980 report the NAS, while recognizing the potential lack of therapeutic efficacy associated with treating tetracycline-resistant *Salmonella* cases, the committee reported that the available data neither proved nor disproved human health effects from subtherapeutic uses in livestock (NAS, 1980). The U.S. House Appropriations Committee funded an FDA study in 1981, which was completed in conjunction with the Seattle-King County Department of Public Health in 1984. This study concluded that, "isolates from human cases and those from retail poultry had similar antimicrobial susceptibility patterns, including prevalence of 29.7% and 32.8%, respectively, for tetracycline resistance, which was found to be plasmid-mediated." During this year, the Secretary for Health and Human Services was petitioned by the Natural Resources Defense Council for suspension of subtherapeutic uses that posed an "imminent hazard." This was followed by hearings at the House committee level and by the FDA Commissioner as well as a review of the 1984 FDA study. As a result, in 1985, the Secretary denied the petition and in 1987 the FDA requested that the NAS initiate a quantitative risk assessment of the human health consequences associated with the use of penicillin and the tetracyclines at subtherapeutic concentrations in animal

feeds. The task was undertaken by the Institute of Medicine (IOM), which concluded that there was no definitive evidence of adverse effects to human health from subtherapeutic uses of antimicrobials in food animals, although they believed such effects could exist (IOM, 1989).

In 1988, Uttley et al. were the first to describe human infection with vancomycin-resistant enterococci (VRE), and so commenced an increased recognition of the importance of emerging Gram-positive bacteria as human pathogens. Enterococci are commensal bacteria, playing a vital, beneficial and usually innocuous role as a minor constituent of the bacterial flora of the large intestine of humans and most animal species. However, under special circumstances, especially in the critically ill or immunocompromised patient, enterococci (especially *E. faecium* and *E. faecalis*) can translocate from the intestine across the intestinal wall to cause bloodstream, urinary tract, and other infections, as well as colonize heart valves and implants. Among the healthcare-associated infections (HAIs) reported to the National Healthcare Safety Network, January 2006–October 2007, enterococcal infections ranked third in frequency in the United States (Hidron et al., 2008). Public health concern focused on antimicrobial growth promoters when it was realized in the early 1990s that many of the agents in widespread use are active against enterococci, select resistant strains that can be recovered from meat products, and have counterparts in human antimicrobial therapy.

A World Health Organization (WHO) Consultation was convened in Berlin in 1997 to (1) obtain an international consensus on priority medical problems arising from the use of antimicrobials in livestock production; and (2) recommend to WHO the next steps toward the development of guidelines for control and containment of the emergence of medically relevant antimicrobial resistance in food animals. The final report recommended that "the use of any antimicrobial agent for growth promotion in animals should be terminated if it is used in human therapeutics or known to select for cross-resistance to antimicrobials used in human medicine" (WHO, 1997).

This recommendation was subsequently modified by WHO in the Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food (WHO, 2000). Two recommendations were specifically targeted to antimicrobial growth promoter use. Recommendation 18 stated, "Use of antimicrobial growth promoters that belong to classes of antimicrobial agents used (or submitted for approval) in humans and animals should be terminated or rapidly phased out in the absence of risk-based evaluations. The termination or phasing-out should be accomplished preferably by voluntary programs of food animal producers, but by legislation if necessary." Recommendation 19 stated, "Risk-based evaluations of all antimicrobial growth promoters should be continued. Characterization of the risk may include consideration of the present and potential future importance of the drug to human medicine, its selection of resistance, the potential exposure to humans from resistant bacteria from food animals, as well as other appropriate scientific factors."

During the late 1990s, as a consequence of the recommendations of WHO (1997), a number of regional and national reviews of antimicrobial resistance and food animal antimicrobial use were conducted by expert panels appointed by various authorities (e.g., Joint Expert Technical Advisory Committee on Antibiotic Resistance [JETACAR], 1999; UK Ministry of Agriculture, Fisheries, and Food, 1998; European Commission SSC, 1999).

The recommendations of the European Commission Directorate for Consumer Policy and Consumer Health Protection's Scientific Steering Committee (SSC) on Antimicrobial Resistance (1999) stated that:

> Regarding the use of antimicrobials as growth promoting agents, the use of agents from classes which are or may be used in human or veterinary medicine (i.e., where there is a risk of selecting for cross-resistance to drugs used to treat bacterial infections) should be phased out as soon as possible and ultimately abolished. Efforts should also be made to replace those antimicrobials promoting growth with no known risk of influencing intestinal bacterial infections by non-antimicrobial alternatives. It is essential that these actions are paralleled by the introduction of changes in animal husbandry practices which will maintain animal health and welfare during the phase-out process. Thus, the phase-out process must be planned and coordinated since precipitous actions could have repercussions for animal health. Meanwhile, it should be reiterated to manufacturers and farmers

that the continuous feeding of antimicrobial growth promoters to food animals for the purpose of disease prevention is a contravention of EU regulations and represents misuse; more effective enforcement measures should be adopted.

The JETACAR recommendations established criteria for approval of growth promoters similar to those of Swann et al, (1969). The criteria included: demonstrable efficacy under local farming conditions, rare use of the antimicrobial as systemic therapy in humans and animals and use of an antimicrobial not considered critical therapy for human use, and low likelihood of impairing the efficacy of any other prescribed therapeutic antimicrobial(s) through the development of resistant strains. The Australian veterinary medicine regulatory authority was charged with using a risk analysis approach, including a cost-benefit analysis, for antimicrobial growth promoters. The prioritized review recommended by the JETACAR report included glycopeptides, streptogramins, and macrolides. Avoparcin was withdrawn from the marketplace worldwide and a risk assessment was not completed; virginiamycin has been reviewed and its continued use with label changes has been recommended; and the macrolide review is still pending in 2012.

Denmark hosted a WHO consultation in Foulum in 2002 that evaluated the Danish experience of removing growth promoters from animal production (WHO, 2003). The Danes declared the experiment in banning products successful, in spite of an increase in weaner pig mortality and no clear reduction in the prevalence of resistance in human pathogens. An independent review of the effects of the removal of antimicrobial growth promoters in Europe concluded that an increase in food animal disease resulted in increased therapeutic use of antimicrobials (Casewell et al., 2003). Phillips et al. (2004) published a critical review of the published literature on risks to human health from antimicrobial use in food animals and concluded that "there is little evidence that resistant enterococci from animals are a risk to human health."

In the United States, the position of the American Veterinary Medical Association (AVMA, 2009) on antimicrobials in livestock feeds calls for a transparent, science-based risk analysis to determine appropriate actions.

The theory and application of risk assessment to resistance selection by veterinary use of antimicrobial drugs and consequent impact on human health is a novel extension of traditional risk assessment methods that has been comprehensively reviewed by Cox (2005). Risk assessment is or should be a scientific and evidence-based process with clear description of all data sources, assumptions, and uncertainties. The ideal risk assessment will be supported by a sensitivity analysis of each assumption, allowing its importance to be evaluated. Key areas for further research should be clearly identified as new hypotheses are generated. The output of the risk assessment will be a description of the likelihood of harm to human health, presented as a range of credible values.

There are several antimicrobial risk assessments that have been published. One is for the streptogramin virginiamycin (Cox and Popken, 2004; Kelly et al., 2004). The U.S. FDA CVM (2004) posted an online draft risk assessment that also examined the likelihood of impaired therapeutic efficacy of human use quinupristin-dalfopristin (QD) as a result of the ingestion of streptogramin-resistant *E. faecium* (SREF) present on food commodities and arising from the use of virginiamycin in livestock and concluded the estimated risk was very low. QD is the sole member of the streptogramin class available for parenteral use in humans and is used to treat vancomycin-resistant *E. faecium* infection. Importantly, there is currently (2012) no approved use in humans of a streptogramin for infection with *Enterococcus faecium*. Pfaller (2006) reviewed bambermycin use and concluded there was no tangible human health issue. Risk assessments of the penicillins, tetracylines and macrolides have determined the likelihood of harm to humans to be exceedingly low (Cox, 2009, 2010; Mathers, 2011).

In spite of recommendations for evidence-based decision making and risk assessment, concerns among some groups persist and a Citizen's Petition was filed in the United States in 2005 by the Natural Resources Defense Council requesting action by FDA CVM on the NOOH for penicillin and tetracylines issued in the 1970s. In 2011, the FDA CVM rejected the Citizens Petition, but in 2012 this rejection was overturned by the United States District Court, Southern District of New York, which directed the FDA to proceed with the hearing process (U.S. District Court, 2012).

Judicious Use

The US FDA CVM Guidance for Industry #209 declares that performance uses of antimicrobials in feed are "in judicious use" and requests drug sponsors to voluntarily withdraw the indication from the product per the instructions in draft Guidance for Industry #213, as noted above. The Animal Health Institute (AHI) estimated that according to its member companies surveyed in the United States, only about 13% of the antimicrobial products sold for food animal use were intended for purposes other than therapeutic indications (AHI, 2012).

Alternatives to Performance Uses

A detailed and comprehensive summary of many of the currently available alternatives is beyond the scope of this chapter, although a more thorough discussion can be found elsewhere (Barug et al., 2006). A brief summary of products used in the feed or via systemic administration is given. In addition, improvements in animal husbandry, genetics and nutrition also have a profound and positive impact on animal production and health.

Dried distillers' grains (DDGs) are used as a supplemental animal feed ingredient that has become a common practice in many areas (University of Minnesota, 2012). Ethanol production from corn and other substrates is frequently contaminated with bacteria and this has led to the use of an antimicrobial to minimize the adverse effects of these bacteria during fermentation. Some of the antimicrobials are the same as those used for performance uses in food animals. The inclusion of small residual amounts of antimicrobial in the DDGs fed to food animals has been evaluated by FDA and the residues determined to pose no public health or food safety hazard and classified as GRAS (generally recognized as safe).

Because of concerns regarding the potential for selection of antimicrobial-resistant bacteria, residues, and environmental effects attributed to the use of antimicrobial growth promoters, a host of non-antimicrobial alternatives are available or are under investigation. In light of the evidence base and quality standards that apply to antimicrobial growth promoters, it is important that alternative products meet equivalent standards of efficacy, safety, and quality of manufacture. Sound decisions on product selection can only be made with confidence if the quality and strength of the evidence in support of claims are available for review and regulatory approval. Since innovative products may not fit traditional regulatory criteria, it is incumbent upon the regulatory agencies to be innovative in their review criteria.

Although not necessarily representative of the safety and quality of alternatives currently in widespread use, caution and the need for appropriate regulation has been underlined by a number of studies. For example, Alcid et al. (1994) recovered an isolate of *E. faecium* bearing the *van*B gene for vancomycin resistance from a probiotic preparation. Wagner and Cerniglia (2005), in a study of antimicrobial drug susceptibilities of anaerobes from a commercially available competitive exclusion product, found resistant *E. coli*, *Bacteroides* spp., and vancomycin-resistant *Lactococcus lactis*, and Ward et al. (2002) found that a variety of herbal products that included garlic and Echinacea caused large increases in the MIC of ampicillin in *E. coli* and *Staphylococcus aureus*. Weese (2002) investigated the composition of a variety of probiotic preparations concluded that most preparations studied were not accurately represented by their label claims.

Resistance to heavy metals is widespread in the environment and resistance genes are commonly situated on plasmids. Two heavy metals, zinc and copper, are widely used for growth promotion. Hasman and Aarestrup (2005) could not exclude the possibility that the use of copper in the diets of pigs in Denmark delayed the elimination of glycopeptide resistance *E. faecium*. Recently, an association between the appearance of MRSA in Danish pigs and the use of zinc has been described (Agersø et al., 2012).

Probiotics and competitive exclusion products (direct-fed microbials; e.g., *Saccharomyces cerevisiae*, Lactobacillus, and other microorganisms) are included in feeds as live microbial supplements. There are multiple modes of action that include competing against pathogens in the gut for binding sites or nutrients, stimulating the gut immune system, and production of bacteriocins such as nisin and lactocidin. Variable improvements in growth responses have been observed.

Prebiotics are indigestible carbohydrates that stimulate "beneficial" intestinal microflora. The best known

examples include the oligosaccharides such as mannanoligosaccharide. Trends toward performance improvements have been observed.

Enzymes such as phytase release phosphorus from orthophosphate groups, improving phosphorus bioavailability and reducing excretion. Other enzymes such as xylanase and glucanase break down plant-based feeds, allowing access to energy from complex carbohydrates. Variable responses in weanling pigs have been reported. A novel enzyme, beta-mannanase, improves digestibility of feeds, resulting in improved performance. Herbal additives such as essential oils, spices, and other plants have not generated a consistent disease prevention or performance response in swine studies.

Organic acids such as propionic, formic, fumaric, citric, and lactic acid can, as acidifiers, be inhibitory to enteric bacteria and improve overall performance by reducing competition for nutrients and reducing subclinical infections or production of bacterial toxins. The best responses are in young pigs. Immune system stimulators such as spray-dried plasma, egg yolk antibodies and conjugated linoleic acid feed supplementation may provide a degree of protection against pathogens.

Beta-adrenergic agonists (such as ractopamine and zilpaterol) act as repartitioning agents to modify carcass composition by shifting nutrient partitioning to increase muscle protein content and reduce fat deposition. Ractopamine, a non-hormonal, non-antimicrobial agent, increased growth rate (9%) and skeletal muscle (12%), and reduced feed:gain (12%) and adipose tissue (14%; Page, 2003). Ractopamine and zilpaterol have been approved by regulatory authorities in many countries, but not Europe.

Recombinant bovine and porcine somatotropins (rbST and rpST) have been associated with large increases in animal productivity. rbST results in increased milk yield and an improvement in efficiency. rpST results in lean muscle deposition. Until sustained delivery systems are available, both products are injected on a daily basis. These products have been approved for use in several countries.

Anabolic hormonal growth implants, such as estrogens, are used as implants in the ears of cattle. Heavy metals such as zinc, copper or chromium have been shown to decrease the incidence of post-weaning scours in pigs, although there are concerns about antimicrobial resistance selection and excretion of the metals into the environment.

Management practice improvements such as providing newborn calves with colostrum, allowing a longer weaning duration for piglets, and management of swine production as an "all-in-all-out" process have resulted in improved health and performance. Improvements to biosecurity, air quality, and stocking densities are now common and are associated with improved production. Improvements in chicken and swine breeds using genetic selection are ongoing and disease-resistant pigs and dairy cattle are being developed. Nutritional improvement practices, especially precision diet formulation, to achieve optimal diets with an appropriate balance of amino acids, vitamins, minerals, and carbohydrates are continuously being refined.

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Antimicrobial Therapy of Selected Organ Systems

Patricia M. Dowling

Antimicrobial Therapy of Osteomyelitis, Septic Arthritis, and Septic Tenosynovitis

Because of the variety of pathogens involved in musculoskeletal infections, appropriate samples must be submitted for microbiological culture and susceptibility testing. Because of the devastating consequences of bone, joint, or tendon sheath infections, aggressive antimicrobial therapy must be initiated as soon as soon there is sufficient evidence of infection. While awaiting culture results, initial antimicrobial selection can be chosen based on the clinical case characteristics and retrospective studies. Increasing rates of antimicrobial resistance in the typical pathogens makes treatment challenging.

Osteomyelitis

Osteomyelitis is acute or chronic inflammation of the bone and its structures secondary to infection with pyogenic organisms. The infection can be limited to a single portion of bone or can involve several regions such as the marrow, cortex, periosteum, the surrounding soft tissue and the synovial structures at the ends of the bone. Osteomyelitis can be hematogenous, traumatic or iatrogenic in origin. Hematogenous infections are seen almost exclusively in septic neonates and typically occur in a joint, epiphysis, or physis. In young animals, the endothelium of capillaries in the epiphysis is discontinuous, which allows extravasation of bacteria. Blood leukocytes are absent from this area, so tissue macrophages are the sole defense against bacterial invasion. The incompetence of these tissue macrophages appears critical to development of hematogenous metaphyseal osteomyelitis in young animals. Traumatic infections are usually secondary to a laceration or puncture wound and can infect bone, joint, tendon sheath and/or bursa and often involve multiple organisms. Iatrogenic infections are usually secondary to surgical procedures with or without implants. Osteomyelitis associated with implants presents the greatest treatment challenge, so broad-spectrum prophylactic antimicrobial therapy is indicated with any procedure that requires surgical implants (Johnson, 1994).

Microbial and host factors are both involved in the development of osteomyelitis. The bacteria involved in osteomyelitis have a range of extracellular and cell-associated factors that contribute to their virulence. Bacterial adhesins promote attachment to extracellular matrix proteins, which is crucial for colonisation of host tissues and implanted biomaterials. *Staphylococcus aureus* expresses several adhesins on its surface, each specifically interacting with one host protein component, such as fibrinogen, fibronectin, collagen, vitronectin, laminin, thrombospondin, bone sialoprotein, elastin, or von Willebrand factor. Other bacterial factors promote evasion from host defences (protein A, some toxins, capsular polysaccharides). A third set promotes invasion or tissue penetration by specifically attacking

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host cells (exotoxins) or degrading components of extracellular matrix (various hydrolases). Some pathogens involved in osteomyelitis produce biofilm, populations of bacteria that attach to a surface or to each other and embedded in a matrix of extracellular polymeric substance. Biofilm bacteria show altered phenotypes in terms of growth, gene expression, and protein production. Biofilms act as a diffusion barrier, slowing down the penetration of antimicrobials. Chronic osteomyelitis is characterised by infected necrotic bone and poor local vascularisation within a compromised soft tissue envelope. Systemic symptoms generally subside, but one or more foci in the bone still contain infected tissue or a sequestrum. The infected foci are surrounded by sclerotic, avascular bone covered by a thickened periosteum and scarred muscle and subcutaneous tissue. This avascular envelope makes systemic antibiotics essentially ineffective. Intermittent exacerbations can occur for years and only respond temporarily to antimicrobials.

Identification of the causative microorganisms is essential for diagnosis and treatment of osteomyelitis. Surgical sampling or needle biopsy of infected tissues are the best methods of diagnosis, as culture from swabs of ulcers or fistulae is often misleading. Sometimes only the histopathological examination of a bone-biopsy specimen with special staining procedures provides an accurate diagnosis of the infection (Lew and Waldvogel, 2004).

In a retrospective study of bacterial culture and susceptibility results from 233 horses and foals with musculoskeletal infections, 91% of the bacteria were aerobic or facultative and 9% were anaerobic (Moore et al., 1992). The common bacteria isolated included Enterobacteriaceae (29%), non-beta-hemolytic streptococci (13%), coagulase-positive staphylococci (12%), beta-hemolytic streptococci (9.4%), and coagulase-negative staphylococci (7.3%). Enterobacteriaceae are the most common bacteria associated with osteomyelitis in septic foals and calves. In septic foals, the most frequently affected bones are the femur, tibia and distal phalanx. With appropriate treatment, many affected foals will go on to have satisfactory athletic potential (Neil et al., 2010). Cases involving *Corynebacterium pseudotuberculosis* infection in horses are increasing in the United States. When *C. pseudotuberculosis* infection results in osteomyelitis or septic arthritis, the prognosis for survival is poor (Nogradi et al., 2012).

Salmonella dublin has been isolated from lesions of septic calves (Healy et al., 1997). *Arcanobacterium (Trueperella) pyogenes* is the most common causative agent of osteomyelitis in adult cattle (Verschooten et al., 2000). *Actinomyces bovis* causes mandibular pyogranulomatous osteomyelitis ("lumpy jaw") in ruminants (Seifi et al., 2003). Infectious pododermatitis ("foot rot") can progress to osteomyelitis, usually *A. pyogenes* and *Fusobacterium necrophorum* are involved (Silva et al., 2004). Osteomyelitis in dogs and cats is often associated with orthopedic surgical procedures (Bergh and Peirone, 2012; Maley et al., 2010). It is most commonly caused by *Staphylococcus pseudintermedius*, but there are increasing reports of *S. aureus* infections and methicillin-resistant strains of both (Schwartz et al., 2009;Weese et al., 2009). With trauma, infections may be polymicrobial, and may include mixtures of streptococci, enterococci, enterobacteriaceae (*E. coli*, *Klebsiella* spp., *Pseudomonas* spp.) and anaerobic bacteria. Hematogenous osteomyelitis is more common in young dogs or cats (Bradley, 2003), and only rarely occurs in adult (Rabillard et al., 2011).

Septic Arthritis and Tenosynovitis

Septic arthritis is inflammation of the joint space caused by a variety of opportunist pathogens that reach the joint hematogenously, by puncture, or by extension from adjacent infected tissues. The normal joint can withstand a large bacterial challenge, but with sufficient virulence and pathogenicity, the synovial defenses are overcome and infection is successfully established. With colonization of the synovium, a variety of enzymes, free radicals, and other inflammatory mediators initiate a marked synovial inflammatory response.

Septic arthritis from Gram-negative bacteria (*E. coli, Salmonella, Pseudomonas, Klebsiella*, etc.) is common in large animal neonates with failure of passive transfer. Involvement of more than one joint occurs in more than 50% of foals with septic arthritis; multiple joint involvement is uncommon in adult horses. In adult animals, septic arthritis and tenosynovitis commonly results from wounds or iatrogenic contamination with bacteria. In cattle, septic arthritis of the distal interphalangeal joint is usually an extension from interdigital pododermatitis ("foot rot"; Starke et al., 2007). In wounds, a variety of Gram-positive and Gram-negative bacteria are typical, whereas *S. aureus* and *S. pseudintermedius* are

the usual isolates from iatrogenic infections. Carriage of methicillin-resistant *S. aureus* and *S. pseudintermedius* is increasingly being reported in small animals, and complicates treatment of septic arthritis and tenosynovitis (Bergstrom et al., 2012; Owen et al., 2004; Weese, 2010).

Culture of synovial fluid is more diagnostic than culture of the synovial membrane. Synovial membrane biopsy can increase the chance of a positive culture result, but positive cultures are obtained from only 75% of cases (Schneider et al., 1992).

It is important that septic arthritis and tenosynovitis be treated as soon as possible to prevent articular cartilage destruction, tendon sheath adhesions and degenerative joint disease. A precise microbiological diagnosis is critical, but treatment can be started on the basis of Gram stain from joint or tendon sheath aspirates while awaiting culture results. Drainage of the joint or tendon sheath is essential to remove bacteria, debris, and inflammatory products that cause cartilage damage and adhesions, as well as to reduce intra-articular pressure that may cause ischemic necrosis (Bubenik, 2005). Repeated closed-needle aspiration (every 12 or 24 hours for 7–10 days) can be done in some veterinary patients. Joint aspiration may be adequate in early stages of septic arthritis but repeated distension irrigation or joint lavage is recommended if clinical improvement does not occur within 24–48 hours.

Prophylactic Antimicrobial Therapy

The use of prophylactic antimicrobials in surgical patients by veterinarians is routine, but much of the use is irrational with respect to choice of antimicrobial, timing, duration, and selection of surgical cases requiring prophylaxis (Dallap Schaer et al., 2012; Knights et al., 2012; Murphy et al., 2010; O'Connor et al., 2011; Weese and Cruz, 2009; Weese and Halling, 2006). Prophylactic antimicrobials are extensively evaluated in human medicine and veterinarians should follow their recommendations (see chapter 21 for a more complete discussion): (1) antimicrobials are only indicated in clean-contaminated, contaminated, or dirty procedures, not clean procedures (except with surgical implants are placed); (2) antimicrobials should be administered preoperatively and intravenously, ideally within 1 hour of incision; (3) antimicrobials should target the predicted bacterial contaminants; (4) antimicrobials should be restricted to a single dose, unless there is contamination of the surgical site or known preexisting infection; and (5) dosing can be repeated intraoperatively if more than 2 drug half-lives have passed after the first dose (Bratzler and Houck, 2005). There is limited data on the most appropriate choices for prophylaxis in veterinary patients. Factors such as cost, elimination half-life, safety, and antimicrobial resistance favor the use of older, relatively narrow-spectrum drugs. The use of newer, broad-spectrum drugs (e.g., third-generation cephalosporins such as cefovecin and ceftiofur) should be avoided in surgical prophylaxis to limit emergence of bacteria resistant to these antimicrobial agents. Attention to intraoperative temperature control and supplemental oxygen administration, along with aggressive fluid resuscitation, may decrease infection rates. Prophylactic antimicrobial therapy should be followed by close observation and treatment with appropriate antimicrobials and surgery if post-operative infection is diagnosed.

In the only published veterinary controlled trial of prophylactic antimicrobial therapy in dogs undergoing elective orthopedic surgery, prophylaxis decreased post-operative infection rate, but potassium penicillin G was as efficacious as cefazolin (Whittem et al., 1999). Equine surgeons prefer a combination of penicillin G (potassium or sodium) and gentamicin, but their dosing regimens commonly deviate from standard recommendations for surgical prophylaxis (Weese and Cruz, 2009).

Sytemic Antimicrobial Therapy

In acute osteomyelitis, parenteral treatment should start as soon as culture specimens are taken and be administered in high doses for at least 3 weeks and changed if necessary depending on susceptibility test results. Oral antimicrobial therapy is often ineffective. Antimicrobial therapy alone is adequate for the treatment of most cases of acute osteomyelitis, but chronic osteomyelitis requires aggressive and prolonged treatment that achieves pharmacodynamically appropriate, local concentrations of bactericidal drugs. Apart from antimicrobial therapy, the cornerstones of osteomyelitis treatment include debridement and sequestrectomy, open wound drainage, fracture stabilization, and grafting of bone deficits (Bergh and Peirone, 2012; Maley et al., 2010; Rahal et al., 2003; Schwartz et al., 2009; Weese et al., 2009). Thorough debridement of bone and soft tissue to remove necrotic debris, purulent material, and avascular bone is imperative for treatment success.

Wound debridement should be combined with appropriate stabilization of unstable fractures and mineralization or removal of metallic implants. Stable fractures can heal in the face of infection. At the time of debridement, affected tissue should be obtained for culture and sensitivity to assist the clinician in choosing the most appropriate antimicrobial drug.

Antimicrobial therapy should be with bactericidal drugs, ideally administered parenterally for 2 weeks with subsequent orally administered drugs for a further 4–6 weeks. Most antimicrobials traverse the capillary membrane in normal and infected bone, and concentrations in bone closely parallel plasma concentrations. Vascular thrombosis and ischemia of infected bone and synovium can limit the delivery of systemic antimicrobials in sufficient concentrations to eradicate the infection.

For most bone and joint infections caused by beta-lactamase producing staphylococci, cephalosporins, clindamycin or ampicillin-sulbactam will be effective. Newer human macrolide antimicrobials (azithromycin, clarithromycin) may also be efficacious. In small animals, clindamycin and metronidazole are used for anaerobic infections. The aminoglycosides and fluoroquinolones also typically have good activity against staphylococci, along with excellent activity against Gram-negative pathogens. While amikacin usually has good activity against *Pseudomonas* spp., it has poor activity against streptococci compared to gentamicin. Because of nephrotoxicity and ototoxicity related to duration of treatment, the aminoglycosides are often reserved for treatment of musculoskeletal infections by local delivery techniques. The fluoroquinolones have excellent broad-spectrum antimicrobial activity. Good safety profiles and the availability of injectable and oral formulations make them popular choices for treatment musculoskeletal infections in many veterinary patients, but resistance in MRSA and MRSP is an increasing problem (Owen et al., 2004; Weese, 2010).

Local Antimicrobial Drug Delivery

Antimicrobial drug delivery systems (DDS) have been developed for use in human and veterinary patients, providing sustained high local drug concentrations while minimizing systemic toxicity. An antimicrobial DDS can achieve high drug concentrations at the site of infection while maintaining low systemic drug levels and avoiding possible adverse effects (Wang et al., 2002). Local administration of antimicrobials can be done with biodegradable and non-biodegradable implants, constant rate infusion or indwelling catheter systems, local injection and regional limb perfusion either by intravenous or intraosseous routes.

Non-biodegradable Antimicrobial Impregnated Implants

Polymethylmethacrylate (PMMA) is a synthetic polymer product marketed in the powder form in North America. Antimicrobials in powder form can be added to the polymer to make non-biodegradable antimicrobial impregnated implants for the treatment of osteomyelitis and septic arthritis in large and small animals and wildlife. In Europe, PMMA is available in combination with gentamicin in premade beads (Septopal) but are easily compounded in North America. Antimicrobials used to make PMMA beads must be heat stable, as the combination of the liquid monomer and the powder polymer produces an exothermic reaction. The antimicrobial must have adequate elution characteristics to produce a sustained and appropriate release from the bead. Antimicrobial elution from the PMMA bead depends upon the pore size, permeability, size and the shape of the implant, the type of antimicrobial and the amount present in the bead (Weisman et al., 2000). Combinations of antimicrobials in beads may not be as effective as single agent beads (Phillips et al., 2007). The amount and rate of wound exudation also affects the elution kinetics of the antimicrobial from the bead (Streppa et al., 2001). Release of an antimicrobial from PMMA is bimodal. There is a rapid release during the first 24 hours after implantation followed by a continuous sustained release that can last from weeks to years (Calhoun and Mader, 1989). Osteomyelitis in horses, cattle, dogs and exotic animals has been successfully treated using PMMA beads, including osteomyelitis due to methicillin-resistant staphylococci (Butson et al., 1996; Haerdi-Landerer et al., 2010; Hartley and Sanderson, 2003; Hespel et al., 2012; Kelly et al., 2012; Trostle et al., 2001). Due to potential synovial irritation and lameness, PMMA use is not recommended inside joints. Perhaps the most negative aspect of their use is their non-biodegradable nature. Although most tissues do not seem to react to the presence of the beads, tissue irritation is possible and in these cases implant removal is recommended.

Biodegradable Antimicrobial Impregnated Implants

Various biodegradable DDS such as collagen sponges, hydroxyapatite cement, plaster of Paris, polyanhidrides, polylactide-polyglycolide and crosslinked high amylase starch have been explored for use in DDS. Their major advantage over PMMA is that a second surgery for removal is not necessary.

Collagen Sponges. Commercially available gentamicin impregnated collagen sponges are available in Europe but not in North America. Their clinical use has been reported in cattle, horses and dogs (Delfosse et al., 2011; Haerdi-Landerer et al., 2010; Ivester et al., 2006; Owen et al., 2004; Renwick et al., 2010). In contrast to PMMA beads, complete elution from collage sponges occurs in a period of 2 weeks with high elution rates during the first week. The main disadvantages of gentamicin-impregnated collagen are the expense and the potential for adverse reactions to a foreign protein as its source is bovine collagen.

Plaster of Paris. Plaster of Paris (POP) is an inexpensive, readily available material that has been investigated for use as an antimicrobial DDS. Plaster of Paris gentamicin-impregnated beads are inexpensive, biocompatible, biodegradable, osteoconductive, and easily manufactured using liquid antimicrobials and a bead mold (Atilla et al., 2010; Santschi and McGarvey, 2003). The relatively short duration of high concentrations of antimicrobial eluted from POP beads suggest that they may be ideal for antimicrobial prophylaxis in high-risk situations such as fracture repair.

Constant Rate Infusion or Indwelling Systems

Constant delivery of antimicrobials is indicated for infections of synovial cavities such as joints or tendon sheaths. Primarily used in horses, commercial constant rate infusion pumps or "in-house" manufactured delivery systems can be used. Affected structures treated with this system include the distal interphalangeal, metacarpo/tarso-phalangeal, intercarpal, radiocarpal, scapulohumeral, tarsocrural, and medial femoropatellar joints, carpal canal and the digital, tarsal, and extensor carpi radialis tendon sheaths. Horses tolerate the tubing well with no apparent discomfort and with only mild soft tissue swelling as a complication. This method allows frequent administration of high concentrations of the appropriate antimicrobial to the infected site. Daily joint lavage can be carried out through the same system.

Intra-articular Injections

Intra-articular or intrasynovial injection of antimicrobial drugs achieves high synovial fluid and bone concentrations with low doses (Werner et al., 2003). Gentamicin, amikacin, and ceftiofur are most frequent used, frequently in conjunction with chondroprotectives such as hyaluronan. Intra-articular or intrasynovial antimicrobials are usually infused after daily through and through lavage. As intra-articular or intrasynovial injection does not produce similar high concentrations in the surrounding soft tissues, systemic antimicrobials are often used concurrently.

Regional Perfusion

Regional limb perfusion (RLP) techniques are used predominantly in large animals to deliver very high antimicrobial concentrations in the distal extremities using the venous system, which is isolated from the systemic circulation by the controlled application of a tourniquet. Pressurizing the venous system allows diffusion of antimicrobials into ischemic tissues and exudates. The RLP techniques are limited to distal extremity areas as it is impossible to isolate regions of the proximal extremity successfully. Therefore areas above the mid-radius and mid-tibia in the fore and hind limb respectively are not good candidates for this technique. Regional limb perfusion can be done by the intravenous or intraosseous route and is easily done in the standing, sedated horse. Intravenous RLP is carried out by catheterizing the cephalic, saphenous, and palmar or plantar metacarpal/metatarsal veins (Kelmer et al., 2009; Kelmer et al., 2012). However, any visible and accessible vein can be used safely to administer antimicrobials. Alternatively, an antimicrobial solution can be administered by the intraosseous route by infusion into the medullary cavity of the cannon bone, tibia or radius (Butt et al., 2001; Mattson et al., 2004). While both RLP techniques are efficacious, many horses and cattle with septic arthritis and tenosynovitis following trauma have generalized cellulitis of the limb, making localization of a superficial vein extremely difficult. If a superficial vein is localized, its

structure is often disrupted by venipuncture, making repeated catheterization difficult. In addition, digital IV catheters are difficult to maintain in large animal patients. The intraosseous perfusion technique eliminates the need to find a vein, repeated venipuncture or catheterization of distal veins, and enables repeated local perfusion with relative ease.

There are many unanswered questions regarding the appropriate choice and dose of antimicrobial, the best perfusion volume, the optimal number of perfusions, and the appropriate interval between perfusions for horses or cattle with septic conditions in a distal limb. Currently, it is recommended that the distal limb be perfused once daily for a total of 4 days.

Gentamicin, amikacin, and ceftiofur are most frequent used (Butt et al., 2001; Kelmer et al., 2012; Mattson et al., 2004; Pille et al., 2005; Werner et al., 2003; Whithair et al., 1992), but the emergence of antimicrobial resistance has increased the use of enrofloxacin, vancomycin, and imipenem (Fiorello et al., 2008; Parra-Sanchez et al., 2006; Rubio-Martinez et al., 2005; Rubio-Martinez et al., 2006). Adverse effects from regional perfusion are not well investigated, but high doses of aminoglycosides have been reported to cause toxic osteonecrosis secondary to intraosseous perfusion, and enrofloxacin may cause vascultitis with intravenous perfusion (Parker et al., 2010; Parra-Sanchez et al., 2006).

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Infections of the Eyes: Conjunctivitis, Keratitis, and Endophthalmitis

While the eye is relatively impermeable to microorganisms, if structural damage occurs sight-threatening bacterial and fungal infections can easily develop. Ocular antimicrobial therapy differs from treating infections in other tissues because drugs can be administered directly

to the eye, directly achieving high drug concentrations. However, there are only a limited number of veterinary-approved antimicrobials for topical ophthalmic use, so practitioners need to make rational antimicrobial choices and extra-label drug use is sometimes necessary for successful therapy. Practitioners should avoid using antimicrobials to treat non-infectious ocular conditions such as uveitis or allergic conjunctivitis. Unwarranted antimicrobials have no effect on an inflammatory disease process and encourage antimicrobial resistance. If a clear decision cannot be made between using an antimicrobial or an anti-inflammatory drug, consult a veterinary ophthalmologist.

Culture and Susceptibility Testing of Ocular Pathogens

As with an infection in any other tissue, tentative identification of the pathogen(s) involved in ocular infections is essential in choosing appropriate antimicrobial therapy. Perform a Gram stain on corneal ulcer scrapings to initially identify pathogens as Gram-positive or Gram-negative bacteria or as fungi. The immediate information gained from cytology is invaluable in directing initial antimicrobial therapy (Massa et al., 1999). Cytology is essential when therapy has already been initiated and "no growth" culture results are more likely. Microbiological culture and susceptibility results help direct therapy, but the practitioner should be cautious when interpreting susceptibility profiles, as the "S" (susceptible), "I" (intermediate), and "R" (resistant) classification is based on achievable plasma antimicrobial concentrations. Since extremely high local drug concentrations are attained with topical or subconjunctival application, an antimicrobial may be effective despite the pathogen being classified as resistant by the diagnostic laboratory.

In cases of canine infectious conjunctivitis and keratitis, *Staphylococcus pseudintermedius*, *Streptococcus* spp. and *Pseudomonas aeruginosa* are the most commonly identified pathogens (Tolar et al., 2006). Methicillin-resistant *Staphylococcus aureus* has been reported in chronic keratitis in a dog (Tajima et al., 2013). Etiology of infectious keratitis in cats is likely to be similar in dogs, with the addition of *Chlamydophila felis* and *Mycoplasma felis*. In cases of equine infectious keratitis, there is initially an equal distribution of Gram-positive and Gram-negative bacteria. The Gram-positive organisms are predominantly *Staphylococcus* spp. or *Streptococcus zooepidemicus*, but the Gram-negative bacteria most frequently isolated are *Pseudomonas* spp. Therefore, when treating horses, it is important to choose initial antimicrobial therapy that is effective against *Pseudomonas* spp. and other Gram-negative bacteria (Clode, 2010; Keller and Hendrix, 2005; Moore et al., 1995; Sauer et al., 2003). After initial antimicrobial treatment, isolation of Gram-negative bacteria increases and *Pseudomonas* spp. and *S. zooepidemicus* isolates are increasingly resistant to antimicrobials (Sauer et al., 2003).

In ruminants, there are several pathogens that may cause primary infectious keratitis, or "pinkeye." The major pathogens are *Moraxella bovis* in cattle, *Mycoplasma conjunctiva* and *Branhamella ovis* in goats and sheep, and *Chlamydophila psittaci* in sheep.

Fungal infections of the eye are rare in dogs, cats and ruminants, but are frequently encountered in horses. Moore et al. (1995) reported that 38% of equine infectious keratitis cases were infected with fungi. *Aspergillus* and *Fusarium* spp. are the most common fungi isolated from ulcerative keratomycosis in horses (Andrew et al., 1998).

Topical Drug Administration

Topically applied ophthalmic drugs distribute to the eye by three routes: transcorneal penetration, absorption by conjunctival blood vessels that flow into the ciliary body, and drainage and absorption through the nasolacrimal system. Transcorneal penetration is the most important consideration for therapy of ocular infections. Drainage and absorption through the nasolacrimal system contributes little to ocular therapy but is responsible for most adverse systemic effects. Commercial droppers deliver 25–50 μl/drop of solution or suspension, but only 10–25 μl are retained in the conjunctival fornix and tear film after immediate overflow. Therefore application of more than one drop at a time does not increase the drug concentration on the ocular surface. After 5 minutes, only 20% of the drug remains on the ocular surface, the rest is absorbed through the cornea and conjunctiva or removed by the nasolacrimal drainage system. Because the epithelium-stroma-endothelium of the cornea is essentially a lipid-water-lipid sandwich, only drugs like chloramphenicol and fluoroquinolones that have both hydrophilic and lipophilic characteristics can penetrate the intact cornea easily. However, when

trauma or a disease process disrupts normal corneal integrity, most antimicrobials achieve effective concentrations in the infected tissue.

Topical ophthalmologic drugs are formulated as ointments, solutions or suspensions. Deciding which formulation to use depends on several practical considerations. Ocular contact time of ointments is longer than solutions or suspensions, so they are more practical when the owner cannot follow a frequent administration regimen. Solutions and suspensions may be easier for some owners to apply than ointments. Avoid ointments on penetrating wounds or a descemetocele, and prior to intraocular surgery, as their petroleum base elicit a severe granulomatous reaction when in direct contact with intraocular tissues.

The application frequency of topical antimicrobials depends on the disease and the drug formulation. One drop of an antimicrobial solution applied 4 times daily is usually sufficient for antimicrobial therapy of uncomplicated corneal ulcers and bacterial conjunctivitis. When ointments are used, a 5 mm strip is applied to the conjunctiva a minimum of 3 times a day. Severe ocular infections may need to be treated more frequently. Subpalpebral and nasolacrimal lavage systems are not well tolerated in small animals, but work well for intensive topical therapy in horses. If more than one drug is involved in the therapeutic regimen, then 3–5 minutes should be allowed between application of each medication to avoid dilution or chemical incompatibility. Antimicrobial therapy is typically continued for 7 days or until the ocular infection is resolved.

Topical Antimicrobials

There are few veterinary formulated ophthalmic antimicrobials available. There are a number of veterinary ophthalmic antimicrobial-corticosteroid combinations, but most ophthalmologists do not recommend the use of fixed ratio antimicrobial-corticosteroid formulations. Corticosteroids are contraindicated with infectious keratitis, and ocular diseases that require corticosteroids to treat an inflammatory process typically do not require antimicrobial therapy. Many ophthalmology references recommend that practitioners compound drugs for ophthalmic use or "fortify" commercially available ophthalmic antimicrobials. Compounding drugs or adding injectable drugs to ophthalmic products carries the risks of chemical incompatibilities and contamination. This practice is usually unnecessary if an accurate diagnosis is made and an aggressive treatment regimen is instituted with commercially available ophthalmic products.

A first choice antimicrobial for corneal ulcers and bacterial conjunctivitis or prophylaxis against surface infection is a "triple antibiotic." Triple antibiotic ointment or solution contains neomycin, bacitracin and polymixin B. This combination provides broad-spectrum antimicrobial activity. These drugs are not lipid-soluble but penetrate the stroma when the corneal epithelium is disrupted. Neomycin is a typical bactericidal aminoglycoside with good activity against *Staphylococcus* spp. and Gram-negative bacteria. *Pseudomonas* spp. are often resistant to neomycin, but polymixin B is rapidly bactericidal against Gram-negative bacteria including *Pseudomonas* spp. Due to systemic toxicity, polymixin B is only used topically, so it is not typically included on susceptibility reports from microbiology services, but in a retrospective study, 100% of *Pseudomonas aeruginosa* isolates were susceptible to polymixin B (Hariharan et al., 1995). Polymixin B also binds and inactivates endotoxin, reducing inflammation and tissue destruction. The third component of triple antibiotic ointment is bacitracin. Like polymixin B, bacitracin is a topical product not routinely included on susceptibility reports. Bacitracin is active against Gram-positive bacteria, with a mechanism of action similar to the beta-lactam antibiotics. Penicillins and cephalosporins are not used as commercial ophthalmic formulations due to the risk of contact sensitization, so bacitracin is their equivalent. Use of triple antibiotic was associated with selection for bacitracin-resistant *Streptococcus zooepidemicus* in cases of equine keratitis (Keller and Hendrix, 2005). Polymyxin B-containing ophthalmic formulations have been associated with anaphylaxis in cats (Hume-Smith et al., 2011).

Gentamicin is available as an ophthalmic solution and ointment. Because of its pharmacokinetic characteristics, gentamicin does not readily cross lipid membranes, but it readily penetrates the stroma when the corneal epithelium is damaged. Gentamicin is a bactericidal aminoglycoside with activity against many Gram-negative pathogens, including many *Pseudomonas* spp. *Staphylococcus* spp. are usually susceptible to gentamicin. *Pseudomonas* spp. and *Streptococcus zooepidemicus* may become resistant to gentamicin during therapy, so patients should be closely monitored for

appropriate clinical response (Sauer et al., 2003). Non-responsive cases should have microbiological culture and susceptibility testing repeated.

Chloramphenicol is available in veterinary formulations as an ointment. Chloramphenicol is soluble in both water and lipid, so it penetrates intact cornea with topical administration. Therefore, it is a good treatment choice for corneal stromal abscesses covered by intact epithelium. Chloramphenicol is a broad-spectrum, bacteriostatic antimicrobial, with excellent activity against *Chlamydophila* and *Mycoplasma* spp. North American MRSA and MRSP isolates are typically susceptible to chloramphenicol (Tajima et al., 2013). However, it is less effective than the aminoglycosides or fluoroquinolones against some Gram-negative bacteria and typically has poor efficacy against *Pseudomonas* spp. Chloramphenicol is a good first choice antimicrobial for corneal ulcers and bacterial conjunctivitis in small animals. Because of the high incidence of *Pseudomonas* spp. involved in equine infectious keratitis, chloramphenicol is not an ideal choice for empirical therapy in horses.

Tetracycline ointment is a broad-spectrum, lipid-soluble, bacteriostatic antimicrobial with good activity against the pathogens that cause infectious feline conjunctivitis and infectious keratoconjunctivitis in ruminants.

Erythromycin is available as a human-labelled ophthalmic ointment that is well-tolerated in cats. As a macrolide, erythromycin is lipid-soluble and its spectrum of activity includes Gram-positive bacteria and *Mycoplasma* and *Chlamydophila* spp. Staphylococci readily develop resistance to erythromycin.

Intramammary antimicrobial formulations are often used topically to treat infectious keratoconjunctivitis ("pink eye") in cattle.

Non-responsive, progressive corneal ulceration results from infection with antimicrobial-resistant pathogens, including *Staphylococcus* spp. and *Pseudomonas* spp. Cytolytic toxins from staphylococci damage cell membranes and destroy polymorphonuclear leucocytes. *Pseudomonas* spp. exoproteins and enzymes released from neutrophils cause collagenolysis. Severe corneal infections from these pathogens may be treated with human-labelled formulations of tobramycin or a fluoroquinolone. Tobramycin is an aminoglycoside that is effective against most gentamicin-resistant *Pseudomonas* spp. and beta-lactamase-producing staphylococci. Ciprofloxacin and ofloxacin are human-labelled fluoroquinolone antimicrobials, with broad-spectrum bactericidal activity and high lipid solubility. They are effective against beta-lactamase-producing staphylococci and aminoglycoside-resistant *Pseudomonas* spp. Neither tobramycin nor the fluoroquinolones are very effective against streptococci. Because of their spectrum of activity, these antimicrobials should not be used for empirical treatment of ocular infections. Their use should be dictated by microbiological culture and susceptibility results. Vancomycin should only be used when no other options are available (Tajima et al., 2013).

Topical Antifungal Drugs

There are few antifungal drugs available for ophthalmic use, so fungal keratitis often requires compounding of other antifungal formulations. These cases are difficult to manage successfully and referral to a veterinary ophthalmologist is advised.

Miconazole is an imidazole derivative with broad antifungal activity. It is often considered the first choice for treatment of mycotic keratitis in horses because of its activity against *Aspergillus* spp. (Andrew et al., 1998). In the countries where there are available formulations, a 1% intravenous solution (10 mg/ml) is applied directly on the eye. Alternatively, the 2% veterinary dermatological cream may safely be applied directly to the eye. Miconazole lotions or sprays that contain ethyl alcohol should not be applied to the eye.

Under the direction of a veterinary ophthalmologist, other azole derivatives such as fluconazole, clotrimazole, voriconazole, and itraconazole can be formulated for the treatment of equine mycotic keratitis. Amphotericin B may be used as a topical treatment of mycotic keratitis when there is resistance to other antifungal drugs, but this is a difficult drug to formulate properly for ophthalmic use. Natamycin is available in the United States as a 5% ophthalmic suspension. It has broad-spectrum activity against yeast and fungi and is the treatment of choice for *Fusarium* infections.

Antiviral Ocular Drug Therapy

Herpes keratitis has only been well documented in the cat, but there are anecdotal reports in dogs and horses. Corticosteroids can accelerate the spread of viral infections so they should not be administered concurrently. Clinically, some cats do appear to respond to antiviral

drugs (Andrew, 2001; Malik et al., 2009). However, herpes infections can go into remission without treatment and it is difficult to determine a specific antiviral treatment regimen that is clinically superior. All of the antiviral drugs are labelled for human use. The topical antivirals are static in action and topically irritating, so frequent administration is necessary and client compliance and patient tolerance is an issue. Trifluridine is incorporated in place of thymidine into viral DNA, resulting in faulty DNA and the inability to replicate or destroy tissue. Trifluridine does penetrate the intact cornea, and ulceration and uveitis increases trifluridine's intraocular penetration. Trifluridine is administered 4–9 times daily for 2 days and then the frequency is reduced over the next 2–3 weeks. If trifluridine is too irritating, then one of the other products may be tried. Vidarabine ointment interferes with viral DNA synthesis. It is poorly lipid-soluble, so corneal penetration is minimal unless ulceration is present. Suggested treatment is to apply a small amount of ointment 5 times daily until corneal re-epithelialization is complete, then every 12 hours for 7 days. Idoxuridine solution interferes with viral DNA replication by substituting for thymidine in the same manner as trifluridine. Idoxuridine does not penetrate the cornea unless the epithelial barrier is broken. Suggested treatment is to apply 1 drop every 4 hours until corneal re-epithelialization occurs. Idoxuridine inhibits DNA formation in the cornea; therefore, prolonged or too frequent administration may damage the corneal epithelium and prevent ulcer healing. Systemic treatment with antiviral drugs may also be useful in some cats (Malik et al., 2009).

Subconjunctival Antimicrobial Therapy

Drug injection into the bulbar subconjunctival space avoids tear dilution and directly bypasses the conjunctival epithelial barrier and rapidly delivers a high concentration in the anterior segment of the eye. Medications injected into the subconjunctival space reach the anterior segment directly through the ciliary circulation and indirectly by leaking from the injection site to be absorbed through the cornea and the conjunctiva. Antimicrobials should not be placed under the palpebral conjunctiva, as blood circulation in this area is directed away from the eye. Therapeutic antimicrobial concentrations are usually maintained for 3–6 hours after a subconjunctival injection, then taper off slowly over the next 24 hours. Subconjunctival injections are indicated if frequent topical application cannot be done. Severe conjunctival irritation may occur with repeated daily injections. Other potential complications include granuloma formation and inadvertent intraocular or intra scleral injection. The antimicrobials most often used for subconjunctival injection are penicillins, cephalosporins, gentamicin, and miconazole.

Systemic Antimicrobial Therapy

Systemic administration is necessary to achieve therapeutic drug concentrations in the lids, lacrimal system, orbit, and posterior ocular segment. The passage of drugs into the eye is normally limited by the blood-ocular barriers and concentrations attained in the aqueous humour are often similar to that attained in the cerebrospinal fluid. This is due to similarities of the blood-ocular barrier to the blood-brain barrier. However, inflammation disrupts the blood-ocular barriers and improves drug penetration. Peak plasma concentrations promote the passage of the antimicrobial into the eye; therefore intravenous routes of administration are preferable to oral, intramuscular or subcutaneous routes. Initial antimicrobial therapy should be chosen on the basis of cytologic evaluation of fine needle aspirates from the infected eye, eyelid, or orbit. Choice of therapy should be re-evaluated when culture and sensitivity information is available. Bacterial endophthalmitis associated with surgical contamination is often due to Gram-positive bacteria, so cefazolin is appropriate for surgical prophylaxis as well. Traumatic perforations of the eye may involve both Gram-positive and Gram-negative bacteria, so a beta-lactam combined with a fluoroquinolone would be a rational choice. Bacterial blepharitis, dacryocystitis and orbital cellulitis is likely due to skin flora such as *Staphylococcus* spp., so beta-lactamase-resistant antimicrobials such as cephalexin or amoxicillin/clavulanic acid are appropriate first choices for therapy. Systemic administration of most antimicrobials (e.g., tetracyclines, macrolides) approved for the treatment of Bovine Respiratory Disease will produce adequate concentrations in tears for the effective treatment of infectious keratoconjunctivitis in ruminants (Alexander, 2010; Brown et al., 1998).

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Bacterial Meningitis

Sixty years after the introduction of antimicrobials, bacterial meningitis remains an important cause of mortality and morbidity in human beings and animals (Fecteau and George, 2004; Radaelli and Platt, 2002; Smith et al., 2004; Toth et al., 2012; Uiterwijk and Koehler, 2012). Bacterial meningitis is unique among infectious diseases in that clinical outcome is suboptimal despite bacteriologic cure of the infection. Despite advances in the diagnosis and treatment of neonatal bacterial septicemia, mortality rates of 100% for bacterial meningitis are reported in animals (Green and Smith, 1992). Powerful, broad-spectrum antimicrobials have not improved the outcome of bacterial meningitis because the inflammatory host response to bacterial products continues after the bacteria are killed with antimicrobials. The host response damages tissues and contributes significantly to central nervous system (CNS) injury. Treatment of bacterial meningitis in human medicine is now utilizes "partner drugs" to both kill bacteria and limit the detrimental effects of the immune response in the CNS (van der Flier et al., 2003). The optimal approach to treating bacterial meningitis in animals consists of early detection of clinical signs, rapid determination of the pathogen(s) involved and their antimicrobial susceptibility, selection of an antimicrobial that achieves therapeutic concentrations in the cerebrospinal fluid (CSF) and administration of drugs to moderate the potentially destructive immune response (Deghmane et al., 2009). Underlying deficiencies such as failure of passive transfer of antibodies also need to be corrected.

Pathogenesis of Bacterial Meningitis

Meningitis is a complex infection that differs pathophysiologically from peripheral bacterial infections. In large animals, meningitis usually occurs in neonates secondary to septicemia and bacteremia associated with failure of passive transfer of colostral antibodies (Viu et al., 2012). As meningitis is a localized manifestation of septicemia, concurrent problems commonly include omphalophlebitis, panophthalmitis, polyarthritis, pneumonia, and enteritis. In order to cause meningitis, bacterial pathogens must sequentially invade and survive in the intravascular space, cross the blood-brain barrier (BBB), and survive in the CSF (Webb and Muir, 2000). The initial host defense against sustained bacteremia is circulating complement, particularly through the alternative complement pathway that does not require specific antibody for activation. Evasion of the alternative complement pathway allows bacteria to survive in circulation. After successful hematogenous dissemination, bacteria are transported to the CNS and localize in the choroid plexus. Subsequently, bacteria enter the ventricular system and are transferred to the subarachnoid space via normal CSF flow. The least understood step in the pathogenesis of meningitis is the mechanism of

bacterial penetration of the BBB and entry into the CSF (Tuomanen, 1993). Work with bioactive cell wall fragments from pneumococcus suggests a single glycopeptide to be responsible for allowing bacterial penetration of the BBB. For strains of *E coli*, important causes of foal and calf meningitis, fimbriation appears to be an important virulence factor. Once bacteria gain access to the CSF, defenses against bacterial invasion are limited, allowing rapid and unchecked bacterial proliferation. Humoral defenses, particularly immunoglobulins and complement, are virtually absent from the CSF. These components of inflammatory defense must be derived from serum. Opsonic activity is undetectable in normal CSF and increases inconsistently during BBB breakdown. The inflammatory response appears in the CSF only when a threshold amount of bacterial components (approximately 10^5 bacteria) is reached.

Brain injury and neuronal death due to bacterial meningitis involves a combination of altered cerebral metabolism, cerebral edema, increased intracranial pressure, decreased cerebral blood flow, altered CSF dynamics and leukocyte-mediated injury to neuronal tissue. Gram-negative lipopolysaccharide (LPS), Gram-positive peptidoglycan, and cytotoxins engage Toll-like receptors of the endothelia and activate their downstream signaling cascades. The endothelial cells then release mediators, including tumor necrosis factor alpha, nitric oxide, and matrix metalloproteinase-2, which increase endothelial permeability. The endothelium expresses multiple leukocyte adhesion molecules and presents chemotactic factors such as interleukin-8 (IL-8) when activated by inflammatory mediators. This combination promotes neutrophil adherence and transendothelial migration. Up regulation of endothelial tissue factor triggers a procoagulant state and stimulates thrombus formation. Endothelial activation and release of vasoconstrictors, such as the endothelins, and vasodilators, such as nitric oxide, impair autoregulation of cerebral perfusion pressure. Along with systemic hypotension in critically ill patients, these events further decrease cerebral perfusion and exacerbate neuronal death.

Etiology of Meningitis

The etiology and epidemiology of bacterial meningitis varies with species. Bacterial meningitis in dogs and cats usually occurs in adults animals from hematogenous spread from distal infections (enteritis, prostatitis, metritis, pneumonia) or from direct extension of non-CNS infections, such as otitis interna (Cook et al., 2003; Meric, 1988; Radaelli and Platt, 2002; Spangler and Dewey, 2000). A wide range of bacteria have been isolated from feline and canine meningitis cases, including *E. coli*, *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Pasteurella* spp., *Actinomyces* spp., *Nocardia* spp., and various anaerobic species including *Peptostreptococcus*, *Eubacterium, Fusobacterium*, and *Bacteroides* spp. Ehrlichial and rickettesial organisms can also cause meningitis in small animals. Bacterial meningitis commonly occurs in neonatal large animals as a sequela to failure of passive transfer of antibodies. Meningitis is predominantly caused by Gram-negative enteric pathogens (*E. coli* and *Salmonella* spp.) and beta-hemolytic streptococci in septic foals, and *Streptococcus* spp. and anaerobes in older horses (Toth et al., 2012; Viu et al., 2012). The Enterobacteriaceae are the cause of most cases of bacterial meningitis in septic ruminant neonates. Along with polyarthritis and pneumonia, *Mycoplasma bovis* can cause meningitis in young calves (Stipkovits et al., 1993). In adult cattle and sheep, meningoencephalomyelitis is caused by *Histophilus somni* and encephalitis by *Listeria monocytogenes* (Braun et al., 2002; Fecteau and George, 2004). Pituitary abscesses in cattle are caused by *Arcanobacterium (Trueperella) pyogenes* and anaerobic bacteria. While enteric Gram-negative pathogens cause meningitis in septic piglets, the most common cause of infectious meningitis in pigs is *Streptococcus suis* type 2 (Gottschalk and Segura, 2000).

Therapy of Bacterial Meningitis

Infections of the CNS are associated with high morbitiy and mortality. Treatment failure in septic neonates is attributed to failure of passive transfer of colostral antibodies, the advanced state of disease when diagnosed, the limited ability of antimicrobials to cross the BBB, and development of antimicrobial-resistant bacteria. The CSF penetration of an antimicrobial depends on the integrity of the BBB and the physical and chemical characteristics of the drug. In order to achieve therapeutic concentrations, antimicrobials for therapy of CNS infections should be lipid-soluble, of low molecular weight, have a low degree of protein binding, and be weak bases to take advantage of ion trapping. For example, beta-lactam antibiotics poorly penetrate the normal BBB, only achieving concentrations in the CSF that are

0.5–2.0% of peak serum concentrations. These weak organic acids are also actively transported out of the CNS against a concentration gradient. This mechanism is disrupted by meningeal inflammation. Inflammation also increases separation of the intercellular tight junctions and vesicular transport, so that penetration of the BBB is significantly enhanced (up to 55% of peak serum concentrations).

The poor host defence mechanisms in the CNS suggest that only antimicrobials that achieve bactericidal concentrations in the CSF should be used for therapy of meningitis. However, highly bactericidal antimicrobials do not necessarily improve clinical outcome. The bacterial cell wall of Gram-positive bacteria and endotoxin released from Gram-negative bacteria stimulate a dramatic inflammatory response. Reports from human medicine indicate that improved clinical outcome does not come from "better" bactericidal drugs, but from treatments targeting the pathogenesis of CSF inflammation. Newer beta-lactams such as imipenem lyse bacteria in a manner that does not create the same high concentrations of inflammatory debris seen with conventional beta-lactam antibiotics. "Partner drugs" can be administered to decrease the detrimental inflammatory response. Work is being done with antibodies that capture the inflammatory cell wall pieces in order to render them inert. To decrease leukocyte damage during inflammation, antibodies that block leukocyte adhesion to endothelia and prevent accumulation of leukocytes in cerebrospinal fluid are being investigated. Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) down-modulate cerebrospinal fluid leukocytosis, chemical abnormalities, and pressure changes and reduce cerebral edema. The precise dosing and timing of these "partner drugs" is critical. For example, corticosteroids are beneficial in some types of meningitis in children when administered early, but have no effect or a detrimental effect when administered later (Yogev and Guzman-Cottrill, 2005). There is little known about the best use of "partner drugs" in animals, but failure of antibody transfer can be corrected in large animal neonates with plasma transfusions. The risks and benefits of administering corticosteroids or NSAIDs need further investigation in veterinary patients with meningitis.

Antimicrobial choice should be based on CNS penetration and the initial results of Gram stain followed by culture and susceptibility results from CSF or other infected tissues. Antimicrobials should be administered intravenously to attain maximum peak plasma concentrations to provide a concentration gradient to aid passage of drugs into the CNS. For therapy with beta-lactams, aminoglycosides, and fluoroquinolones, there is a significant correlation between increasing the drug concentration in the CSF and increasing bactericidal killing rates (Yogev and Guzman-Cottrill, 2005). For these antimicrobials, maximal bactericidal activity occurs when the CSF concentration is 10–30 times higher than the *in vitro* minimal bactericidal concentration (MBC). The maximum bactericidal activity of vancomycin occurs when CSF concentrations are 5–10 times higher than the MBC. In contrast, increasing the CSF concentrations of rifampin do not increase killing rate. To ensure optimal penetration of antimicrobials into the CNS, intravenous dosing should be maintained for the entire treatment course. As meningeal inflammation decreases with therapy, penetration of some drugs across the BBB diminishes. Apparently effective antimicrobial therapy should be continued for 7–14 days.

Penicillins and first-generation cephalosporins may be effective for bacterial meningitis from sensitive Gram-positive bacteria, such as *Streptococcus* spp., *Listeria monocytogenes* and anaerobes. Because it is highly protein-bound, ceftiofur does not reach therapeutic concentrations in the CSF. As enteric bacteria are often resistant to ampicillin or amoxicillin, these aminopenicillins are not recommended in the treatment of meningitis in large animal neonates. Because of their high bactericidal activity against Enterobacteriaceae, third-generation cephalosporins are the preferred therapy for treatment of meningitis in septic neonates. However, their use in large animals is limited to neonates due to the expense of therapy. Third-generation cephalosporins are more active against Gram-negative bacteria than the earlier generation cephalosporins, but no more active against Gram-positive bacteria. Cefotaxime, ceftazidine, ceftizoxine and ceftriaxone consistently reach effective antibacterial concentrations in the CNS in humans with inflamed meninges.

Sulfonamides are commonly administered in conjunction with a diaminopyrimidine to take advantage of synergistic antimicrobial activity and to reduce the development of antimicrobial resistance. These "potentiated" sulfonamides have broad-spectrum activity, including *Streptococcus* spp., *E. coli, Proteus, Pasteurella,*

Histophilus, and *Salmonella* spp. Staphylococci, anaerobes, *Nocardia, Corynebacterium, Klebsiella*, and *Enterobacter* spp. are initially susceptible but may become resistant. Because of frequent use, resistance to trimethoprim-sulfonamide combinations frequently occurs in bacteria isolated from septicemic foals and calves, so its use is not recommended without confirmation from susceptibility test results. Trimethoprim-sulfonamide combinations are effective for treatment of *S. suis* type 2 meningitis in pigs. Sulfonamides are well distributed throughout the body, and a few penetrate into the CSF, depending on degree of protein binding and pKa values. Ormetoprim and sulfadimethoxine, trimethoprim and sulfadiazine and trimethoprim and sulfamethoxazole are all well distributed into the CSF. Meningeal inflammation does not alter distribution into the CSF. With chronic dosing, sulfamethoxaxole accumulates in the CSF, but trimethoprim does not.

Tetracyclines are lipid-soluble and well distributed to most tissues, but do not readily reach therapeutic concentrations in the CSF for most causes of bacterial meningitis. Doxycycline is the most lipid-soluble tetracycline and has the greatest degree of CSF penetration. High intravenous doses of oxytetracycline may be effective for early treatment of meningitis due to *Listeria monocytogenes* in ruminants, but resistance has been documented (Vela et al., 2001).

Chloramphenicol is a bacteriostatic, broad-spectrum antimicrobial with activity against many Gram-positive, Gram-negative and anaerobic bacteria. Its bacteriostatic action may contribute to its efficacy, as it does not cause an explosive release of endotoxin or cell wall fragments. Due to lipid solubility and low protein binding, chloramphenicol is widely distributed throughout the body and achieves CSF concentrations up to 50% of plasma concentrations when the meninges are normal and more if inflammation is present. Because of human health concerns, chloramphenicol has been replaced for many diseases in veterinary medicine by the fluoroquinolones and availability of veterinary formulations of chloramphenicol is limited. If given intravenously, florfenicol penetrates well into CSF, with concentrations in the CSF 46% of plasma concentrations. The CSF concentrations remain above the MIC for *Histophilus somni* for over 20 hours, but concentrations above the MIC values for Gram-negative enteric pathogens are not achieved (de Craene et al., 1997).

Fluoroquinolones penetrate well into the CSF during meningitis, reaching CSF concentrations 20–50% of plasma concentrations. They are potentially useful for meningitis in patients with resistant Gram-negative bacteria that do not respond to beta-lactam drugs. Enrofloxacin is highly lipid-soluble and may attain therapeutic concentrations in the CSF for Gram-negative pathogens such as *E. coli*, *Salmonella* spp., *Actinobacillus* spp. and *Klebsiella* spp. Fluoroquinolones have variable efficacy against streptococci and no activity against anaerobic bacteria. Enrofloxacin is available in injectable formulations for IM use in small animals and SC use in cattle, but these formulations may also be administered by slow IV injection. Enrofloxacin may be less expensive for therapy of meningitis in large animals than third-generation cephalosporins. Ciprofloxacin is available in human IV formulations, but may be cost prohibitive for use in large animals. In the United States, the extra-label use of fluoroquinolones in food animals is strictly prohibited. Use of enrofloxacin in neonatal foals has been documented to cause arthropathies, but because therapy with enrofloxacin is economical and effective, it may still be the treatment of choice in life-threatening cases of sepsis and meningitis.

The macrolides and lincosamides are typically active against Gram-positive bacteria, Gram-negative respiratory tract pathogens and anaerobes. They are not active against the Enterobacteriacea. Erythromycin, clarithromycin, azithromycin and clindamycin concentrate in leukocytes, making them very effective against intracellular pathogens. Lincomycin and clindamycin penetrate into the CNS better than the macrolides. Erythromycin has been used in children with penicillin-resistant *Streptococcus pneumoniae* meningitis, but resistance is common. Erythromycin and clindamycin are available as human IV formulations. Early treatment of bovine respiratory disease with tilmicosin or tulathromycin may prevent thromboembolic meningoencephalitis from *Histophilus somni* in cattle. Advanced cases with microabscesses and thrombophlebitis in the CNS are unlikely to respond.

Rifampin is a highly lipid-soluble antimicrobial with activity against Gram-positive and anaerobic bacteria, including streptococci, *Rhodococcus equi*, *Staphylococcus aureus*, and *Mycobacterium* spp. Because bacterial resistance rapidly emerges to rifampin, it is commonly used in conjunction with other antimicrobials. Rifampin

widely distributes in tissues and the CSF. It is most commonly used as the oral formulation in combination with a macrolide to treat *Rhodococcus equi* infections in foals. However, there are human intravenous formulations that could be used, providing the dose is corrected for bioavailability and it appears to be useful in controlling deleterious inflammation (Spreer et al., 2009).

Metronidazole is highly effective against anaerobic bacteria, including *Bacteroides fragilis* (penicillin-resistant strains), *Fusobacterium*, and *Clostridium* spp. Metronidizole is very lipid-soluble and readily penetrates into the brain and CSF. Metronidazole is available in human intravenous formulations, but these formulations may be cost prohibitive for large animals. In the United States and Canada, metronidazole is strictly prohibited from use in food animals.

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Urinary Tract Infections

Introduction

Bacterial urinary tract infections (UTIs) commonly cause of disease in veterinary patients. Approximately 14% of all dogs will acquire a bacterial urinary tract infection during their lifetimes and many dogs presented to a veterinarian for other problems will have a concurrent bacterial UTI (Ling, 1984). Studies of feline lower urinary tract disease suggest that the prevalence of bacterial UTI is less than 5% in cats presenting with an initial episode of signs related to urinary tract disease (Buffington et al., 1997; Segev et al., 2011). The incidence of UTI is much higher in older cats, as they are more susceptible to bacterial UTI because of diminished host defenses secondary to aging or concomitant disease (e.g., diabetes mellitus, renal failure, hyperthyroidism; Litster et al., 2011). Bacterial UTIs in ruminants are associated with catheterization or parturition in females and as both a cause and consequence of urolithiasis in males (Otter and Moynan, 2000; Rebhun et al., 1989; Yeruham et al., 2006). In horses, UTIs are uncommon and typically associated with bladder paralysis, urolithiasis, or urethral damage (Frye, 2006).

Antimicrobials are the cornerstone of UTI therapy, and many patients with recurring UTIs are managed empirically with repeated courses of antimicrobial therapy. This approach fails if the underlying pathophysiology

predisposing the animal to the UTI is not addressed and it encourages the selection and spread of antimicrobial-resistant bacteria.

The consequences of bacterial UTI can be significant if the infection goes undiagnosed and untreated. Because many cats and dogs with UTI do not display clinical signs or do not have detectable bacteriuria or pyuria, diagnosis can be incidental. Colonization of any part of the urinary tract with bacteria increases susceptibility to infection in other parts of the urinary tract and body. Some consequences of undiagnosed UTI include infertility, urinary incontinence, discospondylitis, pyelonephritis, and renal failure. Septicemia can occur as a consequence of UTI in immunosuppressed patients. In intact males, the UTI frequently extends to the prostate gland or other accessory sex glands. Due to the blood-prostate barrier, it is difficult to eradicate bacteria from the prostate gland, potentially resulting in re-infection of the urinary tract following appropriate treatment, systemic bacteremia, infection of other parts of the reproductive tract, or local infection within the prostate and eventual abscess formation. In dogs, infection of the urine with urease producing bacteria (*Staphylococcus pseudintermedius* and *Proteus mirabilis*) is associated with the formation of struvite uroliths. *Corynebacterium urealyticum*, also a rapid urease producing organism, is associated with alkaline urine and struvite and calcium phosphate precipitation, which can result in bladder wall encrustations (Bailiff et al., 2005).

Pathogenesis

Infection of the urinary tract primarily depends on the interaction between host defenses and virulence factors of the bacteria. Studies in cats and dogs have shown that when the host defenses are altered by catheterization, surgery or other diseases of the urinary tract (idiopathic cystitis, urolithiasis, polyps, neoplasia, etc.), the incidence of bacterial UTI is high (Barsanti et al., 1985; Stiffler et al., 2006). Abnormalities of host defenses are thought to be the most important factor in the pathogenesis of UTI and the persistence of complicated UTI.

The most common route of infection is by ascent of bacteria within the urethra. Anatomical abnormalities of the lower urinary tract such as vulvar abnormalities, urethrostomies, as well as indwelling catheters and cystotomy tubes are risk factors for ascending bacterial infections (Smarick et al., 2004; Stiffler et al., 2006). Commensal bacteria of the distal urethra compete with invading uropathogenic bacteria by consuming essential nutrients, interfering with bacterial adhesion to the uroepithelium or by secreting bacteriocins. In addition, the urethral surface has intrinsic properties that prevent bacterial colonization. The uroepithelium of the distal urethra and vagina has surface microvilli that allows for the attachment of resident bacteria. In contrast, the surface of the proximal urethra and bladder has microplicae. These folds flatten when the lumen of the urethra is distended during the act of micturition, thus making it difficult for bacteria to adhere. Another host defense of the urethra is production of secretory IgA, which prevents bacterial adherence and colonization. Intrinsic properties of the urethra such as urethral peristalsis and a functional high-pressure zone in the mid-urethra also act to prevent bacterial colonization.

The anatomy and function of the ureters also provide a mechanism of defense against bacterial invasion of the kidneys. The distal ureter courses through the bladder wall at an angle forming a one-way valve preventing vesicoureteral reflux. Peristalsis of the ureters results in unidirectional flow of urine from the kidneys to the bladder. Renal defenses against infection are primarily local and systemic immune responses. The renal cortex is less susceptible to infection than is the medulla, possibly due to increased blood flow in the cortex. Renal tubule epithelial cells express Toll-like receptors of the innate immune system, which trigger the innate immune response to bacterial infection (Ben Mkaddem et al., 2010).

Micturition is an important defense against bacterial colonization of the lower urinary tract. Frequent voiding of urine removes ascending bacteria in the urethra. In addition, the flattening of urethral folds may dislodge adherent bacteria during voiding. Urine dilutes bacterial populations and complete voiding expels bacteria that do gain access to the bladder. The pH extremes and osmolarity of urine inhibit bacterial growth and salts, urea and organic acids in urine reduce bacterial survival. Urine lactoferrin scavenges essential iron from bacteria. Soluble and cell associated factors in the bladder, such as Tamm-Horsfall protein, glycosaminoglycans, secretory IgA and uromucoid act to block bacterial adherence. If bacteria successfully attach to the uroepithelium, additional host defense mechanisms are triggered. The uroepithelium normally has a very slow turnover rate. But in response to intracellular invasion,

bladder cells exfoliate in an apoptosis-like mechanism to clear the bacteria through the urine flow. Intracellular invasion also triggers neutrophil infiltration of the uroepithelium and the bladder lumen and pyuria is a feature of UTI. Diseases of the urinary tract such as bladder atony, urolithiasis and prolonged urine retention predispose to infection because of the presence of residual urine. Dilute urine, glucosuria, and impaired immune response may contribute to the development of UTI in animals with diabetes, hyperadrenocorticism or those receiving corticosteroids or cyclosporin (Forrester et al., 1999; Hess et al., 2000; Ihrke et al., 1985; Peterson et al., 2012; Torres et al., 2005). Animals with these disorders should have their urine cultured even if clinical signs and urinalysis findings are not suggestive of UTI.

Uropathogens

The most frequently isolated bacteria causing UTI in dogs, cats, horses and cattle are *E. coli*. In dogs and cats, *Staphylococcus pseudintermedius*, *Proteus* spp., *Streptococcus* spp., and *Klebsiella* spp. are reported less frequently, and enterococci and *Pseudomonas aeruginosa* tend to be isolated from recurrent or complicated UTIs (Ball et al., 2008; Seguin et al., 2003). Streptococci and enterococci follow *E coli* in prevalence in UTI in horses, while *Corynebacterium renale* follows in cattle (Clark et al., 2008; Yeruham et al., 2006; Yeruham et al., 2004).

Bacterial virulence factors enhance colonization of the urinary epithelium and the development of UTI. Strains of uropathogenic *E. coli* (UPEC) have a number of virulence mechanisms that enable them to invade, survive and multiply within the uroepithelium. These bacteria are responsible for > 90% of cases of UTI and are often found among the fecal flora of the same host (Katouli, 2010). Upon entry into host uroepithelium, UPEC can both multiply and emerge from the host cells or remain latent in membrane-bound vesicles. The multiplying UPEC form intracellular bacterial communities free within the cytoplasm and can bridge host cells without entering the urine. Urine may then culture negative for the pathogen for a time, even though the infection is persisting. The exfoliation of infected uroepithelium and the influx of neutrophils are normal defense mechanisms that work to the advantage of UPECs. Bladder cell exfoliation leaves underlying tissue exposed and susceptible to bacteria within the urine. The shedding of infected host cells into the urine facilitates the spread of UPEC in the environment. The influx of neutrophils compromises the integrity of the uroepithelium and may allow UPEC to penetrate deeper tissues. Other virulence factors include capsules that surround bacteria that limit phagocytosis, antibody coating, and opsonization and the formation of biofilms. In addition, *E. coli* produces factors such as hemolysin and aerobactin that promote bacterial growth. These virulence mechanisms allow UPEC to persist within the uroepithelium in the face of antimicrobial therapy that effectively kills bacteria in the urine (Blango and Mulvey, 2010; Mulvey, 2002; Mulvey et al., 2001; Mulvey et al., 2000) Other uropathogens may have similar strategies to UPEC in establishing tissue reservoirs and persistent infection.

Diagnosis

Urinalysis

Bacterial UTI is diagnosed by examination and microbiological culture of urine. When possible, cystocentesis is the best method of collecting urine for examination. Free catch or catheterized samples must be interpreted in light of possible contamination. With UTI, the urinalysis characteristics are highly variable. Urine sediment should always be evaluated for bacteria and cells. Rod-shaped bacteria may not be visible when their concentration is ≤ 10,000/ml and cocci may not be visible when their concentration is ≤ 100,000/ml. Clinically relevant (> 5 cells per HPF) hematuria and pyuria is not always present with bacterial UTI, so the presence of bacteria without an inflammatory response does not always indicate contamination. Urine dipsticks are unreliable for evaluating white blood cells. Dogs with *E. coli* infections are more likely to have dilute urine (urine specific gravity < 1.025), which may either reflect endotoxin-mediated effects on urinary concentrating ability or the antimicrobial properties of concentrated urine. Prior to obtaining culture and sensitivity results, Gram stain may allow identification of the pathogen as Gram-positive or Gram-negative and is helpful in determining initial antimicrobial therapy. If the urine is persistently alkaline, suspect a urease-producing pathogen; *Staphylococcus* spp. if cocci are present and *Proteus* spp. if rods are present. If urine is persistently acidic, suspect *E coli* if Gram-negative rods are present and streptococcus or enterococcus if Gram-positive cocci are present.

Urine Culture

Because of the consequences of infection and the increases in antimicrobial resistance in uropathogens, urine culture should be performed for the diagnosis of all suspected UTIs. Bacterial identification and susceptibility testing must be performed with adequate biocontainment and properly trained personnel, following protocols standardized by an appropriate organization, such as the Clinical and Laboratory Standards Institute (CLSI) or the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST; Weese et al., 2011).

Treatment

Categorization of the UTI will help in determining appropriate antimicrobial therapy. A simple urinary tract infection is due to a temporary break in the host defenses, responds quickly to appropriate therapy and does not recur. Because most antimicrobials achieve high concentrations in the urinary tract tissues and urine, most cases of simple bacterial UTIs are one-time infections that respond well to appropriate therapy. A complicated UTI is due to a persistent underlying abnormality in the urinary tract or host defenses. A relapse occurs when the original infection is not cleared despite therapy. Re-infection occurs when the patient is infected a new bacterial species or strain after successful therapy documented by a negative urine culture. A superinfection occurs when a different bacterial species or strain colonized the urinary tract while the patient is still on antimicrobial therapy for the original infection. Re-infections are attributed to re-inoculation of the urinary tract from gastrointestinal flora in a host with deficiencies in their immune defense mechanisms. The deficiencies can be intrinsic to the patient (e.g., diabetes mellitus, hyperadrenocorticism) or iatrogenic (e.g., corticosteroid or chemotherapy administration). Relapses are due to infection by uropathogens with enhanced intrinsic virulence (Thompson et al., 2011). In addition, conditions that damage the urothelium such as urolithiasis, neoplasia, catheterization, surgery or cystitis caused by cyclophosphamide or idiopathic causes can predispose to the development of complicated UTI. Other causes of complicated UTI include anatomic defects (ectopic ureters, urachal diverticula), interference of normal micturition (urinary obstruction, damaged nervous innervation causing bladder atony) or changes in urine concentration or composition (glucosuria).

Treatment for a simple UTI may be empirical, based upon knowledge of the commonly isolated pathogen and their typical susceptibility to antimicrobials; however, empirical therapy often fails and is not recommended because of the serious consequences from increasing rates of antimicrobial resistance (Weese et al., 2011). To effectively treat a complicated UTI, further diagnostics must be carried to identify and address the underlying pathology.

The choice of an antimicrobial must consider the pharmacokinetics and pharmacodynamics of the drug, appreciation of the potential adverse effects (for both animal and owner), ease of administration and cost. Urine concentrations of antimicrobials are more important than serum concentrations during the treatment of simple UTI but susceptibility testing results normally reflect achievable serum concentrations. In general, urine concentrations will exceed those of serum if the antimicrobial is excreted in an active form in the urine. If the urine concentration is 4 times (or greater) than the minimum inhibitory concentration (MIC), it will most likely be effective for treatment of UTI caused by that pathogen (90% effective; Ling, 1984). Therefore, despite a susceptibility testing result of "R" for amoxicillin for first time UTI caused by *E. coli* or *Staphylococcus pseudintermedius* in dogs or cats, the extremely high urine concentrations attained make amoxicillin the clear first choice for therapy (Weese et al., 2011). Similarly, injectable penicillin G is efficacious as first-line therapy in the treatment of UTI in horses and cattle.

Pharmacokinetic/pharmacodynamic integration should be considered in determining the appropriate dosage regimen. For beta-lactam antimicrobials, there is a significant correlation between the T > MIC in serum, urine, or renal tissue, and the effect measured as colony counts in either urine or urinary tract tissue. The importance of the T > MIC for treatment of UTI may explain the poor efficacy results of beta-lactam antibiotics in treatment of UTIs, as they have probably not been dosed frequently enough. So while the label dose of amoxicillin is sufficient, the label frequency of every 12 hours needs to be reduced to every 8 hours. Obviously, this impacts on client compliance with increased daily dosing. Highly protein-bound beta-lactams, such as cefovecin, overcome this limitation, as the protein-bound drug acts as a depot to provide 14 days of therapy after a single injection. As their bacterial killing effect is

concentration-dependent, fluoroquinolone and aminoglycoside efficacy correlates best to AUC:MIC ratios. In the murine model, gentamicin and fluoroquinolone treatment results in significantly lower bacterial counts than the beta-lactam antimicrobials, indicating that rapid bacterial kill is important in the treatment of UTI. Therefore, client compliance during therapy for UTI is imperative. This makes single daily dose administration (e.g., fluoroquinolones, cefpodoxime) or long-acting injectables (e.g., cefovecin, ceftiofur) attractive, and is the basis for much of the irrational first-line use of fluoroquinolones and third-generation cephalosporins. For dogs, antimicrobials should be administered just before bedtime or confining the dog, to maintain a high urine concentrations for the longest possible time.

Antimicrobial Treatment Choices

Amoxicillin and ampicillin are bactericidal and relatively non-toxic with a spectrum of antibacterial activity greater than penicillin G. They are easily administered orally to dogs and cats. Injectable ampicillin products are available for large animals. Initially, they have excellent activity against staphylococci, streptococci, enterococci, and *Proteus* spp., and may achieve high enough urinary concentrations to be effective against *E. coli* and *Klebsiella* spp. *Pseudomonas* spp. and *Enterobacter* spp. are resistant. Absorption of ampicillin is affected by food, so therapeutic success may be easier to achieve with amoxicillin. As penicillins, they are weak acids with a low volume of distribution, so do not achieve therapeutic concentrations in prostatic fluid.

Amoxicillin/clavulanic acid is used orally in small animals. It has an increased spectrum of activity against Gram-negative bacteria due to the presence of the "suicide" drug, clavulanic acid. Clavulanic acid irreversibly binds to beta-lactamases, allowing the amoxicillin fraction to interact with the bacterial pathogen. This combination usually has excellent bactericidal activity against beta-lactamase-producing staphylococci, *E. coli*, and *Klebsiella* spp. *Pseudomonas* spp. and *Enterobacter* spp. remain resistant. However, clavulanic acid undergoes some hepatic metabolism and excretion, so the antimicrobial activity in urine may be due primarily to the high concentrations of amoxicillin achieved in urine. It is not clear that amoxicillin/clavulanic acid is more efficacious for uncomplicated UTIs than amoxicillin.

Cephalexin is a first-generation cephalosporin available in in human and veterinary formulations. In the United States, cefadroxil is available as a veterinary product for dogs and cats. Like the penicillins, they are bactericidal, acidic drugs with a low volume of distribution and are relatively non-toxic. Vomiting and gastrointestinal disturbances may occur in dogs and cats treated with cephalosporins. Cephalosporins have greater stability to beta-lactamases than penicillins, so have greater activity against staphylococci and Gram-negative bacteria. They have excellent activity against staphylococci, streptococci, *E. coli*, *Proteus* spp., and *Klebsiella* spp. *Pseudomonas* spp., enterococci, and *Enterobacter* spp. are resistant. Use of cephalosporins (and fluoroquinolones) predisposes patients to enterococcal infections, including vancomycin-resistant clones (Hayakawa et al., 2013).

Cefovecin is a third-generation cephalosporin approved for the treatment of UTI in dogs due to *E. coli* and *Proteus* spp. With SC dosing, therapeutic concentrations are achieved for 14 days, making this an attractive treatment choice for fractious animals. Cefpodoxime is an oral third-generation cephalosporin approved for use in dogs in the United States for skin infections (wounds and abscesses) but is used extra-label for the treatment of canine UTI. Cefpodoxime has a relatively long half-life in dogs, so it is dosed once daily.

Ceftiofur is a third-generation injectable cephalosporin approved for treatment of canine UTI caused by *E. coli* and *Proteus* spp. It is approved for treatment of respiratory tract infections in horses, cattle, sheep, goats and swine. After injection, ceftiofur is rapidly metabolized to desfuroylceftiofur. Desfuroylceftiofur has equivalent activity to ceftiofur against *E. coli* but is half as potent as ceftiofur against staphylococci and has variable activity against *Proteus* spp. If the microbiology service utilizes ceftiofur when performing susceptibililty testing, a false expectation of therapeutic efficacy may result. *Pseudomonas* spp., enterococci, and *Enterobacter* spp. are resistant to ceftiofur and desfuroylceftiofur. Ceftiofur is associated with a duration and dose-related thrombocytopenia and anemia in dogs that would not be expected with the recommended dosage regimen.

Enrofloxacin, ibafloxacin, orbifloxacin, difloxacin, marbofloxacin and pradofloxacin are all fluoroquinolones approved for UTIs in the dog and some are approved in the cat, but all are used in cats. Large animal injectable formulations are available for the treatment of respiratory tract infections; however, extra-label drug use in food-producing animals in the United States is strictly prohibited. Ciprofloxacin is the human

formulation and may be cheaper to use in very large dogs, but pharmacokinetics differences in veterinary species may result in inefficacy. The fluoroquinolones are bactericidal, amphoteric drugs; they possess acidic and basic properties, but they are very lipid-soluble at physiological pH (pH 6.0–8.0), so have very high tissue distribution. Ciprofloxacin has the greatest antimicrobial activity of all the fluoroquionolones against *Pseudomonas* spp. All fluoroquinolone drugs usually have excellent activity against staphylococci and Gram-negative bacteria, but may have variable activity against streptococci and enterococci. The therapeutic advantage of these drugs is their Gram-negative antimicrobial activity and high degree of lipid solubility. The use of fluoroquinolones should be reserved for UTIs that involve Gram-negative bacteria, especially *Pseudomonas* spp. and UPECs that are potentially intracellular in location and for UTIs in intact male dogs because of excellent penetration into the prostate gland and activity in abscesses. Once-daily, high-dose fluoroquinolone therapy for a short duration is efficacious because these drugs exert concentration-dependent killing and have a long post-antibiotic effect (PAE). The newest fluoroquinolone for dogs and cats, pradofloxacin, requires two genetic mutations for resistance, so MIC values are lower than for other fluoroquinolones and it is hoped that pradofloxacin will be less selective for antimicrobial resistance (Schink et al., 2013) The fluoroquinolones should be avoided for chronic, low-dose therapy, as this encourages the development of bacterial resistance that is often multidrug. Cases that involve *Pseudomonas* spp. should be carefully investigated for underlying pathology and corrected if at all possible. Once *Pseudomonas* spp. becomes resistant to the fluoroquinolones, there are no other patient and client-convenient therapeutic options.

Gentamicin and the other aminoglycosides are basic drugs, but they are very large polar (water-soluble) drugs, so have a low volume of distribution and will not penetrate the blood-prostate barrier. They are not absorbed orally, so must be given by subcutaneous, intramuscular or intravenous injection. The aminoglycosides have a similar spectrum of activity to the fluoroquinolones, but their use for UTIs is limited because of the necessity of parenteral injections and potential for nephrotoxicity and ototoxicity. Like the fluoroquinolones, the aminoglycosides are concentration-dependent, bactericidal killers with a long PAE, so once-daily therapy of short duration is efficacious and minimizes the risk of nephrotoxicity. They can be considered for in-hospital or outpatient treatment of UTI due to fluoroquinolone-resistant pathogens; but again the importance of identifying and correcting underlying pathology must be emphasized.

Nitrofurantoin is approved for treatment of human UTI and is available as tablets, capsules and a pediatric suspension. It is only used for treatment of UTI in humans, as it has a very low volume of distribution and therapeutic concentrations are only attained in urine. It is considered a carcinogen, so it is banned for use in food-producing animals, but its use in small animals is increasing with increasing rates of antimicrobial resistance to veterinary antimicrobials. Nitrofurantoin is used for infections caused by *E. coli*, enterococci, staphylococci, *Klebsiella* spp., and *Enterobacter* spp. (Maaland and Guardabassi, 2011). It is increasingly indicated for treatment of UTI caused by multidrug-resistant bacteria, which are otherwise difficult to treat using conventional veterinary antimicrobial agents. The pharmacokinetics and adverse effect profile of nitrofurantoin have not been investigated in dogs and cats, and the need for multiple daily dosing makes it inconvenient for clients.

Tetracyclines are bacteriostatic, amphoteric drugs with a high volume of distribution. Tetracyclines are broad-spectrum antimicrobials, but because of plasmid-mediated resistance, variable susceptibility occurs in staphylococci, enterococci, *Enterobacter* spp., *E. coli*, *Klebsiella* spp., and *Proteus* spp. *Pseudomonas* spp. are resistant. Doxycycline is a very lipid-soluble tetracycline that is better tolerated in cats and will achieve therapeutic concentrations in urine and the prostate, so it may be useful for some UTIs (Wilson et al., 2006). Doxycycline may also be effective in the treatment of methicillin-resistant staphylococcal UTI (Rubin and Gaunt, 2011). If capsules or tablets are administered, it is critical to follow the dose with fluids afterward to ensure passage into the stomach. If capsules lodge in the esophagus, severe local necrosis with subsequent esophageal stricture can occur.

Chloramphenicol has a high volume of distribution and is capable of achieving high tissue concentrations, including in the prostate of male dogs. It is active against a wide range of Gram-positive and many Gram-negative bacteria, against which it is usually bacteriostatic. Chloramphenicol is typically active against *Enterococcus* spp., *Staphylococcus* spp., *Streptococcus* spp., *E. coli,*

Klebsiella spp., and *Proteus* spp. *Pseudomonas* spp. are resistant. North American isolates of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* are typically susceptible. Well known for causing idiosyncratic (non-dose dependent) anemia in humans and dose-dependent bone marrow suppression in animals, its use in both human and veterinary medicine is increasing due to antimicrobial resistance rates (Papich, 2012).

Trimethoprim/sulfonamides (TMP/sulfas) are combinations of two very different drugs that act synergistically on different steps in the bacterial folic acid pathway. Trimethoprim is a bacteriostatic, basic drug that has a high volume of distribution and a short elimination half-life, while the sulfonamides are bacteriostatic, acidic drugs with a medium volume of distribution and long half-lives (ranging from 6 to over 24 hours). These drugs are formulated in a 1:5 ratio of TMP to sulfa; however, the optimal bactericidal concentration is a ratio of 1:20 TMP:sulfa. Microbiology services utilize the 1:20 ratio in susceptibility testing; however, the widely varying pharmacokinetic properties of this drug combination make it difficult to determine a therapeutic regimen that achieves the 1:20 ratio at the infection site. Although the combination does penetrate the blood:prostate barrier, the sulfa drugs are ineffective in purulent material because of the freely available PABA from lysed phagocytes. The combination of TMP/sulfa is synergistic and bactericidal against staphylococci, streptococci, *E. coli* and *Proteus* spp. Activity against *Klebsiella* spp. is variable and *Pseudomonas* spp. are resistant. Although enterococci may appear susceptible to TMP/sulfas *in vitro*, they escape the antifolate activity of the drug combination in vivo by incorporating preformed exogenous folates, so they should not be considered for treatment. While frequently recommended as a second treatment after amoxicillin for canine UTI, TMP/sulfas are associated with a number of adverse effects, and chronic low-dose therapy may result in bone marrow suppression and keratoconjunctivitis sicca.

Dosage Regimens

Currently, the duration of therapy for UTI is controversial. While animals are routinely treated with antimicrobial drugs for 10–14 days, shorter duration antimicrobial regimens are routinely prescribed in human patients, including single dose fluoroquinolone therapy. A clinical comparison of 3 days of therapy with a once-daily high dose of enrofloxacin with 2 weeks of twice-daily amoxicillin/clavulanic acid showed equivalence in the treatment of simple UTI in dogs (Westropp et al., 2012). However, further studies are needed to determine the optimal dosage regimens for different classes of antimicrobials and it is inappropriate to use fluoroquinolones as first-line therapy for simple UTI. Patients with complicated UTI may require longer courses of therapy and underlying pathology must be addressed. Chronic complicated cases of UTI, pyelonephritis and prostatitis may require antimicrobial treatment for 4–6 weeks, with the risk of selecting for antimicrobial resistance. A follow up urine culture should be performed after 4–7 days of therapy to determine efficacy. If the same or a different pathogen is observed, then an alternative therapy should be chosen and the culture repeated again after 4–7 days. Urine should also be cultured 7–10 days after completing antimicrobial therapy to determine if the UTI is cured or has recurred.

Recurrent Urinary Tract Infections

In dogs and cats, if UTI occurs only once or twice yearly, each episode may be treated as an acute uncomplicated UTI. If they occur more often, and predisposing causes of UTI cannot be identified or corrected, chronic low-dose therapy may be necessary to manage the patient. Low antimicrobial concentrations in the urine may interfere with fimbriae production by some pathogens and prevent their adhesion to the uroepithelium. In dogs, recurrent UTIs are due to a different strain or species of bacteria about 80% of the time, therefore antimicrobial culture and sensitivity is still indicated. Initiate therapy as before, and then when urine culture is negative, continue antimicrobial therapy once daily at one-third of the total daily dose. The antimicrobial should be administered last thing at night to ensure that the bladder contains urine with a high antimicrobial concentration for as long as possible. Appropriate antimicrobials for chronic, low-dose therapy include amoxicillin, ampicillin, amoxicillin-clavulanic acid, doxycycline, cephalexin, cefadroxil, and nitrofurantoin. A trimethoprim/sulfonamide can be used, but folate supplementation should be provided (15 mg/kg q 12 h) to prevent bone marrow suppression and there is the risk of keratoconjunctivitis sicca developing with chronic use. While

attractive for client convenience, third-generation cephalosporins such as cefpodoxime and cefovecin should not be used for chronic therapy. During chronic therapy, urine culture should be repeated every 4–6 weeks. As long as the culture is negative, therapy is continued for 6 months. If bacteriuria occurs, the infection is treated as an acute episode with an appropriate antimicrobial. After 6 months of bacteria-free urine, the chronic low-dose antimicrobial therapy may be discontinued and many patients will not have additional recurrences. In some cases, chronic therapy may be continued for years in patients that continue to have recurrent UTI.

Treatment Failure

Treatment failures may be due to poor owner compliance, inappropriate choice of antimicrobials, inappropriate dose or duration of treatment, antimicrobial resistance, superinfection or an underlying predisposing cause (e.g., urolithiasis, neoplasia, urachal diverticula). If treatment for a simple or complicated UTI fails, a thorough evaluation should be carried out to determine and when possible, address the cause of failure. When faced with a therapeutic failure, the practitioner needs to consider if the UTI is due to a relapse or a re-infection. Relapses due to infection by uropathogens with enhanced intrinsic virulence occur with what should be effective antimicrobial therapy. Strains of UPEC have a number of virulence mechanisms that enable them to invade, survive and multiply within the uroepithelium. The sequestration of UPEC within the bladder uroepithelium presents a great therapeutic challenge in human and veterinary patients (Thompson et al., 2011). Currently, there is no clear consensus from the human literature on how to approach these recurrent and persistent UTIs.

Antimicrobial Resistance in Uropathogens

Acquired resistance to antimicrobials by uropathogens is of great concern in human and veterinary medicine. The prevalence of multidrug resistance (MDR) in uropathogens is increasing, particularly in canine and feline infections (Dierikx et al., 2012; Gibson et al., 2008; Hubka and Boothe, 2011; Ogeer-Gyles et al., 2006; Thompson et al., 2011). Extended-spectrum beta-lactamase (ESBL) genes are increasingly identified in *E. coli* isolates from companion animals (O'Keefe et al., 2010; Pomba et al., 2009). Increases in the occurrence of fluoroquionolone-resistant *E. coli* in dogs have been widely reported (Aly et al., 2012; Craven et al., 2010; Gebru et al., 2011, 2012; Sato et al., 2012; Shaheen et al., 2011). As the mechanism of resistance to fluoroquinolones frequently involves efflux pumps, it also conveys multidrug resistance (Aly et al., 2012). Fluoroquinolone resistance is also increasing in other uropathogens, including enterococci, *Proteus mirabilis* and *Staphylococcus pseudintermedius* isolates (Cohn et al., 2003; Ghosh et al., 2011; Jackson et al., 2010). There is increasing evidence that animals are an important reservoir of antimicrobial-resistant bacteria causing infections in humans (Platell et al., 2012). Enterococci isolated from canine UTIs have been associated with several different resistant phenotypes, with the majority exhibiting resistance to three or more antimicrobials. One *E. faecium* isolate displayed high-level resistance to vancomycin and gentamicin. Sequence analysis suggested that resistance was due to gene exchange between human and canine enterococci (Simjee et al., 2002).

The use of "last resort" human antimicrobials in veterinary patients with resistant infections is controversial. Vancomycin, imipenem-cilastatin, meropenem, fosfomycin, quinupristin-dalfopristin, and tigecycline should not be used routinely in the treatment of UTI in animals. Non-antimicrobial control of infection should be considered whenever feasible. Custom-made vaccines, cranberry juice/extract, probiotics and adherence/colonization inhibitors, and establishment of asymptomatic bacteriuria may be useful in preserving the efficacy of antimicrobials (Thompson et al., 2012).

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24

Antimicrobial Therapy of Selected Bacterial Infections

Steeve Giguère

This chapter discusses special considerations required when treating selected bacterial infections (anaerobic, atypical mycobacterial, *Brucella*, leptospirosis, mycoplasma, and *Nocardia*).

Anaerobic Infections

Obligate anaerobic bacteria (anaerobes) are those that are unable to grow in the presence of molecular oxygen. They can be Gram-negative or Gram-positive rods or cocci. Anaerobic bacteria are important pathogens in many different types of infections. Only a few of the several hundred different species produce primary disease. These include members of the genera *Clostridium* (e.g., *C. difficile*, *C. perfringens*), enterotoxigenic *Bacteroides fragilis*, and the pathogenic anaerobic spirochetes (e.g., *Brachyspira* spp.). The great majority of other anaerobes that cause disease in animals are opportunistic pathogens. The most commonly encountered infectious process involving anaerobes are those stemming from inoculation (infection) of a normally sterile site by a member of the relatively pathogenic species of the genera of normal flora (*Actinomyces*, *Bacteroides*, *Clostridium*, *Eubacterium*, *Peptostreptococcus*, *Porphyromonas*, etc) occupying the mucosal surface contiguous to the compromised site.

In vitro Activity

In vitro susceptibility testing of all anaerobic bacteria is time consuming and often unnecessary. The selection of antimicrobial agents for the empirical treatment of pure or mixed anaerobic infections is often based on surveillance data at the local or national level. Such data are scant as it relates to anaerobes isolated from most veterinary species. The CLSI has recently established rigorously standardized methodologies for MIC determination by the agar dilution method. The disk diffusion test is not accepted for the *in vitro* susceptibility testing of anaerobes. The E-test (chapter 2) represents a simple approach to testing for a limited (because of high cost) number of drugs. Activity of various antimicrobial agents against common anaerobic bacterial pathogens is summarized in Table 24.1

Metronidazole, chloramphenicol, clindamycin, and some second- (cefoxitin) and third-generation cephalosporins (ceftizoxime) are effective in the treatment of anaerobic infections (Jang et al., 1997a). Penicillins (penicillin G, amoxicillin, ampicillin, ticarcillin) are effective against most anaerobes (except members of the *B. fragilis* group and occasionally other Gram-negative species), but when combined with a beta-lactamase inhibitor (clavulanic acid, sulbactam, or tazobactam), beta-lactams are effective against the majority of anaerobes. Macrolide and tetracyclines have some activity against anaerobes but they are rarely indicated as first line therapy for infections caused by anaerobes.

Resistance

All anaerobes are naturally resistant to the aminoglycosides, since these antibiotics require an oxygen-dependent

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Table 24.1. Activity of antimicrobial agents against anaerobic bacterial species.

Drug	Peptostreptococcus spp.	*Fusobacterium* spp.	*Bacteroides fragilis*	*Clostridium perfringens*	*Prevotella* spp.
Penicillin	++	++	–	++	+/–
Beta-lactam with beta-lactamase inhibitors	++	++	++	++	++
Cefoxitin	+	+	+	+	+
Chloramphenicol	+	+	+	+	+
Clindamycin	+	+	+/– to + *	+	+
Macrolides	+/–	–	–	+	+/–
Metronidazole	+/–	+	++	+	++
Carbapenems	+	+	++	+	++
Newer fluoroquinolones[a]	+	+	+	+	++
Tetracyclines[b]	+/–	+/–	+/–	–	+/–

Level of in vitro activity: –, minimal; +/–, moderate; +, good; ++, excellent.
*Level of resistance varies considerably between studies.
[a]Newer fluoroquinolones include pradofloxacin, levofloxacin, gatifloxacin, trovafloxacin, moxifloxacin, and gemifloxacin.
[b]With the exception of tigecycline, which is active against most anaerobes.
Adapted from Nagy, 2010, and Brooks, 2011.

transport system to get into the bacterial cell. Likewise, anaerobes are inherently resistant to the first- and second-generation fluoroquinolones (e.g., nalidixic acid, norfloxacin, enrofloxacin, ciprofloxacin, etc.), though several newer compounds (e.g., pradofloxacin, levofloxacin, trovafloxacin, moxifloxacin, gemifloxacin) have good *in vitro* activity against many clinically important anaerobes including *B. fragilis* (Stein and Goldstein, 2006). However, their activity against *Bacteroides* group species other than *B. fragilis* is limited and fluoroquinolones have been linked to *C. difficile*–associated diarrhea in people (Stein and Goldstein, 2006).

Resistance to beta-lactam antimicrobial agents is mediated by 1 of 3 major resistance mechanisms: inactivating enzymes (beta-lactamases), low-affinity penicillin-binding proteins, or decreased permeability. Inactivating beta-lactamases are the most common. The most common beta-lactamases found among *Bacteroides* and *Prevotella* spp. are functional class 2e cephalosporinases. These enzymes are all inhibited by beta-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam). Thus, whereas penicillin or ampicillin are not very active against most *B. fragilis* and *Prevotella* species, the beta-lactam/beta-lactamase inhibitor combinations are highly active. Cefoxitin-hydrolyzing proteins inactivating cefoxitin and cefotaxime, such as those encoded by cepA and cfxA, are far less common but they have been observed in many species in the *B. fragilis* group (Nagy, 2010). Much of the data on resistance comes from human rather than veterinary sources but the findings are probably reasonably applicable to animals.

Resistance to the tetracyclines is unpredictable because of acquired resistance. With the exception of the newly developed drug tigecycline, tetracyclines are of limited clinical use for the treatment of anaerobic infections. The effectiveness of trimethoprim-sulfonamides is also unpredictable for the treatment of infectious processes involving anaerobes. This is because some anaerobes (and there is no way to predict which) are able to scavenge thymidine from necrotic material and thereby bypass the block in the production of this chemical by trimethoprim-sulfonamides (Indiveri and Hirsh, 1992). So even though *in vitro* tests (done under controlled thymidine-less conditions) predict effectiveness *in vivo*, trimethoprim-sulfonamide combinations are not recommended for treatment of infectious processes involving anaerobes.

Resistance to metronidazole is uncommon among Gram-negative anaerobic bacteria. Metronidazole resistance is more common among Gram-positive anaerobic bacteria including *Actinomyces* spp. some anaerobic streptococci. An isolated report of metronidazole resistance has been reported for *C. difficile*–associated diarrhea affecting horses in a teaching hospital (Jang et al., 1997b). Although clindamycin has long been considered a gold

Table 24.2. Choice of antimicrobial drugs to treat non-spore-forming anaerobic infections in animals.

Type of Infection	Single Agent	Combination of Agents
Relatively non-serious; e.g., bite infections	Amoxicillin, ampicillin, azithromycin, chloramphenicol, clindamycin	Amoxicillin–clavulanic acid, sulbactam-ampicillin
Serious infections, including intra-abdominal infections	Cefoxitin; carbapenem	Piperacillin-tazobactam; ticarcillin-clavulanic acid; aminoglycoside plus metronidazole or clindamycin; third-/fourth-generation cephalosporin plus metronidazole or clindamycin; fluoroquinolone plus metronidazole

standard for treatment of anaerobic infections, resistance to clindamycin has increased steadily over the past 20 years, with resistance ranging between 10% and 40% among *B. fragilis* group strains (Nagy, 2010).

Clinical Application

Infectious processes involving normally sterile sites are usually a mixture of anaerobes and aerobes (facultative as well as obligate species). Many anaerobic bacterial infections are mixed, but attempted elimination of all the organisms may not be necessary. This is because the unique synergism that sometimes occurs between aerobic and anaerobic bacteria is such that elimination of only some of the species present in the mixed bacterial infection will result in removal of the synergistic effect and clearance of the infection. The two major approaches to the treatment of anaerobic infections are appropriate antimicrobial therapy and surgical management. Debridement of necrotic tissue and drainage of abscesses are important whenever possible. In human medicine, no consensus has been reached regarding the specific agents, dosage, and duration of therapy for anaerobic infections (Nagy, 2010), so that clinical judgment is required in making these choices. Whether the clinician chooses an antimicrobial combination or a single antimicrobial drug will depend on assessment of the seriousness of the infection and of its consequences.

Empiric treatment (usually the case since susceptibility test results of aerobic organisms are unavailable for at least 48 hours; for anaerobic species, at least 5 days) is usually initiated based on likely microorganisms and their typical *in vitro* susceptibility profile. The severity of the infection is another important factor dictating the choice of antimicrobial drugs (Table 24.2). For mild infection, a single antimicrobial agent with adequate spectrum against both aerobes and anaerobes is typically selected. For serious infectious, a combination of drugs highly effective against the aerobic component with other drugs highly effective against the anaerobic component may be chosen. Examples include an aminoglycoside or a fluoroquinolone with amoxicillin-clavulanic acid, clindamycin, or metronidazole. The use of such combinations is necessary in the treatment of peritonitis resulting from spillage of intestinal contents into the intestine because mixed infection with anaerobes and enteric Gram-negative bacteria is common. Septic pleuritis in horses is another condition in which it is usual to combine treatement against the aerobic (*Streptococcus equi* subsp. *zooepidemicus* and Gram-negative aerobes) component and the likely non-spore-forming anaerobic bacterial component that may be a consequence of the infection. One typical combination is penicillin-gentamicin for the aerobes and metronidazole for the anerobes.

Treatment of anaerobic intestinal infections (enterotoxigenic *B. fragilis, Brachyspira hyodysenteriae, B. pilosicoli*, *C. difficile*, *C. perfringens*) involves a range of choices. Diarrhea associated with *C. difficile* is typically treated with metronidazole in non-food-producing veterinary species. Treatment of disease produced by *B. hyodysenteriae* is discussed in chapter 33.

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Brucella

Brucellosis is the disease produced by members of the genus *Brucella*. The genus contains ten species: *B. abortus*, *B. canis*, *B. ceti*, *B. inopinata*, *B. melitensis*, *B. microti*, *B. neotomae*, *B. ovis, B. pinnipedialis* and *B.suis* (Pappas, 2010). Treatment of brucellosis is usually restricted to affected companion animals, that is, dogs and horses, because the disease in food-producing livestock is controlled by national eradication programs. Treatment strategies are expensive and involve long-term administration of antibiotics that may not be approved for use in food-producing animals. Brucellae are facultative intracellular pathogens that survive within macrophages. This fact is important in predicting clinical efficacy when using the results of *in vitro* susceptibility tests. Therapy with two antimicrobials is indicated because of recurrence of disease after cessation of single antimicrobial therapy (Solera et al., 1997). Experimental evidence and clinical experience treating human patients has shown that at least one of the antibiotics should have intracellular distribution (Solera et al., 1997).

Despite *in vitro* activity against *Brucella* spp., relapses are common when monotherapy with the tetracyclines, rifampins, and trimethoprim-sulfonamides is used (Solera et al., 1997). *Brucella* spp. are also very susceptible *in vitro* to the fluoroquinolones, but clinical data show that treatment of human patients with ciprofloxacin alone is ineffective, perhaps because fluoroquinolones are less active at the acid pH of the phagolysosome (Garcia-Rodriguez et al., 1991). However, combination of a fluroquinolone with rifampin had an 85% cure rate in a small group of human patients (Agalar et al., 1999). A critical review of the literature concluded that use of quinolones alone is associated with unacceptably high rates of relapse and, when used in combination with rifampin or doxycycline, does not lead to improved outcomes over those associated with conventional regimens (Falagas and Bliziotis, 2006). Conversely, in a clinical trial, 12 dogs infected with *B. canis* were given 5 mg/kg of enrofloxacin orally q 12 h for 30 days. Enrofloxacin did not eradicate the pathogen in all dogs but fertility was maintained and the recurrence of abortions, transmission of the disease to the puppies, and dissemination of microorganisms during parturition were prevented (Wanke et al., 2006).

The treatments that have been found to control brucellosis in human patients involve the use of two agents: doxycycline plus an aminoglycoside (e.g., gentamicin) or doxycycline plus rifampin (Solera et al., 1997). The combination doxycycline-rifampin is the most commonly used regimen in people due to the convenience or oral therapy (Demirtürk et al., 2008). However, a meta-analysis has shown that systemic streptomycin with oral doxycycline or another tetracycline results in a higher cure rate and fewer relapses than oral doxycycline-rifampin (Solera et al., 1994).

For children, because of the tooth-staining effects of tetracyclines, rifampin plus trimethoprim-sulfonamide or rifampin plus an aminoglycoside are recommended alternatives (Solera et al., 1997). Therapies showing promise (effective in rodent models of brucellosis) include the newer macrolide azithromycin (Atkins et al., 2010) and liposomal formulations containing gentamicin (Hernández-Caselles et al., 1989). Because *Brucella* spp. have zoonotic potential, careful consideration should be given to the appropriateness of treatment. There are no current published recommendations for the treatment of brucellosis in companion animals, but clinical data acquired from human experience indicate that tetracycline plus rifampin should be given together for at least 6 weeks.

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Atypical Mycobacteria

For convenience, members of the genus *Mycobacterium* are categorized into those that produce tuberculosis (*M. tuberculosis, M. bovis*), leprosy (*M. leprae*), and the atypical mycobacteria. The atypical mycobacteria are composed of those species that are so-called slow growers (taking weeks to months to form visible colonies *in vitro*: e.g., *M. avium* complex, *M. genavense*, *M. gordona*, *M. kansasii*, *M. marinum*, *M. simiae*, *M. szulgui*, *M. ulcerans*, and *M. xenopi*) and those that are called rapid growers (days to weeks to form visible colonies *in vitro*: e.g., *M. chelonei*, *M. fortuitum*, *M. phlei*, *M. smegmatis*, and *M. vaccae*). The distinction between rapid growers and slow growers is sometimes important when trying to formulate a treatment strategy since there are differences in susceptibility between members of these two groups (Brown-Elliott et al., 2012).

Members of the *M. avium* complex are the main atypical mycobacteria affecting human patients with acquired immunodeficiency syndrome, in birds (second to *M. genavense* in pet birds), swine, and rarely in horses and sheep. Dogs and cats are highly resistant to disease caused by members of the *M. avium* complex (though disseminated disease has been described in previously normal cats), being affected most often by other atypical strains such as *M. chelonei*, *M. fortuitum*, *M. lepraemurium* (cats), *M. phlei*, *M. smegmatis*, and *M. xenopi*. Almost all of the atypical mycobacteria are environmental dwellers, and as such the environment is the major source of infection, rather than an infected patient (Heifets, 1996). Some form of immunosuppression is often, but not always, a prerequisite for disease.

Numerous trials involving human patients have demonstrated that monotherapy leads to the development of resistance to the drug being used (Heifets, 1996; Alangaden and Lerner, 1997). Consequently, most regimens recommended for the treatment of atypical mycobacteriosis involve the use of at least two and preferably 3 antimicrobial drugs. In addition, mycobacteria are facultative intracellular parasites, able to survive within the phagolysosome. Thus, it is important when choosing an antibiotic that drugs be used that penetrate into cells.

Resistance

Mycobacteria are naturally resistant to all of the antibiotics that affect the cell wall (penicillins and cephalosporins), probably because of the high lipid content of the mycobacterial cell wall. Resistance rapidly occurs subsequent to use of a single antimicrobial to which the bacterium was originally susceptible. Resistance results from mutations in the chromosomal gene encoding the target of the antibiotic.

Susceptibility

There are no firm rules for treating infectious processes that involve atypical mycobacteria in veterinary medicine. The American Thoracic Society (ATS) and the Infectious Diseases Society of America have published guidelines for the treatment of atypical or nontuberculous mycobacterial disease in people (Griffith et al., 2007). Most strains of atypical mycobacteria are susceptible to clarithromycin and azithromycin, and macrolides remain the cornerstone of multidrug therapy. For infection caused by the *M. avium* complex and for most (but not all) other atypical mycobacterial species, a daily regimen of clarithromycin (or azithromycin), rifampin, and ethambutol is recommended. Other drugs that have shown effectiveness as added partners to clarithromycin in various drug combination regimens include: clofazimine; fluoroquinolones (members of the *M. avium* complex are unpredictable; *M. chelonae* is resistant); and amikacin (most predictably active against rapid growers; Khardori et al., 1994; Heifets, 1996; Yajko et al., 1996; Alangaden and Lerner, 1997; Watt, 1997).

Clinical Application

The first clues that an atypical mycobacterium may be involved is the presence of chronically occurring lesions that include draining tracts, lack of response to a variety of antimicrobial agents, and the lack of growth on media after 24–48 hours of incubation. In addition to historical clues, if portions of the affected area are stained with either a Romanovsky-type stain (Giemsa, Wright's) or with Gram's, atypical mycobacterial cells have characteristic properties. In the former, the bacterial cells may appear as "ghosts," and in the latter, they may appear as rods with "speckles." Such clues should prompt the use of the acid-fast stain, and the inoculation of appropriate media to be incubated for a suitable length of time. If an acid-fast bacterium is present, then appropriate antibiotic therapy should be started. If an isolate is obtained, it should be sent to an appropriate reference laboratory for susceptibility testing. Treatment should involve surgical drainage wherever possible and prolonged antimicorbial treatment, which might last for months, is usually required depending on clinical response and the nature of the infection.

Treatment of Individual Animals with Johne's Disease

Johne's disease, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a common cause of diarrhea, weight loss, and edema due to hypoproteinemia in ruminants and camelids. Johne's disease is better controlled at the herd level rather than by the treatment of individual animals (Sweeney et al., 2012). Treatment of occasional valuable animals or pets is aimed at reducing clinical signs rather than completely preventing shedding of the microorganism. *In vitro*, amikacin, streptomycin, ciprofloxacin, rifabutin, rifampin, and monensin are active against MAP (Brumbaugh et al., 2004; Zanetti et al., 2006; Krishnan et al., 2009). Azithromycin and clarithromycin were highly active in some but not all studies (Krishnan et al., 2009; Zanetti et al., 2006). Monensin significantly reduces the number of hepatic granulomas a mouse model of infection (Brumbaugh et al., 2004) and reduces fecal shedding in cattle (Hendrick et al., 2006). Other drugs that have been used in various clinical reports or experimental studies include rifampin, isoniazid, clofazimine, and gallium nitrate.

With the exception of monensin in some countries, none of these drugs are approved for use in cattle. There are no drugs approved for the treatment of Johne's disease. In Canada, monensin is approved the reduction in fecal shedding of MAP in mature cattle in high-risk Johne's disease herds as an aid in the herd control of Johne's disease as one component of a multicomponent Johne's disease control program. Based on a consensus statement from the American College of Veterinary Medicine, the recommended treatment protocol for a cow, sheep, goat, or camelid with clinical signs of Johne's disease is rifampin (10–20 mg/kg PO q 24 h) and isoniazid (10–20 mg/kg PO q 24 h; Sweeney et al., 2012). Monensin should be included if it can be legally administered for its label claims (Sweeney et al., 2012).

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Mycoplasma

The class Mollicutes is comprised of a diverse group of small bacteria that lack the capacity to produce a cell wall. The family Mycoplasmataceae is comprised of two cholesterol-requiring genera; *Mycoplasma* and *Ureaplasma*. Within this family, the genus *Mycoplasma* contains about 124 species and the genus *Ureaplasma* contains 7 species. Most animal pathogens are members of the genus *Mycoplasma*. Microorganisms classified until recently as obligate intracellular pathogens within the family Anaplasmataceae have recently been recognized as belonging to the genus *Mycoplasma*. There are increasing numbers of host-adapted species of haemoplasma being identified, in some cases causing only clinically inapparent bacteremias. These infections are often spread by vectors (lice, fleas). Mycoplasmas infections are associated with the respiratory tract, arthritis, mastitis, septicemia, and the urogenital tract of many animal species.

In vitro Activity

It is difficult to ascertain susceptibility since *in vitro* testing of isolates is difficult, and is usually not performed except by specialized laboratories. There are currently no MIC testing control standards for veterinary mycoplasmas and breakpoints have not yet been determined by the CLSI; as a result; MIC data cannot be defined as susceptible, intermediate, or resistant. There is need to examine *in vitro* activity of animal-derived mycoplasma more frequently than has been done in the past. In general, the macrolides (in particular azithromycin, clarithromycin, erythromycin, tylosin, tiamulin), florfenicol, and the fluoroquinolones appear to be the most active (Kobayashi et al. 1996; Thomas et al., 2003; Francoz et al. 2005; Assunção et al., 2007). Aminoglycosides, chloramphenicol, lincosamides, and tetracyclines are also active against *Mycoplasma* spp. Ketolides (e.g., telithromycin) are highly active against *Mycoplasma* species affecting people. MICs of tulathromycin for *M. bovis* isolates range from 0.125 to > 64 μg/mL (Godinho, 2008). However, tulathromycin was efficacious in the treatment of calves infected with a strain of *M. bovis* that had an MIC of > 64 μg/mL, so the clinical relevance of tulathromycin MIC values is unknown (Godinho, 2004). With the exception of the fluoroquinolones, which are bactericidal, the bacteriostatic activity of mycoplasma-active antibiotics may be another factor that makes mycoplasma infections often only slowly responsive to treatment.

Because of their inability to synthesize a cell wall, all mycoplasmas are resistant to antimicrobial agents acting on the cell wall (penicillins, cephalosporins, glycopeptides, etc). In addition, mycoplasmas are resistant to rifampin. Some species, such as *M. bovis* and *M. hyopneumoniae*, are intrinsically resistant to 14-membered macrolides such as erythromycin. Strains of mycoplasma from farm animals are increasingly frequently resistant to the tetracyclines, although the genetic basis of resistance of mycoplasmas to tetracyclines and other antimicrobial drugs has not been well characterized (Rosenbusch et al., 2005; Aarestrup and Kempf, 2006). In Denmark, the progressive development of resistance to tylosin over 2 decades by *M. hyopneumoniae* was linked to the extensive use of this drug in swine during this period (Aarestrup and Friis, 1998).

Clinical Application

Mycoplasmas are often both hard to isolate and slow growing. As a consequence, treatment of mycoplasmas infections is usually empirical rather than based on *in vitro* susceptibility. Elimination from tissues is often slow, since most antibiotics have only a bacteriostatic effect against mycoplasma. Despite excellent activity *in vitro*, treatment of established mycoplasma infections in animals has sometimes been disappointing, perhaps because effective treatment may require a 2- to 3-week rather than a shorter course. There is a paucity of data on the clinical efficacy of treatment of many mycoplasma infections in animals, which contrasts with the proven efficacy in human medicine of tetracycline or macrolide treatment of *Mycoplasma pneumoniae*. The guiding general principle required for effective treatment of a mycoplasma infection is therefore to choose

an antimicrobial agent that penetrates cells well (florfenicol, fluoroquinolone, lincosamide, macrolide, or tetracycline) and to administer the drug for a prolonged period of time, with isolation and *in vitro* susceptibility testing in cases of failure of clinical response. In food-producing animal, selection of an antimicrobial agent and duration of therapy must comply with country-specific regulations regarding antimicrobial drug use.

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Nocardia

Nocardiosis has been reported to occur in a variety of animal species, but of the domesticated variety, cattle, horses, dogs, and cats are most commonly affected (Beaman and Beaman, 1994). There are currently 99 Nocardia spp. *Nocardia nova* is the species most often isolated from dogs and cats (localized lesions most often associated with an extremity), whereas *N. asteroides* is most often isolated from cattle and horses (Biberstein et al., 1985).

The clinical findings in nocardiosis are non-specific and may be mistaken for a variety of other bacterial infections, fungal infections and malignancies. Nocardiosis can be suspected when moderately acid-fast branching filaments are seen in a sample collected from the affected site. The definitive diagnosis of *Nocardia* requires the isolation and identification of the organisms from a clinical specimen. Since nocardial colonies may take up to 2 weeks to appear, it is important to notify the laboratory when *Nocardia* infection is suspected, so that appropriate measures can be taken to optimize the of the microorganism.

Because nocardiosis is a rare disease, the best therapeutic agent, administration route, and treatment duration have not been well established in clinical trials. Recommendations are typically based on the results of *in vitro* susceptibility testing, animal models, and clinical expert opinions. Drugs active against most *Nocardia* spp. *in vitro* include trimethoprim-sulfonamide combinations, tetracyclines (doxycycline, minocycline, tigecycline), aminoglycosides (particularly amikacin), carbapenems (e.g., imipenem, meropenem, doripenem), and linezolid (Table 24.3; Lai et al., 2009; Conville et al.,

Table 24.3. Comparison of the susceptibility of *Nocardia asteroides* with that of *N. nova*.

Antimicrobial Drug	*Nocardia asteroides* (% susceptible)	*Nocardia nova* (% susceptible)
Ampicillin	27	44
Amoxicillin-clavulanate	67	6
Cefuroxime, cefotaxime, ceftriaxone	94–98	Cefuroxime (100 %); other third generation (83–94%)
Ciprofloxacin	38	0
Dapsone	92	94
Doxycycline	88	94
Minocycline	94	100
Amikacin	90–95	100
Erythromycin	60	100
Clarithromycin	–	100
Trimethoprim-sulfa	100	89
Imipenem	77	100
Tobramycin	–	33

2012). Fluoroquinolones (particularly moxifloxacin) and macrolides are also active agains some *Nocardia* spp. (Lai et al., 2009; Conville et al., 2012).

Trimethoprim-sulfonamides combinations have been the agents of choice for the treatment of nocardiosis in people and in animals for several decades. In some species, long-term treatment with this class of antimicrobial is sometimes associated with undesirable adverse effects (chapter 17). Occasional *Nocardia* spp. may be resistant to trimethoprim-sulfonamides. As a result, an initial combination therapy with two or more active agents is recommended for human patients with disseminated or severe nocardiosis (Ambrosioni et al., 2010). Examples of drugs commonly added to trimethoprim sulfonamides in people include amikacin, ceftriaxone, moxifloxacin, or imipenem (Ambrosioni et al., 2010). The duration of therapy is variable and depends on the site of the lesions and the immune status of the patient. Surgical treatment may be necessary depending on the clinical presentation and the body site involved.

Nocardioform Placentitis

Nocardioform placentitis, a common cause of placentitis in mares, is not caused by *Nocardia* spp. but rather by *Amycolatopsis* spp. (*A. kentuckyensis*, *A. lexingtonensis*, *A. pretoriensis*), *Crossiella equi*, or *Cellulosimicrobium cellulans* (Labeda et al., 2003; Bolin et al., 2004). Antimicrobial agents active against *Amycolatopsis* and *C. equi in vitro* include trimethoprim-sulfonamides and ceftriaxone (active against both species), doxycycline and minocycline (active particularly against *C. equi*), and amikacin (active particularly against *Amycolatopsis* spp.; Erol et al., 2012).

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Leptospira and Leptospirosis

In vitro susceptibility testing show leptospires to be susceptible to a wide variety of antimicrobial drugs including penicillin G, ampicillin, amoxicillin, third- (ceftriaxone, cefotaxime) and fourth- (cefepime) generation cephalosporins, imipenem, macrolides, tetracyclines, streptomycin, tiamulin, and fluoroquinolones (Ressner et al., 2008). They are relatively resistant to cephalothin, chloramphenicol and sulfonamides. Acquired resistance has not been reported.

Experimental infections with laboratory animals have established the value of penicillin G, macrolides, streptomycin, and tetracyclines in treatment of leptospirosis. The efficacy of fluoroquinolones is questionable. In a hamster model of leptospirosis, fluoroquinolones (iprofloxacin, gatifloxacin, or levofloxacin) resulted in similar survival as doxycycline but required much higher dosages (≥ 25 mg/kg/day for fluoroquinolones versus 5 mg/kg/day for doxycycline; Griffith et al., 2007). Cephalexin, cefadroxil, and cefoperazone had little activity, although cefotaxime was effective. First- and second-generation cephalosporins should therefore not be used for treatment. Treatment of human patients has established the value of penicillin G, ceftriaxone, cefotaxime, or doxycycline therapy in leptospirosis. In a hamster model of leptospirosis, minocycline or tigecycline were significantly more effective than doxycycline (Tully et al., 2011). In acute leptospirosis, recommended treatments in animals include ampicillin or amoxicillin, penicillin G, streptomycin, doxycycline or other tetracylines, or erythromycin. Amoxicillin (or ampicillin) or doxycycline are probably the drugs of choice.

Infection of dogs with leptospires results in illness of varying severity, depending on the infecting strain,

geographical location, and host immune response. In general, leptospirosis should be considered as a differential diagnosis in dogs with signs of renal or hepatic failure, uveitis, pulmonary hemorrhage, acute febrile illness, or abortion.

Based on a consensus statement from the American College of Veterinary Medicine, the recommended treatment protocol for canine leptospirosis is doxycycline is 5 mg/kg PO or IV q 12 h for 2 weeks (Sykes JE et al., 2011). If vomiting or other adverse reactions preclude doxycycline administration, dogs with leptospirosis should be treated with ampicillin, 20 mg/kg IV q 6 h (Sykes JE et al., 2011). Penicillin G (25,000–40,000 U/kg IV q 12 h) also could be used.

Chronic leptospirosis is characterized by abortion and stillbirth, recurrent iridocyclitis, repeat breeding in pigs and possibly cattle, and subclinical meningeal infection, depending on the serovar involved and the animal species affected. Many studies of *pomona* infection in swine and cattle have established the value of a single IM injection of 25 mg/kg of dihydostreptomycin or streptomycin in removing the kidney carrier state. It did not, however, remove serovar *hardjo* from the genital tract and kidney of bovine carriers in one study (Ellis et al., 1985). In outbreaks of leptospiral abortion in cattle, the usual recommendation has been to vaccinate after treating once with streptomycin. Because streptomycin is often difficult to obtain and its use is prohibited or discouraged for use in food animals in some countries, attempts have been made to find alternatives. Injection of 1 or 2 (q 48 h) doses of 15 mg/kg of amoxicillin was found to remove the kidney carrier state of serovar *hardjo* in cattle (Smith et al., 1997). After experimental inoculation of cattle with serovar *hardjo*, a single injection of oxytetracycline (20 mg/kg, IM), tilmicosin (10 mg/kg, SC), or multiple injections of ceftiofur sodium (2.2 mg/kg, IM, once daily for 5 days) resulted in elimination of urinary shedding of leptospires (Alt et al., 2001). In another study odf experimentally infected cattle, a single dose of tulathromycin resulted in clearance of the leptospires from the urine and kidney tissue in 9 of 9 animals whereas a single dose of ceftiofur crystalline free acid resulted in clearance of the leptospires from the urine in 8 of 10 and from the kidney tissue in all 10 animals (Cortese et al., 2007).

In swine, oral treatment with tetracyclines (800 g/ton for 8–11 days) will control leptospirosis but cannot be relied on to eradicate renal carriage. Tylosin (44 mg/kg 5 days), erythromycin (25 mg/kg 5 days), and tetracycline (40 mg/kg for 3 or 5 days) all given IM q 24 h effectively removed kidney carriage of serovar *pomona* in swine. Ceftiofur and ampicillin at standard dosages for 3–5 days was not effective (Alt and Bolin, 1996). Studies are needed to determine whether and what antimicrobial treatments are effective in therapy of periodic ophthalmia of horses.

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25

Antimicrobial Drug Residues in Foods of Animal Origin

Patricia M. Dowling

There is increasing concern over the adverse effects of antimicrobial drugs on human intestinal flora, including selection of resistant bacteria and disruption of the barrier effect of the normal resident intestinal flora. Currently, there is no documented evidence that antimicrobial drug residues in foods of animal origin cause adverse human health effects (e.g., prolonging antimicrobial therapy, prolonging hospitalization, predisposition to infection, treatment failure) when present at concentrations currently recognized as safe by regulatory agencies.

Regulation of Veterinary Drug Residues

Livestock and poultry production depends on drugs and other chemicals to protect animal health. To protect consumers from adverse health effects, federal programs are charged with the regulation of chemicals and drugs and the detection of chemical and drug residues in foods of animal origin. The United States Food and Drug Administration's (FDA) Center for Veterinary Medicine (CVM) and the Health Canada's Veterinary Drugs Directorate (VDD) approve veterinary drugs and establish the acceptable concentrations of drug residues in animal-origin food products. Drug approval requirements are available for the United States from the FDA in guidance documents at www.fda.gov and in Canada from the VDD at http://www.hc-sc.gc.ca/dhp-mps/vet/index-eng.php. The United States Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS) and the Canadian Food Inspection Agency (CFIA) monitor meat, poultry, eggs and honey for residues of drugs and chemicals. Monitoring of antimicrobial residues in milk and dairy products is mainly carried out on a state or provincial basis at the processor level. The U.S. and Canadian agencies use hazard analysis and critical control point (HACCP)-based systems consistent with the principles of risk analysis. The Codex Alimentarius Committee on Residues of Veterinary Drugs in Foods is a subsidiary body of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). This Codex committee facilitates world trade in agricultural commodities through the establishment of internationally recognized standards, codes of practice, guidelines, and recommendations that are based on the consensus of expert scientific opinion. A primary function is the establishment of internationally acceptable concentrations of veterinary drugs in food animal products.

Before any drug can be approved in the United States or Canada for use in a food-producing animal, an extensive toxicologic evaluation of the drug and its metabolites is undertaken. This ensures that any drug residues in animal-derived foods do not harm the consumer. A battery of four toxicologic tests are required to satisfy human food-safety requirements for any drug intended for use in a food-producing animal

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species: (1) Metabolism studies for identification of residues for toxicological testing. This includes metabolite identification in the target species and metabolite identification in a laboratory animal species. (2) Toxicological testing in laboratory animals, including genetic toxicity tests, acute toxicity tests, subchronic (90-day) toxicity tests, and a two- to three-generation reproduction study with a teratology component in rats. Lifetime carcinogenicity studies in two rodent species are required only if genetic toxicity tests indicate that the drug or metabolites are potentially carcinogenic (the decision by FDA to require lifetime carcinogenicity studies is based on a decision tree process referred to as *threshold assessment*). Other specific toxicity tests are required as needed. (3) Residue depletion studies in the target species. (4) Regulatory analytical methodology for identification and quantitation of marker residues in animal tissues, milk, honey or eggs.

Based on the results of toxicity tests, regulatory agencies establish an acceptable daily intake (ADI). The ADI represents a level of daily intake of a chemical that, during an entire lifetime, appears to be without appreciable risk to the health of the consumer. The ADI is used to determine the maximum concentration of a marker residue in edible tissues, honey, milk, or eggs that is legally permitted or recognized as acceptable. In the United States, these acceptable concentrations are termed *tolerances* while in Canada and the European Union they are termed *maximum residue limits* (MRLs). The MRL is calculated such that daily intake of food with residues at the MRL will result in a total daily consumption of residues in quantities at or below the ADI. ADIs are based on the total residue of a chemical present in food (parent compound and all metabolites) whereas MRLs are based on a single, measurable marker residue, which may be the parent compound or any of its metabolites. The toxicological significance of all residues must be known, and any residue that cannot be definitively determined as being without toxicological concern is assumed to have the same toxicity as the parent compound or metabolite upon which the ADI is based (Brynes, 2005). In establishing MRLs, consumption estimates for the various foods are taken into account so that foods consumed infrequently or in small amounts are allowed greater MRL values than those foods likely to be consumed daily or that represent a major component of the diet. Because of differences in consumption factors, MRLs and label withdrawal times may differ between countries, even though ADIs are equivalent (Fitzpatrick et al., 1995; Fitzpatrick et al., 1996; Table 25.1). International values for MRLs can be searched for on the International Maximum Residue Level Database at www.mrldatabase.com.

Table 25.1. Estimated consumption of animal products by the extreme (90th percentile) consumer.*

Food	United States (g/day)	Canada (g/day)
Beef muscle	155	206
Beef liver	20	20
Swine muscle	95	98
Chicken muscle	54	84
Fluid milk	690	677

*Fitzpatrick et al., 1996.

Veterinary drug sponsors first began to be required to account for the potential impact of ingested antimicrobial drug residues on the human intestinal flora in the 1980s. There is increasing emphasis in the antimicrobial approval process on evaluating antimicrobial drug residues capable of reaching the human colon and establishing microbiological ADIs. As *in vivo* models for safety evaluations relevant to humans are not available, *in vitro* minimal inhibitory concentrations (MICs) for relevant intestinal bacteria are used. Guidelines now require two endpoints of concern in determining a microbiologic ADI: (1) reduction or elimination of the barrier effect of the normal intestinal flora; and (2) development of and/or increase in the pool of antimicrobial-resistant strains of potential pathogens. The European Medicines Evaluation Agency's Committee for Medicinal Products for Veterinary Use (CVMP) calculates and publishes both toxicologic and microbiologic ADIs for antimicrobial drugs (Cerniglia and Kotarski, 2005). The most relevant ADI (usually the lowest) is used to determine the ADI in European veterinary drug approvals. The U.S. FDA CVM's Guidance for Industry #52, "Microbiological Testing of Antimicrobial Drug Residues in Food," recommends that antimicrobial drug sponsors use a "decision tree" approach to address the human food safety of antimicrobial residues and establish microbiological ADIs. In 2004, the VDD adopted the guidelines of the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary

Medicinal Products (aka, VICH): Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to Establish a Microbiological ADI. These documents are not regulations, but science-based processes drug sponsors may use when they seek approval of an antimicrobial for use in food-producing animals.

Despite the regulatory concerns regarding the impact on human health from veterinary antimicrobial residues, evidence of such effects is difficult to obtain. The assignments of ADIs, MRLs (tolerances), and antimicrobial drug withdrawal times are designed to be very conservative to ensure consumer safety, so antimicrobial residues in food are only a negligible fraction of the total amount of antimicrobials to which humans are exposed. Therefore, it is unlikely they contribute significantly to the emergence of antimicrobial resistance or disruption of the normal intestinal microflora in humans.

Residue Monitoring Programs

The United States National Residue Program (NRP), administered by the USDA FSIS, is an interagency program designed to identify, rank and test for chemical contaminants in meat, poultry, and egg products. The program screens for chemical residues from approved and unapproved veterinary drugs, pesticides, and environmental compounds. The NRP is designed to: (1) provide a structured process for identifying and evaluating residues of concern in food animal products; (2) analyze chemical compounds of concern; (3) collect, analyze, and report results; and (4) identify the need for regulatory follow-up subsequent to the detection of violative concentrations of residues.

When a violation is detected, the FSIS or the CFIA condemns the carcass or adulterated product. If the product has been distributed into commerce, it is subject to a voluntary recall. FSIS notifies the FDA of residue violations and assists in obtaining the names of producers and, in the case of food animal products, other parties involved in offering the animals or products for sale. The federal agencies take appropriate action when a violation is detected. These actions include follow-up inspections, further directed sampling according to a surveillance plan, or even seizure and recall of products when the human health risk is considered unacceptable. Follow-up actions vary according to the magnitude of the health risk; regulatory emphasis is on preventing repeat violations and preventing distribution of contaminated products into the public food supply. As a deterrent, the FSIS posts a Residue Repeat Violator List on its web site. The list identifies producers with more than one residue violation in the last 12 months. The list is useful to processors and producers who are working to avoid violative residues.

With increasing public concern over the risks of chemical contamination, there has been greater focus on strengthening the identification, ranking, and testing for chemical hazards in meat, poultry, and egg products in the United States. In 2012, the FSIS began using multianalytic methods that analyze more compounds per sample while using fewer samples. The new multiresidue methods (MRM) approach (1) screens for a variety of analytes, not just antimicrobials; (2) has been validated at concentrations appropriate to tolerances; (3) uses mass spectrometry to forensically distinguish individual analytes, even if multiple drugs are present in the same sample; (4) mitigates unknown microbial inhibition responses; and (5) reduces the time and personnel needed to obtain results. The new system uses a three-tiered sampling system that includes scheduled sampling (Tier 1), targeted sampling at the production or compound class level (Tier 2), and targeted sampling at the herd/flock or compound class level (Tier 3). The new program analyzes approximately 800 random samples per chemical compound class for each of the production classes (beef cows, bob veal, dairy cows, steers, heifers, market hogs, sows, young chickens, and young turkeys) tested in Tier 1. By increasing the number of samples analyzed, the FSIS increases the probability of detecting a residue violation to 99% if the violation rate is equal to or greater than 1% in the population of animals being sampled.

Tier 2 includes the inspector-generated sampling program at the establishment level. When FSIS Inspection Program Personnel (IPP) detect evidence of disease or drug use in an animal carcass, they hold and test samples from those carcasses. An animal may be suspect because of historical information on a production class, or appearance on ante- and post-mortem inspections. Typical suspect animals include culled dairy cows, bob veal calves (calves < 3 weeks of age and weighing < 68 kg), any animal with visible evidence of an injection site,

any animal showing evidence of an infectious disease, or animals of a given production class for which a high incidence of residue violations has been detected through the monitoring program. The Tier 2 program also includes targeted testing at the production and compound class level for show animals and bob veal calves. The FSIS will adjust targeted sampling plans in response to information about misuse of animal drugs and/or exposure to environmental chemicals gained from other agencies (such as the FDA and the Environmental Protection Agency), as well as Tier 1 sampling data. The Tier 3 level, still to be implemented, will encompass targeted testing at a herd or flock level.

The CFIA's National Chemical Residue Monitoring Program (NCRMP) has operated annually since 1978. The NCRM consists of monitoring sampling and directed sampling, which detects post-processing residues in food animal products in the marketplace. The NCRMP prioritizes sampling on the basis of estimated risk. Food items that are consumed in greater quantities by Canadians, those that are most likely to be contaminated, or those potentially contaminated with the most toxic compounds, are sampled and tested to the greatest extent. Testing for a specific drug or chemical may be temporarily discontinued if the test results show no positive residue finding in three consecutive years of at least 300 test samples. As in the United States, producers and distributors of food who violate Canadian standards are placed on enhanced inspection in order to identify the causes and reduce or prevent reoccurrences of violations.

Animal and egg products imported to the United States or Canada have passed inspection in their country of origin; therefore, import sampling is re-inspection. The level of re-inspection by the FSIS or CFIA depends on the exporting country's performance history. Import sampling is designed to verify the equivalence of chemical residue programs in countries exporting meat, poultry, honey, and egg products to the United States or Canada.

Causes and Incidence of Residue Violations

Drugs, pesticides, environmental contaminants, and naturally occurring toxicants can leave residues in meat, milk, eggs and honey. Of these, drugs are the most frequently detected chemicals and the overwhelming majority of violations are from antimicrobials. Each year, FSIS and the CFIA analyze samples from all market classes of food-producing animals. The highest priority for detection programs are the antimicrobials banned for use (or extra-label use) under the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) in the United States and the Food and Drugs Act in Canada (chapter 26).

When approved veterinary drugs are administered according to their label directions, the prevalence of violative drug residues in animal products should be less than 1%. Residue violation rates greater than 1% indicate that a drug has been used in a manner inconsistent with label directions. From 1960 to 1972, the prevalence of violative antimicrobial drug residues in swine, lambs, calves, and fat cattle slaughtered in the United States was 30%, 21%, 18%, and 7%, respectively. Prior to 1962, approximately 13% of all milk produced in the United States contained residues of antimicrobial drugs (Huber, 1971). Since the 1960s, the prevalence of residues in food animal products has declined significantly but there are still some problems. Several factors contribute to the drug residue problem, but most violations result from use of veterinary drugs in some manner that is inconsistent with the labeling. Analysis of the probable causes for violative residues in the United States reveal that failure to observe withdrawal times, drugs administered in error, treatment of animals with greater than labeled doses, failure to use the appropriate route of administration, and improper maintenance of medication records are identifiable risk factors (Paige et al., 1999). Medicated feeds are a frequent cause of residue violations in market hogs and poultry. Adherence to medicated feed withdrawal times may be burdensome, inconvenient, and expensive in that non-medicated feed must be provided during the withdrawal period and this requires the changing of feed programs and containers for a short time at the end of the feeding period. Lack of treatment records or failure to adequately identify treated animals can lead to insufficient withdrawal periods. When drugs are administered to animals at higher than label dosages, or when drugs are used in species for which they are not approved, the prescribing veterinarian is responsible for withdrawal recommendations. Recommendations made by veterinarians are often rough estimates and may be inadequate for depletion of drug residues from the carcass, milk, honey or eggs.

Salvaging diseased animals for slaughter that have been treated with antimicrobial drugs is a common cause of violative drug residues, especially in cull dairy cows and veal calves. The 2010, the US NRP examined food samples of animal origin for the presence of 128 chemical compounds, including 78 veterinary drugs, 45 pesticides, and 5 environmental contaminants. The majority of violations detected were veterinary drugs, particularly sulfonamides and antimicrobials. Of the 211,733 samples analyzed in 2010, there were 1,632 violations: 23 from scheduled sampling (now referred to as Tier 1) and 1,609 from the inspector-generated program (now referred to as Tier 2). From inspector-generated sampling, FSIS labs reported 2,043 residue violations in the 1,609 animals (a single animal may have multiple violations): beef cows (84), bob veal (765), bulls (8), dairy cows (700), formula fed veal (3), goat (1), heavy calves (5), heifers (10), market hogs (3), non-formula-fed veal (7), and steers (23). Neomycin accounted for the most residue violations (520 or 25%), followed by penicillin (281 or 14%). The high rate of neomycin violations in veal calves is mainly due to neomycin-medicated milk replacers fed to calves with enteritis. In normal calves, the oral bioavailability of aminoglycosides such as neomycin is very poor. But with inflammation and damage to the mucosal barriers with enteritis, sufficient quantities of neomycin are absorbed systemically and result in violative kidney residues. In 2008, the FDA issued over 30 warning letters to dairies and farms that sold animals as food that contained approved and unapproved drug residues in excess of FDA tolerance levels. Many of the drugs were used in an extra-label manner that was inconsistent with the regulations of the Animal Medicinal Drug Use Clarification Act (AMDUCA). Educational intervention during follow-up investigations by regulatory authorities prevents similar events from recurring in the future.

In 2010, the United States imported over 3 billion pounds of fresh and processed meat, poultry, and egg products, from 29 of the 33 countries eligible for exportation to the United States. The import testing program included analysis of approximately 121 chemical residues from 13 compound classes of veterinary drugs and pesticides. No residue violations for antimicrobials were detected in 2010.

In the 2009–2010 Canadian NCRMP, over 160,000 tests for residues of veterinary drugs, agricultural chemicals, environmental contaminants, mycotoxins, and metals were performed on monitoring samples of domestic and imported dairy, eggs, honey, meat and poultry products, fresh fruit and vegetables, processed products, and maple products. Foods of animal origin (dairy, eggs, honey, meat and poultry) were tested for veterinary drug residues, and overall compliance rates (by test) ranged from 98.03% to 99.93%. The majority of violations observed were for drugs for which there is no established MRL, so the detection of any amount constituted a violation. For approved veterinary drugs, oxytetracycline and penicillin G residues in beef and pork were the most common causes of violations.

Residues in Milk and Dairy Products

Milk that is contaminated with antimicrobials is considered a public health hazard because of adverse reactions and antimicrobial resistance. Antimicrobials are known to interfere with the manufacture of dairy products; concentrations of 1 ppb delay starter activity for cheese, butter, and yogurt. Antimicrobials also decrease the acid and flavor production associated with butter manufacture, and they reduce the curdling of milk and cause improper ripening of cheeses. The odds that a violative antimicrobial residue will be found in bulk tank milk increases with increasing milk production and an increase in the somatic cell count (SCC) status of the herd. Higher producing herds may have more problems with management, as there are typically more employees responsible for treatments and more cow records to maintain. The SCC is an indicator of the prevalence of mastitis within a herd and such infections are routinely treated with antimicrobials in order to lower the SCC to acceptable levels (Ruegg and Tabone, 2000; Saville et al., 2000).

In the United States, the National Milk Drug Residue Data Base is a voluntary industry reporting program. Mandatory reporting is required by State regulatory agencies under that National Conference on Interstate Milk Shipments. The Pasteurized Milk Ordinance requires all bulk milk tankers to be sampled and analyzed for animal drug residues before the milk is processed. In addition, a minimum of four samples from pasteurized fluid milk and milk products must be tested from each plant every 6 months and each producer must be tested at least 4 times every 6 months. In 2011, 3,787,251 milk samples were analyzed for animal drug

residues, and 1,079 were positive for a drug residue. A total of 3,796,684 tests were reported on the samples for eight different groups of families or individual drugs. Twenty-six testing methods were used to analyze the samples for drug residues. Of the positive samples, 671 were from milk tankers, none were from pasteurized fluid milk or milk products and 395 were from producer samples. The violations resulted in the discard of 28,174,000 pounds of milk. The majority of residue violations were due to beta-lactam antibiotics, tetracyclines, and sulfonamides. The most frequently used residue tests were the Charm SL tests, the Delvotest P 5 Pack, IDEXX SNAP tests, and the Charm II Tablet Competitive tests. In Canada, regulation of milk and dairy products is done on a provincial basis. Drug residues statistics from individual provinces is not available. In 2002/2003 in the federal program, the CFIA tested 3,577 milk and cheese products with no antimicrobial or sulfonamide violations detected.

Drug residues in milk are tested for by several methods, such as microbial growth inhibition assays, microbial receptor assays, receptor binding assays, immunologic assays, enzymatic assays, and chromatographic analysis (Mitchell et al., 1998). Because of the problems and penalties associated with antimicrobial residues in milk and dairy products, a number of rapid antimicrobial screening tests have been developed for testing bulk tank or tanker milk. Despite brand names that include the term *cowside*, none of the tests are currently validated for testing individual cows. The milk withdrawal time (WDT) for a drug with a lactating dairy cow claim is based on the time required after treatment for milk residues to fall below the MRL in 99% of animals, 95% of the time. The milk WDT is not the point at which residues can no longer be detected. Differences may occur between label milk WDTs for the same product in Canada and the United States. In Canada there is no assumption regarding dilution of drug residues in the bulk tank, so milk from an individual cow must be below the legal MRL to establish the WDT. The FDA assumes that no more than one-third of the milk in the bulk tank will come from treated cows. Therefore the label WDT in the United States is determined so that the milk from any treated cow will be less than 3 times the legal MRL. Currently, milk WDTs are established using a quantitative chemical test as milk screening tests do not have the required analytical characteristics to establish official WDTs. The commercially available screening tests have excellent sensitivities for the antimicrobials they are designed to detect and excellent negative predictive values but they have poor positive predictive values. So a negative test on an individual cow is excellent insurance that a violation on the bulk tank milk will not be detected, but a positive test on an individual cow will not necessarily result in bulk tank drug concentrations above the legal MRL (Gibbons-Burgener et al., 2001). Because 90/95 levels must be below the MRL, screening tests can produce a positive result when the drug concentration is below the legal MRL. These "subviolative" positive test results are positive test results on a milk sample in which the actual drug concentration is at or above the detectable concentration of the test, but below the established MRL. With all of the tests, there is a characteristic response curve, which means that as the drug concentration increases in the milk, there is a corresponding increase in the percentage of positive tests until a plateau is reached and all samples test positive. Even if two different tests have the same 90/95 results at the MRL, the responses at less than MRL concentrations can differ. If the contract between a producer and the milk processor states that there shall be "no drugs" in the milk, then the processor is free to use any validated residue detection test, even if its 90/95 sensitivity level is far below what is safe for human consumption (the legal MRL) and the label WDTs for drugs used in dairy cattle are essentially meaningless. This is problematic for some drugs like ceftiofur and cephapirin (intrauterine formulation) that have zero milk WDTs on the label, but for which screening test sensitivity can be far below the MRL that was used to establish the zero WDT. Also, rapid testing methods incorporating semi-quantitative visual detectors will give a range of actual readings at any single drug concentration. For example, when screening a sample that truly contains a residue at 6 ppb, repeating the test could give a range of readings from 4 to 12 ppb. The rejection of subviolative but "safe" milk is an economic issue for veterinarians and dairy producers, who may not understand how they can use an approved drug according to label directions, follow the label WDT, and still have a residue violation. The regulatory authorities and processors know that these testing methods will result in a very small percentage of milk being dumped for testing positive, even though the drug residues are safe for human consumption (below the MRL). Identifying the specific drug and quantity present in a milk sample requires more specific

chemical analysis, such as high-performance liquid chromatography (HPLC) and/or mass spectrometry. This is not feasible for every milk sample that tests positive with a rapid screening test due to the time and expense of withholding a positive milk tanker from processing until quantitative results are obtained. The regulating authorities accept the imprecision of the screening tests for the sake of the public good and the efficient delivery of milk products to consumers.

The issue with subviolative positives becomes more complicated when using multiresidue tests such as the beta-lactam, tetracycline, or sulfonamide screening tests. Each multiresidue test detects one or more drugs at concentrations below their respective MRL, but is not ideal for detecting all drugs (especially cloxacillin). When testing for a known or suspected drug in milk, it is best to use a test that is designed specifically for that drug. When testing milk from cows where the treatment history is unknown, it is better to use a multidrug screening test. However, a positive result on a multidrug test will not identify which specific drug is present.

Even though "cowside" tests are only validated for bulk tank milk, Sischo et al. (1997) reported that the use of antimicrobial residue screening tests for evaluating an individual cow's milk was associated with a reduction in the risk of residue violations. Screening milk for residues in milk post-partum following prepartum intramammary therapy in heifers is recommended to reduce the risk for contamination of bulk tank milk (Andrew et al., 2009). In addition, the Milk and Dairy Beef Residue Prevention Protocol of the Dairy Quality Assurance Program recommends that milk from individual cows be tested for antimicrobial residues following extra-label use of antimicrobials. Testing milk from treated cows following an appropriate milk-withholding period allows the dairy producer to make informed decisions about milk withholding and reduces the risk of contamination of commingled milk.

In Table 25.2, three commonly used "cowside" screening tests are compared according to their sensitivities for specific antimicrobials against the MRL values in the United States, Canada, and the European Union. For some tests, the sensitivity is far below the legal MRL (e.g., ceftiofur, cephapirin), and can result in "subviolative positives" with unnecessary milk discard. For others, the sensitivity is above the legal MRLs, resulting in false negatives (e.g., cloxacillin, erythromycin). Screen tests need to be interpreted with caution. High levels of natural inhibitors are present in mastitic milk and in colostrum, and they can cause false positive results in the microbial growth inhibition assays. Heat treatment of milk to 82°C for 5 min inactivates natural inhibitors and can be used to prove false-positive results in the microbial growth inhibition assays (Kang et al., 2005). High concentrations of milk protein and milk fat can adversely affect antimicrobial residue test performance, but the degree of the effect depends upon the analytical method of the screening test (Andrew, 2000). Higher concentrations of immunoglobulins and milk protein can also cause false positives with screening tests used on samples from recently freshened heifers or cows (Andrew, 2001).

Other Effects of Drug Residues in Food on Human Health

Residues of antimicrobial animal drug raise special human safety concerns with regard to allergic reactions and carcinogenicity. Ordinary cooking procedures for meat, even to "well done," cannot be relied on to inactivate drug residues. More severe heating for canning or prolonged cooking with moist heat can inactivate the more heat sensitive compounds, such as penicillins and tetracyclines, but the nature of the degradation products is unknown in most cases (Moats, 1999). Allergic reactions are manifested in many ways, from life-threatening anaphylactic reactions to lesser reactions such as rashes. Veterinary drug residues do not cause primary sensitization of individuals because exposures are too low and for short duration. However, violative residues of animal drugs in food have the potential to cause allergic reactions in sensitive individuals. Reports of acute adverse reactions in humans from ingestion of drug residues are rare. Of the few reports that document adverse reactions in people consuming residue-contaminated foods, the overwhelming majority are allergic reactions to penicillin. In reference to these allergic reactions, Burgat-Sacaze et al. (1986) stress the following: (1) Involvement of residues constitutes a small percentage of food allergies. The major allergens involved are natural food constituents or human food additives. (2) The clinical observations report rashes the most frequently, but anaphylactic shock has not been reported. (3) In most cases, residues are implicated without sufficient diagnostic evidence. Most suspicions

Table 25.2. Comparison of commercially available on-farm milk screening tests for antimicrobials.

Drug	Test	Sensitivity (ppb)	US MRL (ppb)	CDN MRL (ppb)	EU MRL (ppb)
Amoxicillin	Charm Farm Cowside	6	10	NE	4
	Delvotest P	6			
	IDEXX SNAP B-lactam	7.3			
Ampicillin	Charm Farm Cowside	5	10	10	4
	Delvotest P	5.8			
	IDEXX SNAP B-lactam	5.8			
Ceftiofur	Charm Farm Cowside	300	50	100	100
	Delvotest P	50–70			
	IDEXX SNAP B-lactam	5.4			
Cephapirin	Charm Farm Cowside	10	20	20	10
	Delvotest P	7			
	IDEXX SNAP B-lactam	11.7			
Chlortetracycline	Charm Farm Cowside	300	300	100	100
	Delvotest P	300			
	IDEXX SNAP Tetracycline	30			
Cloxacillin	Charm Farm Cowside	30	10	NE	30
	Delvotest SP	20			
	IDEXX SNAP B-lactam	50			
Dicloxacillin	Charm Farm Cowside	25	NE	NE	30
	Delvotest P/SP	100			
	IDEXX SNAP B-lactam	50			
Erythromycin	Charm Farm Cowside	150	50	50	40
	Delvotest SP	250			
Gentamicin	Charm Farm Cowside	300	30	100	100
	IDEXX SNAP Gentamicin	30			
	Delvotest SP	400			
Lincomycin	Charm Farm Cowside	200	150	NE	150
	Delvotest SP	400			
Neomycin	Delvotest P	150	150	150	500
Novobiocin	Delvotest P/SP	600	100	100	100
Oxytetracycline	Charm Farm Cowside	300	300	100	100
	Delvotest P	300			
	IDEXX SNAP Tetracycline	30			
Penicillin	Charm Farm Cowside	4	5	10*	4
	Delvotest SP	2.7			
	IDEXX SNAP B-lactam	3.0			
Pirlimycin	Charm Farm Cowside	200	400	400	100
	Delvotest SP	50			
Polymixin B	Delvotest P	30	0	4	NE
Sulfonamides	Charm Farm Cowside	20–200	10	10	100
	Delvotest SP	100			
Tetracycline	Charm Farm Cowside	100	300	100	100
	Delvotest P	300			
	IDEXX SNAP Tetracycline	20			
Tilmicosin	Charm Farm Cowside	100	0	NE	40
Tylosin	Charm Farm Cowside	100	50	NE	50
	Delvotest P/SP	100			

*MRL value is in IU/mL.
NE: no legal maximum residue limit established.

are based on an observed hypersensitivity reaction following food intake and tests that demonstrate that the individual is not allergic to the food eaten but is to some drugs, and hence the possibility of the presence of residues of these drugs in the food, without actual demonstration of drug residues in the food. Thus "circumstantial evidence" is often the only criterion and residue involvement is anecdotal. Nearly all reports of acute adverse reactions from foodborne residues implicate penicillin as the offending agent, and the source of penicillin residues is most often milk or dairy products. These milk residues likely originated from intramammary infusion of penicillin used for the treatment of mastitis (Siegel, 1959). Although a substantial number of farm milk samples have been found to contain small amounts of penicillin, there have been relatively few published reports of adverse reactions from milk residues (Boonk and van Ketel, 1982; Borrie and Barrett, 1961; Erskine, 1958; Vickers, 1964; Vickers et al., 1958; Wicher et al., 1969; Zimmerman, 1959). In all instances, the victims reported a history of penicillin allergy or skin disease unrelated to penicillin allergy. Symptoms varied in intensity from mild skin rashes to exfoliative dermatitis. In an investigation of 252 patients with chronic recurrent urticaria, 70 (27.8%) were determined to be allergic to penicillin by dermal testing. When 52 of these penicillin-allergic patients were restricted to a diet containing no milk or dairy products, 30 had remission of symptoms, whereas only 2 out of a group of 40 patients with chronic urticaria and negative skin tests responded favorably to the milk-free diet. Many drugs other than penicillin, including other beta-lactams, streptomycin (and other aminoglycosides), sulfonamides, and to a lesser extent, novobiocin and tetracyclines, are known to cause allergic reactions in sensitive persons; however, there is only a single report of a reaction to meat suspected of containing streptomycin residues (Tinkelman and Bock, 1984).

Other potential adverse human effects from antimicrobial residues in food animal products include carcinogenicity and bone marrow suppression. While there is no evidence that consuming residue-containing food animal products affects human health, a number of antimicrobials are banned from veterinary use in many countries because of concerns. Idiosyncratic (non-dose-dependent) aplastic anemia can occur in humans exposed to chloramphenicol. Nitroimidazoles (e.g., metronidazole), nitrofurans (e.g., nitrofurazone) and carbadox are banned for veterinary use in many jurisdictions due to carcinogenicity potential. Ironically, all of these banned antimicrobials are still used therapeutically in humans.

Preventing Residues: The Food Animal Residue Avoidance Databank

The Food Animal Residue Avoidance Databank (FARAD) was established in 1982 as a cooperative project between North Carolina State University, the University of California, the University of Florida and the USDA FSIS as a way to reduce the rate of residue violations in animal products through education and information. The founding philosophy of FARAD was that information about residue avoidance from all sources should be immediately available from a scientific source. The FARAD was developed to not only contain information related to approved animal drugs but to also include information on extra-label drug use and environmental toxins. For this "one-stop shopping" information service to work, the FARAD information was collated into a searchable computer database, with residue and pharmacokinetic data analyzed and interpreted by veterinary pharmacologists and toxicologists. The FARAD database includes over 1200 drugs and chemicals and over 20,000 pharmacokinetic records extracted from over 11,000 citations. The FARAD system focuses on published pharmacokinetic information such as the tissue elimination half-lives, clearance rates, and volumes of distribution for those drugs, pesticides, and environmental contaminants that have the greatest potential for persisting in tissues of livestock at slaughter. From these pharmacokinetic values, mathematical models are developed to estimate residue depletion times. For over 25 years, the US FARAD centers have been providing accurate and timely information to veterinarians to protect the US food supply. In 2002, the Canadian global FARAD was established at the Western College of Veterinary Medicine at the University of Saskatchewan and a second center has been established at the Ontario Veterinary College at the University of Guelph. Supported by the Canadian food animal commodity groups and veterinary pharmaceutical companies, the Canadian gFARAD provides similar services to Canadian veterinarians.

When using drugs in an extra-label manner in food animals in the United States or Canada, or in the event of animal exposure to pesticides, herbicides and other toxic chemicals, veterinarians can request withdrawal recommendations from their FARAD (www.farad.org in the United States and www.cgfarad.usask.ca in Canada). When contacting a FARAD center, the veterinarian should be prepared to provide information regarding the brand name and generic name of the drug, the dose, the type and number of animals treated and the disease condition prompting treatment.

Conclusion

Food safety is one of the most significant issues facing animal agriculture. Consumer concerns about drug and chemical residues continue to erode the demand for animal-derived foods. Globally, concerns over food safety have resulted in disruption of international trade. Formal training in the area of residue prevention has been limited at a time when advances are rapidly reshaping the way that food safety programs operate. The development of multiresidue tests allows for extensive monitoring of large numbers of animal products prior to reaching the food supply. Quality assurance programs require livestock producers, processors and packers to verify that their animals and animal products are wholesome and free of drug residues. HACCP programs are in place at federally inspected abattoirs. Failure of the veterinary profession and the livestock industry to embrace "farm to plate" programs will ultimately undermine the public's confidence in the safety of the food supply. Clearly, at a time when consumer demand for a safe and wholesome food supply has never been greater, the need for national regulatory authorities, the veterinary profession and the livestock industry to assert strong leadership in food safety has never been more critical.

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26

Regulation of Antimicrobial Use in Animals

Karolina Törneke and Christopher Boland

Approval and licensing of antimicrobials for use in animals, particularly food-producing animals, is a complex process that strives to ensure that products are effective and safe. It also involves management of the risks of adverse consequences from antimicrobial use. To foster responsible use of antimicrobials and facilitate their safe use (including containment of antimicrobial resistance) regulatory authorities may give specific guidance or apply specific restrictions of use. Pharmacovigilance is also applied in many countries and many jurisdictions also monitor antimicrobial sales and use and trends in antimicrobial resistance.

The OIE Terrestrial Animal Health Code sets out standards for the improvement of animal health and welfare and veterinary public health worldwide, and refers to the responsibility of regulatory authorities and pharmaceutical industry in this respect (chapter 6.9.3, OIE 2011):

> The pharmaceutical industry has to submit the data requested for the granting of the marketing authorization. The marketing authorization is granted only if the criteria of safety, quality and efficacy are met. An assessment of the potential risks and benefits to both animals and humans resulting from the use of antimicrobial agents in food-producing animals should be carried out. The evaluation should focus on each individual antimicrobial product and the findings not be generalized to the class of antimicrobials to which the particular active principle belongs. Guidance on usage should be provided for all dose ranges or different durations of treatment that are proposed.

The risks considered in the approval of veterinary antimicrobial products include:

- Harm due to uncontrolled quality of the antimicrobial product.
- Harm to people directly exposed (human occupational safety).
- Harm to organisms inadvertently exposed (environmental safety).
- Harm to the treated animals caused by the product and the way it is used (target animal safety).
- Harm to the treated animal due to failure of the product to achieve its claims (efficacy).
- Harm to people exposed to the product or residues of the product through consumption of food products of animal origin (human food safety).
- Harm to people exposed to microorganisms resistant to the product's antimicrobial ingredients or metabolites either via contamination of food or direct contact with animals shedding resistant microorganisms.

For most veterinary products, harm due to exposure to the product focuses on the potential toxicity of residues of the product itself (parent drug or metabolites).

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In the case of antimicrobial products intended for use in food-producing animals, risk evaluation also includes harm to humans due to the effect that the antimicrobial drug may have on microorganisms.

In most countries, antimicrobials undergo comprehensive, in-depth testing prior to receiving marketing approval as detailed in the text below. Many products produced for animals are marketed globally and many countries have similar data requirements for approval. Effort has been expended to promote international harmonization of animal drug regulatory requirements. The International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH, www.vichsec.org) was formed by government and industry participants from the European Union, Japan, and the United States and have been joined by observers and interested parties from Canada, Australia, and New Zealand. The working groups of VICH have developed and harmonized study protocols, criteria, and standards for the registration of new veterinary pharmaceutical and immunological products as well as standards for post-marketing surveillance and reporting. These standards now include guidelines concerning microbiological affects and the potential development, emergence and spread of antimicrobial resistance. The general standards apply to products destined for national markets as well as for products destined for multiple markets around the world. Individual countries have further refined their pre-market-assessment guidance to give more detail on preferred antimicrobial assessment (e.g., vol. 3, part 10 vetMORAG issued by the Australian Pesticides and Veterinary Medicines Authority, or the US Food and Drug Administration Guidance for Industry #152). Risk assessment guidance is being refined in other jurisdictions as antimicrobial-resistance issues are clarified.

Assessment guidance on approving veterinary antimicrobials focuses on adequate risk assessment, including these aspects:

- Potential increase in number of resistant bacteria in the gastrointestinal tract or on the skin/mucosa of exposed animals due to the use of the antimicrobial product.
- The probability that humans will be exposed to the resistant bacteria.
- The probability that human exposure to resistant bacteria will result in adverse human health consequences.

The following are the areas that are most commonly considered in pre-market assessment of a veterinary medicinal product in general and an antimicrobial product in particular.

Demonstration of Quality

Ensuring product quality (i.e., compliance to approved product and manufacturing specification) is the essential starting point in the veterinary medicinal product assessment of risks because any assumptions of hazards and probable risks must be based on a consistent, uniform product. Adequate quality of the product ensures batch-to-batch consistency and that the product fulfills the established specifications to the end of the authorized shelf life. For these reasons, all veterinary medicinal products should be manufactured to the appropriate quality and purity and produced in compliance with the provisions of Good Manufacturing Practice (GMP). Conditions on market approvals are set by regulators to ensure safety and efficacy, given the product specifications and approved uses. The company's ongoing ability to produce the product to approved specifications is also a cornerstone in monitoring and compliance.

Quality was the first discipline on which international harmonization was achieved via the VICH program aimed at harmonizing technical requirements for veterinary product registration. In addition, many regional quality guidelines continue to exist. For further information, refer to VICH guidance documents GL1-GL5, GL8, GL10-11, and GL17-18.

Demonstration of Safety

The manufacturer should demonstrate that the drug is sufficiently safe for use under the conditions described in the proposed labeling. The requirements for demonstration of safety can be separated into environmental safety, user safety, consumer safety and target animal safety and should include not only risks related to exposure to the product itself, but also exposure to residues of the product in food and, with regard to antimicrobial resistance, exposure to resistant microorganisms following exposure to the product. A package of

pharmacological, toxicological, and microbiological and epidemiological data, based on studies *in vitro* or in laboratory animals, target animal field trials, and risk modeling, is required for all pharmacologically active substances in antimicrobial veterinary products.

User Safety

A veterinary medicinal product must be safe for person(s) who will administer the product to animals and other people in contact with the product. The most well-known risk related to exposure to the substance itself is the risk of hypersensitivity reactions and other toxicological reactions. Since some injectable products cause severe tissue irritation or adverse systemic effects (e.g., tilmicosin), accidental self-injection is also of concern. Safety of the veterinary drug to the user is considered by evaluating the toxicity profile of the drug, the route of administration, the packaging, and instructions to the veterinarian or animal caregiver.

Treatment with antimicrobials increases antimicrobial resistance in the commensal flora of the gastrointestinal tract and the skin/mucosa, so there is an increased risk for exposure of humans to antimicrobial-resistant bacteria due to direct contact with treated animals. This is a concern with antimicrobial use in both food-producing and companion animals. Zoonotic strains of methicillin resistant *Staphylococcus aureus* (MRSA) have been acknowledged as a prominent public health hazard (Catry et al., 2010). Although few jurisdictions have guidance available for such risk analysis today, the risk for spread of antimicrobial resistance between animals and humans via direct contact or exposure to fecal material, secretions and exudates may increase regulatory requirements for non-food animal drug approvals. In New Zealand, companies are requested to submit a specific risk assessment when applying for marketing authorization for antimicrobials for companion animals.

Environmental Safety

Environmental safety evaluation includes risk to water (ground and drinking), plant, animal, and microbial species in water (ground and drinking) or soil that may be exposed.

An exposure threshold approach is generally used to determine when environmental fate and effect studies are needed. When an environmental assessment is required, the drug sponsor conducts laboratory-based toxicity studies with invertebrates, plants and microorganisms representative of the environmental compartment of concern.

VICH has developed extensive guidance to assess the potential for veterinary medical products to affect non-target species in the environment, including both aquatic and terrestrial species. Evaluation of environmental effects is carried out in two phases. Phase I guidance describes criteria for determining whether an environmental impact assessment should be undertaken (VICH GL6). According to the guidelines, environmental studies are not necessary for compounds that have limited environmental distribution (e.g., antimicrobial products used to treat companion animals). If the exposure limits set are exceeded in Phase I, the Phase II assessment is needed to obtain data on environmental fate, metabolism, and toxicity of the active substance, using the test methods described in the Phase II guideline (VICH GL38). The VICH Phase II guidance contains sections for aquaculture, intensively reared terrestrial animals, and pasture animals. Current VICH guidance does not include consideration of the risk to animal or public health due to spread of resistant bacteria in the environment. However, this is an area of growing concern. Resistant bacteria in efflux water from drug manufacturing plants may result from high concentrations of active components in such water unless accurate residue management is applied (Li et al., 2011).

Tolerance in the Target Animal Species

Regulatory jurisdictions provide guidance on how to document possible safety concerns of the veterinary medicinal product for the target animal. This guidance is often based on VICH guidelines. By documenting signs of toxicological and secondary pharmacological effects in the target animal species under laboratory conditions where dose levels excessive (with regard to level and time) of the dose intended for approval are administered, the targets of toxicity are mapped. Besides clinical signs, clinical pathology and necropsy results are normally documented. The results obtained are used to establish a margin of safety and provide adequate product information.

Human Food Safety Related to Hazards from Veterinary Drug Residues

To determine the food safety of residues of an antimicrobial, the drug sponsor conducts a standard battery of animal-based toxicology and microbiology tests. The battery of animal studies for antimicrobial agents to be used in food-producing animals includes tests for repeat-dose toxicity), reproduction toxicity, developmental toxicity, genotoxicity, and effects on human intestinal flora. For reference, the reader is recommended to refer to VICH guidance documents GL22-23, GL31-33, and GL36-37.

These tests must provide adequate data to ensure human food safety. The toxicology studies are designed to determine the minimum dose that causes a toxic effect and the maximum dose that causes no observed adverse effect (NOEL). These endpoints are then used to calculate an acceptable daily intake (toxicological ADI). The toxicological ADI is established in a similar way for all substances intended to be included in veterinary pharmaceutical products, whereas the effects on human intestinal biota (the so called microbiological ADI) is considered specifically for substances with antimicrobial properties. Antimicrobial drug residues may disrupt the human gastrointestinal flora and increase the population of resistant bacteria. VICH GL36 outlines how to determine the need for establishing a microbiological ADI, recommends test systems and methods for determining NOELs for the endpoints of health concern, and recommends a procedure to derive a microbiological ADI. The normal intestinal biota limits colonization by exogenous (potentially pathogenic) microorganisms. To ensure that this colonic barrier is not disrupted following ingestion of drug residues in food, data should be provided to show that the potentially active concentration in the intestinal tract are well below MIC of a set of bacteria in the normal human intestinal biota. A second endpoint to be considered when establishing a microbiological ADI is the possible increase of the population(s) of resistant bacteria in the human intestinal tract, either due to de novo development of resistance or selection of resistant strains that were previously present in low numbers. The lowest of the pharmacological, toxicological and microbiological ADI provides the basis for determining the maximum residue limits (MRLs).

Human Food Safety Related to Risk for Spread of Antimicrobial Resistance from Animals

A concern to be addressed in relation to antimicrobial resistance is the contribution of antimicrobial drug use in food-producing animals to the emergence of antimicrobial drug-resistant bacteria that causes disease in humans (Figure 26.1). This may occur directly in case of resistant zoonotic pathogens or indirectly due to gene transfer from animal commensals to human pathogens in the intestine of a person. The drug sponsor is required to carry out a risk assessment that addresses the probabilities of resistance developing, the transfer of resistant bacteria to humans and the development of unresponsive disease in humans and estimating an overall antimicrobial-resistance risk. While the sponsor may propose options for risk reduction or mitigation, it is the regulators responsibility to select and impose risk management measures, carry out the risk communication, and establish the risk-monitoring system (see below).

The sponsor should document the risk assessment of a veterinary antimicrobial for the spread of antimicrobial resistance. The VICH document GL27 provides instructions for gathering information on the drug, its mode of action and spectrum of activity, including MICs of target animal pathogens and foodborne and commensal organisms, the mechanism of resistance development, and other related information. However, the VICH GL27 does not provide guidance as to how the assessment should be carried out and to cover this aspect some regulatory jurisdictions have issued more detailed guidance (e.g., U.S. FDA Guidance for Industry #152 and APVMA part 10, vetMORAG, NZ Antimicrobial Resistance Registration Information Guidelines) to complement the VICH guidance. Although specific guidelines may not be available in other countries/regions, similar approaches are followed on a case-by-case basis.

Demonstration of Efficacy

Drug efficacy studies include preclinical (microbiological data and pharmacokinetics) and clinical studies (experimental and field trials). The number and types of studies required to demonstrate drug effectiveness at the proposed dose or dose range differs between jurisdictions and type of application. The drug sponsor must provide a sufficient number of studies of adequate quality to allow assessment of the drug's efficacy. The quality of a study's design and conduct includes factors of rigor,

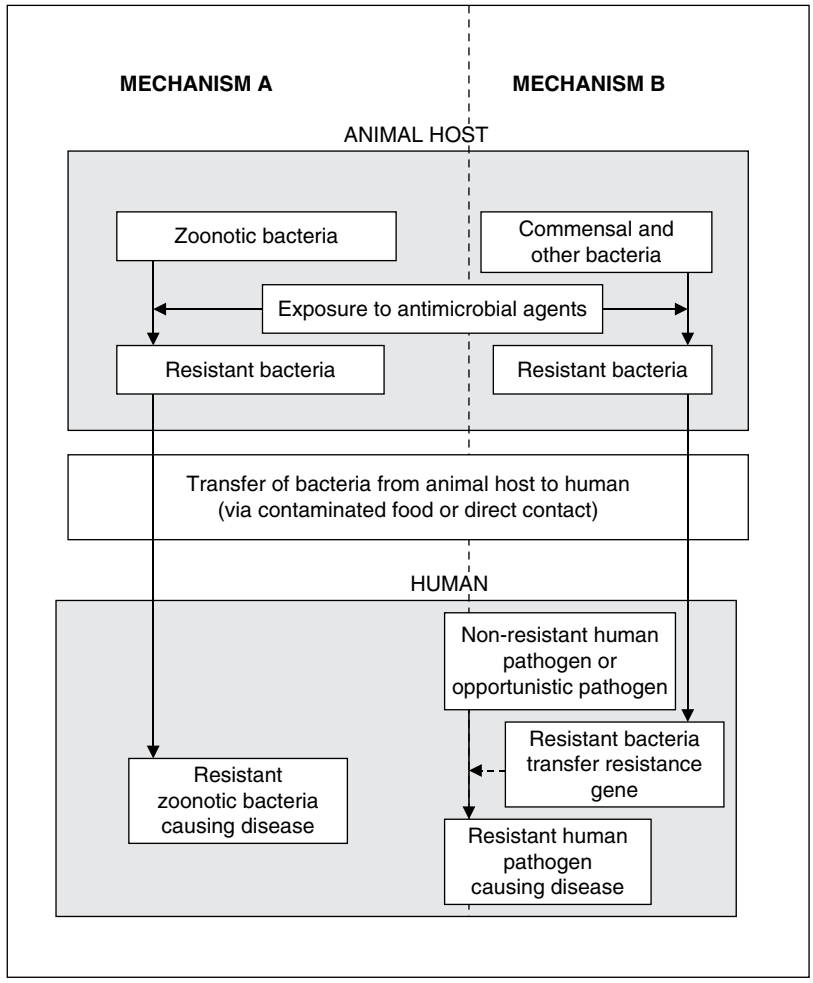

Figure 26.1. Resistance development in zoonotic pathogenic bacteria (Mechanism A) and animal-origin commensal bacteria (Mechanism B). Mechanism A is the scenario in which animals are treated with antimicrobial agents and the zoonotic bacteria present (e.g., *Salmonella*) in the animal develop resistance. Humans are exposed to the resistant zoonotic bacteria, resulting in colonization and possibly disease that is less responsive to antimicrobial therapy. Mechanism B is the more complicated scenario. In this scenario animals are treated with antibacterial products and commensal bacteria (e.g., *Enterococcus* species or a [non-patogenic] *E. coli*) develop resistance. The commensal bacteria could be transferred to humans and either colonize in the human intestine or at least remain long enough to transfer the resistance genetic material to bacteria (possibly pathogenic) in humans.

statistical power, and scope. Most jurisdictions have research/trial guidelines and some even require the trial and analysis to be carried out under good clinical practice (GLP) and/or good laboratory practice (GLP) accreditation. Preclinical studies, including pharmacokinetic and microbiological data, are usually generated to establish an appropriate dosage regimen necessary to ensure the efficacy of the antimicrobial product. Important information about the antimicrobial includes the mode of action, the spectrum of antimicrobial activity, and identification of bacterial species that are intrinsically resistant. Bacterial kill curve data for the target pathogen(s)

should be used to determine whether the antimicrobial exerts time-dependent or concentration-dependent killing activity.

Important pharmacokinetic information includes bioavailability applicable to the route of administration, concentration of the active antimicrobial in the plasma/serum and preferably at the site of infection, volume of distribution and parameters related to elimination and excretion. In addition, pharmacokinetics may be used to establish the bioequivalence of a generic product with the pioneer product.

Definitive proof of the efficacy of an antimicrobial product is based upon a demonstration of effectiveness in clinical trials. VICH has established a Good Clinical Practice guidance (VICH GL9) that provides information on the design and conduct of clinical studies of veterinary drugs in the target species. The goal is to ensure the accuracy, integrity, and correctness of the data submitted to the regulatory authority for product registration. The guidance sets out detailed requirements for the clinical investigator, study monitor, and drug sponsor, including instructions on study design, animal selection, animal housing and feeding, and study treatments. Emphasis is placed on developing a comprehensive study protocol in order to help ensure that a well-designed study is developed and executed.

It should be noted that evidence of efficacy does not necessarily imply that the use of the product would be prudent. Many jurisdictions publish treatment guidelines listing products as "first-" or "second-line" (see chapter 7 on responsible use and "Management of Antimicrobial Resistance" below).

Benefit/Risk Balance

The regulatory authority responsible for the approval of veterinary medicinal products evaluates all data submitted in the application in order to explore the balance of the benefits and risks associated with the use of the products. This process is complex, as different weight needs to be put on different parts. Efficacy and safety for the target animal needs to be balanced, implying a higher acceptance for intolerance in case of highly efficacious, life saving medicines. Risks to public health related to exposure to drug residues are very important, as there is little consumer tolerance for exposure to hazardous chemicals in food. This risk is controlled by applying withdrawal/withholding periods that adequately reduce exposure. Risks to the user and environment from exposure to the drug substance could also be mitigated although such risk mitigation might not be fully quantitative. Risks for development, emergence and spread of antimicrobial resistance are the most difficult to balance as all use of antimicrobials increases resistance and thus a certain level of risk acceptance is a prerequisite for approval of antimicrobials. In addition, the hazard (resistant bacterial population) is subject to the whims of a biological system faced with multiple influencing factors with different levels of exposure and varying capacity of the microorganisms to take advantage of opportunities under different circumstances. Factors to be considered:

- The probability that the population of resistant bacteria will increase due to the use of the antimicrobial product.
- The probability that humans will be exposed to the resistant bacteria.
- The probability that human exposure to resistant bacteria will result in adverse health consequences.

While risk mitigation may be applied at a product level, there are aspects that must be covered at a substance/class level (see "Management of Antimicrobial Resistance" below).

Risk Mitigation Strategies

The regulator must consider the information available in the dossier in a risk management context and decide if marketing approval should be granted. If some of the risks need to be managed to an acceptable level or if there is significant uncertainty surrounding the estimate of some risks, the regulating agency will impose risk reduction measures. Different jurisdictions employ different risk reduction measures (or combinations of measures) but the most common are:

- Setting conditions for manufacturing and distribution.
- Controlling the supply chain by specifying who can sell the product and who can authorize its use.
- Dictating what instructions, warning or advice must be put on the label of the product.
- Requiring reporting of how much product is used and what it is used for.

- Restricting how the product can be used.
- Imposing compliance to codes of practice.

Pharmacovigilance

To document the continued safety and efficacy of the products, many jurisdictions have pharmacovigilance systems to detect and assess instances of adverse events, including adverse drug reactions and inefficacy in live animals and residues in animal carcasses and foodstuffs (see chapter 7). For antimicrobials, the reports of suspected lack of efficacy are important because these cases may provide early signals of emerging antimicrobial resistance. Pharmacovigilance may also involve monitoring the prevalence of antimicrobial-resistant organisms and potential environmental impact of drug use.

Extra-Label Drug Use

The regulatory agencies, such as the US Food and Drug Administration (FDA) and the Canadian Veterinary Drugs Directorate (VDD), approves the labeling of veterinary drugs and establishes the acceptable concentrations of drug residues in animal-origin food products. Unfortunately, there are many disease conditions that require veterinarians to use of drugs in a manner inconsistent with their labeling. This extra-label drug use (ELDU) may involve administration of a drug: (1) to a species for which there is no specific veterinary drug approval; (2) by a non-approved route; (3) using a non-approved drug dose or dose frequency; (4) for a disease that is not listed on the label; or (5) for humans to animals. *Off-label* is a term commonly used in foreign countries and by physicians in the United States. It is also sometimes used in veterinary medicine as a synonym for *extra-label*, but the term has no legal or regulatory definition.

ELDU in the United States

In the United States, the Animal Drug Use Clarification Act (AMDUCA) of 1994 codified ELDU in animals by veterinarians. Under AMDUCA, ELDU is limited to drug treatment when the health of the animal(s) is threatened or suffering or death may result from failure to treat. Under AMDUCA, a veterinarian must select, prescribe and/or dispense drugs that are to be used in an extra-label manner within the context of a valid veterinary-client-patient relationship (VCPR). A valid VCPR is established when a veterinarian is responsible for making medical judgments regarding the health of the animal(s) and their need for drug treatment, and the owner of the animal(s) has agreed to follow the veterinarian's instructions; the veterinarian has sufficient knowledge of the animal(s) to initiate at least a preliminary diagnosis of the condition requiring treatment; and the veterinarian is readily available for follow-up in case of adverse reactions or failure of the treatment regimen. A valid VCPR exist only when the veterinarian personally examined the animal(s), or is familiar with the health status of the herd or flock from timely visits to the premises where the animal(s) are kept. It is not legal for producers to use drugs in an extra-label manner without a veterinarian's prescription. AMDUCA requires that animal drugs approved for a particular use are to be used when they are available. The FDA does not view cost as an acceptable reason for ELDU. Extra-label use of drugs in treating food-producing animals for improving rate of weight gain, feed efficiency, or other production purposes (including reproductive management) is prohibited under AMDUCA. And due to the issues surrounding regulation of feed mills, ELDU in animal feeds is not allowed. The FDA will use regulatory discretion in the case of minor species that are difficult to medicate in any other manner but only when the animal(s) are farmed or confined and the health or life of animal(s) is in danger.

AMDUCA requires that only FDA approved human or veterinary drugs be used can be used in an extra-label manner. The use of compounded drugs in food-producing species is permitted under AMDUCA only when there is not an approved product and only from approved human or animal drug products. If an approved veterinary drug can be used for the compounding, it is not permissible to compound from an approved human drug. The compounding must be performed by a licensed pharmacist upon the prescription of a veterinarian or by a veterinarian if allowed by their state's pharmacy law. The compounded product must be safe and effective and the compounding operation must be consistent with providing small quantities of product for very specific patient needs. A number of publications have demonstrated problems with stability and quality of compounded veterinary drug products. The FDA has been very clear that compounding of non-approved drugs from bulk "active pharmaceutical ingredients" in food animals will not be tolerated without specific

written approval. The only exceptions to this rule are antidotes for use in food animals that are not available as approved products. The FDA will use regulatory discretion to permit compounded formulations of ammonium molybdate, ammonium tetrathiomolybdate, ferric ferocyanide, methylene blue, pilocarpine, picrotoxin, sodium nitrite, sodium thiosulfate, and tannic acid to be used as antidotes.

AMDUCA requires specific record keeping and labeling requirements for drugs that are dispensed or prescribed for extra-label use. The records must be kept for a minimum of 2 years after treatment and the FDA must be allowed access to the veterinarian's records to evaluate risk to public health.

Record requirements:

- Identify the animals, either as individuals or a group.
- Species of animal(s) treated.
- Numbers of animals treated.
- Medical conditions being treated.
- Brand name of the drug and generic name of active ingredient(s).
- Dosage prescribed or used.
- Duration of treatment.
- Specified withdrawal, withholding, or discard time(s), if applicable, for meat, milk, eggs, or other animal-derived food products.

Label Requirements:

- Name and address of the prescribing veterinarian.
- Established name of the drug.
- Any specified directions for use including the class/species or identification of the animal or herd, flock, pen, lot, or other group.
- The dosage frequency, and route of administration and the duration of therapy.
- Cautionary statements restricting use to a licensed veterinarian ("CAUTION: Federal law restricts this drug to use by or on the order of a licensed veterinarian").
- The specified withdrawal, withholding, or discard time for meat, milk, eggs, or any other food product originating from the treated animal(s).

The FDA Center for Veterinary Medicine regulates ELDU and enforces the regulations of AMDUCA. In cases of AMDUCA violations, FDA regulatory actions may include warning letters, seizure of product, misdemeanor fines, injunction or criminal prosecution. The FDA may prohibit ELDU of an approved new animal or human drug or class of drugs in animals if the FDA determines that an acceptable analytical method for residue detection has not been established or cannot be established, or the extra-label use of the drug or class of drugs presents a risk to public health. The prohibition may be a general ban on the extra-label use of the drug or class of drugs or may be limited to a specific species, indication, dosage form, route of administration, or combination of these factors. Currently, the following drugs are prohibited from ELDU in food-producing:

- Chloramphenicol.
- Clenbuterol.
- Diethylstilbestrol (DES).
- Dimetridazole.
- Ipronidazole.
- Other nitroimidazoles.
- Furazolidone.
- Nitrofurazone.
- Sulfonamide drugs in lactating dairy cattle (except approved use of sulfadimethoxine, sulfabromomethazine, and sulfaethoxypyridazine).
- Fluoroquinolones.
- Glycopeptides.
- Gentian Violet.
- Phenylbutazone in female dairy cattle 20 months of age or older.
- Cephalosporins (except cephapirin in cattle).

The FDA recently enacted the cephalosporin prohibition to preserve the effectiveness of cephalosporin drugs for treating humans by reducing the risk of cephalosporin resistance in certain bacterial pathogens. The order specifically prohibits using cephalosporin drugs at unapproved dose levels, frequencies, durations, or routes of administration; using cephalosporin drugs in cattle, swine, chickens or turkeys that are not approved for use in that species (e.g., human or companion animal formulations); and using cephalosporin drugs for disease prevention. The order does not limit the use of cephapirin, because the FDA does not believe that this cephalosporin used for mastitis or uterine infections in cows contributes significantly to antimicrobial resistance.

The antiviral drugs, adamantanes and neuraminidase inhibitors, are approved for treating or preventing

influenza A in humans, and are prohibited from ELDU in chickens, turkeys, and ducks regardless of whether or not ELDU criteria are met. Vaccines are considered "veterinary biologics" and fall under regulation by the USDA Center for Veterinary Biologics (USDA CVB). Veterinarians are allowed discretionary use of vaccines. For example, if a minor species requires a vaccine not labeled for that species, the veterinarian can use a particular vaccine as they see fit. Veterinarians should check their state regulations to ensure that it is acceptable to vaccinate animals with vaccines labeled for another animal species. The use of pesticides by a veterinarian is under the jurisdiction of the Environmental Protection Agency (EPA). While their use is not subject to ELDU regulations, pesticides must always be used according to the instructions on the label.

In Canada, ELDU of veterinary drugs is not codified as it is in the United States. While the approval of drugs for sale in Canada is under federal jurisdiction, the practice of veterinary medicine falls within provincial jurisdiction and the legislation and regulations governing the practice of veterinary medicine vary from province to province. In Canada, ELDU is not specifically confined to veterinarians and may legally be performed by multiple users including intermediate health professionals (e.g., pharmacists, animal health technicians) and laypersons (e.g., animal owners, livestock producers). According to Health Canada, ELDU is a recognized tool in the practice of veterinary medicine and ELDU in food-producing animals by anyone other than licensed veterinarians is not recommended except when such use is conducted under the supervision of a veterinarian within the context of a valid veterinarian-client-patient relationship. Furthermore, ELDU is not recommended with antimicrobial drugs of very high importance to human health and should only be undertaken in compliance with the Food and Drugs Act and its Regulations, which includes banned substances, medicated feeds and violative residues.

In the European Union, the Veterinary Medicinal Products (VMR) Directive 2001/82/EC sets out the controls on the manufacture, authorization, marketing, distribution and post-authorization surveillance of veterinary medicines applicable in all European Member States. The VMR generally prohibits ELDU but in order to avoid unacceptable suffering, a veterinarian responsible for an animal may treat that animal in accordance with a sequence referred to as the "prescribing cascade" or simply "the cascade." The provisions of the cascade are set out in the VMR, and differ depending on whether the animal requiring treatment is a food-producing animal or a non-food-producing animal. When using the cascade there are also a number of requirements that need to be fulfilled with regard to record keeping, labeling, and storage.

Management of Antimicrobial Resistance

Besides risk management measures applied within the framework of marketing authorization for specific veterinary medicinal products, many authorities provide general guidance on how to use antimicrobials to minimize risks related to antimicrobial resistance. At a joint meeting in Rome in 2007 (Joint FAO/WHO/OIE Expert Meeting on Critically Important Antimicrobials), WHO and OIE with FAO agreed on a joint list of antimicrobial classes that are critically important for human use, are widely used in veterinary medicine and where resistance might be zoonotic (i.e., there is evidence or a reasonable likelihood that zoonotic bacteria or resistance determinants may be transmitted to humans through the food chain when/if those antimicrobials are used in animals). The organizations agreed that fluoroquinolones, third- and fourth-generation cephalosporins and macrolides are the three groups of highest concern and they recommend that regulatory authorities give these three groups the highest priority for risk analysis.

Another organization that has provided guidance on antimicrobial resistance risk analysis is Codex Alimentarius (www.codexalimentarius.org), an organization founded by WHO and FAO to develop food standards, guidelines and related texts such as codes of practice. A code of practice to minimize and contain antimicrobial resistance (CAC/RCP 61–2005) and a guideline for risk analysis of foodborne antimicrobial resistance (CAC/GL 77, 2011) are available. These documents focus on risk for spread of antimicrobial resistance in a global food trade perspective. A list of risk management options are presented in the Codex document guideline for risk analysis of foodborne antimicrobial resistance (CAC/GL 77, 2011). These cover a full range of activities from information campaigns and treatment guidelines to promote responsible use of antimicrobials, to banning certain drugs or uses. Some examples of such risk mitigation measures applied in different jurisdictions are:

Restrictions Related to Prescription of Antimicrobials

Different measures to control the way antimicrobials are made available are applied in different jurisdictions. For instance, the use of antimicrobials for non-therapeutic indications such as growth promoters have been extensively discussed worldwide (see chapter 22). Some countries have banned such use and other countries also may soon impose bans as both Codex and OIE recommend such use to be phased out. Many countries now limit antimicrobials as prescription only medicines. Further to restrictions to prescription only status, extra-label use could be prohibited and requirement of direct veterinary oversight could be applied, such as the recent FDA restrictions on the ELDU of cephalosporins in the United States. The possibility to separate prescription and distribution of antimicrobials has also been discussed. Countries like Sweden and Denmark restrict veterinarians' right to sell antimicrobials to avoid economic incentives for increased prescription.

Withdrawals and Refusals of Market Approval

There are examples of withdrawals of marketing authorization in cases where a risk analysis pointed at an unacceptable risk for antimicrobial resistance. In the United States, the FDA withdrew approval of enrofloxacin and sarafloxacin products intended for poultry because of evidence that the use of fluoroquinolones in poultry causes the development of fluoroquinolone-resistant *Campylobacter* that may be transferred to humans and cause resistant infections. New Zealand adjusted its market authorizations for antimicrobials in 2001 to refuse approval of mass medication using critically important antimicrobial agents for which there was evidence that resistance could develop and be transferred to humans. The relative importance of the agents targeted was confirmed by the WHO/OIE lists. A similar decision covering all food-producing animal species was taken in Australia in 2006.

Targets to Reduce Consumption

In Europe, some countries have set targets for reduction of total consumption of antimicrobials by animals. Denmark is a country where authorities have presented a more complex model to ensure compliance with responsible use principles by applying special provisions for the reduction of the consumption of antimicrobials in pig holdings. In their pig production, farms are contracted and the use of antimicrobials is mapped linked to a "yellow card" (Government Order No. 1319 of December 1st 2010) which puts financial pressure on non-compliant farms.

Non-statutory Risk Reduction Initiatives

The regulatory agencies often augment the compulsory risk reduction measures with education and awareness programs, usually with voluntary cooperation from the affected industry sectors or professional health care groups such as the veterinary profession. Recommendations for responsible use of antimicrobials are a cornerstone of antimicrobial risk management (see chapter 7).

Monitoring and Surveillance

In order to track the current levels of antimicrobial use and antimicrobial resistance, monitoring programs are applied in many jurisdictions. Due to differences in methodology, data is usually not directly comparable between countries and regions and there is a need for harmonization of methodology. With regard to monitoring of antimicrobial resistance, OIE has reviewed methods focusing on international harmonization (chapter 6.7, OIE 2010) and many countries have built surveillance programs for zoonotic bacteria and commensals based on these methods. Official monitoring programs for target animal pathogens is still uncommon but will probably be more comprehensively performed in the future. Monitoring of sales is still a less common practice and there are difficulties in translating sold tons of antimicrobials into a measure that can be used for comparison of different countries, production forms and antimicrobial products. One initiative to overcome these hurdles is the European Medicines Agency's European Surveillance of Veterinary Antimicrobial Consumption (ESVAC), a monitoring system covering the entire EU. A first report of historical data from nine countries was published in 2010. A similar report is available from New Zealand. The combination of monitoring of drug sales and rates of antimicrobial resistance could be a valuable tool when assessing the impact of different risk mitigation measures.

For instance, restrictions of use of a certain antimicrobial from a previously high level might correlate with reduced resistance rates. In Canada, a voluntary ban against cephalosporins in chicken production correlated well with resistant *Salmonella enterica* Serovar Heidelberg in humans (Dutil et al., 2010).

Conclusion

Veterinary antimicrobial products provide special challenges both for drug sponsors and regulatory authorities. Antimicrobial resistance not only endangers the efficacy of products in the treatment of animal diseases but can also impact human health. Transfer of resistant zoonotic or commensal bacteria or transfer of resistance determinants, either directly from treated animals to humans or indirectly via food, is of growing concern. Regulators in countries around the world have developed standards for market approval requirements and post-marketing control and surveillance. Besides measures taken at the product level, risk analyses for groups of antimicrobials important in human medicine have been conducted and risk mitigation measures have been aimed at ensuring that veterinary antimicrobials are used in a responsible manner. The goal of these efforts is to balance the need to minimize the impact on human health while having appropriate veterinary medicinal products available to meet the health and welfare needs of animals.

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Section IV

Antimicrobial Drug Use in Selected Animal Species

27

Antimicrobial Drug Use in Horses

Steeve Giguère and Tiago Afonso

Rational drug therapy has been defined as the selection of the proper drug to be administered according to a dosage regimen appropriate to the patient after due appraisal of potential benefits and risk of that therapy. The first step in this decision-making process is to determine whether an infectious agent is the likely cause of the disease, and if so, that the animal is unlikely to efficiently eliminate the infection without antibiotic therapy. In choosing the appropriate antimicrobial agent, the veterinarian must consider: (1) the likely identity of the infecting microorganism(s); (2) its *in vitro* antimicrobial susceptibility pattern or the clinical response in equine patients infected with the same pathogen; (3) the nature and site of the infectious disease process; (4) the pharmacokinetic characteristics of the chosen antimicrobial agent in horses such as bioavailability, tissue distribution, and rate of elimination; (5) the pharmacodynamic properties of the antimicrobial agent selected; (6) its safety in horses; and (7) the cost of therapy.

Common Bacterial Pathogens of Horses and Their Typical Susceptibility Patterns

Because the identity and *in vitro* susceptibility of an infecting microorganism are rarely known when therapy is begun, initial therapy is usually empirical and is based on knowledge of the agents likely to be present and their historical susceptibility (Tables 27.1 and 27.2). In some cases, the most likely etiologic agent can be highly suspected simply based on the clinical presentation and the horse's history. For example, abscessation of the submandibular and retropharyngeal lymph nodes is most likely caused by *Steptococcus equi* subspecies *equi*. On the other hand, pleuropneumonia in an adult horse may be caused by any one or combinations of a number of bacteria and thus requires bacteriologic culture of a tracheobronchial aspirate and pleural fluid to determine the etiologic agent(s). Similarly, cellulitis, mastitis, musculoskeletal infections, peritonitis, and urinary tract infections, may be caused by a variety of bacteria. A Gram stain of properly collected material is a simple, rapid and inexpensive means of identifying the presence and morphological features of microorganisms in body fluids that are normally sterile. A negative Gram stain is, however, not sufficient to confirm the absence of microorganisms. Although seeing bacteria on a Gram stain rarely reveals their identity, it can provide useful information regarding therapy while awaiting bacterial culture and antimicrobial susceptibility testing. For example, Gram-positive cocci in chains suggest *Streptococcus* spp. *Streptococcus* spp. isolated from a purulent lesion in a horse are likely group C streptococci, which are usually susceptible to penicillin. On the other hand, the presence of both Gram-positive and Gram-negative bacteria indicate a mixed infection that will require broad-spectrum antimicrobial agents at least until bacterial culture reveals the etiologic agents and their *in vitro* susceptibility

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Table 27.1. *In vitro* antimicrobial susceptibility of selected bacterial isolates from horses.[a]

	Antimicrobials[b]												
Microorganisms (n[c])	AMP	AMI	XNL	CHL	ENR	E	GM	KA	OX	P	TE	TMS	RIF
Gram-positive													
Staphylococcus aureus (211)	41	96	57[d]	95	89	76	89	87	74	44	83	81	95
Other staphylococci (149)	32	95	62[d]	95	93	86	75	68	86	32	85	76	95
Enterococcus spp. (179)	86	1	5	74	24	35	10	3	–	75	64	9	30
Streptococcus equi (132)	99	0	100	90	60	96	78	0	–	100	96	82	99
S. zooepidemicus (758)	99	1	100	88	50	94	55	1	–	99	16	63	98
S. dysgalactiae subsp. equisimilis (104)	96	9	99	86	75	78	68	7	–	93	47	84	90
Gram-negative													
Acinetobacter spp. (94)	59	84	45	67	88	47	84	75	–	–	78	60	–
Actinobacillus equuli (140)	62	50	99	71	96	19	80	54	–	46	80	60	–
Actinobacillus spp. (42)	79	71	100	86	95	15	91	59	–	54	88	71	–
Bordetella bronchiseptica (14)	7	100	0	100	93	31	100	92	–	–	100	71	–
Citrobacter spp. (30)	3	95	87	75	97	0	70	71	–	–	73	50	–
Enterobacter spp. (132)	25	83	64	78	85	2	65	63	–	–	74	54	–
Escherichia coli (362)	52	95	92	88	94	1	85	83	–	–	71	53	–
Klebsiella spp. (130)	2	91	82	74	88	1	71	70	–	–	76	60	–
Pasteurella spp. (28)	78	100	89	94	89	36	100	84	–	47	93	75	–
Proteus spp. (24)	25	100	92	20	70	0	75	80	–	–	17	38	–
Pseudomonas spp. (232)	4	88	17	9	54	4	71	31	–	–	41	22	–
Salmonella spp. (185)	20	–	74	67	58	1	24	25	–	–	83	25	–

[a]Data from the Animal Health Laboratory (AHL) of the University of Guelph (2005–2012) kindly provided by Dr. Durda Slavic and Beverly McEwen.
[b]Percent susceptible isolates.
[c]Approximate number of isolates (some isolates were not tested against every antimicrobial agent).
[d]*In vivo*, ceftiofur is rapidly metabolized to desfuroylceftiofur. Desfuroylceftiofur is as effective as ceftiofur against most bacterial pathogens but most coagulase-positive *Staphylococcus* spp. are resistant. Therefore, despite *in vitro* susceptibility, ceftiofur is not the ideal choice for the treatment of staphylococcal infections.
– = Not tested or testing not indicated.
AMP, ampicillin; AMI, amikacin; XNL, ceftiofur; CHL, chloramphenicol; ENR, enrofloxacin; E, erythromycin; GM, gentamicin; KA, kanamycin; OX, oxacillin; P, penicillin; TE, tetracycline; TMS, trimethoprim-sulfonamide; RIF, rifampin.

pattern. The initial selection of the antimicrobial agent and route of administration will depend on the severity of the disease and the site of infection. A combination of gentamicin for Gram-negative coverage and penicillin for Gram-positive and anaerobic coverage is commonly used as initial broad-spectrum therapy for severe bacterial infections in adult horses. Enrofloxacin can be used as a substitute to gentamicin in adult horses, whereas ampicillin or cefazolin can replace penicillin. Addition of metronidazole is recommended for disease processes where *Bacteroides fragilis* is commonly isolated such as pleuropneumonia and peritonitis.

Many infectious diseases involving the neonatal foal such as pneumonia, peritonitis, meningitis, osteomyelitis, septic arthritis, and omphalophlebitis are the sequelae of bacteremia. Gram-negative bacteria account for 70–95% of the microorganisms isolated from cultures of blood samples in equine neonates, with *Escherichia coli* being by far the most common isolate. Gram-positive cocci account for approximately 25% of isolates. Treatment protocols for equine neonates must include antimicrobials with a high level of activity against enteric Gram-negative bacteria while providing adequate coverage against Gram-positive microorganisms. Bactericidal agents are preferred because neonatal foals have a naive immune system and their defense mechanisms against bacterial pathogens are often compromised. The combination of an aminoglycoside (amikacin or gentamicin)

Table 27.2. Antimicrobial drug selection in infection of horses.

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
Upper Respiratory Tract	Strangles	*Streptococcus equi*	Treatment of a horse with strangles depends on the stage of the disease. While the organism is susceptible to penicillin, parenteral antibiotics given after abscess formation may prolong the disease. Horses with severe systemic signs or internal abscesses require antibiotics.	Penicillin G[a]	Ceftiofur
	Guttural pouch empyema	*Streptococcus equi*, *S. zooepidemicus*, rarely Gram-negatives	Local irrigation with saline is the treatment of choice. Lowering the horse's head facilitates drainage and reduces the risks of aspiration. Systemic or topical antimicrobials rarely indicated unless infection is spreading.	Penicillin G[a]	Ceftiofur
	Guttural pouch mycosis	*Emericella nidulans*, *A. fumigatus*, other opportunistic fungi	Surgical occlusion of the affected artery is the treatment of choice. Even when successful, medical therapy may be too slow to prevent several bouts of hemorrhage.	Topical enilconazole; Systemic antifungal agents usually not required	Topical natamycin
	Fungal rhinitis	*Aspergillus* spp. Other opportunistic fungi	Surgical removal of the mycotic plaque and associated necrotic tissue, combined with topical antifungal therapy.	Topical enilconazole	Topical natamycin; Topical amphotericin B
	Sinusitis, primary	*S. zooepidemicus*	Treatment may consist of a daily lavage of sinus with saline (± antiseptics or antimicrobial agents) combined with systemic antimicrobial agents. Non-responsive cases may require sinusotomy.	Penicillin G[a]	Ceftiofur; trimethoprim-sulfonamide[b]
	Sinusitis, secondary	Mixed opportunistic aerobic[c] and anaerobic[d] infection	Usually requires treatment of primary problem; i.e., removal of diseased tooth.	Penicillin G[a]	Ceftiofur; trimethoprim-sulfonamide[b] and metronidazole; chloramphenicol
Lung	Bacterial pneumonia or lung abscesses; adults	*S. zooepidemicus*, Opportunistic aerobic pathogens[c], *S. pneumoniae*, *Mycoplasma* spp.	*S. zooepidemicus* is most commonly isolated.	Ceftiofur; penicillin G[a] is drug of choice if streptococcal infection is confirmed	Broad-spectrum antibiotics[e]; trimethoprim-sulfonamide[b]
				Oxytetracycline	Enrofloxacin[f]; chloramphenicol
	Bacterial pneumonia or lung abscesses	*S. zooepidemicus*	Most common cause of pneumonia/bronchitis in older foals.	Penicillin G	Ceftiofur; macrolide ± rifampin for refractory abscesses
	Older foals	Opportunistic aerobic		Ceftiofur	Broad-spectrum antibiotics[e]
		R. equi	Treatment must be a minimum of 3–4 weeks.	Rifampin and macrolide (erythromycin, azithromycin, or clarithromycin).	Doxycycline[h] rifampin; trimethoprim-sulfonamide rifampin

(*continued*)

Table 27.2. Antimicrobial drug selection in infection of horses. (*continued*)

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
		Pneumocystis jiroveci	May be found in immunocompromised foals or in association with *R. equi.*	Trimethoprim-sulfonamide	
	Bacterial pneumonia; neonatal foals	Opportunistic aerobic pathogens[c]	Neonatal pneumonia often a part of a generalized infection affecting many different organ systems.	Broad-spectrum antibiotics[e] (amikacin preferred over gentamicin)	Third-generation cephalosporins; ticarcillin-clavulanic acid
	Pleuropneumonia	Opportunistic aerobic[c] and anaerobic pathogens[d]	While systemic antimicrobial agents are most essential treatment for bacterial pleuropneumonia, thoracic drainage and nursing care are important.	Broad-spectrum antibiotics[e] ± metronidazole	Ceftiofur ± metronidazole; penicillinG[a] and enrofloxacin[f] ± metronidazole; trimethoprim sulfonamide[b] and metronidazole
		Mycoplasma felis		Oxytetracycline	Enrofloxacin;[f] chloramphenicol
	Fungal pneumonia	Opportunistic fungi: *Aspergillus* spp., *Candida* spp., *Mucor* spp.	If fungal pneumonia is secondary to severe primary disease (i.e., liver failure, enterocolitis, peritonitis, etc.), treatment is difficult and prognosis is poor. If fungal pneumonia is secondary to aggressive antibiotic therapy (i.e., neonatal foal) then prognosis is guarded.	Amphotericin B	Itraconazole; voriconazole
	Tuberculosis	*Mycobacterium*	Treatment is not usually attempted. Public health concern. Reportable disease.	See chap. 24	
Gastrointestinal	Oral, gastric candidiasis	*Candida* spp.	Seen in immunosuppressed animals or ones on long-term antibiotic therapy. May just require discontinuation of antibiotic therapy.	Fluconazole	Voriconazole; itraconazole; Amphotericin B
	Acute enterocolitis; salmonellosis	*S. typhimurium*, other serovars	Systemic antimicrobials indicated in animals showing signs of or at risk for septicemia (foals, immunocompromised animals, aged animals, aged animals). Treatment with antibiotic is not thought to alter the course of the disease.	Broad-spectrum antibiotics[e]; enrofloxacin[f]	Third-generation cephalosporins; susceptibility variable
	Acute enterocolitis; clostridiosis	*C. difficile*, *C. perfringens* type A *C. perfringens* type C	The first approach in therapy is to stop the precipitating antimicrobial agent when applicable.	Metronidazole	Oral bacitracin (22 mg/kg PO BID day 1, then SID); oral vancomycin[g]
	Potomac horse fever (equine ehrlichial colitis)	*Neorickettsia risticii*		Oxytetracycline	Oral doxycycline; [h] rifampin and erythromycin
	Proliferative enteropathy	*Lawsonia intracellularis*	Proliferative illeitis and diarrhea in foals.	Macrolide ± rifampin	Oxytetracycline; chloramphenicol
	Abdominal abscess	*S. equi*, *S. zooepidemicus*, *Corynebacterium pseudotuberculosis*	Most commonly a complication of strangles. Long-term treatment frequently required.	Penicillin G[a] ± rifampin	Macrolide ± rifampin; chloramphenicol; trimethoprim-sulfonamide[b]

		R. equi (foals)	Abdominal abscess(es) and ulcerative enterocolitis. Peritonitis may be present. Pneumonia, diarrhea, septic physitis, or arthritis may occur concurrently.	Rifampin and macrolide (erythromycin, clarithromycin, or azithromycin)	Doxycycline[h] ± rifampin; Trimethoprim-sulfonamide. Rifampin
	Peritonitis	Mixed opportunistic aerobic[c] and anaerobic pathogens[d] *Actinobacillus equuli*	Obtaining culture and sensitivity of peritoneal fluid highly recommended. Peritoneal lavage may be beneficial in some cases.	Broad-spectrum antibiotics[e] and metronidazole	Third- or fourth-generation cephalosporine and metronidazole; penicillin G[a] + enrofloxacin[f] + metronidazole
	Tyzzer's disease	*Clostridium piliforme*	Treatment is usually not successful.	Erythromycin ± rifampin; penicillin G and aminoglycoside	Oxytetracycline
	Liver abscess	β-hemolytic Streptococci, *C. pseudotuberculosis*, opportunistic aerobic[c] or anaerobic[d] pathogens	Ultrasonography may be helpful in diagnosis. May occur concurrently with other abdominal abscess(es). Long-term treatment required.	PenicillinG[a] and enrofloxacin[f] ± metronidazole	Broad-spectrum antibiotics[e] ± metronidazole
	Cholangiohepatitis	Gram-negative enteric organisms	May be difficult to identify the offending organism(s). Long-term therapy required. Prognosis is poor when several obstructing calculi are present. For obstructing stones, choledocholitotomy may be indicated.	Trimethoprim-sulfonamide	Ceftiofur; enrofloxacin[f]
Soft Tissue	Candidiasis	*Candida* spp.	Infection of multiple systems may occur. Fungemia, although uncommon, has been seen in immunocompromised foals on aggressive, broad-spectrum antibiotic therapy.	Fluconazole	Voriconazole; itraconazole; amphotericin B
	Bacterial septicemia	*E. coli*, opportunistic aerobic[c] pathogens (mostly Gram-negatives)	Neonate is most commonly affected. Parenteral administration of antibiotics recommended, at least initially. Treatment required for a minimum of at least 2 weeks.	Broad-spectrum antibiotics[e] (amikacin preferred over gentamicin)	Third- or fourth-generation cephalosporins; ticarcillin-clavulanic acid
	Omphalophlebitis	Opportunistic aerobic[c] pathogens	Ultrasonography is useful when external signs of infection are not apparent. Surgical resection may be the treatment of choice in some cases.	Broad-spectrum antibiotic[e] (amikacin preferred over gentamicin)	Third- or fourth-generation cephalosporins; ticarcillin-clavulanic acid
	Fistulous withers	*Brucella abortus, Actinomyces bovis*	Public health concern with brucellosis. Treatment regimen using killed *Brucella* vaccine may be effective.	Oxytetracycline and streptomycin or gentamicin	Oral doxycycline[h] or trimethoprim-sulfonamide and gentamicin or rifampin
		Opportunistic aerobic[c] and anaerobic[d] pathogens		Broad-spectrum antibiotics[e]	Ceftiofur; trimethoprim-sulfonamide
	Traumatic and contaminated wounds	Opportunistic aerobic[c] and anaerobic[d] pathogens	Exploration, lavage, debridement, and local therapy are more important than systemic antimicrobial agents.	Trimethoprim-sulfonamide (superficial wound); broad-spectrum antibiotics[e] (deep contaminated wound)	Ceftiofur

(*continued*)

Table 27.2. Antimicrobial drug selection in infection of horses. (*continued*)

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
	Ulcerative lymphangitis	*C. pseudotuberculosis*	Drainage of *C. pseudotuberculosis* subcutaneous abscesses is preferred over antibiotic therapy. Systemic antibiotics required for ulcerative lymphangitis, internal abscesses, or in horses with signs of systemic illness.	Penicillin G[a]	Trimethoprim-sulfonamide; erythromycin ± rifampin; chloramphenicol
	Subcutaneous abscesses	β-hemolytic *Streptococcus* spp.	Drainage of abscesses preferred over antibiotic therapy. Systemic antibiotics required for internal abscesses or in horses with signs of systemic illness.	Penicillin G[a]	Ceftiofur; chloramphenicol
	Burns	*P. aeruginosa, S. aureus*; other opportunistic aerobic[c] pathogens	Care of burn wounds includes thorough cleansing, surgical debridement, daily hydrotherapy, and topical antimicrobials. Systemic antibiotics are not effective in preventing local burn wound infections and may permit the growth of resistant bacteria. Systemic anitibiotics only if signs of systemic infection.	Topical: silver sulfadiazine cream. Systemic: broad-spectrum antibiotics[e]	Ticarcillin-clavulanic acid; third-generation cephalosporins
	Clostridium myonecrosis	*C. perfringens, C. septicum, C. chauvoei*, other spp.	Surgical debridement, including fasciotomy, and supportive care are essential. Poor prognosis.	Penicillin G (IV) + metronidazole	Tetracycline; chloramphenicol
Bone and Joint	Osteomyelitis; septic arthritis neonates	Opportunistic aerobic[c] pathogens *Salmonella* spp. *R. equi*	In foals, osteomyelitis and septic arthritis are seen secondary to septicemia. Antibiotics and surgical debridement are required for osteomyelitis. Antibiotics and joint lavage are required for septic arthritis. Intra-articular antibiotics as well as IV regional or intraosseous perfusion with antimicrobial may be beneficial.		Broad-spectrum antibiotics[e] Third- or fourth-generation cephalosporins; (amikacin preferred over gentamicin); see above for *R. equi*
	Osteomyelitis adults	Opportunistic aerobic[c] pathogens	Usually secondary to traumatic and contaminated wounds. Antibiotics and surgical debridement are required.	Broad-spectrum antibiotics[e]	Third- or fourth-generation cephalosporins; trimethoprim-sulfa
	Septic arthritis or tenosynovitis sulfonamide adults	*Staphylococcus* spp. Opportunistic aerobic[c] pathogens	In adults, septic arthritis is usually associated with intra-articular injection or wounds. Joint/tendon sheath drainage and lavage are highly recommended. Intra-articular antibiotics as well as regional IV or intraosseous perfusion with antimicrobials may be beneficial. *In vitro* susceptibility testing highly recommended.	First-generation cephalosporin and amikacin or gentamicin	Broad-spectrum antibiotics[e]; trimethoprim-sulfonamide
	Lyme disease	*Borrelia burgdorferi*	Definitive diagnosis is difficult; presence of serum antibody does not indicate disease.		Oxytetracycline; Oral doxycycline;[h] ceftriaxone; ceftiofur

Skin	Dermatophilosis Ampicillin (streptothricosis, rain rot)	*D. congolensis*	Systemic therapy often unnecessary and generally reserved for severe or generalized cases. Infected animals should be groomed and bathed daily with providone-iodine shampoo or chlorhexidine solution (Novalsan 2%). If treated systemically a short course of antibiotics is often effective (3–5 days)	Procaine penicillin G	
	Folliculitis/ furunculosis	*Staphylococcus* spp., *Streptococcus* spp., *C. pseudotuberculosis*	Same as dermatophilosis. Antibiotics, if required, should be based on culture/ sensitivity.	Broad-spectrum antibiotics[e]	Trimethoprim-sulfonamide
	Staphylococcal cellulitis	*S. aureus*, *S. intermedius*	Requires aggressive systemic antibiotics	First-generation cephalosporin and gentamicin or amikacin (amikacin preferred)	Broad-spectrum antibiotics;[e] trimethoprim-sulfonamide; chloramphenicol
	Dermatophytosis	*Trychophyton equinum*, *T. mentagrophytes*, *Microsporum gypsum*, *M. equinum*, etc.	Disease may spontaneously regress but therapy shortens the recovery period and may decrease the spread of the disease. Topical therapy is sufficient. Treat the whole body of all contact animals.	5% lime sulfur or 0.5% sodium hypochloride solution or providone-iodine topically daily for 3–5 days and reapply weekly until resolution of infection	Topical natamycin; topical enilconazole; topical miconazole
	Sporotrichosis	*Sporothrix schenckii*	Treatment is often effective. Continue treatment for several weeks after lesions disappear or relapse will occur. Systemic iodides may cause abortion in pregnant mares.	Itraconazole and sodium iodide: 40 mg/kg of 20% solution IV for 2–5 days followed by oral potassium iodide: 2 mg/kg SID PO until lesions regress	Amphotericin B; fluconazole
	Pythiosis (phycomycosis, swamp cancer, Florida horse leech, bursattii, gulf coast fungus)	*Pythium insidiosum*	Immediate radical surgical removal of all infected tissues is essential for effective treatment. Early immunotherapy with soluble *Pythium* antigens[i] is effective, especially when combined to surgical removal.	Intralesional amphotericin B; amphotericin B (distal limb) systemic iodides (see sporotrichosis)	Topical or intralesional miconazole; systemic fluconazole
Renal	Cystitis	Opportunistic aerobic[c] bacteria, *Candida* spp.	Cystitis is usually secondary to urolithiasis, bladder neoplasia, or bladder paralysis. Treat for 7–10 days and reculture urine.	Trimethoprim-sulfonamide; fluconazole for *Candida* spp.	Ceftiofur; broad-spectrum antibiotics[e]
	Pyelonephritis	Opportunistic aerobic[c] bacteria	Same predisposing factors as cystitis Usually chronic and insidious, may be difficult to treat. Use aminoglycosides cautiously in face of renal disease. Treat a minimum of 2–3 weeks; duration required is variable and may be longer.	Trimethoprim-sulfonamide; third-generation cephalosporin	Penicillin G and enrofloxacin[f]
Cardiovascular	Bacterial endocarditis	*Streptococcus* spp., opportunistic aerobic[c] pathogens	Prognosis is poor to grave. Long-term treatment is required (several months). Antibiotic choice should be based on blood culture.	Broad-spectrum antibiotics[e] ± rifampin	Third- or fourth-generation cephalosporin; penicillin G and enrofloxacin[f]
	Bacterial pericarditis	*Streptococcus* spp., mixed opportunistic aerobic[c] and anaerbic[d] pathogens	Prognosis is guarded. Culture of peri- fluid is recommended. Drainage and lavage of the pericardial sac are also recommended.	Broad-spectrum antibiotics[e]	Third- or fourth-generation cephalosporin; penicillin G and enrofloxacin[f]

(*continued*)

Table 27.2. Antimicrobial drug selection in infection of horses. (*continued*)

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
	Thrombophlebitis	Mixed opportunistic aerobic and anaerobic pathogens	Blood culture recommended.	Broad-spectrum antibiotics[e] ± metronidazole	Ceftiofur; trimethoprim-sulfonamide[b]
Nervous	Bacterial meningitis or spinal abscess	Opportunistic aerobic[c] pathogens	Most often associated with nenonatal septicemia. Prognosis is poor.	Third- or fourth-generation cephalopsorin[j] ± aminoglycoside (amikacin preferred)	Broad-spectrum antibiotics penicillin G and enrofloxacin; trimethoprim-sulfonamide;[e]
	Mycotic meningitis/ encephalitis	*Cryptococcus neoformans*	Prognosis is grave.	Fluconazole	Amphotericin B
		Aspergillus spp.	Prognosis is grave.	Amphotericin B	Itraconazole; voriconazole
	Brain abscess	*Streptococcus equi*, *Streptococcus* spp.	Prognosis grave.	Penicillin G[a] ± rifampin	Third-generation cephalosporin[j]
	Tetanus	*Closridium tetani*	Antibiotics to eliminate the infection but tetanus antitoxin to neutralize the unbound toxin.	Peniclin G[a]	Ampicillin
	Botulism	*Clostridium botulinum*	Antitoxin to neutralize unbound toxin. Antibiotics if suspected wound contamination or to prevent complications such as aspiration pneumonia.	Penicillin G[a]	Ampicillin
	Otitis media/interna	*Actinobacillus* spp., *Staphylococcus* spp., *Streptococcus* spp., opportunistic aerobic[c] pathogens	Cause vestibulocochlear and/or facial nerve dysfunction as well as head shaking.	Trimethoprim-sulfonamide	Chloramphenicol; third-generation cephalosporin
	Equine protozoal myeloencephalitis	*Sarcocystis neurona*	Treatment may stop progression of disease and occasionally reverse clinical signs. Long-term therapy required.	Ponazuril; diclazuril	Sulfadiazine (24 mg/kg PO SID) and pyrimethamine (1 mg/kg PO SID)
Ophthalmic	Bacterial keratitis; Mild corneal ulceration	Gram-negative or Gram-positive opportunistic bacteria	Topical application.	Topical bacitracin-neomycin-polymixin B combinations	Topical gentamicin; topical ofloxacin
	Bacterial keratitis; severe melting keratitis	*P. aeruginosa*	Topical (or subconjunctival when appropriate) application (see chap. 22).	Topical tobramycin; topical ofloxacin	Topical ciprofloxacin
	Fungal keratitis	*Aspergillus* spp. *Alternaria* spp., *Mucor* spp., *Fusarium* spp., *Candida* spp.	Topical application.	Natamycin; voriconazole	Miconazole; itraconazole- DMSO ointment
	Foreign body penetration	Gram-negative or Gram-positive bacteria, fungal agents	Topical broad-spectrum coverage Systemic antimicrobials indicated if anterior chamber penetrated and/or if peri-orbital tissues are infected.	Topical gentamicin; systemic broad-spectrum antibiotics[e]	Topical tobramycin; systemic trimethoprim-sulfonamide; penicillin G and enrofloxacin[f]
	Manifestation of systemic disease	Bacterial: *A. equuli*, leptospirosis, *R. equi*	Ocular signs may be immune mediated. Primary treatment is aimed at systemic disease.	See specific infection	
		Fungal: *Crypto-coccus* spp., *Histoplasma* spp., *Aspergillus* spp.	Often associated with optic neuritis, chorioretinitis, anterior uveitis, blepharitis, purulent conjunctivitis.	See specific infection	

Reproductive Tract	Retained placenta	*S. zooepidemicus*, coliforms	Bacterial infections are commonly associated with prolonged (> 6–8 h) retention of membranes. Systemic antimicrobials recommended if early treatment with oxytocin fails.	Broad-spectrum antibiotics[e]	Trimethoprim-sulfonamide; third-generation cephalosporin
	Endometritis, metritis, and pyometra	*S. zooepidemicus, E. coli, P. aeruginosa*	Control of pneumovagina (Caslick's) is indicated in most cases. Urovagina and peritoneal lacerations also predispose to infection. Antiseptics used by the intrauterine route may induce a chemical irritation. Uterine lavage and hormonal therapy (e.g., oxytocin, PGF_2) are adjunct treatments. Systemic antibiotics are indicated primarily when endometrial biopsy suggests a deep endometrial infection or in cases of septic metritis with systemic clinical signs. Therapy based on *in vitro* susceptibility testing.	Choice of agent based on culture and sensitivity.	
	Fungal endometritis	*Candida* spp., *Aspergillus* spp.	Systemic antifungal agents are usually not warranted.	Intrauterine: clotrimazole (cream or suspension 500 mg daily for 7 days)	Intrauterine: nystatin (500,000 IU); amphotericin B (50–100 mg)
	Placentitis	Highly variable. *S. zooepidemicus*, *E. coli*, *Klebsiella* spp. are most common	Culture and sensitivity of discharge is highly recommended as organism(s) involved is unpredictable. It may be difficult to obtain effective antibiotic levels at the site of infection and resolution of the infection may not be possible until after paturition.	Trimethoprim-sulfonamide	Broad-spectrum antibiotics[e]
	Contagious equine metritis	*Taylorella equigenitalis*	Mares may become carriers once infected. Stallions are asymptomatic carriers. Reportable disease.	Mares: intrauterine potassium penicillin, cleansing of vulva and clitoral fossa with 4% chlorhexidine solution followed with packing of the clitoral fossa with chlorhexidine or nitrofurazone ointment. Stallions: potassium penicillin G 2000 IU/ml of semen extender. Wash penis daily with chlorhexidine solution and pack with nitrofurazone ointment	
	Mastitis	*S. zooepidemicus*, *Staphylococcus* spp., other opportunistic aerobic[c] pathogens, *Mycoplasma* spp.	Systemic antimicrobial therapy is recommended. Intramammary preparations for cows may also be used.	Trimethoprim-sulfonamide[b]; oxytetracycline for *Mycoplasma* spp.	Broad-spectrum antibiotics[e]

(*continued*)

Table 27.2. Antimicrobial drug selection in infection of horses. (*continued*)

Site	Diagnosis	Common Infecting Organism(s)	Comments	Suggested Drug(s)	Alternative Drug(s)
	Balanoposthitis	*S. zooepidemicus*, *Pseudomonas* spp., *Klebsiella* spp.	Bacterial balanoposthitis as a clinical problem is uncommon. Antimicrobial therapy is directed at infected semen or the recipient mare through the use of antimicrobials in semen extender. immediately prior to natural service. Washing of penis and prepuce with a mild soap is recommended. Disinfectant or topical antibiotics should not be used routinely as recolonization may occur and this treatment may displace commensals and allow pathogens to become established.	Potassium penicillin G 1000 IU and amikacin 1000 g per ml of semen extender	Ticarcillin 1000 g per ml of semen extender
	Seminal vesiculitis	*P. aeruginosa*, *K. pneumonia*, *Streptococcus* spp., *Staphylococcus* spp.	Systemic antibiotics based on *in vitro* susceptibility testing. Antibiotics can also be deposited in the seminal vesicle using a flexible endoscope. If infection cannot be eradicated, appropriate semen extender must be used for breeding (see recommendations for balanoposthitis).	Broad-spectrum antibiotics[e]	Ticarcillin-clavulanic acid; penicillin G[a] and ciprofloxacin[f] or enrofloxacin[f]
	Orchitis, epididymitis	*S. zooepidemicus*, *K. pneumoniae*	*In vitro* susceptibility testing is recommended.	Broad-spectrum antibiotics[e]	Third-generation cephalosporins; trimethoprim-sulfonamide[b]
Systemic Diseases	Leptospirosis serovar *bratislava*, *pomona*, and others	*L. interrogans*	Uveitis, nephritis, abortions, pyrexia, liver dysfunction.	Oxytetracycline	Ampicillin; doxycycline; penicillin
	Equine ehrlichiosis	*Anaplasma phagocytophilum*	Fever, limb edema, petechiation, ataxia, anemia, leukopenia, thrombocytopenia.	Oxytetracycline	Oral doxycycline[h]
	Systemic mycosis	*Histoplasma capsulatum*, *Blastomyces dermatidis*		Itraconazole	Histoplasma Fluconazole
		Coccidioides immitis		Fluconazole	Itraconazole

[a]Penicillin G (potassium, sodium, or procaine).
[b]Trimethoprim-sulfonamide may not be effective against streptococci *in vivo*, regardless of *in vitro* susceptibility results.
[c]Includes *Actinobacillus* spp., *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Pasteurella* spp., *Pseudomonas aeruginosa*, *Proteus* spp., *Staphylococcus aureus*, *S. zooepidemicus*.
[d]Includes *Bacteroides* spp., *Clostridium* spp., *Fusobacterium* spp., *Peptostreptococcus* spp., and others.
[e]Combination of a beta-lactam (penicillin G, ampicillin, or a first-generation cephalosporin) and an aminoglycoside (gentamicin or amikacin).
[f]Should not be used in young growing horses because of the risk of arthropathy.
[g]The use of vancomycin should be restricted for severely ill cases with confirmed *Clostridium* spp. infection with documented resistance to conventional antimicrobials.
[h]Administer orally only. Intravenous doxycycline has resulted in severe cardiovascular effects including collapse and death in some horses.
[i]Pan American Veterinary Labs (www.pavlab.com), Hutto, Texas.
[j]As opposed to most other third-generation cephalosporins, ceftiofur does not cross the normal blood-brain barrier.

with penicillin, ampicillin, or ceftiofur is often initiated until culture results are available. Such combination provides adequate coverage against approximately 90% of bacterial isolates recovered from blood cultures. Amikacin, although more expensive, is preferred to gentamicin because of its lower frequency of resistance amongst Enterobacteriaceae. Similarly, ampicillin is preferred to penicillin because of its higher activity against enterococci. In situations when an aminoglycosides should not be used such as renal failure, a similar coverage may be provided by a third- or fourth-generation cephalosporin such as cefotaxime or cefepime, respectively. Alternatively, ceftiofur may be used to provide coverage against approximately 80% of isolates recovered from blood cultures from equine neonates. If blood or other cultures are positive, antibiotic therapy can then be adjusted according to the susceptibility test results. If the animal has a positive blood culture, a minimum of 2 weeks of antibiotic therapy is recommended; if the infection is well established in an organ, such as the lung, joints, or bones, then antibiotics should be provided on a long-term basis.

Bacteria that are typically considered to be contaminants or part of the normal microflora need not be tested for susceptibility. However, when pathogenic bacteria are identified, selection of an antimicrobial agent is often simplified because some common equine pathogens have predictable *in vitro* susceptibility profiles. For example, most β-hemolytic streptococci isolated from horses are susceptible to penicillin G, as are most anaerobes except for *Bacteroides fragilis*. *Pasteurella* spp. isolated from horses also have a predictable susceptibility profile (Table 27.1). In contrast, Enterobacteraceae, *Enterococcus* spp., *Pseudomonas* spp., and *Staphylococcus* spp. have unpredictable susceptibility (Table 27.1). *In vitro* susceptibility testing is particularly important for these bacterial species.

Minimum Inhibitory Concentration versus Breakpoint—What Is the Difference?

In vitro bacterial susceptibility is determined by disk diffusion, concentration-gradient, or dilution methodologies (chapter 2). Disk diffusion provides qualitative susceptibility data whereas broth-dilution methods and the concentration-gradient test (E-test) generate a minimum inhibitory concentration (MIC) expressed quantitatively in μg/ml. All of the tests assess inhibition of bacterial growth rather than killing of the pathogen as the endpoint. Susceptibility designations are determined by comparing the microorganism's MIC (or zone of inhibition if the disk diffusion method is used) to clinical breakpoints established by the Clinical Laboratory Standards Institute (CLSI).

Simply stated, an antimicrobial's clinical breakpoint is the concentration above and below which specific bacterial isolates are categorized as susceptible, intermediate, or resistant. Clinical breakpoints take an antimicrobial's minimum inhibitory concentration (MIC) into consideration, but are based on additional interpretive criteria. Specifically, clinical breakpoints are determined by (1) the range of *in vitro* MICs of an antimicrobial for representative populations of specific bacterial pathogens; (2) pharmacokinetic parameters of the antimicrobial in target animal species (e.g., drug distribution at the site of infection); and when available, (3) results of clinical trials in the target species, the ultimate standard of efficacy. Clinical breakpoints are relevant for the specific bacteria, specific drug, and specific organ system infected only. As an example, the breakpoint for ceftiofur in horses is only relevant to *S. zooepidemicus* in the respiratory tract, and infections in other organs caused by aberrant *S. zooepidemicus* infections would not necessarily have the same clinical breakpoint.

Results of *in vitro* susceptibility tests are typically presented to the clinician by designating the pathogen as susceptible, intermediate, or resistant. The CLSI defines the three susceptibility designations as follows:

- Susceptible: An infection caused by the specific isolate can be effectively treated with the recommended antimicrobial and dosage regimen. CLSI generally requires clinical response rates of at least 80% at a specific MIC before organisms are categorized as susceptible.
- Intermediate: An infection by the isolate can be treated at body sites where drugs are physiologically concentrated or when a high dosage can be used; also indicates a "buffer zone" that should prevent minor technical factors from causing major discrepancies in interpretations.
- Resistant: An infecting isolate is not inhibited by achievable concentrations of the drug with normal dosage schedules; clinical efficacy has not been reliable in treatment studies.

Interpreting MIC Values

When species-specific breakpoints are used, pathogens with an MIC below an antimicrobial's susceptibility breakpoint have a higher probability for treatment success, and organisms with an MIC above the resistance breakpoint have a lower probability of treatment success. However, there is no evidence that efficacy increases the further the MIC is below the breakpoint. Conversely, it should be noted that a relatively high MIC in itself is not necessarily an indicator of resistance. Some resistance breakpoints have been set at > 32 μg/ml or higher (e.g., the resistance breakpoint for *Pasteurella multocida* against tulathromycin for bovine respiratory disease is 64 μg/ml).

For the equine practitioner, an important limitation in interpreting the results of *in vitro* susceptibility data is that breakpoints for only a small number of drugs (ampicillin, ceftiofur, gentamicin) have been established for specific infections in horses. For all other antimicrobials, the breakpoints have been adapted from human or other domestic animal species data. For these antimicrobials, a result indicating susceptibility is unquestionably preferable to one indicating resistance. However, there are no data correlating the results to clinical efficacy and there is no guarantee that the breakpoint is valid for a given pathogen or site of infection in horses. For example, the CLSI breakpoint for susceptibility to doxycycline is ≤ 4 μg/ml based on human pharmacokinetic and clinical efficacy data. Administration of oral doxycyline to an adult horse at the recommended dosage of 10 mg/kg results in peak serum, synovial fluid and peritoneal fluid concentrations of approximately 0.5 μg/ml (Bryant et al., 2000). A pathogen isolated from the synovial fluid of a horse with a MIC of 4 μg/ml would be reported as susceptible even though such concentrations are far from achievable in horses. Based on pharmacokinetic data in horses, a breakpoint of ≤ 0.25 μg/ml would be more appropriate as a susceptibility standard for doxycycline (Bryant et al., 2000; Davis et al., 2006)). Thus the lack of equine- and disease-specific interpretive criteria is one factor that may explain discrepancies between *in vitro* susceptibility and clinical response.

Causes of Treatment Failure

By itself, *in vitro* susceptibility of a specific pathogen does not guarantee clinical outcome. Other factors, such as the animal's age, immune status, and presence of mixed infections can contribute to individual clinical response.

Therapeutic failure may occur when the disease process does not have a bacterial etiology, when there is a change in the bacterial population at the site of infection, or when the pathogens have become resistant to the chosen antimicrobial agent. While the etiologic agent may be susceptible to several antimicrobial agents, not all such agents may reach therapeutic concentrations at the site of infection. The rate and extent of penetration of a drug into sites outside the vascular space are determined by the drug's concentration in plasma, molecular charge and size, lipid solubility and extent of plasma protein binding (chapter 4). It can also be affected by specific uptake by cells, cellular barriers (e.g., blood-brain barrier) and tissue blood flow.

Therapeutic failure may occur when the microenvironment at the site of infection is not conductive to antimicrobial activity. For example, gentamicin requires an oxidative transport system to penetrate the bacterial membrane. Therefore, a given microorganism may be susceptible to gentamicin *in vitro* but the drug may be ineffective in an anaerobic microenvironment. Similarly, the acidic environment of infected tissues may reduce the efficacy of macrolides, fluoroquinolones, and aminoglycosides. Thus, the goal of antimicrobial therapy is to select an antibiotic that, in addition to exhibiting good antimicrobial activity against the infecting microorganism, will achieve therapeutic concentrations in the infected area.

Pharmacodynamic Properties of Antimicrobial Agents

Determination of the appropriate dose and dosing interval of an antimicrobial agent requires knowledge and integration of both its pharmacokinetics and pharmacodynamic properties. The pharmacokinetic properties of a drug describe its disposition within the body and includes the process of drug absorption, distribution, metabolism, and excretion (chapter 4). On the other hand, pharmacodynamic properties of a drug address the relationship between drug concentration and antimicrobial activity (chapter 5). The most significant factor determining the efficacy of beta-lactams, chloramphenicol, glycopeptides, macrolides, tetracyclines, and trimethoprim-sulfonamide combinations is the length

of time that serum levels exceed the MIC of the pathogen. Increasing the concentration of the drug severalfold above the MIC does not significantly increase the rate of microbial killing. Rather, it is the length of time that bacteria are exposed to concentrations of these drugs above the MIC that dictates their rate of killing. Optimal dosing of such antimicrobial agents involves frequent administration. Other antimicrobial agents such as the aminoglycosides, fluoroquinolones, and metronidazole exert concentration-dependent killing characteristics. Their rate of killing increases as the drug concentration increases above the MIC for the pathogen and it is not necessary or even beneficial to maintain drug levels above the MIC between doses. Thus, optimal dosing of aminoglycosides and fluoroquinolones involve administration of high doses with long dosing intervals.

Route of Administration

The route of administration and the antimicrobial preparations available also greatly influence the choice of an antimicrobial agent for use in the horse. Intravenous medication is usually restricted to hospitalized horses or those under the direct care of a veterinarian. Maintenance of an intravenous catheter in the field, while certainly possible, is not advisable under most circumstances. Intramuscular administration of antibiotics to the horse is restricted by duration of treatment and volume of the preparation (total dose) to be administered. Repeated injection of large volumes of medication results in local muscle necrosis and pain. Even well behaved horses object to repeated injections. Novice horse owners can rarely use the IM site of injection in the rump and thus are limited to rotation of injection sites on both sides of the neck. For this reason, oral administration of antibiotics is the most popular route of administration to horses.

Adverse Effects

Unfortunately, several antimicrobial agents commonly used orally in other monogastric species, such as penicillin G, amoxicillin, cefadroxil and ciprofloxacin are poorly absorbed, particularly in adult horses, and therefore cannot be used orally. The large bowel of the horse makes this species particularly susceptible to antimicrobial-induced enterocolitis secondary to disruption of the normal colonic microflora and overgrowth of pathogenic microorganisms, most likely *Clostridium* spp. including *C. difficile*. The onset of acute and sometimes fatal diarrhea in the horse has been anecdotally associated with the use of almost every oral and parenteral antimicrobial agent. However, orally administered antimicrobials with low bioavailability and good activity against anaerobes are most likely to induce diarrhea. For this reason, oral beta-lactam antimicrobials should be used with caution in the horse.

Antimicrobials that are partially excreted in the bile after parenteral administration should also be used with caution. Certain antibiotics such as lincomycin and clindamycin are associated with well-recognized enterocolitis syndromes and their use must be avoided in horses. Other antibiotics such as oral trimethoprim-sulfonamide combinations, macrolides, chloramphenicol, metronidazole, fluoroquinolones, tetracycline, and cephalosporins have been occasionally linked to enterocolitis in horses. Anecdotally, some antibiotics are known to induce diarrhea in some parts of the world while used extensively without evidence of such adverse effect in others. This possible geographic variation in the incidence of antibiotic-induced diarrhea likely results from differences in colonic microflora. Foals seem less susceptible to antibiotic-induced enterocolitis than adult horses. In a retrospective study conducted at 3 referral hospitals in the United States, 32 of 5251 (0.6%) horses treated with antimicrobial agents for disorders not related to the gastrointestinal tract developed antimicrobial-associated diarrhea (Barr et al., 2012). The most frequently used antimicrobials in the 32, horses with antimicrobial-associated diarrhea were gentamicin in combination with penicillin (n = 7; 3%), enrofloxacin (n = 7; 5%), and doxycycline (n = 4; 1%; Barr et al., 2012).

Recommendations for Specific Disorders of Microorganisms

The remainder of this chapter outlines major infectious diseases of horses by organ system and provides recommendations for initial selection of antimicrobial agents while awaiting culture and sensitivity results (Table 27.2). Suggested drug dosages are shown in Table 27.3. Once an antimicrobial agent has been selected, the reader

Table 27.3. Common antimicrobial drug dosage in horses.[a]

Drug Preparation	Dose (mg/kg)	Dose interval (h)	Route of administration
Beta-lactams			
Benzyl penicillins:			
Penicillin G (Na, K)	25,000 IU/kg	6	IV
Penicillin G (procaine)	25,000 IU/kg	12	IM
Aminobenzyl penicillins:			
Ampicillin sodium	20	6–8	IV or IM
Ampicillin trihydrate	20	12	IM
	20	8	PO (foals only)
Amoxicillin trihydrate	30	8	PO (foals only)
Bacampicillin	25	12	PO
Pivampicillin	25	12	PO
Antistaphylococcal penicillins:			
Oxacillin	25	8–12	IM
	25	6	IV
Antipseudomonal penicillins:			
Ticarcillin	50	6	IV
Ticarcillin-clavulanic acid	50	6	IV
First-generation cephalosporins:			
Cefazolin	20	8	IM
	20	6–8	IV
Cephalothin	20	8	IM
	20–30	6	IV
Cephapirin	20	8	IM
	20–30	6	IV
Cephalexin	10	6	IV
	30	8	PO
Cephradine	25	6	IV
	25	6–8	PO (foals only)
Cefadroxil	20–40	8	PO (foals only)
Second-generation cephalosporins:			
Cefoxitin	20	6	IV or IM
Third-generation cephalosporins:			
Cefoperazone	30	6–8	IV or IM
Cefotaxime	40	6	IV
Ceftiofur sodium	2.2–4.4	24	IM
	5	12	IV or IM (foals)
Ceftiofur crystalline free acid	6.6	repeat in 4 days[m] IM	
Ceftriaxone	25	12	IV or IM
Cefpodoxime	10	8	PO
Fourth-generation cephalosporins:			
Cefepime	11	8	IV (foals)
	2.2		IV (adults)
Cefquinome	4.5	12	IV or IM (foals)
	1	24	IV or IM (adults)
Carbapenems			
Imipenem[g]	15	6	IV[c]
Aminoglycosides			
Amikacin	10	24	IV or IM (adults)
	25	24	IV or IM (foals)
Gentamicin	6.6	24	IV or IM (adults)
	12	36	IV or IM (foals < 2 weeks)
Fluoroquinolones			
Ciprofloxacin[b]	5.5	24	IV[h]
Enrofloxacin[b]	5.5	24	IV

(*continued*)

Table 27.3. Common antimicrobial drug dosage in horses.[a] (*continued*)

Drug Preparation	Dose (mg/kg)	Dose interval (h)	Route of administration
	7.5	24	PO
Orbifloxacin[b]	7.5	24	PO
Marbofloxacin[b]	2	24	IV
	3.5	24	PO
Difloxacin[b]	7.5	24	PO
Moxifloxacin[b]	5.8	24	PO
Fleroxacin[b]	5	24	IV or PO
Levofloxacin[b]	4	24	IV or IM
Tetracyclines			
Oxytetracycline	5	12	IV[c]
Doxycycline	10	12	PO[d]
Minocycline	4	12	PO
	2.2	12	IV
Macrolides			
Erythromycin (phosphate, stearate, ethylsuccinate, estolate)	25	6–8 PO	
Erythromycin (lactobionate, gluceptate)	5	6 IV[c]	
Azithromycin	10	24–48[e]	PO
Clarithromycin	7.5	12	PO
Other			
Chloramphenicol (palmitate or base)	50	6 or 12[f] PO	
Chloramphenicol (sodium succinate)	25–50	6 or 12[f] IV	
Metronidazole	25	12	PO
	35	12	Per rectum
Tinidazole	15	12	PO
Rifampin	5	12	PO
Sulfadiazine	24	12–24	PO
Trimethoprim-sulfonamide	30 (combined)	12	PO or IV
Pyrimethamine	1	24	PO
Vancomycin[g]	4.5–7.5	8	IV[h]
Sodium iodide (20 % solution)	20–40[j]	24	IV[i]
Potassium iodide	10–40[j]	24	PO[i]
Antifungal agents			
Amphotericin B	0.5–0.9[j]	24	IV[k]
Fluconazole	14	loading dose	PO
	5	24	PO
Itraconazole	5	24	PO[n]
Voriconazole	4	24	PO
Ketoconazole	30 (in 0.2N HCl)	12	Intra-gastric[l]

[a]Pharmakokinetics data are available for horses but, in most cases, safety studies have not been performed in the equine species.
[b]Enrofloxacin should not be used in young growing horses because of the risk of arthropathy. The problem might occur with other fluoroquinolones.
[c]Dilute and give by slow IV infusion.
[d]Administer orally only. Intravenous doxycycline has resulted in severe cardiovascular effects including collapse and death in some horses.
[e]Once a day for 5 days followed by q 48 h therapy.
[f]Administer BID in foals less than 5 days of age and QID thereafter.
[g]Should be used only for the treatment of serious bacterial infections caused by microorganisms resistant to all other antimicrobial agents.
[h]Dilute and administer slowly.
[i]May cause abortion in pregnant mares.
[j]Pharmacokinetic studies are not available. Empirical dose based on human dose, measurement of serum levels in clinical cases, or anecdotal observation of positive clinical response in equine patients.
[k]Dilute in 5% dextrose and give over 2–4 hours.
[l]Administer by nasogastric tube to prevent irritation by 0.2N HCl.
[m]Administer every 7 days thereafter if prolonged treatment is necessary.
[n]Oral solution has significantly better bioavailability than capsules.

Table 27.4. Suggested doses for intrauterine antimicrobial therapy in mares.

Drug	Spectrum	Dose[a]
Amikacin sulfate	Excellent Gram-negative coverage (including most *P. aeruginosa*)	2 g[b]
Ceftiofur	Broad-spectrum (not effective against *P. aeruginosa*)	1 g
Gentamicin sulfate	Gram-negative	2 g[b]
Penicillin G (potassium)	Gram-positive	5×10^6 IU
Ticarcillin	Broad-spectrum (not effective against *Klebsiella*)	6 g
Ticarcillin-clavulanic acid	Broad-spectrum	6 g

[a]Administer daily for 4–6 days. The volume infused is determined by the size of the uterus (35–150 ml is usually sufficient).
[b]Buffered with equal volume of 7.5% bicarbonate.

should consult the appropriate chapter for potential toxicities and specific contraindications. Very few of the antimicrobial agents mentioned in this chapter have been approved for use in horses. Those that have been approved are often recommended at higher dosages or to treat a disease other than that for which the compound is approved. Therefore, for most antibiotics, controlled safety studies involving administration of the drug to a large number of horses have not been performed. It must also be remembered that, although this chapter deals strictly with antimicrobial therapy, supportive, local or surgical therapy may in some cases be as important as the antibiotic in resolution of the infection. Recommendations for intrauterine therapy are presented in Table 27.4.

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28

Antimicrobial Drug Use in Dogs and Cats

Jane E. Sykes

Veterinarians who treat dogs and cats work in a wide variety of situations, from rural one-person practices with limited small animal caseloads to specialized multi-veterinarian canine and feline hospitals and advanced referral centers. The range of facilities and services available, and the pet owners' ability to pay, is correspondingly varied, although the veterinarian's goal remains the same—to provide effective, safe and economical attention for patients in their care. Antimicrobial drugs often form part of the treatment regimen chosen, but the decision to use them must not be made lightly: these are not placebos or antipyretic agents, nor do they replace the fundamental diagnostic skills of history taking, physical examination, and logical analysis of clinical findings.

Concerns have increased dramatically in recent years regarding the use and misuse of antimicrobial drugs in companion animals, including the emergence of methicillin-resistant staphylococci and multidrug-resistant Gram-negative bacteria in companion animals, and the potential for spread of these bacteria to humans. Evidence is accumulating that some bacterial strains that inhabit dogs and cats have the potential to be shared with, and cause disease in humans. Bacterial strains found in humans can also be transmitted to dogs and cats. A policy of selective and restricted use of antimicrobial agents is important to avoid potential criticisms and externally imposed restrictions on the use of antimicrobial drugs. Widespread, inappropriate use of these agents can reduce efficacy and create problems with infections that are more difficult and expensive to treat. This has been demonstrated repeatedly by outbreaks of nosocomial infections in human and veterinary hospitals, attributable in part to selection pressure applied by overuse of antimicrobials, especially for prophylaxis in surgical and non-surgical patients. Overuse of antimicrobial drugs can also lead to other unanticipated adverse effects such as hepatotoxicity, esophageal stricture formation, or immune-mediated reactions. At the time of writing, guidelines have been developed by the International Society for Companion Animal Infectious Diseases (ISCAID) for treatment of urinary tract infections in dogs and cats (Weese et al., 2011), and are in the process of development for superficial pyoderma, respiratory infections, and bloodstream infections.

Antimicrobial Drug Chemotherapy

The principles that govern selection and therapeutic use of antimicrobial drugs are outlined in chapter 6 and apply to all animal species, which includes dogs and cats. An adequate clinical assessment is of prime importance in deciding (1) whether or not to treat with antimicrobial drugs; (2) the choice of antimicrobial drug; and (3) the estimated duration of treatment. This should identify the likely presence or absence of infection, the body systems involved, and the pathogens likely to be responsible. There are many causes of an increased rectal temperature

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other than bacterial infection. Increased rectal temperatures also occur in other pathologic conditions (viral infections, neoplasia, drug reactions, immune-mediated disorders, other non-specific inflammatory diseases such as pancreatitis, heat stroke and pathologically increased muscle activity) and some physiologic states (exercise, excitement, high ambient temperature and humidity). Conversely, dogs or cats with normal rectal temperatures may have life-threatening systemic infections, and cats with septic shock are frequently hypothermic. Leukocytosis is also not indicative of infection, and occurs commonly with non-specific inflammatory processes such as pancreatitis, immune-mediated diseases, neoplasia, trauma, excitement, stress, and glucocorticoid administration. Wherever possible, treatment with broad-spectrum antimicrobial drugs should be postponed until the results of further laboratory tests confirm that infection is present. The one exception to this rule is if signs of severe sepsis or septic shock are present, such as fever together with prolonged capillary refill time, tachycardia, tachypnea and brick red mucous membranes in dogs; and fever or hypothermia, bradycardia, and/or tachypnea in cats. In this case, initial blood cultures should be collected, followed by immediate parenteral treatment with broad-spectrum antimicrobial drugs.

If the infection site is accessible for specimen collection, the most rapid and inexpensive way to identify the presence of infection is to examine smears of fine needle aspirates treated with Gram stain and/or a Romanovsky method (Giemsa, Diff-Quik). This can indicate whether bacteria are present and whether they are rods or cocci, as well as whether they are Gram-positive or Gram-negative. In the absence of Gram staining, most cocci that infect dogs or cats are Gram-positive and (with a few exceptions) rods are usually Gram-negative. A decision on possible treatments can then be made by (1) considering these findings alongside the known spectrum of activity of various antimicrobial drugs; (2) the ability of these antimicrobial drugs to penetrate the site of infection; and (3) information on the regional prevalence of drug resistance, which should be based on the results of culture and susceptibility testing for other animals seen in the practice in the recent past (e.g., for staphylococci, whether methicillin resistance is widespread or rare). If the site of infection is not accessible for specimen collection, initial drug selection can be based on knowledge of the site of infection, the pathogens more frequently implicated there, and the likely drug susceptibility of those organisms. This process can be based on personal experience or on published information like that shown in Table 28.1 for dogs and Table 28.2 for cats. In many routine or less serious infections, treatment on this "best-guess" basis will prove satisfactory, without the need for additional investigation. The drug with the narrowest antimicrobial spectrum should be selected, so as to reduce effects on untargeted microflora. Cost, route of administration, and the potential for adverse effects are other factors to consider. Examples of adverse effects that might lead to selection of alternate drugs include, although rare, the potential for development of acute blindness in cats treated with enrofloxacin, and the prevalence of hypersensitivity reactions in doberman pinschers treated with trimethoprim-sulfonamide combinations. Drug selection may also have to be modified in renal failure, liver failure, pregnancy, or neonatal patients (Table 28.3; chapter 4).

If a bacterial infection is suspected, another important early decision is whether to undertake culture and susceptibility testing before treatment is initiated (see chapter 2). This usually provides the best guide to the pathogens present and their antimicrobial drug susceptibility. Bacterial culture and susceptibility testing, when performed and interpreted properly, is never wrong, but (1) it may not be affordable for some pet owners; and (2) for anatomic sites that are not normally sterile (such as the nasal cavity), results must be considered in light of the usual commensal bacterial species that are present at those sites, and in some cases, interpretation of these results may be difficult. For clients that lack financial resources, it should also be kept in mind that prescription of an inappropriate antimicrobial drug wastes client time and financial resources and may be associated with progression of disease. Culture and susceptibility testing is essential for very serious infections; recurrent or non-responsive infections; or whenever the susceptibility profile of the likely pathogens is unknown or unpredictable. In these situations, minimum inhibitory concentrations (MICs) should ideally be sought to help select the most appropriate antimicrobial drug. For lower urinary tract infections, serum MICs may overestimate the likelihood of resistance because many antimicrobial drugs concentrate in the urine at levels many-fold higher than those present in serum. However, use of urine MICs may lead to inappropriate treatment decisions if unrecognized infection of the renal parenchyma or deep-seated infections of the bladder wall are present. Susceptibility testing is not always essential:

Table 28.1. Antimicrobial drug selection for selected infections in dogs.[a]

Site	Diagnosis	Common Infecting Organisms	Comments	Suggested drugs
Skin and subcutis	Superficial pyoderma	*Staphylococcus* spp. (especially *S. pseudintermedius*).	Attempt to identify underlying causes (most often allergic dermatitis, but also endocrinopathies). Prolonged treatment may be needed. Culture of pustules is indicated in regions where methicillin-resistant *S. pseudintermedius* is widespread or if disease is refractory or recurrent.	Consider topical treatment, e.g., with antiseptic shampoos, as an alternative to systemic antimicrobial drugs. Clindamycin or first-generation cephalosporins (e.g., cephalexin, cefadroxil). Alternatives include amoxicillin-clavulanate, trimethoprim and ormetoprim-potentiated sulphonamides, lincomycin, or erythromycin. Use of other drugs should be based on culture and susceptibility.
	Deep pyoderma	*Staphylococcus* spp., *E. coli*, *Proteus*, *Pseudomonas*.	Attempt to identify underlying causes. Prolonged treatment may be needed. Culture of skin lesions strongly recommended.	See superficial pyoderma, but drugs that are active against Gram-negative bacteria may be required based on culture and susceptibility.
	Surface pyoderma	*Staphylococcus*, *Streptococcus*.	Often secondary to skin folds or self-trauma. Local cleansing and topical antibacterials are sufficient.	
	Malassezia dermatitis	*M. pachydermatis*.	Identify and eliminate underlying causes. Topical treatment with shampoos is recommended.	Itraconazole or fluconazole. Ketoconazole is an alternative but is more likely to cause adverse effects.
	Dermatophytosis	*Microsporum*, *Trichophyton*.	Topical treatment and environmental clean-up required. Localized lesions may not require systemic treatment.	Itraconazole or fluconazole. Alternatives include griseofulvin or terbinafine.
	Bite wounds, traumatic and contaminated wounds	*Staphylococcus*, *Streptococcus*, *Enterococcus*, *Pasteurella*, *Escherichia coli*, *Pseudomonas*, anaerobes.	Wound irrigation and debridement. Antibiotics of questionable prophylactic benefit for contaminated wounds.	Clavulanic acid–amoxicillin or ampicillin-sulbactam. For serious infections that may involve resistant Gram-positive and Gram-negative bacteria, consider a combination of an aminoglycoside and ampicillin-sulbactam.
	Anal sac inflammation/ abscessation	*E. coli*, *Enterococcus* spp., *Proteus* spp., anaerobes.	Local treatment is usually indicated. Systemic antimicrobials can be used if severe infection is present.	Clavulanic acid–amoxicillin.
Ear	Otitis externa	*Staphylococcus* spp., and less often streptococci, *Pseudomonas*, *E. coli* or *Proteus* spp.; *Malassezia*.	Identify and address underlying causes (allergic dermatitis, foreign bodies, ear mites). Ear cleaning. Consider topical glucocorticoid or analgesic.	Choice should be based on ear cytology and if possible, integrity of the tympanic membrane. Topical enrofloxacin solutions may be considered; or if rods are present, topical preparation that contains aminoglycosides, polymixin B, or ticarcillin-clavulanate. Ointments that contain clotrimazole, miconazole, or posaconazole may be required if *Malassezia* is present.
	Otitis media and interna	As for otitis externa.	Otitis externa also often present. Identify and address underlying causes. Treat as for otitis externa but additional systemic treatment indicated. Avoid ototoxic drugs.	Treatment should be based on ear cytology and culture and susceptibility. If cocci are present, cephalexin is recommended, but if rods are present, consider a fluoroquinolone. Systemic antifungal drug treatment is indicated if *Malassezia* is present.
Eye	Superficial ocular infection	*Staphylococcus* spp., *Streptococcus* spp., *E. coli*, *Proteus*.	Identify and correct underlying causes (poor eyelid conformation, dystichiasis, allergies, keratoconjunctivitis sicca).	Topical neomycin-polymixin-bacitracin.

(*continued*)

Table 28.1. Antimicrobial drug selection for selected infections in dogs.[a] (*continued*)

Site	Diagnosis	Common Infecting Organisms	Comments	Suggested drugs
Upper respiratory	Bacterial rhinitis	Usually resident bacteria that invade opportunistically. In crowded environments, *Bordetella bronchiseptica*, *Streptococcus equi* subspecies *zooepidemicus*, or *Mycoplasma* spp. (especially *M. cynos*) may be involved.	Treatment with antibiotics alone is rarely curative unless infection is caused by transmissible bacteria (e.g., shelter environments). For other situations, underlying causes (neoplasia, aspergillosis, foreign bodies, nasal mites) must be identified and addressed.	If transmissible bacterial infections are suspected, doxycycline is the treatment of choice because it is active against *Bordetella*, *Streptococcus*, and *Mycoplasma* spp. Amoxicillin-clavulanate is an alternative but is not active against *Mycoplasma* spp.
	Fungal rhinitis	Usually *Aspergillus* spp.	Rule out nasal neoplasia. Secondary bacterial infection may be present.	Debridement and topical clotrimazole or enilconazole. Systemic itraconazole or voriconazole are alternatives if cribriform plate destruction is present.
	Canine infectious respiratory disease complex	Viruses, *Bordetella bronchiseptica*, *Streptococcus equi* subsp. *zooepidemicus*, *Mycoplasma* spp. (especially *Mycoplasma cynos*).	Most recover untreated in 7–10 days. Treat if mucopurulent discharges are severe or systemic illness is present. Consider the possibility of distemper pneumonia.	Doxycycline. Clavulanic acid–amoxicillin is an alternative but is not active against *Mycoplasma* spp.; consider nebulized gentamicin if refractory to treatment and infection with *B. bronchiseptica* has been confirmed with culture.
	Bacterial pneumonia	Single or mixed infections that involve various facultative (especially Gram-negative) bacteria and anaerobes if aspiration pneumonia is present.	Aerobic culture and susceptibility testing on transtracheal wash or bronchoalveolar lavage indicated. Consider anaerobic culture if aspiration suspected.	A combination of clindamycin and enrofloxacin is a suitable initial choice pending the results of culture and susceptibility testing. If anaerobes are suspected, a beta-lactam/beta-lactamase inhibitor combination may be more appropriate (such as ampicillin-sulbactam and enrofloxacin).
	Pneumocytis jiroveci pneumonia		Secondary to inherited or acquired immunodeficiency.	Trimethoprim-sulfonamide combinations.
	Pyothorax	Various and often mixed, which includes anaerobes, *Actinomyces*, Gram-negative and Gram-positive bacteria, *Nocardia* spp.	Culture and susceptibility testing on pleural fluid indicated. Chest tube placement required to drain pus; surgery may be indicated.	Ampicillin-sulbactam and enrofloxacin are suitable initial choices pending the results of culture and susceptibility testing. If *Nocardia* is suspected based on history or cytology, trimethoprim-sulfamethoxazole should be used.
Gastrointestinal and abdominal	Periodontitis, gingivitis	Resident anaerobic and facultative bacteria.	Dental cleaning, scaling, other dental treatment may be needed.	Clindamycin or amoxicillin-clavulanate.
	Malar or carnassial abscess	Resident oral flora.	Dental extractions, alveolar bone curettage, drainage.	Clindamycin or amoxicillin-clavulanate.
	Gastric helicobacteriosis	*Helicobacter* spp., gastric helicobacter-like organisms.	Relationship between infection and disease often unclear.	Amoxicillin, clarithromycin and bismuth salicylate or amoxicillin, metronidazole and bismuth salicylate.
	Bacterial enteritis	*Salmonella* spp.	Can be found in healthy and sick dogs. When present with diarrhea and systemic illness is not present, treatment is not indicated.	If systemic infection is present (i.e., with fever, lethargy, changes on the CBC, positive blood cultures), parenteral fluoroquinolones indicated.
		Campylobacter spp.	Often present in healthy dogs	If diarrhea is present and no other cause of illness can be identified, consider treatment with a macrolide.
		Clostridium perfringens, C. difficile.	Present in many healthy dogs. Diagnosis of clostridial diarrhea requires demonstration of toxin production by toxin ELISA assays in association with diarrhea. Significance may still be unclear even when toxin is detected.	Metronidazole.

	Giardiasis	*Giardia* spp.	Infection often subclinical. Some assemblages/ species may be zoonotic.	Fenbendazole. Alternatives are metronidazole, tinidazole, or ronidazole.
	Coccidiosis	*Isospora* spp.	Clinical illness usually associated with young age or co-infections with other enteric pathogens.	Sulfonamide +/- trimethoprim. Alternatives are ponazuril or toltrazuril (Europe).
	Parvoviral enteritis	Secondary facultative and anaerobic bacteria from the gastrointestinal tract.	Parenteral antimicrobial drug treatment is important to counteract opportunistic bacterial invasion.	Ampicillin-sulbactam, cefazolin (mild disease); ampicillin-sulbactam and a fluoroquinolone (severe disease).
	Cholecystitis, cholangiohepatitis	*Escherichia*, *Salmonella*, *Enterococcus* anaerobes.	Address underlying causes (e.g., disrupted bile flow). Consider ultrasound-guided collection of bile for aerobic and anaerobic culture and susceptibility.	Beta-lactam and beta-lactamase inhibitor combination with an aminoglycoside or a fluoroquinolone; narrow spectrum based on culture results.
	Bacterial peritonitis	Mixed anaerobes and facultative enteric bacteria.	Surgical exploration and lavage may be needed. Culture and susceptibility testing indicated.	As for cholecystitis/cholangiohepatitis.
Urinary and urogenital	Lower urinary tract infection/bacterial cystitis	*E. coli*, *Staphylococcus* spp., *Proteus*, *Streptococcus*, *Enterococcus*, *Enterobacter*, *Klebsiella*, *Pseudomonas*	Identify and address underlying cause whenever possible (calculi, tumor, incontinence, hyperadrenocorticism).	Trimethoprim-sulfamethoxazole or amoxicillin. Amoxicillin-clavulanate could be used where the regional prevalence of beta-lactamase production is high.
	Pyelonephritis	See lower urinary tract infection.	Culture and susceptibility recommended. Prolonged treatment required.	Amoxicillin and a fluoroquinolone pending culture results.
	Prostatitis	See lower urinary tract infection.	Culture and susceptibility recommended. Prolonged treatment required. Surgery may be needed for prostatic abscessation or for castration.	Trimethoprim-sulfonamide or a fluoroquinolone. Chloramphenicol is an alternative.
	Orchitis/epididymitis	*E. coli*, *Brucella* spp.	May be associated with urinary tract infection and prostatitis. Castration may be required.	Trimethoprim-sulfonamide or a fluoroquinolone. Doxycycline and an aminoglycoside (streptomycin or gentamicin) for *Brucella*; addition of a fluoroquinolone and rifampin could also be considered for treatment of brucellosis.
	Vaginitis/ balanoposthitis	Resident bacteria, herpesvirus, *Mycoplasma*, *Brucella*.	Identify predisposing factors. Local cleaning usually sufficient. Puppy vaginitis resolves with maturity.	
	Metritis, pyometra	*E. coli*, *Streptococcus*, *Staphylococcus*, other Gram-negative bacteria, sometimes anaerobes.	Ovariohysterectomy recommended. Culture uterine contents at surgery. Prostaglandin and antibiotic treatment may be successful for open pyometra.	Ampicillin-sulbactam and either a fluoroquinolone or an aminoglycoside.
	Mastitis	*E. coli*, *Staphylococcus*, *Streptococcus*.	Do cytology and culture and susceptibility testing.	If weaning possible, use chloramphenicol (unaffected by milk pH). Otherwise, amoxicillin-clavulanate pending results of culture and susceptibility.
Musculoskeletal	Osteomyelitis, septic arthritis	*Staphylococcus* and to a lesser extent *Streptococcus, Enterococcus, E. coli*, *Proteus*, *Pseudomonas, Klebsiella*, anaerobes.	Culture and susceptibility strongly recommended. Requires debridement and drainage and prolonged treatment with antimicrobial drugs. Local antimicrobial treatment (impregnated beads) may also be useful.	Withhold treatment until results of culture and susceptibility are available. If treatment is considered necessary, clindamycin or clindamycin and an aminoglycoside (if Gram-negative bacteria or methicillin-resistant staphylococci) could be considered. Chloramphenicol is an alternative in regions where the prevalence of methicillin-resistant staphylococci is high, but some may be resistant to chloramphenicol.

(*continued*)

Table 28.1. Antimicrobial drug selection for selected infections in dogs.[a] (*continued*)

Site	Diagnosis	Common Infecting Organisms	Comments	Suggested drugs
	Diskospondylitis	*Staphylococcus*, *Streptococcus*, *Brucella*, *E. coli*, *Aspergillus*.	Blood culture and susceptibility recommended, also *Aspergillus* antigen and *Brucella* antibody serology. Disk aspiration or urine culture may also yield a causative organism.	Clavulanic acid–amoxicillin or cephalexin; consider addition of a fluoroquinolone if there is no response after a week, and diagnostic test results are negative.
Nervous system	Bacterial meningitis	*Staphylococcus*, *Pasteurella*, *Actinomyces*, *Nocardia*, sometimes anaerobes.	CSF culture and susceptibility recommended.	Consider a combination of ampicillin and metronidazole (which has improved penetration). Alternatives are trimethoprim-sulfamethoxazole or chloramphenicol.
	Tetanus	*Clostridium tetani*.	Nursing case, antitoxin, wound debridement.	Metronidazole or penicillin.
	Botulism	*Clostridium botulinum*.	Nursing care.	Not indicated.
	Hepatic encephalopathy	Normal intestinal flora.	Oral antimicrobial drugs to suppress ammonia production by gastrointestinal bacteria; add lactulose and restricted protein diet.	Ampicillin or neomycin.
Other bacterial	Actinomycosis	*Actinomyces* spp.	Mostly with other bacteria in infections of the subcutaneous tissues, thorax, abdomen, retroperitoneum. Drainage, debridement, prolonged treatment needed. Identify and remove any plant foreign bodies.	Penicillin G or ampicillin.
	Bacteremia, bacterial endocarditis	Various Gram-positive and Gram-negative facultative bacteria, *Bartonella*, rarely anaerobes.	Blood culture and susceptibility testing indicated. Treat parenterally for 7–10 days (or as long as possible) then switch to oral treatment for 4–6 weeks.	Penicillin and an aminoglycoside pending the results of culture and susceptibility.
	Bartonellosis	*Bartonella vinsonii* subsp. *berkhoffii*, *Bartonella henselae*.	*Bartonella* serology and culture (low sensitivity) indicated. Significance as a cause of disease may be unclear unless endocarditis is present.	Penicillin and an aminoglycoside for endocarditis. Prognosis guarded to poor as valve replacement often required.
	Brucellosis	*Brucella canis*.	Potential zoonosis.	Doxycycline plus dihydrostreptomycin or gentamicin; consider addition of rifampin.
	Leptospirosis	Various serovars of *Leptospira interrogans*.	Potential zoonosis. Fluid therapy essential, dialysis may be required.	Penicillin, ampicillin, or doxycycline; oral doxycycline recommended once vomiting ceases for elimination of the carrier state.
	Lyme borreliosis	*Borrelia burgdorferi*	Consider non-steroidal anti-inflammatory drugs for analgesia.	Doxycycline. Amoxicillin is a possible alternative.
	Nocardiosis	*Nocardia* spp.	Pulmonary, systemic, or cutaneous lesions.	Trimethoprim-sulfonamide.
	Neonatal septicemia	*Streptococcus* spp., *E. coli*, *Staphylococcus* spp.		Ampicillin-sulbactam, first-generation cephalosporin. Consider cautious use of an aminoglycoside if Gram-negative bacteria are suspected.
	Rapidly growing mycobacteria	*Mycobacterium fortuitum*, *Mycobacterium smegmatis*.	Cutaneous-subcutaneous and less often systemic infections.	High-dose doxycycline or a fluoroquinolone; aminoglycosides could also be considered.
	Slow-growing opportunistic mycobacteria	*Mycobacterium avium*.	Usually systemic infections in immunocompromised dogs.	Three-drug combination of a macrolide (such as clarithromycin) with rifampin, ethambutol, doxycycline, and/or a fluoroquinolone suggested.
	Tuberculous mycobacteria	*Mycobacterium tuberculosis*, *Mycobacterium bovis*.	Prolonged combination drug treatment; potential zoonosis.	Combination of isoniazid, rifampin, and clarithromycin, with or without ethambutol. Isoniazid may cause seizures.

Other protozoal	Babesiosis	*Babesia canis.*		Imidocarb dipropionate or a combination of atovaquone and azithromycin.
		Babesia gibsoni.		Atovaquone and azithromycin.
		Babesia conradae.		Atovaquone and azithromycin.
	Cryptosporidiosis	*Cryptosporidium* spp.	Infection often subclinical and self-limiting. Potential zoonosis.	No uniformly successful treatment; some improvement may be seen with paramomycin or azithromycin.
	Hepatozoonosis	*Hepatozoon americanum, Hepatozoon canis.*	Treatment may reduce signs without resolving infection. Use non-steroidal anti-inflammatory drugs to control inflammation and pain.	*H. americanum*: acute—clindamycin, sulfonamide, trimethoprim, pyrimethamine; chronic—decoquinate *H. canis*: imidocarb dipropionate.
	Leishmaniasis	*Leishmania* spp.	Complete resolution of infection may not occur.	Meglumine antimonate and allopurinol. Alternatives are amphotericin B or miltefosine.
	Neosporosis	*Neospora caninum.*		Clindamycin. Alternative is sulfonamide plus pyrimethamine.
	Toxoplasmosis	*Toxoplasma gondii.*		Clindamycin. Alternative is sulfonamide plus pyrimethamine or azithromycin.
	Trypanosomiasis, American	*Trypanosoma cruzi.*	Potential public health risk.	Nifurtimox or benznidazole.
Rickettsial, ehrlichial, and hemotropic mycoplasma infections	Rocky Mountain Spotted Fever	*Rickettsia rickettsia.*		Doxycycline.
	Ehrlichiosis, anaplasmosis	*Ehrlichia canis, Ehrlichia ewingii, Anaplasma phagocytophilum, Anaplasma platys.*	*Ehrlichia canis* infections require prolonged treatment (6–8 weeks). Dogs with chronic *E. canis* infections may not respond to treatment.	Doxycycline.
	Hemoplasmosis	*Mycoplasma haemocanis, Mycoplasma haematoparvum.*	Usually only of pathogenic significance in splenectomized or immunocompromised dogs.	Doxycycline or a fluoroquinolone.
Systemic mycoses	Aspergillosis, disseminated	*Aspergillus terreus, A. deflectus.*	Genetic immunodeficiency suspected in German Shepherds and Rhodesian Ridgebacks. Any immunosuppression should be removed if possible.	Itraconazole or itraconazole and amphotericin B; voriconazole or posaconazole are alternatives but may be expensive. Consider addition of terbinafine for refractory cases. Do not use fluconazole.
	Blastomycosis	*Blastomyces dermatitidis.*		Itraconazole or fluconazole, with or without amphotericin B.
	Coccidioidomycosis	*Coccidioides* spp.		Itraconazole or fluconazole, with or without amphotericin B. Voriconazole may also be effective and has CNS penetration but is expensive.
	Cryptococcosis	*Cryptococcus neoformans* or *Cryptococcus gattii.*	Dogs often develop severe disseminated disease with *C. neoformans*, possibly due to an underlying immunodeficiency.	Fluconazole with or without amphotericin B; itraconazole may be effective when fluconazole fails.
	Histoplasmosis	*Histoplasma capsulatum.*		Itraconazole with or without amphotericin B.
	Sporotrichosis	*Sporothrix schenckii.*		Itraconazole with or without amphotericin B; supersaturated potassium iodide is an alternative.

[a]These selections reflect personal opinion based on review of the literature, discussion with colleagues, and clinical experience. They are intended to guide drug selection when laboratory data are lacking. Laboratory data (Gram stain of exudate or aspirate, or culture and susceptibility test) should be used to guide drug selection if available. Selection may change once culture and drug susceptibility test results are known. See Greene, 2012, for additional information. (Greene C. Infectious Diseases of the Dog and Cat, 4th ed. St. Louis: Elsevier Saunders.)

Table 28.2. Antimicrobial drug selection for selected infections in cats.[a]

Site	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Skin and subcutis	Bacterial pyoderma	*Staphylococcus* spp., *Streptococcus* spp.	Attempt to identify underlying causes (most often allergic dermatitis, but also endocrinopathies). Prolonged treatment may be needed. Culture of skin lesions is indicated in regions where methicillin-resistant *S. aureus* is widespread or if disease is refractory or recurrent.	Clindamycin or first-generation cephalosporins (e.g., cephalexin, cefadroxil). Alternatives include amoxicillin-clavulanate. Use of other drugs should be based on culture and susceptibility.
	Cat fight abscesses	*Pasteurella*, anaerobes.	Drainage is most important.	Clavulanic acid–amoxicillin.
	Surface pyoderma	*Staphylococcus, Streptococcus*.	Often secondary to skin folds or self-trauma. Local cleansing and topical antibacterials are sufficient.	
	Malassezia dermatitis	*M. pachydermatis*.	Identify and eliminate underlying causes. Topical treatment with shampoos is recommended.	Itraconazole or fluconazole. Ketoconazole is an alternative but is more likely to cause adverse effects.
	Dermatophytosis	*Microsporum, Trichophyton*.	Topical treatment and environmental clean-up required. Localized lesions may not require systemic treatment.	Itraconazole or fluconazole. Alternatives include griseofulvin or terbinafine.
	Feline leprosy	*Mycobacterium lepraemurium*, others.	Surgical removal preferred if possible.	Clofazamine and clarithromycin.
	Rapidly growing opportunistic mycobacterial infections	*Mycobacterium fortuitum, M. smegmatis, M. chelonae, M. abscessus*.	Culture and susceptibility testing recommended if possible. Early surgical resection may lead to dehiscence.	High-dose doxycycline or a fluoroquinolone; consider aminoglycosides.
	Tuberculosis mycobacteriosis	*Mycobacterium microti, Mycobacterium bovis*.	Primarily occurs in the UK.	Clarithromycin with rifampin and a fluoroquinolone.
Ear	Otitis externa	*Staphylococcus* spp., and less often streptococci; *Malassezia*.	Identify and address underlying causes (allergic dermatitis, foreign bodies, polyps, retrovirus infections, ear mites). Ear cleaning.	Choice should be based on ear cytology and, if possible, integrity of the tympanic membrane. Topical enrofloxacin solutions may be considered; or if rods are present, topical preparation that contains aminoglycosides, polymixin B, or ticarcillin-clavulanate. Ointments that contain clotrimazole, miconazole, or posaconazole may be required if *Malassezia* is present.
	Otitis media and interna	As for otitis externa.	Otitis externa also often present. Identify and address underlying causes. Treat as for otitis externa but additional systemic treatment indicated. Avoid ototoxic drugs.	Treatment should be based on ear cytology and culture and susceptibility. If cocci are present, cephalexin is recommended, but if rods are present, consider a fluoroquinolone. Systemic antifungal drug treatment is indicated if *Malassezia* is present.
Eye	Conjunctivitis	Feline herpesvirus-1 or calicivirus, *Chlamydophila felis*, *Mycoplasma* spp., *Bordetella bronchiseptica* (kittens).	The presence of feline herpesvirus-1 is likely if keratitis is also present.	Doxycycline. Famciclovir or topical cidofovir may be indicated for severe herpesviral infections.

Upper respiratory	Feline upper respiratory tract disease/rhinitis	Usually resident bacteria (*Staphylococcus*, *Streptococcus*, *Pasteurella*, *Mycoplasma* that invade opportunistically). Resistant *Pseudomonas aeruginosa* rhinitis may occasionally be involved. In crowded environments and young animals, *Bordetella bronchiseptica*, *Streptococcus canis* or *Streptococcus equi* subsp. *zooepidemicus*, or *Mycoplasma* spp. (especially *M. felis*) may be primary pathogens.	Consider underlying causes (viral infections, nasal neoplasia, foreign bodies, oronasal fistulas).	If transmissible bacterial infections are suspected, doxycycline is the treatment of choice because it is active against *Bordetella*, *Streptococcus*, and *Mycoplasma* spp. Amoxicillin-clavulanate is an alternative but is not active against *Mycoplasma* spp. Cats with chronic idiopathic rhinosinusitis may require repeated treatment with antimicrobial drugs; culture and susceptibility should be performed wherever possible.
	Fungal rhinitis	Usually *Cryptococcus* spp. but occasionally *Aspergillus* spp.	Rule out nasal neoplasia. Secondary bacterial infection may be present.	Fluconazole, itraconazole, or ketoconazole for cryptococcosis; for refractory disease, addition of flucytosine or amphotericin B should be considered. Aspergillosis may be treated with itraconazole or posaconazole but complete resolution of infection may not be achievable.
	Bacterial pneumonia	Single or mixed infections that involve various facultative (especially Gram-negative) bacteria and anaerobes if aspiration pneumonia is present; *Bordetella bronchiseptica* may be involved in young kittens.	Aerobic culture and susceptibility testing on endotracheal wash indicated. Consider underlying causes such as feline inflammatory airway disease.	A combination of clindamycin and a fluoroquinolone is a suitable initial choice pending the results of culture and susceptibility testing. If anaerobes are suspected, a beta-lactam/beta-lactamase inhibitor combination may be more appropriate (such as ampicillin-sulbactam and a fluoroquinolone). Doxycycline is the treatment of choice if *Bordetella* pneumonia is suspected.
	Pyothorax	Various and often mixed, which includes anaerobes, *Actinomyces*, *Pasteurella*, and sometimes *Mycoplasma*.	Culture and susceptibility testing on pleural fluid indicated. Chest tube placement required to drain pus; surgery may be indicated.	Ampicillin-sulbactam or penicillin G and metronidazole (some anaerobes may produce beta-lactamase enzymes).
Gastrointestinal and abdominal	Periodontitis, gingivitis	Resident anaerobic and facultative bacteria.	Dental cleaning, scaling, other dental treatment may be needed.	Clindamycin or amoxicillin-clavulanate.
	Lymphoplasmacytic (caudal) gingivostomatitis	Resident oral flora	Complete dental extraction may be required for refractory cases; immunomodulators such as prednisolone or recombinant feline interferon omega could also be considered; chlorhexidine oral rinses.	Clindamycin.
	Gastric helicobacteriosis	*Helicobacter* spp., gastric helicobacter-like organisms.	Relationship between infection and disease often unclear.	Amoxicillin, clarithromycin and bismuth salicylate or amoxicillin, metronidazole and bismuth salicylate.
	Bacterial enteritis	*Salmonella* spp.	Primarily causes disease in immunocompromised cats or kittens. Treat only if systemic illness is present.	If systemic infection is present (i.e., with fever, lethargy, changes on the CBC, positive blood cultures), parenteral fluoroquinolones indicated.

(*continued*)

Table 28.2. Antimicrobial drug selection for selected infections in cats.[a] (*continued*)

Site	Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
		Campylobacter spp.	No clear association with diarrhea.	If diarrhea is present and no other cause of illness can be identified, consider treatment with a macrolide.
		Clostridium perfringens, C. difficile.	No clear association with diarrhea. Diagnosis of clostridial diarrhea requires demonstration of toxin production by toxin ELISA assays in association with diarrhea. Significance may still be unclear even when toxin is detected.	Metronidazole.
	Giardiasis	*Giardia* spp.	Infection often subclinical. Some assemblages/species may be zoonotic.	Fenbendazole. Alternatives are metronidazole, tinidazole, or ronidazole.
	Coccidiosis	*Isospora* spp.	Clinical illness usually associated with young age or co-infections with other enteric pathogens.	Sulfonamide +/- trimethoprim. Alternatives are ponazuril or toltrazuril (Europe).
	Parvoviral enteritis	Secondary facultative and anaerobic bacteria from the gastrointestinal tract	Parenteral antimicrobial drug treatment is important to counteract opportunistic bacterial invasion.	Ampicillin-sulbactam, cefazolin (mild disease); ampicillin-sulbactam and a fluoroquinolone (severe disease).
	Cholecystitis, cholangiohepatitis	*Escherichia*, *Salmonella*, *Enterococcus*, anaerobes.	Address underlying causes (e.g., disrupted bile flow). Consider ultrasound-guided collection of bile for aerobic and anaerobic culture and susceptibility.	Beta-lactam and beta-lactamase inhibitor combination with an aminoglycoside or a fluoroquinolone; narrow spectrum based on culture results.
	Bacterial peritonitis	Mixed anaerobes and facultative enteric bacteria.	Surgical exploration and lavage may be needed. Culture and susceptibility testing indicated.	As for cholecystitis/cholangiohepatitis.
Urinary and urogenital	Lower urinary tract infection/bacterial cystitis	*E. coli*, *Staphylococcus* spp., *Proteus*, *Streptococcus*, *Enterococcus*, *Enterobacter*, *Klebsiella*, *Pseudomonas*.	Rare in cats unless underlying disease such as renal failure, hyperthyroidism, or diabetes mellitus is present.	Trimethoprim-sulfamethoxazole or amoxicillin. Amoxicillin-clavulanate could be used where the regional prevalence of beta-lactamase production is high.
	Pyelonephritis	See lower urinary tract infection.	Culture and susceptibility recommended. Prolonged treatment required.	Amoxicillin and a fluoroquinolone pending culture results.
	Metritis, pyometra	*E. coli*, *Streptococcus*, *Staphylococcus*, other Gram-negative bacteria, sometimes anaerobes.	Ovariohysterectomy recommended. Culture uterine contents at surgery.	Ampicillin-sulbactam and either a fluoroquinolone or an aminoglycoside.
Musculoskeletal	Osteomyelitis, septic arthritis	*Staphylococcus* and to a lesser extent *Streptococcus, Enterococcus*, anaerobes; *Mycoplasma* may cause arthritis.	Culture and susceptibility strongly recommended. Requires debridement and drainage and prolonged treatment with antimicrobial drugs.	Withhold treatment until results of aerobic, anaerobic, and mycoplasma culture and susceptibility are available. If treatment is considered necessary, clindamycin or clindamycin and a fluoroquinolone (if Gram-negative bacteria or mycoplasmas suspected) could be considered.
Nervous system	Bacterial meningitis	*Staphylococcus*, *Streptococcus, Pasteurella*, rarely *Mycoplasma*.	CSF culture and susceptibility recommended.	Ampicillin or penicillin G; an alternative is trimethoprim-sulfamethoxazole. Doxycycline or a fluoroquinolone are indicated if mycoplasmas are suspected.

	Tetanus	*Clostridium tetani.*	Nursing case, antitoxin, wound debridement. Rare in cats.	Metronidazole or penicillin.
	Hepatic encephalopathy	Normal intestinal flora.	Oral antimicrobial drugs to suppress ammonia production by gastrointestinal bacteria; add lactulose and restricted protein diet.	Ampicillin or neomycin.
Other bacterial	Bartonellosis	*Bartonella henselae*, *Bartonella clarridgeiae*.	*Bartonella* serology and culture indicated. Significance as a cause of disease may be unclear because subclinical bacteremia is widespread.	Doxycycline or azithromycin.
	Plague	*Yersinia pestis.*	Human health risk. Treat fleas and lance bubos.	Aminoglycoside; doxycycline or fluoroquinolones are less optimal alternatives.
	Tularemia	*Francisella tularensis.*	Potential zoonosis through biting.	Aminoglycoside.
	Nocardiosis	*Nocardia* spp.	Pulmonary, systemic, or cutaneous lesions.	Trimethoprim-sulfonamide.
Other protozoal	Cytauxzoonosis	*Cytauxzoon felis.*	High mortality but treatment may be effective.	Combination of atovaquone and azithromycin.
	Toxoplasmosis	*Toxoplasma gondii.*		Clindamycin. Alternative is sulfonamide plus pyrimethamine or azithromycin.
	Cryptosporidiosis	*Cryptosporidium* spp.	Infection often subclinical and self-limiting. Potential zoonosis.	No uniformly successful treatment; some improvement may be seen with paramomycin, nitazoxanide, or azithromycin.
	Leishmaniasis	*Leishmania* spp.	Complete resolution of infection may not occur.	Meglumine antimonate and allopurinol. Alternatives are amphotericin B or miltefosine.
Rickettsial, ehrlichial and hemotropic mycoplasma infections	Ehrlichiosis, anaplasmosis	*Ehrlichia* spp., *Anaplasma phagocytophilum.*	Uncommonly reported in cats.	Doxycycline.
	Hemoplasmosis	*Mycoplasma haemofelis*, "*Candidatus* Mycoplasma haemominutum," "*Candidatus* Mycoplasma turicensis."	*M. haemofelis* is most likely to be associated with anemia.	Doxycycline or a fluoroquinolone. "*Candidatus* M. haemominutum" may be refractory to antimicrobial treatment; fluoroquinolones may be more active against this species.
Systemic mycoses	Aspergillosis, sinonasal or sinoorbital	*Aspergillus* spp. (especially *Aspergillus fumigatus*) *Neosartorya* spp.	Prognosis better for sinonasal disease	Itraconazole or itraconazole with amphotericin B; posaconazole. Do not use voriconazole to treat cats.
	Histoplasmosis	*Histoplasma capsulatum.*		Itraconazole with or without amphotericin B.
	Sporotrichosis	*Sporothrix schenckii.*		Itraconazole with or without amphotericin B; supersaturated potassium iodide is an alternative.

[a]These selections reflect personal opinion based on review of the literature, discussion with colleagues, and clinical experience. They are intended to guide drug selection when laboratory data are lacking. Laboratory data (Gram stain of exudate or aspirate, or culture and susceptibility test) should be used to guide drug selection if available. Selection may change once culture and drug susceptibility test results are known. See Greene, 2012, for additional information. (Greene C. Infectious Diseases of the Dog and Cat, 4th ed. St. Louis: Elsevier Saunders.)

Table 28.3. Antimicrobial drugs that are potentially hazardous in renal failure, liver failure, pregnancy, or neonates.

Renal Failure[a]	Liver Failure[b]	Pregnancy[a]	Neonates[a]
Aminoglycosides	Chloramphenicol	Aminoglycosides	Aminoglycosides
Amphotericin B	Clindamycin	Amphotericin B	Chloramphenicol
Chloramphenicol (cat)	Griseofulvin (cat)	Azithromycin	Fluoroquinolones
Clarithromycin	Ketoconazole	Chloramphenicol	Metronidazole
Flucytosine	Lincomycin	Fluconazole	Nitrofurantoin
Fluoroquinolones	Macrolides	Flucytosine	Sulfonamides
Lincomycin	Metronidazole	Fluoroquinolones	Rifampin
Nitrofurantoin	Rifampin	Griseofulvin	Tetracyclines
Sulfonamides	Sulfonamides	Itraconazole	Trimethoprim
Sulfonamide-trimethoprim (cat)	Sulfonamide-trimethoprim (dog)	Ketoconazole	
Tetracyclines (except doxycycline)	Tetracyclines	Metronidazole	
		Nitrofurantoin	
		Sulfonamides	
		Tetracyclines	
		Trimethoprim	

[a]See chapter 4.
[b]There is little information on effects of liver failure on antimicrobial drug therapy. Some listed agents are potentially hepatotoxic, others might accumulate to toxic levels in hepatopathy: in general these warnings constitute relative rather than absolute contraindications.

for example, obligate anaerobic bacterial pathogens and *Streptococcus* spp. have a relatively predictable pattern of susceptibility, so testing is not generally required for these microorganisms. Anaerobes are also difficult to isolate, so when anaerobes are suspected (such as when foreign bodies are present, infections involve the oral cavity or gastrointestinal tract, or there are abscesses, a foul odor, or gas production), treatment for anaerobes is usually indicated regardless of the results of culture and susceptibility. If the infection is serious or life threatening, treatment should be initiates as soon as possible, with the option of changing the medication (to the most narrow-spectrum option) when results become available. Alternatively, antimicrobial drug treatment could be withheld for 1–2 days pending results, provided the delay is unlikely to be harmful.

Antimicrobial Drug Classes and Treatment of Resistant Bacterial Infections

Antibacterial drugs have also been classified for veterinary use as first-line, second-line, and third-line drugs (Weese et al., 2006). First-line drugs are those that could be used for empirical selection in the absence of or pending the results of culture and susceptibility testing, and include amoxicillin, cephalexin, doxycycline, and trimethoprim-sulfonamides. Second-line drugs are those to be used on the basis of culture and susceptibility testing and because of the lack of any appropriate first-line options, and include ticarcillin, piperacillin, amikacin, and third-generation cephalosporins. Fluoroquinolones have also been included in this group because, in human medicine, excessive fluoroquinolone use has been associated with emergence of antimicrobial resistance and treatment failures (Bakken, 2004). Fluoroquinolones could be considered as first-line drugs for dogs and cats suspected to have serious Gram-negative bacterial infections that require treatment pending the results of culture and susceptibility testing. The use of third-line drugs, including vancomycin, linezolid, and carbapenems such as imipenem and meropenem, is usually reserved for situations when certain criteria are met (Weese et al., 2006):

1. Infection must be documented based on clinical abnormalities and culture
2. The infection is serious and has the potential to be life-threatening if left untreated.
3. Resistance is documented to all other reasonable first- and second-line options.

4. The infection must be potentially treatable. The use of critical drugs in situations where there is little realistic chance of elimination of the infection (such as failure to remove the underlying cause) is not supported.
5. The clinician may seek advice from an infectious disease clinician or a clinical microbiologist to discuss antimicrobial susceptibility test results, and to discuss the use of these agents if there is unfamiliarity with their use. In some instances, there may be other viable options (e.g., topical therapy).

Drug Formulations

Previous problems with dosage forms not adapted for use in dogs and cats are easing as more manufacturers introduce products specifically for veterinary use, and with the increased access to compounding pharmacies. There remains a need, however, for more oral formulations that are easier to administer to difficult patients, and also for drug preparations with pharmacokinetic characteristics that allow extended dosage intervals (24 hours or more). Transdermal antimicrobial preparations may appear attractive, but their use is not recommended without supportive pharmacokinetic data, as the use of inadequate products may promote development of antimicrobial resistance. Azithromycin is extensively concentrated within cells and is retained in tissues for prolonged periods, with a half-life of 30 hours in the dog and 35 hours in the cat; it is excreted in bile so is not useful for treatment of urinary tract infections. In human patients, a 5-day course can provide therapeutic tissue concentrations for at least 10 days. However, azithromycin is a bacteriostatic drug and although azithromycin has improved spectrum against Gram-negative bacteria, it has less activity against Gram-positive bacteria than erythromycin (Sivapalasingham et al., 2010; Piscitelli et al., 1992). Cefpodoxime proxetil has a half-life longer than other oral cephalosporins and it can be administered only once per day, so is well suited for dogs or cats with susceptible infections when owner compliance may be a problem. Cefovecin has an extremely long half-life in dogs and cats and can be effective for some infections when administered at 14-day intervals. However, concerns have been raised about the potential for subtherapeutic concentrations of such drugs to persist for long periods and select for the presence of resistant bacteria.

Generic drug preparations are frequently favored in larger patients for reasons of cost. Less concern is expressed about veterinary generics than their human counterparts and there is little evidence of a problem at present. However, the potential for differences in bioavailability exists. As these could affect drug efficacy and safety, vigilance is advisable when changing from one brand or formulation of a drug to another.

Route of Administration

The route of administration is sometimes dictated by the drug chosen. For example, if an aminoglycoside, vancomycin or amphotericin B is selected to treat a systemic infection, it must be given parenterally because absorption from the gastrointestinal tract is poor. More often, several routes of administration are possible and the one chosen may depend on the disease being treated, the likely duration of therapy, and the capability of the owner to administer the drug preparation.

Parenteral Administration

Parenteral administration can be valuable to initiate treatment in severe infections where rapid systemic delivery of high drug concentrations to systemic sites is important. Other indications include fractious, unconscious, or vomiting patients, those with oral pain, or infections susceptible only to antimicrobials that must be given parenterally.

The intravenous (IV) route should be used if maximum plasma drug concentrations are desired immediately after dosing, as with life-threatening infections. IV use might also be preferable in dehydrated or hypotensive patients, as poor peripheral perfusion may impede drug absorption from other sites. Long-term intravenous administration for conditions such as endocarditis or diskospondylitis can be achieved through extended hospitalization or, for selected and properly educated clients, in the home environment through the use of vascular access ports and careful catheter management.

Intramuscular (IM) or subcutaneous (SC) administration is usually safer and satisfactory in less demanding circumstances. These routes give similar bioavailability with most antimicrobial preparations, but SC administration is easier and generally causes less pain. Many formulations recommended for IM use can be given to dogs and cats by SC injection, but unfamiliar

preparations should be assessed in a few animals first to check for possible injection site reactions.

For IM injections, the lumbar musculature may be better site for drug administration than the thigh muscles. The preferred location lies midway between iliac crest and last rib, and halfway between dorsal spinous processes and the lateral border of the muscle. Injection here is less likely to be intermuscular, is usually well tolerated, and avoids the risk of major nerve damage.

Oral Administration

Dosage by the oral route is adequate in most infections and is generally the best method for home treatment. The potential for zoonotic transmission of the infection being treated (or other infections) through bite or scratch wounds should be considered if oral medications are to be administered. Clients should also be instructed to wear disposable gloves when administering medications wherever possible and wash their hands thoroughly afterward. Individual dogs and many cats are difficult to dose with solid dosage forms and some owners find it easier to use liquid formulations. With doxycycline, non-solid formulations may be preferred to tablets to minimize risks of esophageal irritation and ulceration; alternatively tablet administration must be followed by a bolus of water administered by syringe. In hospitalized patients, naso-esophageal, esophageal or gastrostomy tubes can be used as alternatives. For fractious animals or animal with oral pain where prolonged courses of antimicrobial drug therapy are likely to be necessary, the advantages of long-term placement of esophageal or gastrotomy tubes should be considered even if solely for the purpose of proper antimicrobial drug administration.

Administration of liquids, powders, or crushed tablets mixed in food or pill-pockets may be possible. Some patients reject medicated food, but may be fooled into swallowing morsels of food containing a tablet or capsule if first offered unmedicated pieces. However, with all forms of oral or enteral administration, the potential effect of ingesta on drug bioavailability should be considered (see below).

Influence of Food on Systemic Availability of Drugs Given Orally

Drug-food interactions that affect drug absorption are common in human patients but are often overlooked in veterinary medicine. The most frequent outcome is reduced or delayed absorption of the drug, although sometimes it is increased or unaffected. The mechanisms responsible are complex and involve food-induced changes in gut physiology and direct interactions between food components and drugs. The composition of the meal, the volume of fluid ingested, and specific formulation of the drug may affect the outcome. Because of these complexities, it is not possible to give conclusive recommendations that cover all situations. It is also difficult to assess the importance of drug-food interactions as studies comparing therapeutic efficacy under fasting and non-fasting conditions in dogs or cats are lacking. However, it may be prudent to fast patients for 1–2 hours before and 1–2 hours after administration of agents for which absorption can be impaired substantially by food, such as most penicillins and tetracyclines other than doxycycline. An alternative would be to give a higher dose with food, but the increase required is difficult to predict. Some antimicrobial drugs can be given without regard to feeding, while others might be better given with food to improve absorption or reduce gastric irritation associated with dosage. Current suggestions are shown in Table 28.4.

Table 28.4. Suggested oral administration of selected antimicrobial drugs in relation to feeding.

Better when Fasting[a]	Better with Food	Indifferent to Feeding
Azithromycin	Cefadroxil[b]	Cephalexin[b]
Most erythromycin reparations[b]	Chloramphenicol palmitate[d]	Chloramphenicol capsules, tablets[b,d]
	Doxycycline[e]	
Most fluoroquinolones[c]	Griseofulvin	Chloramphenicol palmitate[b]
Isoniazid	Itraconazole (capsules)	Clarithromycin[b]
Lincomycin	Ketoconazole	Clindamycin
Most penicillins[b]	Metronidazole[e]	Ethambutol
Rifampin	Nitrofurantoin[e]	Fluconazole
Most sulfonamides		
Most tetracyclines		
Itraconazole (suspension)		

Source: Data are from human studies, except as indicated.
[a]Absorption of these drugs may be reduced or delayed by ingesta. Fasting means no food for 1–2 hours before and 1–2 hours after dosing.
[b]Canine data.
[c]Enrofloxacin availability is reduced by ingesta in dogs. Effects of ingesta on fluoroquinolones are generally mild, but absorption may be delayed slightly. Dairy foods (and products containing multivalent cations) should be avoided.
[d]Feline data.
[e]Food may reduce gut irritation without hindering absorption importantly.

Dosing Rate and Duration of Therapy

Conventional dosage regimens for antimicrobial drugs in dogs and cats are presented in Table 28.5. These should be regarded as guidelines only. The optimum dosage regimen will vary somewhat with the case, depending on the susceptibility of the pathogen, ability of the drug to reach the infection site, and competence of the patient's immune defenses. Higher doses (concentration-dependent drugs) or more frequent dosages (time-dependent drugs) may be required for relatively resistant pathogens or lesions in tissues where drug penetration is poor. Use of the lower end of the dosage range may be satisfactory for lower urinary tract infections if the drug (or its active metabolites) become highly concentrated in urine during the excretory process.

For most aerobic or anaerobic bacterial infections, it is usually apparent within 24–48 hours whether treatment is having the desired effect. Longer periods of treatment (such as 1 week) may be required to assess the response to treatment for slow growing organisms such as mycobacteria or fungal organisms. If a response has not occurred, the diagnosis and treatment regimen should be re-evaluated, including whether an infectious process was the likely cause of illness if infection was not previously confirmed. If infection remains the most likely cause, culture and susceptibility testing should be considered. A different drug could then be selected or an increased dosage of the original agent could be considered if underdosing or poor tissue penetration is suspected. The presence of underlying disease that predisposes to infection, such as neoplasia or foreign bodies, should also be considered as a reason for treatment failure.

Studies that examine the optimal duration of antimicrobial treatment for various infections are lacking. For many uncomplicated infections, treatment times of 7–10 days have been traditionally used. However, one study showed that treatment of dogs that had uncomplicated UTIs with high dose enrofloxacin for 3 days was not inferior to treatment with clavulanic acid-amoxicillin for 10 days (Westropp et al., 2012). Additional studies that evaluate short durations of treatment with other antimicrobial drugs such as amoxicillin and trimethoprim-sulfonamide are needed. One suggestion is to treat for a minimum of 3 days and to continue for 2 days after signs of infection have subsided. Serious infections such as pneumonia and pyelonephritis have generally been treated for a minimum of 4 weeks, but it is possible that shorter durations of treatment may be sufficient. Treatment responses may be slower with chronic infections (such as chronic pyoderma), and prolonged administration (4–6 weeks) is often needed because of existing tissue damage, impaired blood supply, and compromised local or systemic immunity. For systemic mycoses, treatment for a minimum of several months (and sometimes years) is usually required.

Therapeutic Compliance

Carefully formulated therapeutic plans may be valueless if the owner does not follow the suggested dosage regimen. Problems may arise because the owner does not understand the importance of the medication or the instructions given. Furthermore, the owner's inexperience, patient's resistance, and suboptimum formulation characteristics (e.g., poor size, shape, taste, consistency) can prevent satisfactory administration and produce an angry animal and frustrated owner. These problems are likely to be greater with cats and some less congenial small dogs.

Issues of therapeutic non-compliance have not been well studied in veterinary medicine, but a few studies demonstrated poor compliance was common during treatment for acute bacterial infections in dogs. Potential difficulties should be addressed by scheduling dosing to suit the owner's routines. Linking dosage times to fixed points in the owner's day (e.g., mealtimes, bedtime) may assist, although the animal's mealtimes might need changing to avoid undesirable drug-food interactions. Other logical measures are to decide with the owners the dosage form they can best manage, demonstrate its use, and provide clear verbal and written instructions. Studies in human medicine have shown that increasing treatment complexity is associated with increased probability that doses will be missed. Thus, if no therapeutic difference exists between two treatment options, the one with the less complex regimen should be prescribed. Likewise, additional medications of questionable value are best avoided, because ensuing complexity could reduce therapeutic compliance with the more important drugs.

Table 28.5. Conventional dosage regimens for systemically administered antimicrobial drugs in dogs and cats. The reader is referred to specific chapters in this book for detailed information on activity and adverse effects.

Drug	Route	Dose (mg/kg except as indicated)	Dose interval (h)	Comments
Penicillins				
Flucloxacillin	PO	15	8	Penicillinase-resistant penicillin. Avoid ingesta.
Penicillin G, sodium or potassium	IV, IM, SC	20,000–40,000 U/kg	4–6	May increase plasma sodium or potassium concentration.
Ampicillin sodium	IV, IM, SC	10–20	6–8	Administer IV over 3 minutes.
Ampicillin sulbactam	IV, IM	10–20	8	Administer IV over 3 minutes.
Amoxicillin	PO	10–20	8–12	
Amoxicillin-clavulanate	PO	12.5–25	12	More frequent or higher doses recommended for Gram-negative infections.
Ticarcillin disodium, ticarcillin-clavulanate	IV	33–50	4–6	
Piperacillin sodium/piperacillin-tazobactam	IV	40	6	
Cephalosporins				
Cephalexin	PO	20–30	6–12	Use 30 mg/kg q 12 h for pyoderma. More frequent dosing (q 6–8 h) is required for Gram-negative bacterial infections.
Cefadroxil	PO	22	12	
Cefazolin sodium	IV, IM	20–35	8	22 mg/kg q 2 h during surgery if needed.
Cefpodoxime proxetil	PO	5–10 (dogs)	24	Of all third-generation cephalosporins, most active against staphylococci. Less active against Gram-negative and anaerobic infections.
Cefaclor	PO	15–20	8	For otherwise resistant bacterial infections.
Cefixime	PO	10	12	Use 5 mg/kg q12–24 h for resistant bacterial urinary tract infections.
Cefotetan disodium	IV, SC	30	8	For serious and resistant infections. May cause pain on injection.
Cefoxitin sodium	IV, IM	30	6–8	For serious and resistant infections. May cause pain on injection.
Ceftiofur sodium	SC	2.2–4.4 (dogs)	24	For treatment of otherwise resistant urinary tract infections.
Cefotaxime sodium	IV, IM	20–50	6–8	For serious and resistant infections.
Ceftazidime	IV	30	6	For serious and resistant infections. Potent antipseudomonal activity.
Cefepime	IV, IM	40 (dogs)	6	For serious and resistant infections. Reduce dose in renal failure.
Cefovecin	SC	8	14 days	Approved for use for skin and soft tissue infections and in some countries, urinary tract infections. Efficacy for treatment of infections in other sites not established.
Carbapenems				
Imipenem-cilastatin	IV, IM, SC	5	6–8	250–500 mg of reconstituted drug should be added to no less than 100 mL of fluids and administered IV over 30–60 minutes.
Meropenem	IV	8.5	12	SC injections may cause alopecia at the injection site. For *Pseudomonas aeruginosa* or infections with MIC values approaching the breakpoint, use 12 mg/kg q 8 h SC or 24 mg/kg q 8 h IV.
		24	12	

Glycopeptides				
Vancomycin	IV	15	8	Reconstituted drug is added to 0.9% NaCl or 5% dextrose and administered over 30–60 minutes. Therapeutic drug monitoring recommended.
Fluoroquinolones				
Enrofloxacin	PO, IV, IM IM, PO	5–20 (dogs) 5 (cats)	24	Avoid use of enrofloxacin in cats. Reduce dose or increase dosage interval with renal failure. Dilute in fluids (e.g., 1:10 dilution) and infuse IV over 30 minutes.
Ciprofloxacin hydrochloride	PO IV	20–30 10	24	Oral absorption may be limited.
Marbofloxacin	PO	2.75–5.5	24	
Orbifloxacin	PO	2.5–7.5	24	Orbifloxacin suspension provides lower and more variable plasma levels than the tablets. A dose of 7.5 mg/kg should be used.
Difloxacin hydrochloride	PO	5–10	24	Urine concentrations may not be sufficient for treating UTIs.
Moxifloxacin	PO	10	24	Moxifloxacin has enhanced activity against Gram-positive bacteria and anaerobes.
Pradofloxacin	PO	3–5 (dogs) 5–10 (cats)	24	Pradofloxacin has enhanced activity against Gram-positive bacteria and anaerobes. May not be available in some countries.
Nitroimidazoles				
Metronidazole	PO	15 (dogs) 10–15 (cats)	12 24	Reduce dose by 50% in animals with hepatic dysfunction. An intravenous solution, metronidazole hydrochloride, is available.
Metronidazole benzoate	PO	25 (cats)	12	More palatable formulation for cats that may be available from some compounding pharmacies.
Tinidazole	PO	15	12 (dogs) 24 (cats)	Antiprotozoal drug.
Ronidazole	PO	30	24	For *Tritrichomonas foetus* infections.
Rifamycins				
Rifampin	PO	5	12	Preferably use in combination with other drugs. Avoid in animals with hepatic dysfunction. Do not administer with fatty meals. Drug interactions may occur. Monitor liver enzymes with prolonged treatment. May impart a red-orange color to the urine and tears.
Trimethoprim-sulfonamides				
Sulfadimethoxine	PO	55 on day 1, 27.5 thereafter	24	Isosporiasis with or without a dihydrofolate reductase inhibitor.
Trimethoprim-sulfamethoxazole, trimethoprim-sulfadiazine	PO, IV	30	12	Dose listed is for combined components, i.e., 5 mg/kg of trimethoprim and 25 mg/kg of sulfonamide. Avoid in animals with hepatic failure. Reduce dose in animals with renal insufficiency. Obtain baseline Schirmer tear test and monitor this and the CBC with prolonged treatment. Each 5-mL vial of the injectable preparation should be diluted in 75–125 mL of 5% dextrose and administered IV over 1 hour.
Ormetroprim-sulfadimethoxime	PO	55 on first day, then 27.5 (dogs)	24	Dose listed is for combined components. Avoid in animals with hepatic failure. Reduce dose in animals with renal insufficiency. Obtain baseline Schirmer tear test and monitor this and the CBC with prolonged treatment.

(*continued*)

Table 28.5. Conventional dosage regimens for systemically administered antimicrobial drugs in dogs and cats. The reader is referred to specific chapters in this book for detailed information on activity and adverse effects. (*continued*)

Drug	Route	Dose (mg/kg except as indicated)	Dose interval (h)	Comments
Aminoglycosides				
Gentamicin sulfate	IV, IM, SC	9–14 (dogs) 5–8 (cats)	24	Ensure adequate fluid and electrolyte balance during treatment. Avoid in animals with renal impairment. Monitor BUN, creatinine, and urinalysis for casts and proteinuria.
Amikacin	IV, IM, SC	15–30 (dogs) 10–14 (cats)	24	See gentamicin.
Tobramycin sulfate	IV, IM, SC	3-6	24	See gentamicin. Reserve for infections resistant to other aminoglycosides.
Kanamycin sulfate	IV, IM, SC	20	24	See gentamicin. Less active than gentamicin and amikacin.
Streptomycin,	PO	20	6	
dihydrostreptomycin	IM, SC	20–30	24	
Neomycin	PO	10–20	6–12	Not absorbed systemically; used to treat hepatic encephalopathy. Use cautiously in animals with renal disease and avoid use for > 14 days.
Chloramphenicol and Related Drugs				
Chloramphenicol	PO, IV, IM	40–50 (dogs) 12.5–20 (cats)	6–8 12	Avoid long-term use in cats. Monitor CBC with long-term use. Warn owners that human exposure to chloramphenicol may cause bone marrow disease. Drug interactions may occur. Avoid in animals with hepatic failure.
Macrolides and Lincosamides				
Erythromycin	PO	10–20	8	Do not administer to rabbits or rodents, as this may cause fatal diarrhea.
Tylosin	PO	7–15	12–24	See erythromycin. For colitis in dogs, use 20 mg/kg q 8h with food, and taper to q 24h if a response occurs. 20 mg/kg is equal to 1/8 teaspoon of tylosin phosphate (Tylan) for a 20-kg dog.
	IM	8–11	12	
Clarithromycin	PO	7.5	12	
Azithromycin	PO	5–10	24	Serum MIC values may not predict tissue concentrations.
Lincomycin hydrochloride	PO	15–25	12	See erythromycin. A dose of 10 mg/kg PO q 12h has been suggested for pyoderma.
Clindamycin hydrochloride,	PO	11—33 (dogs)	12	See erythromycin. For IV administration, dilute 1:10 in 0.9% saline and administer over 30–60 minutes. The oral suspension may be unpalatable to cats. For toxoplasmosis, use 25 mg/kg q 12h PO.
clindamycin phosphate	IV, IM	11 (cats)	24	
		10	12	
Oxazolidinones				
Linezolid	PO, IV	10 (dogs)	8	The use of linezolid should be reserved for Gram-positive infections that are susceptible to linezolid but resistant to all other reasonable alternatives, on the basis of culture and susceptibility testing.
Tetracyclines				
Tetracycline	PO	15–20	8	Do not mix with food containing cations such as calcium, zinc, magnesium, iron, aluminum.
	IV, IM	4.4–10		
Oxytetracycline	PO	20	12	
	IV	7.5–10		
Doxycycline	PO, IV	5	12	For IV administration, dilute in 100 to 1000 mL of LRS or 5% dextrose and administer over 1–2 hours.
		10	24	

Minocycline	PO	5–12.5	12	
Antimycobacterial Drugs				
Clofazimine	PO	4–8 (dog) 25 mg/cat	24	May not be available in some countries.
Ethambutol	PO	15 (dog)	24	
Isoniazid	PO	5–10 (dog max 300 mg daily)	24	May cause neurologic toxicity.
Other Antiprotozoal Drugs				
Allopurinol	PO	10	12	For leishmaniasis in combination with meglumine or miltefosine.
Amprolium	PO	1.25 g of 20% powder	24	
Atovaquone	PO	13.3 (dogs) 15 (cats)	8	Babesiosis and cytauxzoonosis with azithromycin. Administer with food. Drug interactions may occur.
Benznidazole	PO	5–10	12	For Chagas' disease.
Decoquinate	PO	10–20	12	American hepatozoonosis and sarcocystosis. Powder (6% decoquinate; 60 mg active ingredient per gram) is mixed with food. This equates to 0.5–1 tablespoon/10 kg body weight q 12 h.
Diminazene aceturate	Deep IM	3–5	Once	Babesiosis and trypanosomiasis. Narrow therapeutic range.
Fenbendazole	PO	50	24	
Imidocarb dipropionate	Deep IM	6.6 (dogs) 5 (cats)	Once, repeat in 14 days	Large *Babesia* spp. infections. Caution with hepatic or renal insufficiency. Avoid use in conjunction with other cholinesterase inhibitors.
Nitazoxanide	PO	100 mg/animal	12	Cryptosporidiosis. Efficacy and safety unclear. Vomiting common in cats.
Paramomycin	PO	10	8	Cryptosporidiosis. Caution in animals with diarrhea due to possible systemic absorption. Avoid in cats.
Ponazuril	PO	20–50 (dogs)	12–24	Toxoplasmosis, neosporosis, isosporiasis. Optimal dose, duration, efficacy, and adverse effects unknown.
Pyrimethamine	PO	1	24	Primarily neosporosis, toxoplasmosis, and American hepatozoonosis. Use with a sulfonamide. Use caution with hepatic and renal insufficiency. Monitor CBC. Folinic acid supplementation (5.0 mg/day) may be required.
Meglumine antimonate	SC	75–100	24	For leishmaniasis in combination with allopurinol.
Miltefosine	PO	2	24	For leishmaniasis in combination with allopurinol.
Toltrazuril	PO	5–10 (dogs) 18 (cats)	12–24	Hepatozoonosis, isosporiasis. One dose may be effective for isosporiasis. Optimal dose and duration for hepatozoonosis unknown.
Antifungal Drugs				
Amphotericin B deoxycholate	IV	0.5 mg/kg (dogs) 0.25 mg/kg (cats)	Administer on a Monday-Wednesday-Friday basis for 4 weeks or until azotemia develops	Dilute in large volume of 5% dextrose in water. Obtain baseline CBC, kidney panel, and UA and ensure adequate hydration before starting treatment. Recheck kidney panels before each treatment.

(*continued*)

Table 28.5. Conventional dosage regimens for systemically administered antimicrobial drugs in dogs and cats. The reader is referred to specific chapters in this book for detailed information on activity and adverse effects. (*continued*)

Drug	Route	Dose (mg/kg except as indicated)	Dose interval (h)	Comments
Lipid complexed amphotericin B (Abelcet)	IV	3 mg/kg (dogs) 1 mg/kg (cats	Administer on a Monday-Wednesday-Friday basis for 4 weeks or until azotemia develops	Dilute to 1 mg/mL in D5W. Administer calculated dose IV over 1–2 hours.
Ketoconazole	PO	10–15 (dogs) 5–10 (cats)	12	Do not administer to pregnant animals. Monitor liver enzymes monthly during treatment. Drug interactions may occur. Inhibits adrenal function. Antacids impair absorption.
Fluconazole	PO	5–10 50 mg/cat	12	Do not administer to pregnant animals. Monitor liver enzymes monthly during treatment. Drug interactions may occur.
Itraconazole	PO	5	12–24	Do not administer to pregnant animals. Monitor liver enzymes monthly during treatment. Drug interactions may occur. Use of the oral suspension warrants dose reduction to 3 mg/kg. Monitor serum drug levels after 2 weeks if there is inadequate response to treatment. Compounded formulations are unstable.
Voriconazole	PO	4 (dogs only)	12	Do not use in cats. Use cautiously in animals with liver disease. Also see fluconazole. Consider therapeutic drug monitoring.
Posaconazole	PO	5–10 (dogs) 5 (cats)	12–24 24	Absorption may be improved when daily dose is split into 2–4 doses. Consider therapeutic drug monitoring. Also see fluconazole. Antacids impair absorption.
Flucytosine	PO	25–50	6–8	Monitor CBC. Use cautiously in animals with impaired renal function. Avoid use in dogs. Serum concentration monitoring is recommended in humans.
Griseofulvin (microsized)	PO	25	12	Administration with fatty food improves absorption. Dose may be increased to 50 mg/kg q 12 h for refractory infections. Avoid in cats with FIV infections. Do not use in pregnancy. Drug interactions possible. Monitor CBC.
Griseofulvin (ultramicrosized)	PO	15	12	Avoid in cats with FIV infections. Do not use in pregnancy. Drug interactions possible. Monitor CBC.
Terbinafine	PO	30–40	24	Administer with food.

Table 28.6. Surgical procedures that may warrant antimicrobial prophylaxis.

Gastrointestinal tract	Dental procedures combined with other surgery; biliary surgery if infection present; resection of: esophagus, stomach in gastric dilation-volvulus, intestine in obstruction; colonic, rectal, and anal surgery.
Orthopedic	Extensive internal fracture fixation, open fracture repair, total hip prosthesis.
Other procedures	Perineal herniorrhaphy, hernia repairs with non-absorbable mesh, pacemaker implantation, lobectomy in infection, extensive neurosurgery, prolonged (> 2 hours) surgery with much tissue manipulation.

Outcome

The response to antimicrobial therapy can be most favorable when the correct drug is used to treat an uncomplicated microbial infection in a patient that is otherwise healthy, or if an underlying cause of opportunistic infection can be removed or resolved. By contrast, the outcome is likely to be disappointing if the wrong drug is chosen, if bacteria are not responsible for the condition, or if complicating factors have not been addressed. Additional specific and supportive measures, such as nursing, fluid therapy, and surgery, are often very important. If the response to appropriate therapy is poor or repeated relapses occur, an underlying maintaining cause should be considered, including retroviral infections in cats, other immunodeficiency disorders, tumors, and foreign bodies.

Antimicrobial Prophylaxis

The principles and practice of antimicrobial prophylaxis are described in chapter 21.

Antimicrobial Prophylaxis in Surgery

There are few situations where prophylaxis is warranted in small animal surgery, but Table 28.6 lists some potential indications. The drugs selected should be effective against coagulase-positive staphylococci and *Escherichia coli*, the microbes most likely to cause post-operative wound infections in dogs and cats. Cefazolin usually has excellent activity against susceptible staphylococci and *E. coli*, and has low toxicity. It is also active against many obligate anaerobes, which might be preferred if anaerobes are of particular concern, as in colonic or rectal surgery. Where available, injectable ampicillin-sulbactam preparations are economical alternatives to cephalosporins and may have improved activity against anaerobes. If used, these antimicrobials should be administered every 2 hours during surgery and treatment should not be continued beyond the peri-operative period.

Unfortunately, the emergence of methicillin-resistant staphylococci (and especially *Staphylococcus pseudintermedius*) in some geographic locations (North America and Europe) has meant that prophylactic treatment with beta-lactam drugs such as cephalosporins has the potential to select for these bacteria when sterile surgical technique is not optimal. These drugs have no efficacy against methicillin-resistant bacteria or the majority of multidrug-resistant Gram-negative bacteria. Thus, antimicrobial drugs should never be used as a substitute for careful infection control measures, which should include proper patient preparation, proper use of scrubbing and sterile drapes, attention to hemostasis, and minimization of surgical time.

Antimicrobial Prophylaxis in Non-surgical Patients

Prophylactic use of antimicrobial drugs in non-surgical patients is controversial and veterinary data are limited, but it is not generally warranted. Chemoprophylaxis might be effective if the period of risk is brief (a few hours or days), as with some chemotherapy-induced myelosuppression, or the target is a single drug-susceptible species. For example, trimethoprim-sulfonamides have been used successfully for prophylaxis in dogs treated with chemotherapeutics such as doxorubicin (Chretin et al., 2007). However, attempts at long-term chemoprophylaxis are liable to simply select for bacteria that are resistant, especially if host defenses remain compromised. Prophylactic antimicrobial drug treatment of animals with indwelling urinary catheters is strongly discouraged as it increases the risk of infection with resistant bacteria. If indwelling urinary catheterization is required, a closed sterile collection system should always be used, and the catheter should be removed as soon as it is no longer required, because the risk of ascending infection increases with every day the catheter is left in place. Instead of using prophylactic

antimicrobials, a better approach is to carefully monitor individuals at risk for signs of infection and to treat promptly and appropriately if infection occurs, as well as resolving underlying causes of compromised host defenses whenever possible. Routine culture of catheters at the time of catheter removal is not recommended, because they become contaminated with ascending bacteria (which does not equate to infection). If infection is suspected (based on the presence of pyuria or hematuria) and a catheter is in place, the catheter should be removed and the urine collected for culture by cystocentesis or through a newly placed catheter, which should then be withdrawn if possible. Routine treatment of dogs and cats with antimicrobials after removal of a catheter is controversial, but could be considered if the consequences of infection are likely to be severe (such as reobstruction in cats with urethral obstructions). Urine culture should never use specimens from the collection bag, and there is no indication to culture the catheter tip (Weese et al., 2011).

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29

Antimicrobial Drug Use in Cattle

Michael D. Apley and Johann F. Coetzee

Antimicrobial options for cattle have dramatically changed since the 1970s. Novel properties of new drug groups, changes in route of administration, and advances in drug formulations have dramatically altered characteristics of treatment regimens. Many of the newer antimicrobials have single injection regimens based on extended duration or pharmacodynamic properties. These new regimens are used in an environment of increased regulatory and political pressure, an expanding array of branded food product lines, and increased scrutiny by consumer and antianimal agricultural special interest groups. This chapter addresses important areas of consideration in constructing antimicrobial regimens in cattle within this context, including reasonable antimicrobials for selected diseases and extended discussions of some common therapeutic challenges.

General Considerations of Antimicrobial Use in Cattle

When giving treatment instructions to clients, especially in large-scale production facilities where lay personnel will be identifying and treating ill animals, the veterinarian is obligated to provide written treatment guidelines. The treatment guidelines should be constructed to contain the following information, where appropriate.

- Case definition for initial treatment.
- Initial regimen:
  - Drug(s), dose, route, duration, frequency, slaughter withdrawal.
  - Specific administration instructions: injection site, volume per site, needle size, injection technique.
  - Environmental management during treatment: housing, water, feed.
  - Safety precautions or warnings.
  - Case definitions for treatment success and failure.
- Secondary regimen for treatment of animals failing the initial treatment regimen.
- Any additional regimens for animals not responding after the first and second regimens.
- Disposition of animals not responding to therapy.

It is essential that the treatment protocols not be altered except after agreement by all parties involved. Consistency of protocol application is an absolute necessity in order to evaluate therapeutic and preventive programs in production systems.

In Constructing These Regimens, the Veterinarian Must Make Several Key Decisions.

One or Multiple Antimicrobials in Each Regimen

The search for antimicrobial synergy is prevalent in all branches of medicine. However, there is little evidence that this is achieved in cattle. Anecdotal reports often

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claim that the preferred combination reduces relapses or improves initial treatment response. Arguments that combination therapy will suppress resistance development must be evaluated in light of considering that the bacterial population will also be exposed to a wider variety of antimicrobials.

Different Therapy or Continued Therapy

If the animal did not respond to the initial antimicrobial regimen, was it because of pathogen resistance or the animal being incapable of responding in a short time? In the example of undifferentiated fever, which is often interpreted as respiratory disease in large production systems, the animal may have from 3 to 10 days to respond to the initial regimen before being classified as a treatment failure. Newer antimicrobials give extended durations of antimicrobial coverage such as approximately 7 days for ceftiofur crystalline free acid (Excede, Pfizer Animal Health), and 300 mg/ml long-acting oxytetracylcine (Tetradure 300, Merial), up to approximately 2 weeks for tulathromycin (Draxxin, Pfizer Animal Health) and gamithromycin (Zactran, Merial), and a claimed 28 days for tildipirosin (Zuprevo, Merck Animal Health). These longer durations of therapy bring forth the challenge of deciding when concentrations have reached a low enough level that non-responders should receive additional therapy.

It is reasonable to conclude, in the case of successful response by the majority of animals, that individuals not responding in a short time frame are in need of continued therapy as opposed to necessarily requiring alternate therapy. In the first author's personal experience with randomized, controlled respiratory disease trials, repeating the first regimen as continued therapy in first treatment failures resulted in similar second treatment response as trials where changes were made in continued therapy selections. This is dependent on the first treatment providing satisfactory treatment response and isolates with similar susceptibility profiles being present in all cases.

Quality Assurance

The National Cattlemen's Beef Association beef quality assurance audits awoke the cattle industry in the United States to the need to carefully consider where and what we inject into cattle. It is reasonable to give priority to antimicrobials with subcutaneous, intravenous, or oral administration routes. Current quality assurance guidelines call for intramuscular injection in the neck when this route is necessary. It is reasonable to avoid intramuscular injections whenever possible in cattle. Injections should never go in the high-value muscles of the back, especially in the hip region, and should go in the hind leg only as a last resort.

Extra-Label Drug Use (ELDU)

In the United States, regulations for ELDU were promulgated as directed by the Animal Medicinal Drug Use Clarification Act (AMDUCA, 1996). The regulations should be consulted for actual guidance, but the overall order of expected use may be summarized as follows.

1. Use of an antimicrobial according to label directions.
2. Use of a food animal-labeled drug in an extra-label manner according to requirements set forth in the AMDUCA regulations.
3. Use of a veterinary non-food-animal-labeled drug or human labeled drug according to requirements set forth in the AMDUCA regulations.
4. Use of a compounded product meeting the requirements of the AMDUCA regulations. It is well advised to consult the Food and Drug Administration/ Center for Veterinary Medicine (FDA/CVM) compliance policy guideline on compounding.

Part of the AMDUCA regulation requirements is that the veterinarian must determine an extended slaughter withdrawal time for animals subjected to ELDU. In the United States, this information may be obtained from the Food Animal Residue Avoidance Databank (FARAD). If adequate information for construction of an extra-label slaughter withdrawal time is not available, then the drug may not be used in food animals. In some other countries this information is available through Global FARAD (gFARAD).

In the United States, the FDA/CVM has banned the following antimicrobials from any extra-label use in food animals; chloramphenicol, fluoroquinolones, nitroimidazoles, nitrofurans, and glycopeptides. These regulations also prohibit the extra-label use of sulfonamides in lactating dairy cows (FDA/CVM, 2005). In 2012, The FDA/CVM also prohibited the extra-label use of cephalosporins at unapproved dosages, frequencies, durations, or routes of administration in cattle, swine, chickens or

turkeys (21 CFR Part 530, 2012). Furthermore, use of cephalosporins that are not approved for use in one of these species, and for extra-label disease prevention are also prohibited. The extra-label use of cephapirin in food-producing animals is exempt from these restrictions. The extra-label use of cephalosporins in these species is permitted for the treatment or control of an extra-label disease indication provided this use adheres to a labeled dosage regimen approved for that particular species and production class. Extralabel use of a cephalosporin in a food-producing minor species, such as ducks or rabbits is also allowed. Veterinarians should be familiar with regulations in their respective country in order to protect the interests of their clients and the consuming public.

Is Susceptibility Testing Useful in Selecting Antimicrobials for Use in Cattle?

The answer to this question depends on whether the susceptible and resistant breakpoints have been correlated to clinical efficacy. The Clinical Laboratory Standards Institute (CLSI), formerly the National Committee on Clinical Laboratory Standards (NCCLS), has approved veterinary-specific breakpoints for bovine respiratory disease (BRD) and mastitis for some antimicrobials (CLSI, 2008). These breakpoints have been established after reviewing pharmacokinetic, pharmacodynamic, wild-type isolate MIC distribution, and clinical trial data presented by the drug sponsor. Approved BRD-specific breakpoints have been established for ceftiofur sodium, ceftiofur hydrochloride, ceftiofur crystalline free acid, danofloxacin, enrofloxacin, florfenicol, spectinomycin sulfate, tulathromycin, and tilmicosin phosphate. Approved bovine mastitis breakpoints have been established for intramammary preparations of ceftiofur hydrochloride, penicillin/novobiocin, and pirlimycin. These breakpoints apply only when the antimicrobial is used according to label directions and the susceptibility testing is performed using CLSI approved methods and interpretive criteria.

For other antimicrobials, the breakpoints for bovine indications have been adapted from human interpretive criteria. Examples of this approach include penicillin G, the tetracyclines, potentiated sulfonamides, aminoglycosides, and erythromycin. It should be noted that there are no veterinary approved breakpoints for enteric disease in any species. For in-depth information on the conduct and interpretation of susceptibility testing in cattle, and all veterinary species, the reader is referred to the most recent edition of the Clinical and Laboratory Standards Institute publication M31 (CLSI, 2008).

Arguments that susceptibility testing results have no utility in antimicrobial selection are often based on the fact that animals with "susceptible" organisms have failed to resolve infections and animals with "resistant" pathogens have recovered. It is important to realize that antimicrobial susceptibility testing does not guarantee a specific clinical result in an individual animal. Rather, for veterinary approved breakpoints, it places the animal/drug regimen/pathogen combination in a population where clinical resolution is more or less likely as compared to other categories. The veterinarian must determine when susceptibility testing may be of use in monitoring a population of animals and pathogens.

Judicious Use Guidelines

Concerns about the proper use of antimicrobials in food animals, especially related to resistance development in pathogens with zoonotic potential, have prompted veterinary specialty practice organizations to develop and publish judicious use guidelines. The American Veterinary Medical Association (AVMA) has made prudent use guidelines for the use of antimicrobials in cattle available on their website (AVMA, 2003). These guidelines were developed by the American Association of Bovine Practitioners (AABP) and were then approved by the AVMA Executive Board. While these guidelines do not give specific recommendations for antimicrobial applications, they do provide overall guidance in the approach veterinarians should be taking in designing antimicrobial regimens for cattle.

Some Antimicrobials Have Specific Limitations in Cattle That Either Preclude Their Use or That Require Special Consideration

The extra-label, systemic use of aminoglycosides in cattle has been the subject of resolutions or policy statements by the AVMA, AABP, Academy of Veterinary Consultants (AVC), and the National Cattlemen's Beef Association (NCBA). In general, these statements discourage the

extra-label use of aminoglycosides in cattle due to the prolonged slaughter withdrawal potential. Veterinarians should pay special attention to these statements, especially when a producer organization joins with veterinary organizations in discouraging the extra-label use of a drug in cattle.

Some antimicrobials have significant potential for tissue damage when injected intramuscularly. These include the macrolides (tylosin, erythromycin) and the tetracyclines. Although, as mentioned in the section on quality assurance, a visible lesion is not necessary for an adverse effect on tenderness, persistent visible lesions add to trim loss when primal cuts are fabricated into retail cuts. Intravenous use of tylosin and erythromycin are a possibility, but the non-water-soluble properties of these drugs in commercially available forms combined with the propylene glycol carriers make adverse reactions a possibility. In addition, repeated intravenous injections have become less attractive in light of effective alternatives with less frequent, subcutaneous administration routes.

Antimicrobial Agents Approved for Use in Cattle

Labeled antimicrobial applications and regimens for selected antimicrobials are reported in Table 29.1. While the regimens for labeled pathogens may not be optimal for extra-label use, these regimens give a starting point for consideration. The region for which the label regimen is approved is listed in the table. The reader is cautioned to evaluate locally available antimicrobials for additional indications and/or regimens. They indicate a regimen where patient toxicity is not a concern and for which an adequate slaughter withdrawal has been determined by at least one regulatory agency.

Specific therapeutic antimicrobial application suggestions in cattle are reported in Table 29.2. Where appropriate, justification for drug recommendations are presented in a referenced narration. These suggestions should be considered as starting points for application of evidence-based therapeutic decision processes. Additional discussion for *Mycoplasma bovis*, enteric *Salmonella* spp. and *E. coli*, and *Cryptosporidium parvum* are provided in the text.

Disease-Specific Discussions

Mycoplasma bovis

There has been debate as to whether *M. bovis* is a primary respiratory pathogen in cattle. However, in the United States, *M. bovis* is now listed as a label respiratory pathogen for tulathromycin (Draxxin, Pfizer Animal Health), gamithromycin (Zactran, Merial Ltd.), enrofloxacin (Baytril 100, Bayer Healthcare LLC, Animal Health Division), and florfenicol (NuflorGOLD, Merck Animal Health).

Standardized methods for MIC determination and interpretation of *M. bovis* susceptibility data have not yet been established for any indication. Variations in methods may contribute to variations in MIC results reported in Table 29.3. It is apparent in this table that there is a wide range of MICs determined for each drug, suggesting that some isolates will be refractive to therapy, although the maximum MIC correlated with therapeutic efficacy has not been established.

No clinical trials evaluating antimicrobial therapy of arthritis or tenosynovitis due to *M. bovis* are available. The antimicrobial selected by the veterinarian should have at least some indication of potential efficacy. It is reasonable to begin consideration of those antimicrobials with *M. bovis* on the label for some indication, even though the site of infection may be different. For some cases, the tetracyclines may be appropriate, although it is important to recognize that the pharmacokinetics of injectable and oral tetracyclines are markedly different. The MICs for tilmicosin reported in Table 29.3 are considerably higher than the MICs reported for respiratory pathogens against which this antimicrobial is effective, bringing into doubt the potential for *M. bovis* therapeutic success in other applications as well.

While the fluoroquinolones display good *in vitro* activity against *M. bovis*, and one of the compounds has *M. bovis* on the label for respiratory disease, extra-label use of this class in food animals is illegal in the United States. Therefore, use against arthritis or tenosynovitis would be illegal in the U.S. In countries without this restriction, the fluoroquinolones would be a reasonable consideration for extra-label therapy of musculoskeletal disease due to *M. bovis*, although the pharmacodynamic justification for a single injection approach of the fluoroquinolones for this specific pathogen in a non-respiratory scenario has not been confirmed.

Table 29.1. Antimicrobial Regimens for label indications.

Drug	Region Approval	For Use In	indication(s)	Dose	Regimen
Amoxicillin	U.S.	cattle	Bovine respiratory disease, "shipping fever," pneumonia (*Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*) Acute necrotic pododermatitis, "foot rot" (*Fusobacterium necrophorum*)	6.6–11 mg/kg	q 24 h, 5 days max, IM or SC
	E.U.	cattle, calves	*Actinobacillus equuli, Actinobacillus lignieresi, Actinomyces bovis, Bacillus anthracis, Bordetella bronchiseptica, Clostridium species, Corynebacterium species, Erysipelothrix rhusiopathiae, Escherichia coli, Fusiformis species, Haemophilus species, Moraxella species, Pasteurella species, Proteus mirabilis, Salmonella species, Staphylococci* and *Streptococci*	7 mg/kg	q 24 h, 5 days IM
Amoxicillin/Clavulanic Acid	E.U.	cattle, calves	*Staphylococci, Streptococci, Corynebacteria, Clostridia, Bacillus anthracis, Actinomyces bovis, Escherichia coli, Salmonella spp., Campylobacter spp., Klebsiella spp., Proteus spp., Pasteurella spp., Fusobacterium necrophorum, Bacteroides, Haemophilus spp., Moraxella spp. and Actinobacillus lignieresi.*Respiratory infectionsSoft tissue infections (e.g., joint/navel ill, abscesses etc.)MetritisMastitis	8.75 mg/kg (7 mg/kg amoxicillin and 1.75 mg/kg clavulanic acid)	q 24 h for 3–5 days IM
Ampicillin Trihydrate	U.S.	cattle and calves (including non-ruminating [vea] calves)	Respiratory tract infections, bacterial pneumonia, "shipping fever," calf pneumonia, and bovine pneumonia (*Aerobacter* spp., *Klebsiella* spp., *Staphylococcus* spp., *Pasteurella multocida, Escherichia coli*)	4.4–11 mg/kg	q 24 h, 7 days max, IM
Ampicillin/Sulbactam	Canada	cattle	Bacterial pneumonia, pasteurellosis, "shipping fever" complex caused by or complicated by bacteria resistant to ampicillin	3.3 mg: 6.6 mg/kg	q 24 h, 3+ days, IM
Amprolium	U.S.	calves	Coccidiosis (*Eimeria bovis, Eimeria zuernii*)	prevention: 5 mg/kg; treatment: 10 mg/kg	prevention: 21 days; treatment: 5 days, PO in feed or water
Cefalexin	E.U.	cattle	Metritis, foot rot, wounds, abscesses	7 mg/kg	q 24 h, 5 days max, IM only
Cefquinome	E.U.	cattle	Bovine respiratory disease, pneumonia (*Mannheimia haemolytica*, *Pasteurella multocida*) Acute bovine interdigital necrobacillosis, "foot rot," pododermatitis (*Fusobacterium necrophorum, Bacteroides melaninogenicus*) septicemia in calves (*Escherichia coli*)	1 mg/kg, 2 mg/kg for septicemia in calves	q 24 h, 3–5 days, IM

(*continued*)

Table 29.1. Antimicrobial Regimens for label indications. (*continued*)

Drug	Region Approval	For Use In	indication(s)	Dose	Regimen
Ceftiofur Sodium	U.S.	cattle	Bovine respiratory disease, "shipping fever," pneumonia (*Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*)	1.1–2.2 mg/kg	3–5 days, IM or SC
			Acute bovine interdigital necrobacillosis, "foot rot," pododermatitis (*Fusobacterium necrophorum*, *Bacteroides melaninogenicus*)		
Ceftiofur Hydrochloride	U.S.	cattle	Treatment and control of bovine respiratory disease (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*)	1.1–2.2 mg/kg	3 days; 4–5 days for BRD, foot rot; 5 days for acute metritis; IM or SC
			Acute bovine interdigital necrobacillosis, "foot rot," pododermatitis (*Fusobacterium necrophorum*, *Bacteroides melaninogenicus*)		
			Acute metritis (0–14 days postpartum) associated with bacterial organisms susceptible to ceftiofur		
Ceftiofur Crystalline Free Acid	U.S.	cattle	Bovine respiratory disease, "shipping fever," pneumonia (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*)	6.6 mg/kg	once, SC in base of ear, repeat in 72 hours for acute metritis
			Acute bovine interdigital necrobacillosis, "foot rot," pododermatitis (*Fusobacterium necrophorum*, *Porphyromonas levii*)		
			Acute metritis (0–14 days postpartum) associated with bacterial organisms susceptible to ceftiofur		
Chlortetracycline	U.S.	calves	Bacterial enteritis, "scours" (*Escherichia coli*, *Salmonella* spp.)	22 mg/kg BW/day; for diarrhea prevention: 1.1 mg/kg BW	5 days max, PO in water or PO in milk
			Bacterial pneumonia (*Pasteurella* spp., *Haemophilus* spp., *Klebsiella* spp.)		
Danofloxacin	U.S.	cattle	Bovine respiratory disease (*Mannheimia haemolytica*, *Pasteurella multocida*)	single dose: 8 mg/kg BW; multiple dose: 6 mg/kg BW twice 48 hours apart, SC	once SC (8 mg/kg dose) or twice, 48 hours apart SC
Decoquinate	U.S.	cattle (including veal calves)	Coccidiosis prevention (*Eimeria bovis*, *Eimeria zuernii*)	0.5 mg/kg	at least 28 days, PO in feed and milk
Enrofloxacin	U.S.	cattle	Bovine respiratory disease, "shipping fever," pneumonia (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*)	single dose: 7.5–12.5 mg/kg BW; multiple dose: 2.5–5 mg/kg BW	multiple dose: 3–5 days, SC
Erythromycin	U.S.	cattle	Shipping fever, pneumonia, foot rot, stress	1.1–2.2 mg/kg	q 24 h, as needed, IM
Florfenicol	U.S.	cattle	Bovine respiratory disease (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*)	20 mg/kg IM twice 48 h apart or 40 mg/kg SC once	
			Bovine interdigital phlegmon, "foot rot," acute interdigital necrobacillosis, infectious pododermatitis (*Fusobacterium necrophorum*, *Bacteroides melaninogenicus*)		

Gamithromycin	U.S	cattle	Treatment of bovine respiratory disease (*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*, and *Mycoplasma bovis*). Control of bovine respiratory disease (*Mannheimia haemolytica, Pasteurella multocida*),	6 mg/kg BW	once SC
Gentamicin	U.S.	cattle	Infectious bovine keratoconjunctivitis, "pinkeye" (*Moraxella bovis*)	0.75 mg	3 days max, occular spray
Marbofloxacin	E.U.	cattle, lactating dairy cattle	Pneumonia and shipping fever complex (*Pasteurella* spp., *Histophilus* spp.)	2 mg/kg	q 24 h for 3–5 days IM/IV/SQ
Monensin	U.S.	feedlot cattle, pasture cattle, mature reproducing beef cows, calves (excluding veal calves)	For the prevention and control of coccidiosis due to *Eimeria bovis* and *Eimeria zuernii*.	feedlot cattle/veal calves: 0.14–0.42 mg/lb per day up to a maximum of 360 mg/day. Pasture cattle/beef cows: same dose but not more than 100 mg/day for first 5 days.	feed continuously
Neomycin	U.S.	cattle, not for use in veal calves	Colibacillosis, bacterial enteritis (*Escherichia coli*)	22 mg/kg BW/day	up to 14 days, PO in water
Neomycin/ Oxytetracycline	U.S.	calves	Prevention and treatment of bacterial enteritis, "scours"	doses vary greatly depending on label; values for prevention and treatment of disease reported	PO in feed or milk replacer
Oxytetracycline (200 mg/ml)	U.S.	beef cattle, calves (including non-ruminating [veal] calves); bolus: not for use in veal calves	Pneumonia and shipping fever complex (*Pasteurella* spp., *Haemophilus* spp) Infectious bovine keratoconjunctivitis, "pinkeye" (*Moraxella bovis*) Foot rot and diphtheria (*Fusobacterium necrophorum*) Bacterial enteritis, "scours" (*Escherichia coli*) Wooden tongue (*Actinobacillus lignieresii*) Leptospirosis (*Leptospira pomona*) Wound infections, acute metritis (*Streptococcus* spp., *Staphylococcus* spp.)	20 mg/kg for pneumonia in calves and yearlings; 6.6–11 mg/kg for all indications; 11 mg/kg for foot rot and advanced disease stages	20 mg/kg once; 6.6–11 mg/kg q 24 h, max 4 days (IM, IM/SC, or SC depending on individual product label)
Oxytetracycline (300 mg/ml)	U.S.	cattle, (pre-ruminating) veal calves	Bovine respiratory disease, "shipping fever," pneumonia (*Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*) Infectious bovine keratoconjunctivitis, "pinkeye" (*Moraxella bovis*) Acute bovine interdigital necrobacillosis, "foot rot," pododermatitis (*Fusobacterium necrophorum*) Bacterial enteritis, "scours" (*Escherichia coli*) Wooden tongue (*Actinobacillus lignieresii*) Leptospirosis (*Leptospira pomona*) Wound infections, acute metritis (*Streptococcus* spp., *Staphylococcus* spp.)	30 mg/kg, for BRD; 19.8–30 mg/kg for pinkeye; 11 mg/kg for all others	once, IM or SC for BRD and pinkeye; q 24 h, 4 days max, IM, IV, or SC

(*continued*)

Table 29.1. Antimicrobial Regimens for label indications. (*continued*)

Drug	Region Approval	For Use In	indication(s)	Dose	Regimen
Pen G Procaine	U.S.	cattle	Bacterial pneumonia, "shipping fever" (*Pasteurella multocida*)	6,600 IU/kg	q 24 h, SC
Pen G Procaine + Benzathine Combinations ("long-acting penicillin")	U.S.	cattle	Bacterial pneumonia, "shipping fever" (*Streptococcus* spp., *Corynebacterium pyogenes, Staphylococcus aureus*) Upper respiratory infections, rhinitis, or pharyngitis (*Corynebacterium pyogenes*) Blackleg (*Clostridium chauvoei*)	4,400 IU/kg procaine pen + 4,400 IU/kg benzathine pen G	twice, 48 hours apart, SC
Penicillin/ Dihydrostreptomycin	E.U.	cattle	Respiratory tract infections, listeriosis, septicemia, urogenital tract infections, enteritis	8 mg procaine penicillin and 10 mg dihydrostreptomycin sulphate per kg	q 24 h, 3 days, IM
Spectinomycin	U.S.	cattle	Bovine respiratory disease, pneumonia (*Pasteurella haemolytica, Pasteurella multocida, Histophilus somni*)	10–15 mg/kg	3–5 days, SC
Streptomycin and/or Dihydrostreptomycin	E.U.	cattle	Wooden tongue (*Actinobacillus lignieresii*) Leptospirosis (*Leptospira pomona*)	10 mg/kg	q 24 h, 3 days, IM
Sulfachlorpyridazine	U.S.	calves under 1 month	Diarrhea, colibacillosis (*Escherichia coli*)	66–99 mg/kg BW/day	q 12 h for 1–5 days, PO **or** IV depending on label
Sulfadiazine/ Trimethoprim	E.U	cattle	Urogenital tract infections (*Corynebacterium pyogenes*); respiratory tract infections including rhinitis, pneumonia, bronchitis; pododermatitis	12.5 mg: 2.4 mg/kg	once, or q 24 h, 5 days max, IM or slow IV
Sulfamethazine	U.S.	ruminating replacement calves; sulfamethazine sodium drinking water solution: cattle	Bacterial pneumonia (*Pasteurella* spp.), colibacillosis, bacterial scours (*Escherichia coli*), calf diphtheria (*Fusobacterium necrophorum*). Some labels include coccidiosis (*Eimeria bovis, Eimeria zuernii*), acute metritis (*Streptococcus* spp.)	1 bolus/22.5 kg BW (363 mg/kg) or 1 bolus/91 kg BW or 0.1 g/4.5 kg BW first day, then 0.05 g/4.5 kg BW depending on label; drinking water solution: 247.5 mg/kg on day 1 then 135 mg/kg or 238 mg/kg on day 1 then 119 mg/kg	q 72 h, twice maximum or q 24 h max 5 days depending on label; drinking water solution: 4 days
Sulfamethazine/ Chlortetracycline	U.S.	cattle	Respiratory disease, "shipping fever"	350 mg each of CTC and SMZ/day	28 days continuously, PO in feed
Sulfadimethoxine (injectable)	U.S.	cattle	Bovine respiratory disease complex, "shipping feve" ", bacterial pneumonia (*Pasteurella* spp.) Necrotic pododermatitis, "foot rot," calf diphtheria (*Fusobacterium necrophorum*)	55 mg/kg first dose, then 27.5 mg/kg	q 24 h, IV

Sulfaquinoxaline	U.S.	cattle	Control and treatment of coccidiosis (*Eimeria bovis, Eimeria zuernii*)	6 mg/kg	3–5 days, PO in water
Tetracycline	U.S.	calves	Bacterial enteritis, "scours" (*Escherichia coli*) Bacterial pneumonia (*Pasteurella* spp., *Haemophilus* spp., and *Klebsiella* spp.) "Shipping fever," hemorrhagic septicemia	22 mg/kg BW/day; soluble powder: for disease prevention: 100–200 mg/gallon; for treatment: 200–400 mg/gallon	2–5 days, PO **or** PO in water
Tildipirosin	U.S.	cattle	Bovine respiratory disease treatment and control (*Mannheimia haemolytica, Pasteurella multocida*, and *Histophilus somni*)	4 mg/kg	once, SC
Tilmicosin	U.S.	cattle	Bovine respiratory disease (*Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni*)	10 mg/kg	once, SC
Tulathromycin	U.S.	cattle	Treatment and control of bovine respiratory disease (*Mannheimia haemolytica, Pasteurella multocida, Histophilus somni*, and *Mycoplasma bovis*). Infectious bovine keratoconjunctivitis (*Moraxella bovis*), interdigital necrobacillosis (*Fusobacterium necrophorum* and *Porphyromonas levii*)	2.5 mg/kg	once, SC
Tylosin	U.S.	cattle	Bovine respiratory complex, "shipping fever," pneumonia (*Pasteurella multocida*, *Actinomyces pyogenes*) Necrotic pododermatitis, "foot rot," diphtheria (*Fusobacterium necrophorum*) Metritis (*Actinomyces pyogenes*)	4–10 mg/kg	q 24 h, 5 days max, IM

Table 29.2. Specific therapeutic antimicrobial application suggestions.

	Label applications are U.S. labels except for those in bold print, which are E.U. labels.				
Category	Disease/Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials that are a reasonable choice	Unreasonable extra-label antimicrobal selections for this disease	Comments
Respiratory disease	Pneumonia—*Mannheimia haemolytica., Pasteurella multocida, Histophilus somni*	Ampicillin trihydrate, *ceftiofur (sodium, hydrochloride, and crystalline free acid salts)*, chlortetracycline, *danofloxacin, enrofloxacin, florfenicol, gamithromycin*, oxytetracycline, procaine penicillin G, spectinomycin sulfate, sulfadimethoxine, sulfamethazine, *tildipirosin, tilmicosin, tulathromycin*, tylosin, **cefquinome, trimethoprim/ sulfadiazine. trimethoprim/ sulfadoxine, procaine penicillin/ dihydrostreptomycin, amoxicillin trihydrate, amoxicillin/clavulanic acid**		Gentamicin due to potential toxicity in dehydrated animals and prolonged renal residues in cattle.	Antimicrobials with bovine respiratory disease on the label may be indicated for one or all of these pathogens. The italicized antimicrobials are the author's primary U.S. choices for cattle in advanced stages of the disease or which have experienced extensive stress. Not all of the antimicrobials are labeled for all respiratory pathogens. The labels should be consulted for complete indications.
Respiratory disease	Pneumonia—*Mycoplasma bovis*	Enrofloxacin, gamithromycin, florfenicol, tulathromycin, **tylosin (*Mycoplasma* on label)**	Oxytetracycline, spectinomycin, fluoroquinolones*	Any beta-lactam (penicillins, cephalosporins) due to lack of a cell wall.	See text for comments. *In the USA, fluoroquinolones would only be legal when used for the purpose of respiratory disease due to the primary label pathogens.
Respiratory disease	Diphtheria (necrotic laryngitis)—*Fusobacterium necrophorum*	Oxytetracycline	Ampicillin, ceftiofur, florfenicol, penicillin G, sulfadimethoxine, tylosin and other macrolides such as tulathromycin		Extra-label recommendations are made based on published MIC values that are in the range of other pathogens succesfully treated by these antimicrobials and/or label inclusion of foot rot due to *Fusobacterium necrophorum*. (Baba, 1989; Berg, 1982;

					Druan, 1991; Jousimies-Somer, 1996; Jang, 1994; Lechtenberg, 1998; Mateos, 1997; Piriz, 1990; Samitz, 1996) . All of these isolates were from other sites than necrotic laryngitis. The nature of the site of necrotic laryngitis may make therapy with less lipid soluble antimicrobials more of a challenge.
Infectious enteric disease	Scours, neonatal diarrhea due to *E. coli*	Chlortetracycline, neomycin, oxytetracycline, sulfachlorpyridazine, sulfamethazine, tetracycline (all of these antimicrobials display consistently high MICs that suggest the drugs would be ineffective), A**moxicillin/clavulanic acid bolus, cefquinome (septicemia), danofloxacin, enrofloxacin (septicemia and colibacillosis), marbofloxacin bolus, trimethiprim/ sulfadiazine, trimethiprim/ sulfadoxine**	Ceftiofur, potentiated sulfonamides (all only after susceptibility testing)	(These extra-label indications demonstrated very high MICs to most isolates.) Erythromycin, tylosin, tilmicosin, lincomycin, penicillin, ampicillin, florfenicol.	Recommended extra-label antimicrobials are based on susceptibility data and serum pharmacokinetics and should therefore be interpreted as relating to septicemia associated with enteric disease. See text for additional discussion.
Infectious enteric disease	Scours, neonatal diarrhea due to *Salmonella* spp.	Chlortetracycline, oxytetracycline (these antimicrobials display consistently high MICs that suggest the drugs would be ineffective), E**nrofloxacin, trimethoprim/ sulfadiazine, trimethoprim/ sulfadoxine, procaine penicillin/ dihydrostreptomycin**	Ceftiofur, potentiated sulfonamides (all only after susceptibility testing)	Gentamicin will cause extended withdrawal times that will compromise the ability to slaughter an animal that recovers from the acute disease but does not return to satisfactory production.	Recommended extra-label antimicrobials are based on susceptibility data and serum pharmacokinetics and should therefore be interpreted as relating to septicemia associated with enteric disease. See text for additional discussion.

(*continued*)

Table 29.2. Specific therapeutic antimicrobial application suggestions. (*continued*)

	Label applications are U.S. labels except for those in bold print, which are E.U. labels.				
Category	Disease/Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials that are a reasonable choice	Unreasonable extra-label antimicrobal selections for this disease	Comments
Infectious enteric disease	Enterotoxemia, overeating disease—*Clostridium perfringens* type C,D		Amoxicillin, ampicillin, penicillin G		Antiserum therapy is more likely related to therapeutic success. Septicemia resulting from enterotoxemia may involve multiple gut-related bacteria. Antimicrobial selection should reflect this possibility (see septicemia related to neonatal diarrhea above).
Infectious enteric disease	Hemorrhagic bowel disease—*Clostridium perfringens* type A		Penicillin G, florfenicol		Prognosis of hemorrhagic bowel disease is very guarded, with surgery necessary for resolution in many cases (Dennison, 2002). There is no published evidence that antimicrobial intervention changes the clinical outcome. While there is no published data to support florfenicol efficacy in this disease, the general activity against anaerobes make it a reasonable consideration.
Infectious enteric disease	Cryptosporidiosis—*Cryptosporidium parvum*	**Halofuginone lactate (prevention, and reduction in excretion in affected calves)**	For prevention: lasalocid in calves ≥ 1 week old (toxic in neonates at effective doses!)	Amprolium, sulfas	See text for comments on clinical trial data for cryptosporidiosis. Affected calves have severe acid/base and hydration insults.
Infectious enteric disease	Giardia		Albendazole, fenbendazole, metronidazole (see comments)		The extra-label use of nitroimidazoles (e.g., metronidazole) in food animals is banned in the United States. Fenbendazole regimens of 5 mg/kg q 12H for 3 days or 5 mg/kg q 24H for 5 days, PO, have been suggested (Rings, 1996). Fenbendazole liquid is labeled for giardia in puppies and kittens in the E.U.

Infectious enteric disease	Coccidiosis—*Eimeria bovis, Eimeria zeurnii*	Prevention/control: monensin, lasalocid, amprolium, decoquinate, sulfaquinoxaline; therapy of acute disease: sulfaquinoxaline, sulfamethazine, amprolium	Sulfadimethoxine, sulfadimidine		Amprolium and sulfadimidine were found superior to halofuginone in an induced *Eimeria bareillyi* calf model (Sanyal, 1985). Toltrazuril was found effective in a dose-dependent manner against an induced *Eimeria bovis* model in calves (Mundt, 2003).
Genitourinary	Leptospirosis	Oxytetracycline, **dihydrostreptomycin, tylosin (spirochetes on label)**	Penicillin/dihydro-streptomycin, ceftiofur		Ceftiofur was effective in clearing induced leptospirosis (*hardjo*) in cows at 2.2 and 5.0 mg/kg q24h for 5 days. These regimens were not effective when administered for 3 days. Long-acting 200 mg/ml oxytetracycline (20 mg/kg) and penicillin/dihydrostreptomycin (25 mg/kg) were effective after single doses (Alt, 2001).
Genitourinary	Metritis/endometritis			Intrauterine administration of penicillins, aminoglycosides, and sulfonamides is questionable, as these may undergo enzymatic cleavage, operate poorly in an anaerobic environment, or lose activity in the presence of pus.	Chenault (2004) reported 14-day cure rates of 77%, 65%, and 62% for cows suffering from acute postpartum metritis treated with 2.2 mg/kg IM/SQ ceftiofur HCl (CE) q 24h for 5 days, 1.1 mg/kg CE q 24h for 5 days, and controls, respectively. Königsson (2000) demonstrated that cows treated with 10 mg/kg IM oxytetracycline SID for 5 days demonstrated a shorter time to eradication of intrauterine *A. pyogenes* and *F. necrophorum* than untreated controls ($p < 0.05$).
Genitourinary	Seminal vesiculitis—*Arcanobacterium pyogenes, Brucella abortus., E. coli, Pseudomonas* spp., *Actinobacillus seminis, Actinomyces bovis, Histophilus somni (Haemophilus somnus), Salmonella* spp., *Chlamydia* spp.		Antimicrobial therapy has not been shown to make a difference in clinical outcome. Oxytetracycline in the feed at various doses has been used for prevention. Tilmicosin phosphate, long-acting oxytetracycline, and florfenicol have been used in therapeutic attempts.		*Arcanobacterium pyogenes* is the most common agent in the United States. *Brucella abortus* is the most common in countries with this disease. There is debate as to the role of bacterial or viral pathogens in the pathogenesis of seminal vesiculitis (Larson, 1997).

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Table 29.2. Specific therapeutic antimicrobial application suggestions. (*continued*)

	Label applications are U.S. labels except for those in bold print, which are E.U. labels.				
Category	Disease/Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials that are a reasonable choice	Unreasonable extra-label antimicrobal selections for this disease	Comments
Genitourinary	Nephritis/ pyelonephritis—*Corynebacterium renale, Arcanobacterium pyogenes, E. coli*	**Trimethoprim/ sulfadiazine, trimethoprim/ sulfadoxine**	*For C. renale, Arcanobacterium pyogenes*—penicillin G, ampicillin; *E. coli*—ceftiofur, fluoroquinolones (where legal)		
Genitourinary	Cystitis	**Amoxicillin, trimethoprim/ sulfadiazine, amoxicillin trihydrate**	Amoxicillin, ampicilllin, ceftiofur, oxytetracycline, florfenicol, fluroquinolones (where legal), penicillin G, trimethiprim/sulfa		Antimicrobials for cystitis have traditionally been chosen for their urine concentrations. However, the infection of concern is in the wall of the bladder, not the urine. Therefore, while urine concentrations may be of benefit, lack of significant urine concentrations does not necessarily preclude selection for cystitis.
Musculo/skeletal	Adult arthritis—*Histophilus somni, Mycoplasma bovis*		Oxytetracycline, florfenicol, fluoroquinolones (where allowed by law), tulathromycin, spectinomycin, gamithromycin, lincomycin (given due consideration to potential rumen flora alterations)	If *M. bovis* is suspected, any beta-lactam would be an unreasonable choice. If another organism is confirmed, then ceftiofur and ampicillin may be considered.	Other pathogens may be present as listed for neonatal arthritis. However, therapy of adult bovine arthritis should include consideration of these organisms unless ruled out by culture. Arthritis due to *M. bovis* is often characterized as a tenosynovitis. An extended duration of therapy (1–2 weeks) and a prolonged recovery period are necessary.
Musculo/skeletal	Neonatal arthritis—*E. coli, Arcanobacterium pyogenes, Staphylococcus spp., Streptococcus spp.*	**Amoxicillin trihydrate, amoxicilin/clavulanic acid, procaine penicillin/ dihydrostreptomycin, procaine penicillin G**	Potentiated sulfonamides, flouroquinolones (where allowed by law)		The potential presence of *E. coli* and the varied susceptibility results of ampicillin, florfenicol, and oxytetracycline suggest they are not primary considerations for this disease. The primary metabolite of ceftiofur has a greatly elevated MIC_{90} value for *Staphylococcus* spp. as compared to the parent

					compound (Salmon, 1996), indicating it is not a primary choice where *Staph.* spp. may be part of the infection.
Central nervous system disease	Listeriosis—*Listeria monocytogenes*	**Procaine penicillin/ dihydrostreptomycin, procaine penicillin G**	Penicillin G, oxytetracycline, enrofloxacin (depending on legal status). Therapy durations of 1–2 weeks may be necessary.		Varying results are reported for the recomended drugs. Five of 6 bulls in a case report survived after therapy with oxytetracycline and dexamethasone (Ayars, 1999). A sheep and goat case report indicated poor response to chloramphenicol and oxytetracycline, but 6 of 9 animals recovered when treated with penicillin and gentamicin (Braun, 2002). Enrofloxacin has been reported as effective (Tripathi, 2001) but is illegal in countries with a ban on extra-label use of fluoroquinolones in food animals (e.g., United States).
Central nervous system disease	Thromboembolic meningo-encephalitis (TEME), *Histophilus somni* (*Haemophilus somnus*)		Oxytetracycline, florfenicol		Oxytetracycline is a standard drug of choice for this application. Florfenicol is also suggested due to low MICs for *H. somni* combined with high lipid solubility.
Central nervous system disease	Meningitis—*E. coli* in neonates, multiple other pathogens possible	**Procaine penicillin/ dihydrostreptomycin**	Ceftiofur, fluoroquinolones (where legal), trimethoprim/sulfa	Due to inconsistent coverage of the potential *Enterobacteriacae* component: penicillin G, first-generation cephalosporins, macrolides, tetracyclines, florfenicol	While consideration of penetration of the blood-brain barrier is valid, it is likely that this barrier is disrupted in meningitis, allowing greater penetration of water-soluble compounds. Doxycycline is a lipid-soluble tetracycline, but the high protein binding in serum limits the amount available to the diffusionary pool, and therefore CNS penetration.

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Table 29.2. Specific therapeutic antimicrobial application suggestions. (*continued*)

	Label applications are U.S. labels except for those in bold print, which are E.U. labels.				
Category	Disease/Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials that are a reasonable choice	Unreasonable extra-label antimicrobal selections for this disease	Comments
Central nervous system disease	Otitis media and interna—potential pathogens include respiratory (all ages) and enteric pathogens (neonates) . *Mycoplasma bovis* should be suspected in dairy calves where *M. bovis* mastitis is present in the herd.	**Trimethoprim/sulfadiazine (infections of the ear on the label), tylosin**	In cattle where respiratory pathogens are suspected: macrolides, florfenicol, fluoroquinolones (where legal). Beta-lactams might be expected to have lower concentrations in remote otic tissues.	Aminoglycosides may be expected to have extensive binding to protein debris at the site of infection and are less active in areas with lowered pH.	Without adequate trial data, extra-label recommendations are made on the basis of reported pathogen population MICs and lipid solubility of the compound. Many of the extra-label recommendations would have a hole in the spectrum for at least one possible pathogen (e.g., enrofloxacin—*Strep.* spp., ceftiofur—*Staph.* spp. and *M. bovis*, macrolides and florfenicol—inconsistent against *Enterobacteriacae*, penicillin G and ampicillin—*Enterobacteriacae* and *M. bovis*).
Tissue/integumentary disease	Infectious bovine kerato-conjunctivitis (Pinkeye)-*Moraxella bovis*	oxytetracycline, topical gentamicin, tulathromycin	penicillin G, florfenicol, tilmicosin, topical benzathine cloxacillin		Florfenicol was found to be effective against IBK at either of the label dose regimens (Angelos, 2000, Dueger, 1999). Topical benzathine cloxacillin, 250 or 375 mg/eye, has been shown to be effective in naturally occurring and induced pinkeye models (Daigneault, 1990). Tilmicosin was shown to be effective at both 5 and 10 mg/kg (Zielinski, 1999). Although local penicillin G is a standard treatment, one report indicated no difference in healing of naturally occurring IBK after subconjunctival administration (Allen, 1995).

Tissue/integumentary disease	Infectious pododermatitis (foot rot)—*Fusobacterium necrophorum, Bacteroides melaninogenicus, Porphyromonas levii*	Amoxicillin, ceftiofur (sodium, hydrochloride, crystalline free acid), erythromycin, florfenicol, oxytetracycline, sulfadimethoxine, sulfamethazine, tulathromycin, tylosin, **cefquinome, tilmicosin, sulfadiazine/ trimethoprim**	Procaine penicillin G, ampicillin trihydrate, florfenicol	Different labels will have different pathogens. Severe tissue reactions result from intramuscular use of tylosin and erythromycin.
Tissue/integumentary disease	Actinobacillosus, "wooden tongue"—*Actinobacillus lignieresii*	Long-acting oxytetracyclines (200 and 300 mg/ml), **amoxicillin trihydrate, amoxicillin/clavulanic acid, cefalexin dihydrostreptomycin, trimethoprim/ sulfadiazine (*Actinobacilli* on label)**	Streptomycin, sodium iodide combined with antimicrobial therapy for effect on granulomatous tissue	A case report indicated that cattle receiving IV sodium iodide and intralesional streptomycin regressed lesions faster than negative controls or penicillin-treated cattle (Campbell, 1975). No clinical trials are available.
Tissue/integumentary disease	Actinomycosis, "lumpy jaw"—*Actinomyces bovis*	**Amoxicillin trihydrate, amoxicilin/ clavulanic acid, dihydrostreptomycin, cefalexin, trimethoprim/ sulfadiazine (*Actinomycae* on label)**	Penicillin G, ampicillin trihydrate, oxytetracycline. Sodium iodide may be combined with antimicrobial therapy for effect on granulomatous tissue	No clinical trials are available to confirm efficacy of these antimicrobials. Prolonged therapy is recommended with surgical debridement of the lesion if possible.
Tissue/integumentary disease	Blackleg—*C. chauvoei;* malignant edema—*C. sordellii, C. septicum;* tetanus—*Clostridium tetani*; bacillary hemoglobinuria—*Clostridium hemolyticum*; Black disease—*C. novyi*	**Amoxicillin trihydrate, amoxicillin/clavulanic acid, cefalexin**, procaine penicillin G (*C. chauvoei*), procaine/benzathine penicillin G (*C. chauvoei*), **tylosin**	Penicillin G	All of the approved drugs have "clostridia" on the label without indications for specific clostridial diseases unless indicated. Japanese isolates of *C. perfringens, C. septicum*, and *C. sordellii* displayed phenotypic resistance to oxytetracycline and were confirmed to carry oxytetracycline-resistance genes (Sasaki, 2001).

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Table 29.2. Specific therapeutic antimicrobial application suggestions. (*continued*)

Category	Disease/Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials that are a reasonable choice	Unreasonable extra-label antimicrobal selections for this disease	Comments
	Label applications are U.S. labels except for those in bold print, which are E.U. labels.				
Tissue/integumentary disease	Peritonitis—*Escherichia coli*, *Arcanobacterium pyogenes*, *Clostridium perfringens*, multiple Gram-positive and Gram-negative aerobes and anaerobes. Isolate reports in other species include organisms in all 4 quadrants.		Trimethoprim/sulfa (probably the most consistent for *E. coli*), florfenicol, oxytetracycline (both inconsistent on *E. coli*), ceftiofur for short withdrawal but may not cover *Staph.* spp.	Penicillin/gentamicin is reasonable as to spectrum but gentamicin engenders an extreme withdrawal that precludes salvage slaughter attempts in recovered animals.	No clinical trials are available in cattle. Recommendations are based on wide-spectrum, lipid solubility, and duration of activity. An extended duration of therapy (≥ 1 week) is necessary. Prognosis is extremely poor in advanced cases. Note that the MIC90 of the ceftiofur metabolite against Staph. spp is approximately 8 times that of the parent compound.
Tissue/integumentary disease	Omphalophlebitis (navel ill)	**Amoxicillin trihydrate, amoxicilin/clavulanic acid, procaine penicillin/ dihydrostreptomycin, procaine penicillin G**			
Tissue/integumentary disease	Trichophytosis (ringworm)	Benzalkonium chloride (0.15% topical solution), **enilconazole, natamycin**	Topical iodine solution/scrub, systemic griseofulvin*		*Regulations and availability of extra-label slaughter withdrawal time information should be confirmed prior to using griseofulvin in countries without a label for this application. Griseofulvin is teratogenic.
Tissue/integumentary disease	Rainrot (Dermatophilosis)—*Dermatophilus congolensis*		Penicillin G, oxytetracycline		Penicillin G and oxytetracycline are often cited for therapy of dermatophilosus. A paper evaluating MIC and MBC concentrations, *in vitro* data, and unbound serum concentrations also recommended erythromycin, ampicillin, streptomycin, amoxicillin, and chloramphenicol (Hermoso-de Mendoza, 1994).

					The chloramphenicol results suggest potential for florfenicol efficacy.
Cardiovascular/systemic	Anaplasmosis	Chlortetracycline in the feed for control of active infection	Oxytetracycline, imidocarb diproprionate		Prevention or amelioration of clinical signs with oxytetraycline are well established. However, there are reports in the literature citing both successful and unsuccesful clearance of carriers with oxytetracycline. Recent work has documented unsuccessful clearance of induced anaplasmosis carrier status with the OIE regimen of 22 mg/kg oxytetracycline, IV, q 24h, for 5 days (Coetzee, 2005). Clearance of the carrier state with imidocarb has been documented (Roby, 1972).
Cardiovascular/systemic	Endocarditis—*Arcanobacterium pyogenes* and *Streptococcus* spp. are most common. *Escherichia coli*, other organisms also possible.		Penicillin G, presence of a Gram-negative on blood culture indicates ampicillin, amoxicillin, or cefiofur.		Prolonged therapy is necessary. Addition of rifampin (5 mg/kg, PO, q 12h) has been suggested to improve response. Prolonged therapy (4–6 weeks) has been suggested as an appropriate duration of therapy. (Dowling, 1994; McGuirk, 1991). Lack of clinical efficacy may be due to lack of antimicrobial penetration into vegetative lesions. Florfenicol would be appropriate for pathogens with appropriate MICs (variable on E. coli). In cases where the law and economics permit, fluoroquinolones would be appropriate if an organism other than a *Strep*. spp. was confirmed.

(*continued*)

Table 29.2. Specific therapeutic antimicrobial application suggestions. (*continued*)

	Label applications are U.S. labels except for those in bold print, which are E.U. labels.				
Category	Disease/Pathogen(s)	Drugs for which this disease is a label application (therapy and/or prevention)	Extra-label antimicrobials that are a reasonable choice	Unreasonable extra-label antimicrobal selections for this disease	Comments
Cardiovascular/systemic	Anthrax—*Bacillus anthracis*	**Amoxicillin, amoxicillin/clavulanic acid, tylosin (*Bacillus* on label)**	Penicillin G, oxytetracycline, fluoroquinolones (where legal) doxycycline, first-generation cephalosporins. Chloramphenicol results suggest florfenicol may be an option.		A study evaluating the MICs of 25 geneticially diverse *B. anthracis* isolates from multiple countries reported MIC90 values as follow: ciprofloxacin 0.09 µg/ml, penicillin 0.2 µg/ml, doxycycline 0.34 µg/ml, cefuroxime 32 µg/ml, cephalexin 0.25 µg/ml, cefachlor 1.65 µg/ml, and tobramycin 0.97 µg/ml (Coker, 2002). Except for cefuroxime, and possibly cefachlor, these MIC90 values are in a range where efficacy might be expected with typically used doses. Universally "susceptible" disk diffusion results with unvalidated interpretive criteria have been reported for tetracycline, ampicillin, streptomycin, chloramphenicol, and erythromycin in South African isolates (Odendaal, 1990).

Table 29.3. *Mycoplasma bovis* susceptibility data.

	Rosenbusch (2005) 223 U.S. Isolates			Ayling (2000) 62 British Isolates			Label Data as Indicated by Footnote		
Antimicrobial	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)
Enrofloxacin	0.25	0.5	0.03–4	NA	NA	NA	NA	NA	NA
Danofloxacin	NA	NA	NA	0.5	0.5	0.25–8	NA	NA	NA
Florfenicol	1	4	0.06–8	4	16	4–128	1.0[a]	1.0[a]	0.5–1.0[a]
Chlortetracycline	4	16	0.25 to > 32	NA	NA	NA	NA	NA	NA
Oxytetracycline	2	16	0.125 to > 32	32	64	2 to > 128	NA	NA	NA
Spectinomycin	2	4	1 to > 16	4	> 128	2 to > 128	NA	NA	NA
Tilmicosin	64	> 128	0.5 to > 128	> 128	> 128	16 to > 128	NA	NA	NA
Tulathromycin	NA	NA	NA	NA	NA	NA	0.125[b]	1[b]	≤0.063 to > 64[b]

[a]Nuflor Gold label (2009), 59 U.S. isolates.
[b]Draxxin label (2009), 43 U.S. isolates.
NA = data not available.

Enteric Disease and Septicemia Associated with Escherichia coli and Salmonella spp.

A previous review of the literature showed that there is a paucity of data to support the efficacy of antimicrobial therapy for bacterial enteric disease in calves (Constable, 2004). Little has changed between the time of this review and the writing of this chapter (2012).

The practitioner is hampered by two obstacles. The previously mentioned lack of clinical data from prospective controlled and randomized clinical trials, and the lack of validated susceptibility testing breakpoints for classification of enteric pathogens as susceptible or resistant.

However, the author's discussions with practitioners indicate that few would be willing to forego antimicrobial therapy considering that a proportion of calves with enteric disease are likely septicemic. Also, the potential for septicemia in adult cattle with coliform masitits and salmonellosis call for guidance in reasonable antimicrobial selection.

From an empirical approach, reasonable initial considerations include third-generation cephalosporins, potentiated aminopenicillins, fluoroquinolones (not legal in the United States), potentiated sulfas, and florfenicol. The confirmation of these initial selections would depend on susceptibility testing as presently available.

Susceptibility testing for enteric disease is not based on CLSI-approved breakpoints but rather on breakpoints developed for another veterinary indication or that were adapted from human medicine. There are now also "generic" breakpoints developed, and in development, by the CLSI. However, these generic breakpoints have not been directed toward enteric disease as of the writing of this chapter.

CLSI breakpoints are developed based on a combination of *in vitro* efficacy data coupled with susceptibility testing, "wild-type" isolate MIC profiles, and pharmacokinetic/ pharmacodynamic (PK/PD) data. When applying these breakpoints to other indications, such as enteric disease, it is hoped that the PK/PD indices and the changes in MIC due to a resistance gene are at least similar. Therefore, we might more accurately refer to the process for enteric disease as "resistance testing," where resistant isolates would be considered more likely to possess resistance genes rendering the antimicrobial incapable of having an effect on growth or viability of the pathogen.

Therefore, a reasonable approach is to first rule out any of the potential enteric therapeutics based on legal issues relevant to the practice area. Next, empirical therapy may be guided by accessing enteric culture susceptibility summaries available from your diagnostic laboratory, or by monitoring susceptibility trends within specific production units. A preponderance of resistant classifications for a potential antimicrobial would indicate that the population of pathogens being submitted to that laboratory likely carry some type of resistance gene. This antimicrobial could therefore be moved down on the list of potential selections.

A finding of "susceptible" does not have any validated correlation with the likelihood of therapeutic success in the patient for enteric disease. However, we would assume that in the lack of the presence of resistance genes, the antimicrobial would at least be capable of aiding in clinical recovery to the extent possible with the regimen and site of infection.

There are obviously a lot of assumptions in this discussion, highlighting the need for controlled clinical trials addressing the antimicrobial treatment component of neonatal enteric disease in calves.

Cryptosporidium parvum

Multiple antimicrobials have been evaluated in *Cryptosporidium parvum* calf disease models. Antimicrobials reported as ineffective in calves up to 14 days of age when administered in the milk replacer during a 10-day challenge model include amprolium, sulphadimidine, dimetridazole, metronidazole, ipronidazole, quinacrine, and monensin. Trimethoprim/ sulfadiazine was also ineffective when administered daily as a bolus. Lasalocid was ineffective at 0.8 mg/kg per day. At 8 mg lasalocid/kg per day, 6 of the 10 treated calves died, with 1 of the 4 surviving calves becoming infected (Moon, 1982). Sulfadimethoxine has also been shown to be ineffective against *C. parvum* in a 1- to 7-day-old calf challenge model (Fayer, 1992).

Lasalocid has been used as a preventive or therapeutic agent for cryptosporidium in calves based on anecdotal reports. This use in neonatal calves has resulted in reported toxicities after 100 mg twice daily in milk replacer or 200 mg oral once-daily doses starting at birth, with death occurring after 1–3 administrations (Benson, 1998). The authors confirmed this toxicity experimentally by dosing neonatal calves once at 5 mg/kg.

In other studies, Lasalocid doses of 15 mg/kg have been tolerated in calves ≥ 7 days old with cessation of oocyst shedding 3 days after the last of 3 daily doses of 15 mg/kg (Gobel, 1987). These data would suggest that effective doses of lasalocid are toxic in neonatal calves but may be used in calves of at least 1 week of age.

A trial evaluating decoquinate in a calf *Cryptosporidium parvum* challenge model found a significant decrease in number of days with abnormal stool scores in the treated groups given 875 or 1750 mg (10X label dose) decoquinate per day, but no difference in oocyst shedding or weight gain (Redman, 1994). Another challenge study found no difference in days to diarrhea, days to shedding, or duration of diarrhea or oocyst shedding in calves given 2 mg/kg decoquinate in milk replacer (Moore, 2003).

In naturally occurring *Cryptosporidium parvum* infections, halofuginone lactate administered in the milk replacer at 60 μg/kg per day cleared all shedding of oocysts within 6 days after the start of treatment in 98% of the treated animals. It should be noted that 93% of the untreated controls in this study also cleared the organism within 10 days of arrival at the facility (Villacorta, 1991). In a natural disease model, calves receiving 5 mg of halofuginone lactate daily in milk replacer were 70% less likely to shed *C. parvum* oocysts as compared to untreated controls. Weight gain and milk and starter intakes were not significantly different between groups (Jarvie, 2005). In a challenge study, halofuginone reduced disease at 60 and 120 μg/kg per day but was ineffective at 30 μg/kg per day (Naciri, 1993).

Other antimicrobials such as paromomycin, azithromycin, and clarithromycin, have been demonstrated to be efficacious in murine models or human therapy (Fichtenbaum, 1993; Rehg, 1991, Holmberg, 1998). The prophylactic potential of paromomycin was also demonstrated in a calf model (Fayer, 1993). However, the costs of these 3 agents are prohibitive for food animal applications and have prevented their use.

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30

Antimicrobial Drug Use in Mastitis

Sarah Wagner and Ron Erskine

Introduction

The most common use of antimicrobial drugs on dairy farms is to treat mastitis (Mitchell et al., 1998). The expenses associated with mastitis (decreased milk production, decreased milk quality, drug costs, and discarded milk) may be considerable, and have led many dairy producers to implement management programs focused on mastitis prevention. Money spent on an effective protocol for prevention of mastitis is likely to lead to an overall financial benefit to the dairy. On a farm that is experiencing high somatic cell counts, high rates of clinical mastitis, high levels of subclinical mastitis, or all of these, investigation into the reason for the problem, followed by development and implementation of a program to alleviate the cause and to prevent new occurrence, is recommended. Treatment alone is unlikely to solve herd-level mastitis problems.

Even on well-managed farms with mastitis prevention protocols in place, treatment of clinical mastitis may sometimes be desirable. Subclinical mastitis may be detected through a combination of individual cow Somatic Cell Counts (SCCs) as measured by DHI testing or the California Mastitis Test (CMT), and microbial culture of milk samples. Although the discussion presented here is addressed to clinical cases of mastitis, the principles described are also generally applicable to the treatment of subclinical mastitis.

Mastitis During Lactation

Cow Factors

A number of questions should be asked about the affected cow before deciding how or even whether to initiate treatment of a case of mastitis. Depending on cow factors, one may decide to treat the mastitis using a label-prescribed or extra-label protocol, or it may be more rational not to treat the mastitis, either because treatment is unnecessary or because treatment is unlikely to result in resolution of clinical signs. Risk factors that have been found to decrease therapeutic efficacy include increasing cow age, high SCC before treatment, long duration of infection, multiple infected quarters, and infections caused by *Staphylococcus aureus* (Deluyker et al., 2005; Barkema et al., 2006; Bradley and Green, 2009; Pinzon-Sanchez and Ruegg, 2011). In particular, chronic infections are likely to have poor therapeutic outcomes and may require extended duration of antimicrobial therapy (Owens and Nickerson, 1990; Oliver et al., 2004).

Questions to ask before treatment is instituted include:

1. Is this a new case of mastitis or a relapse? Repeated treatment of a recurrent case of mastitis is frequently unrewarding. If a recurrent case of mastitis is to be treated, the therapeutic regimen should be more extensive than what would be used for a mild, acute case.

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2. How severe is it? A case of mastitis in a cow that has become systemically ill (septic/toxic), will require a therapeutic protocol that includes systemic antibiotic therapy, intramammary therapy, supportive therapy and closer monitoring than a case in which clinical signs are limited to the udder and milk (Erskine et al., 2002).
3. How many quarters are affected? The expense and the likelihood of treatment failure increase as the number of affected quarters increases.
4. What is the cow's stage of lactation? For a cow in late lactation, economic and therapeutic advantages may be gained by treating the cow simultaneously with drying-off.
5. Does the cow have other health problems? It has been established that the likelihood of a cow developing mastitis is increased by the presence of other health problems such as ketosis and hypocalcemia (Kremer et al., 1993). It is reasonable to expect, therefore, that the likelihood of successful therapy of mastitis may be decreased in cows with concurrent illnesses.

Pathogen Factors

Microbial culture of mastitis infections is an invaluable aid in determining whether to initiate drug therapy, and if so, what approach to use. Infections with certain pathogens are likely to respond to antimicrobial drug therapy, while some pathogens may or may not respond to antimicrobial therapy. Some infections are likely to resolve without any treatment. Common mastitis pathogens that are likely to be unresponsive to antimicrobial therapy are listed in Table 30.1. A brief overview of some other commonly encountered pathogens follows.

Streptococcus agalactiae is a contagious mastitis pathogen. It is considered highly responsive to therapy with nearly any antimicrobial drug.

Chronicity decreases the responsiveness of *Staphylococcus aureus* infection to antimicrobial therapy. A new case in one quarter of a young cow is more likely to respond to appropriate therapy than one or more quarters chronically infecting an older cow (Owens and Nickerson, 1990). Other *Staphylococcus* species have shown better response to therapy (Owens et al., 1997). Extra-label extension of the duration of therapy may increase the likelihood of therapeutic success for chronic or recurrent infections with *Streptococcus* or *Staphylococcus* species (Morin et al., 1998; Oliver et al., 2004).

Table 30.1. Common mastitis pathogens unlikely to respond to antimicrobial drug treatment.

Arcanobacterium pyogenes
Bacillus spp.
Mycobacterium spp.
Mycoplasma bovis
Nocardia spp.
Pasteurella spp.
Proteus spp.
Prototheca spp. (algae)
Pseudomonas spp.
Serratia spp.
Yeasts (e.g., *Candida* spp.; antibiotic treatment will delay spontaneous cure)

The Gram-negative coliform organisms (*Escherichia coli, Enterobacter* spp., *Klebsiella* spp.) are variable in their clinical expression and response to antimicrobial therapy. Infections with coliform organisms may cause mild or no clinical signs and occasionally resolve on their own, but they may also cause severe, life-threatening illness or chronic infections. As with other pathogens, treatment decisions should be based on the severity of disease and chronicity of infection; mild acute infections may resolve with no therapy or limited therapy. Recent work has demonstrated that treatment of mild or moderate clinical mastitis due to *E. coli* or *Klebsiella* spp. with intramammary ceftiofur significantly improves the cure rate when compared to untreated controls (Schukken et al., 2011). Chronic infections may require longer term, possibly extra-label therapy, and severely ill cows will require supportive care in addition to treatment with antimicrobial drugs.

Mycoplasma bovis is a unique mastitis pathogen. Mastitis caused by *Mycoplasma* may occasionally resolve without treatment, but antimicrobial therapy will not affect the outcome (Gonzalez and Wilson, 2003).

Selecting an Antimicrobial Drug

Intramammary Antimicrobial Drug Use

After cow and pathogen factors have been weighed and the decision has been made to treat a case of mastitis, a suitable therapeutic regimen must be designed. Components of a therapeutic regimen include the drug

to be used and the drug dose, route of administration, frequency of administration, duration of use, and meat and milk withholding times. For mild to moderate mastitis (abnormal milk with or without mammary swelling), antibiotic therapy is usually administered by the intramammary route, if it is administered at all. Table 30.2 lists antimicrobial drug preparations approved by the U.S. Food and Drug Administration (FDA) for intramammary administration to lactating dairy cows. There are eight antimicrobial drugs available for intramammary use in the United States: amoxicillin, ceftiofur, cephapirin, cloxacillin, erythromycin, hetacillin, penicillin, and pirlimycin.

Intramammary use of drugs or preparations not specifically manufactured for intramammary administration is not recommended; such substances may be irritating to udder tissues and promote inflammation. In addition, compounded preparations are at risk for contamination with infectious pathogens, and milk and meat withholding times recommended for other routes of administration are likely to be inaccurate for intramammary administration. It is also advised not to use 2 different antimicrobial preparations simultaneously in one quarter, since interactions between the two drugs may decrease efficacy. For example, macrolides and lincosamides bind at such close sites on the bacterial ribosome that when they are administered simultaneously, they compete for binding and the net effect of the combination of the two drugs is not additive. Consequently, simultaneous use of the macrolide drug erythromycin and the lincosamide drug pirlimycin, both of which are available in formulations for intramammary use, would not provide additional therapeutic benefit and might actually reduce efficacy as compared to either drug alone.

Spectrum of activity is a key consideration when selecting an antimicrobial drug for intramammary therapy of mastitis. Erythromycin, a macrolide, and pirlimycin, a lincosamide, are the only drugs available as intramammary preparations that are not members of the beta-lactams. Both macrolide and lincosamide drugs have primarily Gram-positive antimicrobial spectra, without activity against coliform mastitis pathogens.

One of the earliest beta-lactam drugs to be developed, benzathine penicillin G, is available for intramammary administration. This drug is active against many streptococci and non-penicillinase-producing staphylococci. The drug is inactive against the Enterobacteriaceae and resistance by staphylococci is likely to be common.

Amoxicillin and hetacillin are aminopenicillins with similar spectrum of activity. The aminopenicillins are active against bacteria susceptible to penicillin G, as well as some Enterobacteriaceae such as *E. coli*. Many *E. coli* isolates are now resistant to the aminopenicillins through beta-lactamases (chapters 8 and 10). Combination with a beta-lactamase inhibitor such as clavulanic acid (chapter 10) is not available for intramammary administration.

Cloxacillin is a penicillinase-resistant penicillin active against penicillinase-producing *S. aureus* strains resistant to the natural penicillins and aminopenicillins but is less active against other penicillin-sensitive organisms (chapter 8).

Cephapirin is a first-generation cephalosporin drug generally active against staphylococci and streptococci, sometimes against Enterobacteriaceae such as *E. coli* and *Klebsiella* spp. but not against *Enterococcus* spp. "Third-generation" cephalosporins such as ceftiofur are less active than first-generation cephalosporins against Gram-positive cocci but more active against the Enterobacteriaceae (chapter 9).

The prognosis for resolution of the intramammary infection may be poor even when a pathogen is considered to be within the spectrum of activity of an antimicrobial drug. For example, *Pasteurella* spp. are within the spectrum of activity of several drugs available for intramammary administration, yet the prognosis for resolution of mastitis caused by *Pasteurella* is always poor (National Mastitis Council, 1999).

All of the drugs currently available as intramammary preparations are time-dependent inhibitors of bacterial growth (chapter 5). From a pharmacodynamic standpoint, efficacy is maximized by keeping the concentration of drug at the site of infection above the level necessary to inhibit microbial growth (minimum inhibitory concentration; MIC) as long as possible between doses of the drug. The drug concentration should be above the MIC for at least half the dosing interval for Gram-positive pathogens and for the entirety of the dosing interval for Gram-negative pathogens (chapter 5).

Once the MIC of the drug is achieved at the site of infection, increased drug concentrations above the MIC are unlikely to improve efficacy for those drugs available as intramammary preparations. Maintaining the concentration at 25% greater than MIC for a certain length

Table 30.2. Intramammary preparations available for lactating cows in the United States.

Drug Name and Class	Product Name	Label Regimen and Indications	Other Label Claims
Amoxicillin Aminopenicillin	Amoxi-Mast (Merck Animal Health)	3 treatments at 12-hour intervals Subclinical *S. aureus* mastitis Subclinical *S. agalactiae* mastitis	Susceptibility shown by *E. coli in vitro* Most *Enterobacter, Klebsiella*, and *Pseudomonas* resistant
Ceftiofur third-generation cephalosporin	Spectramast (Zoetis)	2–8 treatments at 24-hour intervals Clinical coagulase-negative *Staphylococcus* mastitis Clinical *S. dysgalactiae* mastitis Clinical *E. coli* mastitis	
Cephapirin first-generation cephalosporin	Today, Cefa-lak (Fort Dodge Animal Health)	2 treatments at a 12-hour interval Mastitis in lactating cows	Shown to be efficacious against susceptible strains of *S. agalactiae* and *S. aureus*
Cloxacillin penicillin (penicillinase-resistant)	Dariclox (Merck Animal Health)	3 treatments at 12-hour intervals Clinical *S. aureus* mastitis (non-penicillinase producing strains) Clinical *S. agalactiae* mastitis	There is laboratory evidence that indicates cloxacillin is resistant to destruction by penicillinase-producing organisms
Erythromycin macrolide	[1]Gallimycin-36 (Agri-Labs) [2]Gallimycin®-36 (Durvet)	3 treatments at 12-hour intervals Clinical *S. aureus* mastitis Clinical *S. agalactiae* mastitis Clinical *S. dysgalactiae* mastitis Clinical *S. uberis* mastitis	[1]Works against both acute and chronic cases
Hetacillin Aminopenicillin	Hetacin-K Intramammary Infusion (Fort Dodge Animal Health)	3 treatments at 24-hour intervals Acute, chronic, or subclinical mastitis	Shown to be efficacious in treatment of mastitis in lactating cows caused by susceptible strains of *S. agalactiae, S. dysgalactiae, S. aureus*, and *E. coli*
Penicillin penicillin	Masti-Clear (G.C. Hanford)	Not more than 3 treatments at 12-hour intervals Clinical *S. agalactiae* mastitis Clinical *S. dysgalactiae* mastitis Clinical *S. uberis* mastitis	
Pirlimycin Lincosamide	Pirsue Aqueous Gel (Zoetis)	2 treatments at a-24 hour interval Clinical and subclinical mastitis	Has been proven effective only against *Staphylococcus* species such as *S. aureus* and *Streptococcus* species such as *S. dysgalactiae* and *S. uberis*

Note: Although every effort has been made to ensure that the information presented here is accurate and complete, the authors cannot bear responsibility for any errors or omissions. Readers are advised to contact drug manufacturers and/or read package inserts for complete information about the products listed herein.

of time should be as effective as maintaining the drug level at 100% above the MIC for the same time period. Consequently, if one wishes to prescribe extra-label therapy for a case of mastitis that may be difficult to resolve using label dosing regimens, extending the duration of therapy (provided drug concentrations are maintained above the MIC between doses) is expected to be more effective than giving a higher dose at each treatment time without extending the duration of therapy. The only exception to this rule would be if the drug is cleared so slowly that drug accumulation following a higher dose results in the drug concentration remaining above MIC for additional dosing intervals. Available mastitis preparations are unlikely to accumulate in the mammary gland to the point that administering two tubes will result in extension of therapeutic concentrations for one or more additional dosing intervals.

Regardless of the approach, it is critical that extra-label use of any drug in a food animal such as a dairy cow be accompanied by extended milk and meat withholding times. For help in setting extended withholding times following extra-label use, the Food Animal Residue Avoidance Databank provides free assistance. They can be reached in the United States by dialing 1-888-US-FARAD.

Systemic Antimicrobial Drug Use

For acute mild to moderate mastitis, systemic antimicrobial therapy is not generally indicated or undertaken. For severe cases of mastitis (those that involve systemic clinical signs such as fever or depression in addition to abnormal milk and udder swelling), systemic administration of antimicrobial drugs is an appropriate part of therapy. Supportive care by administration of fluids and other methods is also critical in such cases, and has been discussed elsewhere (Morin, 2004). Mastitis with systemic illness is commonly caused by coliform organisms such as *E. coli* and *Klebsiella* spp. An investigation of naturally occurring cases of coliform mastitis with systemic illness has demonstrated that 42% of cows with severe illness due to coliform mastitis had concurrent bacteremia (Wenz et al., 2001) Although systemic illness due to mastitis is frequently caused by Gram-negative pathogens, it may also be caused by Gram-positive pathogens such as *S. aureus* (Erskine et al., 2002). Because microbial culture generally takes 24 hours to yield a preliminary result, therapy of severe mastitis must initially be based on the possibility of a Gram-positive or Gram-negative bacterial etiology. For mastitis due to coliform bacterial infection, research suggests that by the time clinical signs appear, bacterial numbers in the mammary gland have already peaked (Erskine et al., 1989). Consequently, a rational approach to therapy of severe acute mastitis would be to address the possibility of coliform bacteremia by using a systemic drug with a spectrum of activity including Gram-negative pathogens, combined with an intramammary preparation that is active against Gram-positive pathogens.

In the United States, any systemic use of an antimicrobial drug as a therapy for mastitis is an extra-label use, as there is currently no antimicrobial drug approved by the FDA for systemic administration for mastitis. Extra-label drug use in food animals requires extending meat and milk withholding periods. Drugs available for use in lactating dairy cattle, with appropriate spectra of activity against coliform bacteria, include oxytetracycline, sulfadimethoxine, ampicillin, and amoxicillin.

Although tetracyclines have both Gram-positive and Gram-negative activity in their spectrum of activity, some coliforms and *Staphylococcus* species may not be susceptible (chapter 15). The use of sulfadimethoxine to treat mastitis in a lactating cow is illegal in the United States, as mastitis is not a labeled indication for the drug and extra-label use of sulfonamides in lactating dairy cows is prohibited by the regulations codified in the Animal Medicinal Drug Use Clarification Act. Similarly to the tetracyclines, resistance to sulfonamide drugs is now widespread. The aminopenicillins have a spectrum of activity that includes the Enterobacteriaceae but resistance is also widespread (chapters 8 and 10).

Ceftiofur, a third-generation cephalosporin, also has a spectrum of activity that includes coliform mastitis pathogens, and it is relatively resistant to beta-lactamases produced by those bacteria. When used in combination with intramammary antimicrobial drugs, anti-inflammatory drugs, and other supportive therapy, the addition of intramuscular ceftiofur to the treatment regimen for severe acute mastitis decreased the likelihood of a cow subsequently dying or being culled (Erskine et al., 2002). In the United States, the extra-label use of cephalosporins has recently been banned in food animals, with the exception of use of cephapirin mastitis preparations (chapter 9; FDA, 2012).

Questions frequently arise about florfenicol and the lincosamide drug tilmicosin for systemic use against

coliform mastitis, since they are active against the Gram-negative pathogens that cause respiratory disease in cattle. These drugs are not, however, good choices for Gram-negative septicemia associated with severe mastitis. Although the Gram-negative respiratory pathogens *Mannheimia* and *Pasteurella* may be susceptible to these drugs, the Gram-negative coliform organisms that commonly cause mastitis are either entirely resistant to these drugs, or the drug concentration required to inhibit their growth is so high that administration of an impossibly high dose of these drugs would be necessary to obtain any benefit.

Antimicrobial Susceptibility Testing and Mastitis

Antimicrobial susceptibility testing is a way of quantifying the interaction between microbes and antimicrobial drugs in the laboratory (chapter 2). Susceptibility testing may be performed using serial dilution or agar gel diffusion (Kirby-Bauer) methods. For the serial dilution method, the lowest concentration of each drug that inhibits microbial growth is the MIC. This may not be the same as the concentration of drug necessary to sterilize the culture, called the minimum bactericidal concentration (MBC). Using the MIC instead of the MBC to draw conclusions about antimicrobial efficacy is consistent with the therapeutic goal: to assist the cow's own immune system in clearing the infection.

Breakpoints are used to classify MICs as indicators of microbial susceptibility or resistance. For many combinations of drug and microbe, susceptibility testing results are typically reported as "intermediate," "susceptible," or "resistant" (chapter 2). Susceptibility testing is based on the theory that a finding of susceptibility in the laboratory indicates that a favorable outcome of antimicrobial drug therapy is likely, while a finding of resistance in the laboratory is associated with a poor prognosis for therapeutic success.

Validated veterinary breakpoints are specific for a drug, treatment regimen, pathogen, affected species, and disease condition. Validated veterinary breakpoints are developed by a committee of experts in veterinary microbiology and pharmacology in cooperation with the Clinical and Laboratory Standards Institute (chapter 2). Veterinary diagnostic laboratories provide susceptibility testing for combinations of drug, pathogen, species, and/or disease that may or may not have validated breakpoints; when there are no validated breakpoints, breakpoints are used that are derived from data about different pathogens, species, and/or diseases. This extrapolation should be borne in mind when interpreting the results of susceptibility testing. Currently, validated breakpoints are available for two preparations for intramammary treatment of lactating cows (ceftiofur, pirlimycin) and one intramammary preparation for treatment of dry cows (penicillin and novibiocin).

In the Kirby-Bauer method of susceptibility testing, an antibiotic disk containing a known amount of each drug under test is placed onto an agar gel plate inoculated with the pathogen under test. The area around the disk where microbial growth is inhibited is called the zone of inhibition, and the diameter of this zone is used as the breakpoint to classify microbes as susceptible, intermediate, or resistant to the drugs under test (chapter 2). Proper execution of either method of susceptibility testing requires skill, training, attention to detail, and quality control measures. For accurate results, it is recommended that samples for susceptibility testing be submitted to a veterinary diagnostic laboratory that is accredited, for example in the United States by the American Association of Veterinary Laboratory Diagnosticians.

Even when properly executed, susceptibility testing has limited value as an aid to therapeutic decision-making in bovine mastitis. The relationship between susceptibility as determined by laboratory susceptibility testing and the outcome of clinical cases of mastitis appears to be inconsistent at best. Many publications have described patterns of susceptibility seen in the laboratory, but several reports have determined little predictive value of susceptibility testing for clinical outcomes of mastitis (Owens et al., 1997; Constable and Morin, 2003; Hoe and Ruegg, 2005; Apparao et al., 2009.) The issue is further complicated by the use of variable outcomes in trials assessing resolution of clinical mastitis: the achievement of a cure may defined as resolution of clinical signs, or one or more negative microbial cultures, or some combination of these outcomes. A more practical approach to assessing whether a mastitis therapy works is to design farm protocols for treatment of clinical mastitis with selected antimicrobial drugs, then periodically evaluate the protocols for the efficacy of the selected drugs in achieving the farm's therapeutic objectives.

Herd-Based Therapeutic Protocols

Modern management strategies frequently involve a standardized approach to mastitis prevention and therapy. Key benefits to standardizing the farm approach to mastitis therapy are that treatment decisions are made in advance instead of "cow-side" and that a consistent approach is developed. This results in simpler, less time consuming tasks on the farm, from deciding whether or not to treat a case of mastitis to selecting a drug and regimen and assigning an appropriate withholding time for milk and meat from treated cows. Moreover, when treatments are standardized and good records are kept, evaluating whether or not a given treatment is successful on the farm is simplified.

Microbial culture of clinical mastitis cases can be boon to designing a farm protocol for treatment of clinical mastitis. By culturing all new cases of mastitis, the organisms that are causing clinical mastitis on the farm can be identified and an appropriate therapeutic regimen can be developed. In addition, microbial culture results can be used to direct efforts at prevention to appropriate areas. Even if microbiological culture is not performed on every case of mastitis on a particular farm, periodic cultures are still useful as a guide to the development of treatment strategies and protocols. Culture of chronic mastitis cases is also an irreplaceable aid to determining whether a chronic case of mastitis might be resolved by antimicrobial therapy, or if the pathogen causing the mastitis is not amenable to therapy and the greatest financial benefit to the farm would be not to treat.

Some farms have incorporated routine culture of every case of mastitis into their treatment protocol. Farms can use a simple classification system for all cases of mastitis following a 24-hour milk culture of each case using "biplates" which are petri dishes with MacConkey's agar gel, which selects for Gram-negative bacterial growth, on one half, and blood agar gel, which is non-selective, or Factor medium, which is selective for Gram-positive bacterial growth, on the other half. Using these culture media, each case of mastitis may be classified as no growth, Gram-positive growth, Gram-negative growth, or contaminated sample, and treatment regimens may be designed for each possibility. *Mycoplasma* spp. require special media and more stringent incubation conditions than this simple approach provides. On-farm microbial milk culture requires time, training, and organization, but the financial rewards to a farm may be significant. No growth results are typical obtained for 25–50% of all cases of clinical mastitis, and antimicrobial therapy is probably not indicated in such cases (Hess et al., 2003). Decreasing the number of treated cows on farms that have previously treated every case of mastitis may result in financial benefit to the farm, even after the cost of conducting microbial culture of each new case is factored in. Some farms have also elected not to treat cows with Gram-negative pathogens isolated on milk culture; this practice may reduce the percentage of clinical mastitis cases treated to less than half of all new cases, depending on the predominant pathogens on the farm. If this approach is taken, it is imperative that it be undertaken in the knowledge that Gram-negative mastitis, although it will frequently resolve on its own, may also develop into a chronic infection or severe illness. In addition, as mentioned above, recently published research has demonstrated that intramammary antimicrobial drug treatment of clinical mastitis caused by *E. coli* and *Klebsiella* spp. significantly improves the cure rate for such cases (Schukken et al., 2011).

Microbial culture of milk is a practical tool to identify pathogens and design specific therapeutic regimens for mastitis treatment. A recent multistate study found a reduction in antimicrobial usage when culture-based treatments replaced empirical therapy (Lago et al., 2011). Whichever culture-based treatment protocol is adopted, it is prudent to save pretreatment milk samples in the freezer for submission to a diagnostic laboratory for definitive organism identification in the event that the case of mastitis does not resolve.

An example of a herd mastitis treatment protocol is given in Figure 30.1.

Antimicrobial Therapy of Dry Cows

The dry (non-lactating) period of the lactation cycle is a critical time for dairy cattle. The major proportion of calf-growth occurs during this time. Balanced nutrition, especially in the last 2–3 weeks before calving, is essential for prevention of post-parturient metabolic disease. The udder undergoes marked biochemical, cellular and immunological changes during the dry period. Involution

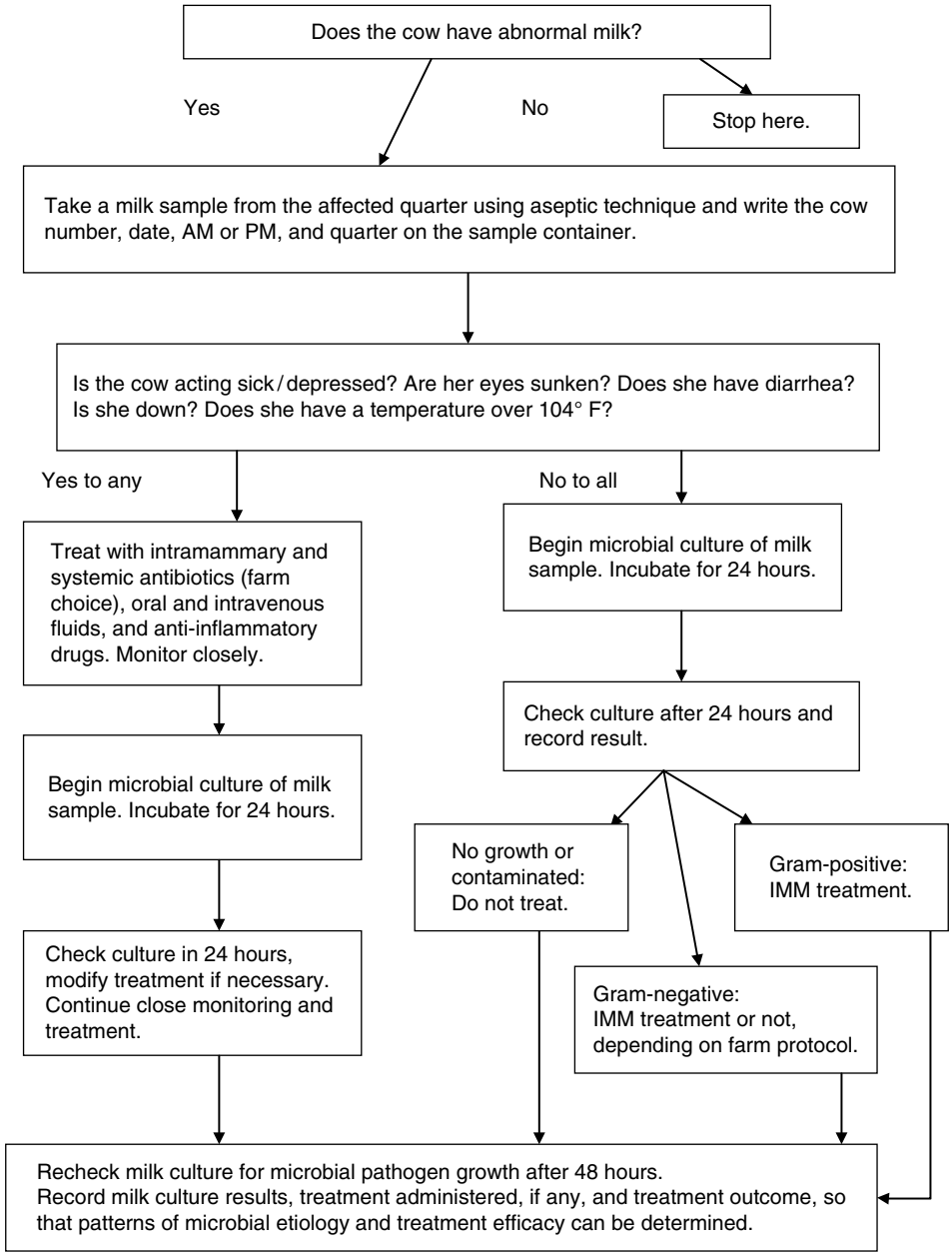

Figure 30.1. Herd mastitis treatment protocol.

of the mammary parenchyma begins 1–2 days after the end of lactation and continues for 10–14 days. During this time, the gland is particularly vulnerable to new intramammary infections (IMI). The periparturient period and the early dry period constitute the times of greatest risk for new IMI in the lactation cycle of the cow. Once involution is complete, however, a more hostile immune environment for bacterial pathogens exists. The most important defense against IMI, as with lactating cows, remains the teat canal. This barrier is enhanced

during the dry period by the formation of a keratin plug. Additionally, during the dry period the mammary gland contains increased numbers of macrophages and lymphocytes and higher concentrations of complement and immunoglobulins that can help orchestrate more efficient phagocytosis. Lactoferrin, a potent iron chelating protein, also markedly increases in dry cow secretions, helping to inhibit growth of Gram-negative bacteria, particularly *E. coli*. Consequently, the dry period is an ideal time to attain synergy between antibacterial therapy and immune function to eliminate pathogens from the gland, without incurring the extensive milk withholding costs typical of lactating cow therapy.

Intramammary administration of antibacterial drugs at the end of lactation has been a standard practice in dairy mastitis management for over 35 years. Cure rates for IMI caused by all Gram-positive cocci (those IMI that existed prior to the dry period, but were not detected following calving) have been reported in numerous studies to average 75% (Nickerson et al., 1999). However, the efficacy of conventional dry cow treatments in eliminating chronic IMI is realistically closer to 15–30% (Sol et al., 1990; Erskine et al., 1994). Most commercial dry cow products have little or no activity against Gram-negative pathogens, so that cure rates for coliform organisms are low. In one study, cows treated with a product with significant activity against Gram-negative bacteria had decreased clinical coliform mastitis during the dry period and early lactation as compared to as compared to cows treated with cloxacillin (Bradley and Green, 2001).

Because of concern regarding overuse of antibacterial drugs and potential effects on antimicrobial resistance of bacteria, selective dry cow therapy (treatment of infected cows only) versus total or blanket dry cow therapy (treatment of all cows) had been discussed. Decisions should be made on an individual herd basis, and results monitored to determine the success of a dry cow mastitis program based on numbers of new IMI during the dry period, cures of existing infections, and effect on the rate of clinical mastitis, particularly in early lactation. An important role of dry cow therapy in addition to eliminating existing IMI is the prevention of new IMI. Intramammary infusion of tilmicosin reduced new infection rates by greater than 33% in a Canadian study (Dingwell et al., 2002). Selective dry cow therapy can result in herd-wide increases in clinical mastitis in the dry period, IMI during the dry period, and clinical mastitis in early lactation, as compared to herds that treat all cows at dry-off (Berry and Hillerton, 2002) Additionally, a recent review of the literature determined that, to date, no evidence exists that supports the concept of emerging antimicrobial resistance in mastitis pathogens (Erskine et al., 2004). Thus, the evidence suggests that, for most herds, intramammary antimicrobial drug treatment of all dry cows is preferred over selective dry cow therapy. In addition, use of an internal teat sealant at dry off in conjunction with antimicrobial therapy reduced new intramammary infections during the dry period by 30%, and clinical mastitis in the first 60 days in milk by 33%, as compared to antimicrobial use alone (Godden et al., 2003).

As with therapy during lactation, systemic antimicrobial drug administration as an adjunct to intramammary administration of dry cow therapy has been investigated. Subcutaneous norfloxacin nicotinate administered at the start of the dry period achieved a better cure rate and lower new infection rate over the dry period for *S. aureus* infections, as compared to untreated cows and cows administered intramammary cephapirin benzathine preparations (Soback et al., 1990). However, the systemic administration of tilmicosin resulted in lower drug concentrations in milk and lower cure rates for *S. aureus* mastitis than intramammary administration (Nickerson et al., 1999). Additionally, cows administered intramuscular oxytetracycline and intramammary cephapirin did not have better cure rates for quarters infected with *S. aureus* than cows treated with cephapirin alone (Erskine et al., 1994). Clinical failure in these trials reflects the importance of designing a therapeutic regimen that will maintain an effective concentration of an appropriate drug at the site of infection for an adequate duration, and the poor prognosis of chronic infections. Systemic therapy should be approached judiciously, using sound pharmacological principles.

In summary, the important considerations for dry cow treatment include: (1) commercial dry cow treatments are generally effective against Gram-positive cocci in preventing and eliminating IMI; (2) because of enhanced immune function and decreased discarded milk costs, dry cows should be preferentially treated as compared to lactating cows for subclinical and chronic IMI; (3) most commercial intramammary products

have little efficacy against Gram-negative pathogens; and (4) treatment of more chronic IMI may include systemic drug regimens, preferably with antimicrobials that distribute well in mammary tissue, such as tetracyclines and macrolides.

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31

Antimicrobial Drug Use in Sheep and Goats

Chris R. Clark

Although sheep and goats are important agricultural animals worldwide, they remain relatively minor species within the North American market and many veterinarians will have had limited exposure to them. In the United States and Canada, there are few licensed veterinary pharmaceutical products available for sheep and goats. For sheep, there are a few approved antimicrobials but there is only 1 antimicrobial approved for use in goats. Furthermore, sheep and goats are classed as separate species by the regulatory authorities. This means that approved drugs are specifically licensed and withdrawal times and maximum residue limits (MRLs) have been set for each species. This situation creates a great deal of confusion for veterinarians as well as sheep and goat producers. Journal articles, textbooks, and the internet provide accessible information from clinical trials and dose regimens for a wide variety of antimicrobials that are not licensed in North America. In many cases, the safety and efficacy of the drug is well documented and the antimicrobial product is actually available in North America; it is just not licensed for sheep or goats.

General Recommendations

Sheep and goats are not simply "small cows"; they react differently to certain medications and suffer from different diseases. A good resource specific for the North American situation is *Sheep and Goat Medicine* (Pugh and Baird, 2011). Prudent antimicrobial use first requires a tentative diagnosis followed by confirmation of the etiological agent by microbiological culture and antimicrobial susceptibility testing before commencing therapy. However, collection of samples from sheep and goats is not always feasible and even if samples are obtained, results usually take at least 2–3 days to process. So empirical therapy is common and should be determined by a thorough physical examination and a presumptive diagnosis, knowledge of the most common pathogens, the expected antimicrobial susceptibility of those organisms, and the pharmacokinetics/pharmacodynamics of the antimicrobial in the species being treated. Tables 31.1 and 31.2 contain information to help make these decisions.

Once an antimicrobial drug is selected, proper administration is important. The label claim (when available) should be followed closely for dose, frequency, route of administration and dose volume. Any deviation from the label constitutes extra-label drug use. For quality assurance, it is also important to administer parenteral antimicrobial drugs in a way that minimizes damage to muscle tissues. Clean syringes and fresh needles should be used. The volume of drug per injection site should generally be limited to five milliliters or less. The subcutaneous, oral or intravenous routes should be selected over the intramuscular route if possible. Intramuscular injections should be given only in the neck. Subcutaneous injections should be given in the neck also. Small volumes

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Table 31.1. Antimicrobial drug selection for common conditions of sheep and goats.

Condition	Species Affected	Etiological Agent(s)	Recommended Treatment	Comments
Infectious abortion				
Enzootic abortion of ewes (EAE)	Sheep and goats	*Chlamydophila abortus*	Tetracycline Oxytetracycline Tylosin	Prophylaxis in high-risk flocks: tetracycline in feed for 6–8 weeks prior to breeding at a dose of 200–400 mg/head/day until lambed. Outbreak: 400–500 mg/head/day tetracycline in feed until lambing finished. Poor efficacy if placental damage already present. Not recommended for dairy goats because of milk withdrawal. Vaccination or biosecurity should be considered. Outbreak or previous diagnosis: long-acting oxytetracycline at label dosage starting 6–8 weeks before start of lambing every 10–14 days until finished.
Campylobacter abortion (Vibrionic)	Sheep	*C. jejuni* *C. fetus* spp. *fetus*	Penicillin G-streptomycin; tetracycline Oxytetracycline (resistance commonly reported) Tylosin Sulfamethazine	Prophylaxis: injections of penicillin-streptomycin for 2–5 days. Antimicrobial susceptibility patterns should be established from any isolates. Vaccination in the face of an outbreak also very successful.
Listeria abortion	Sheep and goats	*L. monocytogenes*	Oxytetracycline	Injectable long-acting tetracycline to all animals at risk in the face of an outbreak.
Toxoplasma abortion	Sheep and goats	*T. gondii*	Monensin Decoquinate	Mixed in feed at a dose of 15 mg/head/day from breeding to lambing. Mixed in feed or premix to feed at a dose of 2 mg/kg/day for last 14 weeks of gestation.
Salmonella abortion	Sheep and goats	*S. typhimurium, S. abortus ovis, S. montevideo, S. dublin*	IM or SC broad-spectrum antimicrobials	Often widespread by the time diagnosis is made. Requires culture and susceptibility testing. Antimicrobials may not eliminate organism; consider culling and environmental management.
Leptospira abortion	Sheep and goats	*L. hardjo, L. pomona*	Penicillin G-streptomycin; tetracyclines	Treat all pregnant animals at risk with injections.
Coxiellosis (Q fever)	Sheep and goats	*C. burnetii*	Tetracycline; (fluoroquinolone where permitted)	Abortions are more common in goats than in sheep. Long-acting injectable oxytetracycline (IM or SC) to all pregnant does every 10–14 days until kidded. Watch withdrawal for milk in dairy goats.
Other infectious reproductive disorders				
Metritis	Sheep and goats	*Trueperella pyogenes, E. coli,* mixed anaerobes including *Clostridium* spp.	Penicillin G; ceftiofur; broad-spectrum antimicrobials	Treat for 3–4 days after clinically normal. Uterine evacuation with prostaglandins and tetanus vaccination should also be considered.
Lamb epididymitis	Sheep	*H. somni, A. seminis, Corynebacterium pseudotuberculosis*	Oxytetracycline	Prophylaxis: low levels in feed in situations where rams intensively managed, or injectable long-acting oxytetracycline (IM or SC). Responds poorly to treatment.
Enzootic posthitis	Sheep and goats	*C. renale* group	Penicillin G; oxytetracycline	Remove from high-protein diet and treat locally with antibiotic ointments. May treat systemically for severe cases.

Brucella ovis ram epididymitis	Sheep	*Brucella ovis*	Oxytetracycline with dihydrostrepto-mycin	20 mg/kg oxytetracycline at 3-day intervals for 5 treatments and 12.5 mg/kg streptomycin 2 x/day for 7 days decreases shedding of bacteria and improves semen quality but may not cure. Should consider culling.
Infectious diseases of lambs and kids, systemic				
Enterotoxemia/pulpy kidney	Sheep and goats	*C. perfringens* type C and D	Oral virginiamycin, penicillin G, or bacitracin	Vaccinate all animals at risk. Withdraw carbohydrate source in diet, give C&D antitoxin and a balanced electrolyte solution (BES) parenterally.
Omphalophlebitis	Sheep and goats	*T. pyogenes, E. coli*, mixed anaerobes	Penicillin G; broad-spectrum antimicrobialsa	Antibiotic therapy alone not often effective. Local drainage and treatment and possibly surgical removal should be considered.
Watery mouth (lambs)	Sheep	Probable *E. coli* endotoxin	Oral amoxicillin; apramycin	Prevention by ensuring clean environment and good colostrum ingestion. Early prophylactic treatment with oral antibiotics.
Metabolic acidosis without dehydration (kids)	Goats	Unknown	Broad-spectrum antimicrobials	Isotonic bicarbonate solutions to correct acid-base deficit followed by balanced electrolyte solution (BES).
Tickborne fever (tick pyemia)	Sheep	*Anaplasma phagocytophilum* and/or *S. aureus*	Long-acting oxytetracycline	At 1–3 weeks of age and repeated at 5–7 weeks, in addition to dipping with an acaricide at those times.
Erysipelothrix polyarthritis	Sheep	*E. rhusiopathiae*	Penicillin G	Treat minimum of 3 days.
Infectious diseases of lambs and kids, digestive				
Colibacillosis	Sheep and goats	Enterotoxigenic *E. coli*	Broad-spectrum anti microbials parenterally	Appropriate diagnosis is necessary (culture and susceptibility testing), also treat with BES. Clean environment and adequate colostrum is important. Consider vaccination. Resistance to antimicrobials is common.
Salmonella dysentery	Sheep and goats	*S. typhimurium* and others	Broad-spectrum antimicrobials	Often poor efficacy due to unpredictable susceptibility patterns. May not eliminate carriers if host-adapted species.
Abomasitis/abomasal hemorrhage	Sheep and goats	*Clostridium* spp.	Oral penicillins	Rarely effective. Should treat symptomatically with antitoxins, non-steroidal anti- inflammatory drugs, and BES. Use polyvalent clostridial vaccine.
Coccidiosis	Sheep and goats	*Eimeria* spp.	Monensin; lasalocid; decoquinate; salinomycin; amprolium; or sulfonamides	Mixing should be done at a feed mill and all feeds pelleted. Some products can be mixed with salt. Dose varies with feed management. Artificially raised lambs/kids can be medicated via milk replacer. Feed from 2 weeks of age until market age. Ionophores toxic to horses and dogs.
Infectious conditions of lambs and kids, respiratory				
Pneumonic pasteurellosis	Sheep and goats	*M. haemolytica, P. multocida*	Tilmicosin; oxytetracycline; ceftiofur; florfenicol	Long-acting oxytetracycline, tilmicosin, or florfenicol can be used as a prophylaxis and during an outbreak therapeutically. Tilmicosin should not be used in goats (therapeutic dose very close to toxic dose). Ceftiofur for daily treatment of affected animals when meat or milk withdrawal is an issue (e.g., market lambs close to slaughter, lactating dairy sheep).
Pasteurella septicemia	Sheep	*Bibersteinia trehalosi*	As with *M. haemolytica*	B. trehalosi shows more resistance and because the disease is peracute, vaccination is recommended for susceptible animals.

(*continued*)

Table 31.1. Antimicrobial drug selection for common conditions of sheep and goats. (*continued*)

Condition	Species Affected	Etiological Agent(s)	Recommended Treatment	Comments
Necrotic laryngitis	Sheep and goats	*Fusobacterium necrophorum*	Penicillin G; oxytetracycline	
Mycoplasma pneumonia	Sheep and goats	*M. ovipneumoniae, M. arginini*	Oxytetracycline; tylosin	Often seen in conjunction with pasteurellosis (atypical pneumonia) or alone.
Mycoplasma mycoides	Goats	*M. mycoides* ss. *mycoides* large colony type	Oxytetracycline; lincomycin; or tylosin	Treatment of peracute septicemia often ineffective. If goat survives, it will probably be a carrier.
Infectious conditions of the integument				
Pinkeye (infectious keratoconjunctivitis)	Sheep and goats	*C. psittaci, M. conjunctivae, R. conjunctivae, Neisseria*	Spiramycin; oxytetracycline; tiamulin IM	Spiramycin or oxytetracycline repeated days 1, 5, and 10; tiamulin repeated days 1, 3, 6, and 9. Oxytetracycline eye ointment. Conjunctival injection of penicillin (least effective).
Secondary infection of contagious ecthyma (Orf)	Sheep and goats	*S. aureus*	Tilmicosin; oxytetracycline; ampicillin	May also try local antimicrobials but wear gloves, as is a zoonosis.
Dermatomycosis (lumpy wool)	Sheep	*Dermatophilus congolensis*	Long-acting oxytetracycline	Decrease humidity (ventilation) if possible, and protect from rain. Powder sheep with powdered alum to help prevent reinfection.
Caseous lymphadenitis	Sheep and goats	*Corynebacterium pseudotuberculosis*	No effective treatment	Although susceptible to penicillin, not effective because of the thick abscess wall. Recommend cull infected animals and avoid opening abscesses as it spreads the pathogen.
Infectious conditions of the foot and musculoskeletal system				
Contagious foot rot	Sheep and goats	*D. nodosus F. necrophorum*	Long-acting oxytetracycline	10–20% zinc sulphate with 2% w/v sodium lauryl sulphate, as a foot bath with or without foot trimming. Must remain in bath 20 minutes. Repeat in 5–7 days. Can use in conjunction with systemic antimicrobials and/or vaccination. Cull chronic non- responders.
Foot scald	Sheep and goats	*F. necrophorum*		Zinc sulfate foot bath as above.
Strawberry foot rot	Sheep and goats	*D. congolensis*	As with lumpy wool	Verify that condition is not chorioptic mange.
Polyarthritis	Sheep and goats	*Chlamydophila pecorum*	Oxytetracycline	Poor response, may relapse.
Polyarthritis	Goats	*Mycoplasma mycoides* subsp. *mycoides*,LC other *Mycoplasma* spp.	Oxytetracycline; tylosin	Poor response, may relapse.
Infectious conditions of the mammary gland				
Gangrenous mastitis	Sheep and goats	*S. aureus, M. haemolytica*	Tilmicosin; broad-spectrum antimicrobials	Gland will be lost if animal survives, so should probably be culled.
Contagious agalactia	Sheep and goats	*M. agalactiae, M. mycoides* ss *mycoides* (goats)	Tetracyclines; tylosin	Probably ineffective, so animal should be culled. Carrier state likely.

Subclinical and clinical mastitis	Sheep and goats	*S. aureus, M. haemolytica*, environmental streptococci, coagulase-negative *Staphylococcus* spp.	Tilmicosin; cloxacillin; cephapirin benzathine; oxytetracycline	Dry treatment to be used at the end of lactation in dairy goats or at weaning for prevention of new infections in high-risk sheep flocks. Do not split tubes. Tilmicosin should not be used in goats (therapeutic dose very close to toxic dose).
Infectious conditions of the oral cavity				
Periodontal disease	Sheep	Many species	No effective treatment	
Tooth root abscess	Sheep and goats	Many species	Oxytetracycline; florfenicol; broad-spectrum antimicrobials	4–6 weeks of therapy. Consider surgical intervention if antimicrobials fail.
Actinobacillosis	Sheep	*Actinobacillus lignieresii*	Sodium iodide	70 mg/kg as 10–20% solution every 2 weeks for 2–3 doses.
Actinomycosis	Sheep	*Actinomyces bovis*	Sodium iodide; sulfadimethoxine; isoniazid	As for actinobacillus. Treat for weeks to months. Prognosis poor.
Infectious conditions of the urinary tract				
Leptospirosis	Sheep and goats	*Leptospira interrogans*	Dihydrostreptomycin; oxytetracycline	Drugs are potentially nephrotoxic, questionable efficacy.
Cystitis	Sheep and goats	*Corynebacterium renale*, other species	Broad-spectrum antimicrobials	Therapy should be based on culture and sensitivity and should be given for 10–14 days.
Infectious conditions of the nervous system				
Bacterial meningitis	Sheep and goats	Many species	Broad-spectrum antimicrobials	Anti-inflammatory drugs important.
Listeriosis	Sheep and goats	*L. monocytogenes*	Oxytetracycline; penicillin G	Injectable long-acting formulation. 22,000–44,000 IU/kg IM twice per day. Broad-spectrum antimicrobials include: ampicillin-sulbactam, ceftiofur, fluoroquinolones, trimethoprim-sulfamethazine, or other potentiated sulfonamide combinations.

Table 31.2 Common antimicrobial dosage regimens for sheep and goats. Many of the drugs listed are not approved for use in sheep and goats in the United States and elsewhere, so that their use constitutes extra-label drug use (ELDU). ELDU of feed additives is prohibited in the United States and fluoroquinolones are banned from ELDU in food-producing animals in the United States.

Drug	Route	Species	Dose Rate	Units	Frequency (h)
Amoxicillin–clavulanic acid	IV, IM	Sheep and goats	20	mg/kg	8
Amoxicillin trihydrate	IM	Sheep and coats	10	mg/kg	8
Ampicillin sodium	IV, IM	Sheep and goats	10–20	mg/kg	12
Amprolium	PO in feed or water	Sheep and goats	10–60	ppm	24, for 5–21 days for control; high dose 5 days for treatment
			10	mg/kg	
Ceftiofur sodium	IM	Sheep*	1.1–2.2	mg/kg	24, for 3 days
		Goats*	1.1–2.2	mg/kg	24, for 3 days
Chlortetracycline	PO	Sheep*	22	ppm	Daily during late gestation to prevent infectious abortion
		Goats	22	ppm	Daily during late gestation to prevent infectious abortion
Decoquinate	PO in feed	Sheep and goats	25–100	ppm	Daily in feed for period of coccidiosis risk
			0.5	mg/kg	Daily during gestation to prevent T. gondii abortion
Danofloxacin	IM, SC	Sheep and goats	1.25	mg/kg	24 3–5 days
Enrofloxacin	IV, IM	Sheep and goats	5	mg/kg	24
Erythromycin	IM	Sheep and goats	3–5	mg/kg	8–12 up to 5 days
Florfenicol	IM, SC	Sheep and goats	20 (IM), 40 (SC)	mg/kg	48(IM) 96(SQ)
Gamithromycin	SQ	Sheep and goats	6	mg/kg	
Lasalocid	PO in feed	Sheep and goats	30	ppm	Daily in feed for period at risk
			1	mg/kg	
Lincomycin hydrochloride	IM	Sheep and goats	10–20	mg/kg	12–24
Marbofloxacin	SC, IM	Sheep and goats	2	mg/kg	24
Monensin	PO in feed	Sheep and goats	11–22	ppm	Daily in feed for period of coccidiosis risk
			1	mg/kg	Daily during gestation for prevention of T.gondii
Neomycin sulfate	PO in feed or water	Sheep and goats	22	mg/kg	24 for up to 14 days

Oxytetracycline	PO in feed	Sheep* and goats	22	ppm	
Oxtetracycline hydrochloride	IV, IM	Sheep* and goats	10	mg/kg	12–24
Oxytetracycline long-acting	IM	Sheep and goats	20	mg/kg	48–72
Penicillin G potassium or sodium	IV	Sheep and goats	20,000–40,000	IU/kg	6
Penicillin G procaine	IM	Sheep* and goats	20,000–40,000	IU/kg	12
Salinomycin	PO in feed	Sheep and goats	11–16	ppm	In feed for period of risk
Sulfonamides	PO in water	Sheep* and goats	50 (loading 100)	mg/kg	24
Tilmicosin	SC	Sheep* Goats	10	mg/kg	Single treatment Do not use due to toxicity
Trimethoprim-sulfonamide	IM	Sheep and goats	24–30	mg/kg	24
Tulathromycin	SC	Sheep and goats	2.5	mg/kg	Single treatment
Tylosin	IM	Sheep and goats	20	mg/kg	12

*Indicates that this product is licensed for use in some areas of North America. For withdrawal times consult the product label or the U.S. FARAD or Canadian gFARAD.

(< 5 ml) can be given subcutaneously in the axilla or the medial aspect of the thigh.

Since owners will often do follow-up treatment, they should be counseled on proper drug handling and administration, and warned of the potential for disease transmission and injection site abscesses if needles are reused. Good record-keeping practices for both the owner and veterinarian, and individual animal identification are key to preventing violative residues in meat and milk. Good records are also necessary so that injection site reactions are not confused with lesions of caseous lymphadenitis, which is an important contagious disease of sheep and goats. Tilmicosin, which is approved for use in sheep, can cause fatal reactions in humans and goats. This drug should not be dispensed without careful consideration of the safety issues. The manufacturer of tilmicosin provides education materials for owners to read and sign, indicating that they understand the potential for toxicity.

Administration of antimicrobials orally via food and water for treatment of infections should be avoided. Intake is hard to control, especially in ill animals in which intake may be decreased. In large flocks with diffuse disease this may be the only practical option despite its limitations; examples include flock and herd outbreaks of coccidiosis and infectious abortion.

Neonatal Enteritis

Like most species, neonatal lambs and kids are prone to enteric infections. These infections can be largely controlled by strict attention to the hygiene of the lambing/kidding area and by ensuring that all newborns receive adequate good quality colostrum. The causes of enteritis in small ruminants are very similar to those seen in cattle. The use of antimicrobials for diarrhea in neonatal calves has been extensively reviewed and is largely applicable in small ruminants (Constable, 2004). Under a week of age, the most likely causes of enteritis are Enterotoxigenic *E. coli* (ETEC), and potentially *Salmonella* spp. Clostridial disease may also be seen but this more commonly results in sudden death and is easily prevented by vaccinating with a multivalent clostridial vaccine in late pregnancy. After kids or lambs are a week of age, enteric viruses, *Cryptosporidium*, and coccidiosis more commonly cause enteritis.

Consequently, antimicrobials have a very limited role in managing neonatal enteritis. The main role of antimicrobials is supportive, and a broad-spectrum antimicrobial will help reduce bacterial overgrowth in the hindgut and treat bacteremia resulting from translocation from the gut through the damaged intestinal wall. If very young animals are affected, the feces should be cultured and the presence of ETEC confirmed. Antimicrobial susceptibility testing is vital as many strains carry multidrug resistance. In the event of a salmonellosis outbreak, culture and susceptibility testing are also vital. When faced with a coccidiosis outbreak, sulfonamides are the treatment of choice for clinical cases. Coccidiostats in a creep feed or milk replacer may be indicated to prevent further outbreaks. The use of antimicrobials in other circumstances is controversial and prudent antimicrobial use guidelines should be followed.

Pneumonia

Pneumonia can be a problem in all ages of sheep and goats, but the etiology changes with age (Scott, 2011). Acute pneumonia characterized by fever, nasal discharge, coughing, dyspnea and in some cases sudden death is most commonly seen in growing lambs and is in many ways analogous to shipping fever seen in growing calves. The most common pathogens include *Mannheimia haemolytica*, *Bibersteinia trehalosi, Mycoplasma ovipneumoniae*, and parainfuenza virus 3 (PI-3). Clinical disease is most commonly the result of infection with a combination of pathogens. Fortunately, there are a number of antimicrobials licensed for sheep pneumonia in North America including: tilmicosin, florfenicol, ceftiofur and short-acting oxytetracycline. Unfortunately, the long-acting oxytetracycline formulations are not licensed for sheep in North America. Only ceftiofur sodium licensed for goats in the USA and although there is evidence to support many of the products approved for sheep in goats, tilmicosin is toxic in goats and should never be used.

Chronic pneumonia most commonly seen in adult sheep and goats due to pathogens such as Maedi-visna (Ovine Progressive Pneumonia), Caprine Arthritis and Encephalitis virus, caseous lymphadenitis and other forms of chronic abscessation. These diseases are typified by weight loss and exercise intolerance. These conditions do not respond to antimicrobial therapy and affected animals should be culled.

Infectious Abortion

Infectious abortion is a very significant problem in sheep and goats. The agents are shed in the vaginal secretions and the maternal behavior of ewes and does in confinement driving them to lick newborn animals means that the diseases can spread very rapidly within a flock/herd resulting in catastrophic abortion storms. With any case of abortion in small ruminants it is vital to define the etiology so that appropriate control measures can be instigated. The fetus and placenta must be submitted to a diagnostic laboratory to confirm the diagnosis (Menzies, 2011). It is also important to warn the client that most causes of abortion in small ruminants are zoonotic.

Enzootic Abortion/*Chlamydophila abortus*

This pathogen is normally brought into the flock/herd by a carrier animal that aborts at end of its first pregnancy. The agent is shed from the vagina and infects many other animals with a potential abortion storm occurring at the next lambing/kidding. The disease is best prevented by good biosecurity practices and the use of a bacterin vaccine.

In event of abortions, the affected animals should be immediately quarantined. The size of the outbreak can be controlled using oxytetracycline to suppress the pathogen growth on the placenta until the lamb/kid can be delivered at term. In small flocks, this may be achieved using extra-label long-acting oxytetracycline at 20 mg/kg IM administered every 3–5 days. In larger flocks, the only option may be to medicate the feed with a tetracycline product at 200 ppm.

Vibrio-abortion/*Campylobacter fetus Subsp fetus* and *Campylobacter jejuni*

This pathogen is normally introduced to the flock/herd by carrier animals or contaminated feed. The pathogen infects and kills the fetus; the fetus is then typically aborted approximately 2 weeks later. The antimicrobial susceptibility of *Campylobacter* can be variable so culture and susceptibility testing is indicated. Since the fetus has typically been dead for some time when abortion occurs, antimicrobial therapy is not useful for controlling the number of future abortions.

Toxoplasmosis

Toxoplasma gondii can cause abortion at any stage of pregnancy. The disease typically comes from feline species but can also circulate within the flock/herd. The disease can be managed by using in feed antiprotozoal medications such as monensin or decoquinate.

Other less common causes of abortion include: Q-fever, listeriosis, and salmonellosis. All animals that abort should be isolated. Only once a diagnosis is made and antimicrobial susceptibility has been confirmed, should mass medication with antimicrobials may be considered to control the outbreak.

Infectious Keratoconjunctivitis ("Pinkeye")

In sheep and goats, infectious keratoconjunctivitis is most commonly caused by *Mycoplasma* spp. and *Chlamydia* spp., not *Moraxella bovis*. Topical antimicrobial therapy with oxtetracycline ophthalmic ointment is indicated; although when large numbers are affected parenteral oxytetracycline therapy is often used.

Bacterial Pododermatitis

Sheep and goats are prone to a number of bacterial foot problems. It is import to determine the exact cause before commencing treatment (Winter, 2011). Interdigital dermatitis or scald is an inflammation of the interdigital skin caused by *Fusobacterium necrophorum* and is associated with wet and dirty conditions. It is simply managed by moving the herd or flock to drier areas and potentially using a zinc sulfate footbath.

Contagious footrot is caused by strains of *Dichelobacter nodosus*. This is a serious condition resulting in significant pain and inflammation. Ideally, this condition should be managed by eradication. Eradication is difficult and typically involves culling of chronically infected animals; foot trimming, zinc sulfate footbaths and moving the animals to pasture that have not been grazed for 2 weeks. Single injections of long-acting oxytetracycline have also been shown to be effective in treating affected animals.

Contagious ovine digital dermatitis has only been diagnosed in sheep in the United Kingdom. If diagnosed promptly, it has been successfully treated with systemic tilmicosin or topical oxytetracycline or tylosin (Winter, 2011).

Mastitis

There is very little specific evidence on the appropriate antimicrobial treatment of mastitis in sheep and goats, but the topic has been well reviewed

(Mavrogianni et al., 2011). In meat producing herds and flocks, acute disease is commonly managed by the administration of systemic antimicrobials and animals are then culled after weaning. The situation is obviously more complex when animals are used for milk production as mastitis is more common, has a great impact on productivity, and there is the issue of antimicrobial residues in milk.

Mastitis in small ruminants is most commonly caused by either *Staphylococcus aureus* or *Mannheimia haemolytica*, with *Staphylococcus* being more common in milking flocks/herds. It is important to know what pathogens are actually involved on a particular farm so routine culture is vital to determining which antimicrobials should be used. Intramammary treatment can be effective and is commonly employed in milk producing animals. Great care should be taken to ensure that the teat is completely clean before administration and particular care should be taken to avoid damaging the teat sphincter when treating. An entire intramammary infusion tube should be used for treatment of the gland. There are no products licensed for intramammary use in either sheep or goats. Systemic therapy is often used in meat producing animals and in cases where there are systemic signs or the disease has become chronic resulting in occlusion of the ducts within the mammary gland due to inflammatory debris. Macrolides, tetracyclines and trimethoprim all penetrate well into the mammary gland when administered systemically. However, the success rate of treating clinical mastitis in small ruminants is poor, similar in many ways to the situation in cattle. Treatment is often started too late, is not continued for long enough, the wrong antimicrobial was used, or the pathology of the disease prevents adequate antimicrobial concentration at the site of infection. Sheep and goats are prone to severe mastitis infections that result in gangrenous mastitis. Such cases are typified by a hard, swollen, cold mammary gland, which develops a characteristic blue color ("blue bag"). Treatment is routinely unsuccessful and if affected animals survive they should be culled. If the doe or ewe has considerable economic value she will require systemic antimicrobial therapy and intensive supportive care and the affected mammary gland should be amputated.

Extra-Label Drug Use and Residue Avoidance

It is difficult to raise sheep and goats without extra-label use of antimicrobials, as sheep and goats are prone to a number of infectious diseases that require treatment to maintain herd/flock productivity and to ensure animal welfare. Producers and practitioners may be unaware that even "pet" sheep and goats are considered food animals by the regulatory authorities and that extra-label drug use (ELDU) regulations apply to their animals as well (Fajt, 2011). It is vital that veterinarians, agricultural producers and owners of small ruminant pets are aware of ELDU and ensure that the appropriate regulations are followed. The U.S. Animal Medicinal Drug Use Clarification Act and Health Canada's Policy on Extra-Label Drug Use provide guidance (see chapter 26). Such ELDU should always be based on a valid veterinarian-client-patient relationship and involve a written prescription in which the prescribing veterinarian sets appropriate withdrawal intervals for meat and/or milk. In the United States and Canada, prescribing veterinarians can contact their Food Animal Residue Avoidance Databank (United States: www.farad.org; Canada: www.cgfarad.usask.ca) for evidence-based withdrawal information.

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32

Antimicrobial Drug Use in New World Camelids

Christopher K. Cebra and Margaret L. Cebra

Over the last 30-plus years, the populations of llamas and alpacas, the two domestic and most common species of New World camelid, have increased rapidly in North America, Australia, and most recently Europe. The combined numbers of these species are roughly seven million in South America (Peru, Chile, Bolivia, and Argentina), 300,000 in North America, 80,000 in Australia, and 30,000 in Europe. Population growth outside of South America has slowed considerably in recent years, but llamas and alpacas continue to be cherished pets whose owners expect quality health care.

Veterinarians in North America have historically found New World camelids to be medically frustrating, because camelids hide disease signs, physical examination and laboratory evaluation often yield no immediate answers, disease pathogenesis and progression is often unique, and reference material lags behind medical advances. One distinct feature of the sick camelid is that it often has impressive leukogram changes, particularly neutrophilia with or without a left shift. These changes may or may not reflect infectious disease—stress neutrophilia is common and can lead to nucleated cell counts as high as 50,000 cells/ul, as well as moderate increases in band cell counts—but in the absence of other definitive diagnostic information, neutrophilia is often used to justify empirical use of antibiotics. In addition to this empirical approach, there is rising recognition of a number of specific infectious conditions affecting camelids.

The choice of antimicrobial medication is also usually empirical, with broad-spectrum coverage desired. This leads to the next frustration: there is a persistent paucity of disease prevalence data and pharmacokinetic data from New World camelids. Camelids are anatomically and physiologically unique, making any sort of extrapolation dangerous. No medications are approved for use in New World camelids, and dosages found in clinical reports can differ from each other as much as 25-fold. Although camelids are generally considered pets in North America, their rising population coupled with economic issues has led to increasing events of reverting to one of their traditional South American roles, as a meat source. This increases the need for veterinarians to be aware of individual circumstances, consider residues and the legality of using certain medications, and potentially discussing these issues with owners.

A variety of antimicrobial agents have been administered to camelids. Some reasonable dosages for these agents can be devised by examining the available information (Table 32.1). However, most have not been studied scientifically, and the attending veterinarian must assume the responsibility for extra-label drug use and potential adverse effects on the animal. As a general rule, antibiotics appear to have longer elimination half-lives in camelids than in domestic ruminants, potentially prolonging their therapeutic effect but also increasing their risk of toxicity. This may be due to a lower rate of urine production in camelids (Lackey,

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Table 32.1. Common antimicrobial drug dosage in adult New World camelids.[a]

Drug Preparation	Dose	Dose Interval (h)	Route of Administration
Beta-lactams[b,c,d]			
Benzyl penicillins:			
Penicillin G (Na, K)	22,000–44,000 IU/kg	6	IV
Penicillin G (procaine)	22,000–44,000 IU/kg	12–24	IM or SC
Aminobenzyl penicillins:			
Ampicillin sodium	10–20 mg/kg	8–12	IV or IM
Ampicillin trihydrate	10–20 mg/kg	12–24	IM or SC
Third-generation cephalosporin:			
Ceftiofur sodium	2.2–4.4 mg/kg (up to 8 mg/kg q12 h in neonates)	12–24	IV,IM,or SC
Ceftiofur HCl	2.2–4.4 mg/kg	12–24	IM or SC
Ceftiofur CFA	6.6 mg/kg	48–120	SC axilla
Aminoglycosides[e]			
Amikacin	18–21 mg/kg	24	IV,IM,or SC
Gentamicin	4.4–6.6 mg/kg	24	IV,IM,or SC
Fluoroquinolones			
Enrofloxacin[f]	5 mg/kg	12–24	IV,IM,or SC
	10 mg/kg	24	PO
Tetracyclines[b,c]			
Oxytetracycline (100 mg/ml)	10 mg/kg	12–24	IV
Oxytetracycline (200 mg/ml)	20 mg/kg	24–72	IM or SC
Other			
Florfenicol[g]	20 mg/kg	24–48	IM or SC
Metronidazole[h]	15–25 mg/kg	8–12	PO or per rectum
Trimethoprim-sulfomethoxazole	18 mg/kg (comb.)	12	IV,IM,or SC

[a]Although these medications at these dosages have been used repeatedly for camelid patients in referral hospitals in North America, pharmakokinetic data are lacking for most of these agents in camelids, as are safety studies. All medications must be used with caution in camelids and the patient must be monitored carefully for adverse reactions or toxic effects.
[b]The higher dosages and/or shorter dosing intervals are indicated in camelids with more severe infections.
[c]The higher dosages and/or shorter dosing intervals may be indicated in alpacas or llamas with lower gastric fill or body fat.
[d]The higher dosages and/or shorter dosing intervals may be indicated in young camelids.
[e]The large differences in volume of distribution between individual camelids and risk of nephrotoxicosis with overdose support caution in the use of this medication at any dose, especially the higher dosages, and especially in camelids with decreased urine production.
[f]Should not be used in young growing camelids because of the risk of arthropathy.
[g]Forty-eight-hour dosing may be adequate for highly sensitive organisms, such as those typically present in tooth root abscesses. For more general antimicrobial coverage, daily dosing may be required.
[h]Oral dosing in juvenile and adult camelids will affect the gastric microbial population. Per rectum administration is preferred in these age groups.

1995), which may increase half-life of antibiotics excreted primarily through the kidneys (e.g., penicillins, aminoglycosides). This slower renal excretion in turn may be affected by concurrent fluid treatments, such that camelids in referral hospital situations may be treated similarly to domestic ruminants, whereas camelids treated in the field must be dosed more conservatively. As another general rule, volume of distribution varies tremendously among individual camelids. Higher dosages are generally recommended to avoid subtherapeutic drug concentrations in some camelids. Thus, the most useful antibiotics are those with a high margin of safety. Pharmacokinetic data for selected antimicrobials in llamas and alpacas are presented in Table 32.2.

A survey of the recent scientific literature suggests that the most commonly used antibiotics in New World camelids are the beta-lactams. The different formulations of ceftiofur predominate, followed by crystalline

Table 32.2. Pharmacokinetic data for selected antimicrobials in llamas and alpacas.

Agent	Species	Dose (mg/kg)	Route	Vol Dist (L/kg)	Clearance (ml/min/kg)	Elim Half-Life (Hours)	AUC (μg h/ml)	Peak Conc (μg/ml)	Time to Peak (Hours)
Ampicillin[a]	Llama	12	IV	0.28 ± 0.09	0.88 ± 0.28	3.33 ± 0.50	228 ± 73		
Ceftiofur[a]	Llama	2.2	IV	0.19 ± 0.02	0.98 ± 0.15	2.19 ± 0.14	38.4 ± 5.8		
Ceftiofur[b]	Llama	2.2	IM	0.61 ± 0.19	1.03 ± 0.41	8.00 ± 1.85	40.1 ± 12.9	5.52 ± 1.11	0.77 ± 0.56
Ceftiofur[b]	Llama	2.62–2.99	IM	0.61 ± 0.20	0.97 ± 0.36	8.81 ± 3.04	54.8 ± 20.8	6.33 ± 2.20	0.91 ± 0.55
Ceftiofur[b]	Alpaca	1	IV	0.54 ± 0.15	1.36 ± 0.39	5.60 ± 1.57	13.4 ± 4.4		
Ceftiofur[b]	Alpaca	1.27–1.44	IV	0.55 ± 0.18	1.44 ± 0.37	4.62 ± 1.18	14.6 ± 3.1		
Ceftiofur[b]	Alpaca	1	IM	0.57 ± 0.12	1.12 ± 0.36	4.31 ± 1.35	15.4 ± 5.1	2.09 ± 0.42	0.49 ± 0.16
Ceftiofur[b]	Alpaca	1.30–1.51	IM	0.64 ± 0.14	1.15 ± 0.27	7.42 ± 1.41	20.9 ± 3.9	3.52 ± 0.47	0.5 ± 0.0
Ceftiofur CFA[c]	Alpaca	6.6	SC	4.06 ± 2.18		64.6 ± 31.4	199 ± 42	2.65 ± 0.85	36
Ceftiofur CFA[c]	Alpaca	6.6	SC q5d	4.18 ± 1.14		52.4	217 ± 85	1.97 ± 0.44	17 ± 16
Enrofloxacin[a]	Llama	5	IV	3.46 ± 0.98	11.67 ± 3.5	3.38 ± 2.13	7.0 ± 2.3		
Enrofloxacin[d]	Alpaca	5	IV	0.44 (0.32-1.07)	84.5 (41.5–115.7)	13.0 (6.3–46.6)	58.4 (43.2–120.6)		
Enrofloxacin[d]	Alpaca	5	SC			7.83 (3.4–15.6)	41.9 (33.5–89.0)	4.2 (1.5–1.7)	6.0 (4.0–8.0)
Enrofloxacin[d]	Alpaca	10	PO			15.3 (8.3–25.0)	32.5 (29.3–42.7)	1.4 (0.8–4.0)	4.0 (0.5–8.0)
Florfenicol[e]	Alpaca	40	SC			99.7 ± 59.9	99.8 ± 23.6	1.95 ± 0.94	2.50 ± 1.07
Florfenicol Gold[e]	Alpaca	40	SC			41.6 ± 21.9	125.2 ± 38.2	7.54 ± 3.62	2.81 ± 1.21
Florfenicol[f]	Alpaca	20	IM	11.1 ± 8.1	6.73 ± 1.55	17.6 ± 11.7	51.8 ± 11.7	4.31 ± 3.03	1.00 ± 0.65
Florfenicol[f]	Alpaca	40	SC	55.7 ± 25.9	7.04 ± 1.75	99.7 ± 59.9	99.8 ± 23.6	1.95 ± 0.94	2.50 ± 1.07
Florfenicol[f]	Alpaca	40	SC q2d			90.2 ± 55.5		4.48 ± 1.28	2.50 ± 0.93
Gentamicin[g]	Llama	4	IV	0.12	0.51	3.03	125.7		
Gentamicin[h]	Llama	5	IV	0.25 ± 0.03	1.10 ± 0.14	2.77 ± 0.34	77.3 ± 10.3	38.2 ± 12.3	
Sulfamethoxazole + TMP[a]	Llama	15	IV	0.46 ± 0.08	1.33 ± 0.47	4.28 ± 0.53	187 ± 47		
Sulfamethoxazole + TMP[i]	Alpaca	12.5	IV	0.35 ± 0.09	1.90 ± 0.77	2.20 ± 0.60	124.4 ± 64	158.3 ± 189.3	
Sulfamethoxazole + TMP[j]	Llama	45	PO			4.0 (3.2-7.2)	34.1 ± 12.8	3.9 ± 1.5	2 (2–4)
Sulfadimethoxine[k]	Llama	55.6–62.4	IV	0.44 ± 0.05	0.73 ± 0.22	9.4 ± 2.0	1403 ± 311	237 ± 27	
Sulfadimethoxine[k]	Llama	50.2–72.4	PO			11.7 ± 6.7	765 ± 210	21.7 ± 14.1	17.6 ± 9.2

(*continued*)

Table 32.2. Pharmacokinetic data for selected antimicrobials in llamas and alpacas. (*continued*)

Agent	Species	Dose (mg/kg)	Route	Vol Dist (L/kg)	Clearance (ml/min/kg)	Elim Half-Life (Hours)	AUC (μg h/ml)	Peak Conc (μg/ml)	Time to Peak (Hours)
Tobramycin[a]	Llama	1	IV	0.14 ± 0.05	0.43 ± 0.07	3.68 ± 1.26	39.5 ± 6.6		
Trimethoprim + SMX[i]	Alpaca	2.5	IV	2.33 ± 1.15	21.63 ± 9.85	0.74 ± 0.1	364 ± 4.45	10.75 ± 2.12	
Trimethoprim + SMX[a]	Llama	3	IV	0.40 ± 0.15	1.4 ± 1.1	3.31 ± 0.56	39.9 ± 16.6		
Trimethoprim + SMX[j]	Llama	9	PO				undetectable	undetectable	
Voriconazole[l]	Alpaca	4	IV	1.19 ± 0.14	1.82 ± 0.42	8.01± 2.88	33.9 ± 5.2	5.93 ± 1.13	
Voriconazole[l]	Alpaca	4	PO	7.11 ± 5.41	9.43 ± 4.57	8.75 ± 4.31	8.76 ± 6.80	1.70 ± 2.71	5.37 ± 3.36

[a]Christensen et al., 1996.
[b]Drew et al., 2004
[c]Dechant et al., 2012.
[d]Gandolf et al., 2005.
[e]Bedenice et al., 2012.
[f]Holmes et al., 2011.
[g]Dowling et al., 1996.
[h]Lackey et al., 1996.
[i]Chakwenya et al., 2002.
[j]Snook et al., 2002.
[k]Junkins et al., 2003.
[l]Chan et al., 2008.

(sodium or potassium) and procaine penicillin. These reports are probably skewed toward referral hospital use, particularly in regards to crystalline penicillin and repeated intravenous dosing, but use of this class of antibiotic is also common in practice. Ampicillin, amoxicillin, and other cephalosporins have seen limited use. The beta-lactam antibiotics, particularly ceftiofur products, are used as single agents, or less frequently in combination, usually with an aminoglycoside. Of the aminoglycosides, gentamicin is most commonly reported, with amikacin used mainly in crias and via regional perfusion. Oxytetracycline, florfenicol, and enrofloxacin account for most of the rest of the reports. Enrofloxacin is used in a variety of situations, usually to replace an aminoglycoside in Gram-negative coverage. Oxytetracycline, florfenicol, and ceftiofur crystalline free acid are most commonly used against selected organisms, such as oxytetracycline versus *Mycoplasma haemolamae*, when a lengthy course makes infrequent injections preferable, or when repeated dosing is impractical.

Ceftiofur sodium has been studied in both llamas and alpacas, and also has the broadest range of dosages in clinical reports (Christensen et al., 1996; Drew et al., 2004). The two main studies provide conflicting information concerning the volume of distribution at steady state and half-lives, but similar information concerning clearance and area under the curve. Additionally, individual camelids in the larger study had volumes of distribution at steady state that differed by up to 100%. Thus while the larger study reports pharmacokinetic parameters similar to those seen in small ruminants, twice-daily dosing to adults at 2.2 mg/kg intravenously or intramuscularly is recommended to avoid subtherapeutic concentrations in the camelids with greater volumes of distribution. Subcutaneous administration at the same dose and interval has become popular, and is empirically successful, but has not been studied scientifically. Higher doses (4–8 mg/kg, IV, IM, or SC q 12 h) have been used in crias up to around 12 weeks of age and in adults for which more aggressive antibiotic protocols were deemed necessary (Buchheit et al., 2010; Simpson et al., 2011); no complications have been reported with these higher doses, but they also have not been studied scientifically. Ceftiofur hydrochloride is also being used somewhat interchangeably with ceftiofur sodium by the intramuscular and subcutaneous routes without reported problems (Lewis et al., 2009). Ceftiofur crystalline free acid is also seeing increasing clinical use where long-acting coverage is desired (Jones et al., 2009). A recent study demonstrated that a single 6.6 mg/kg subcutaneous injection in the axillary region resulted in plasma concentrations of ceftiofur and active metabolites that remained above 0.25 μg/ml for 6 days in adult alpacas, but also suggested that higher concentrations were necessary to be effective against the majority of recent bacterial isolates (Decant et al., 2012). Fifty-four percent of Gram-positive and 27% of Gram-negative isolates from camelids showed sensitivity to ceftiofur concentrations ≤ 0.25 μg/ml, 71% of Gram-positive and 45% of Gram-negative isolates showed sensitivity to concentrations ≤ 0.5 μg/ml, and 88% of Gram-positive and 64% of Gram-negative isolates showed sensitivity at ≤ 1.0 μg/ml. Thus, every 2- to 5-day dosing may be necessary to achieve true broad-spectrum coverage.

Single doses of ceftiofur crystalline free acid were well tolerated, but repeated dosing led to local, non-painful reactions in half the test alpacas. Ceftiofur is usually highly protein-bound, which affects its distribution. Hypoproteinemia is common in sick camelids, but how this affects distribution and other pharmacokinetic parameters has not been tested.

Even though penicillin is commonly used in New World camelids, no pharmacokinetic studies are available, and most dosages are extrapolated from other large animal species. Crystalline penicillin (22,000 U/kg, IV, q 6 h) or procaine penicillin (22,000 U/kg, SC or IM, q 12 h) are the most commonly used products. Doses are often doubled when *Clostridium* or similar pathogens are suspected. Adverse reactions are reported rarely, most commonly either acute procaine-type reactions or rarely hypersensitivity-type reactions after prolonged use. Clinical efficacy appears to be satisfactory. Ampicillin is excreted by similar renal mechanisms and has a similar half-life as penicillin G in other species. However, the half-life of ampicillin in llamas is 2–4 times longer than in horses or sheep, respectively, its volume of distribution at steady state is about 50% greater than sheep and about the same as horses (Christensen et al., 1996). The longer half-life may be the result of low urine production, which could prolong the action of renally excreted medications, and suggests that lower dosages or less frequent dosing intervals of

the penicillins in camelids may achieve sufficient therapeutic effect. Treating the camelid with fluids during the course of antibiotic treatment may negate this effect by enhancing excretion.

Gentamicin sulfate, or the similar compound, tobramycin, has been studied in llamas and camels (Christensen et al., 1996; Dowling et al., 1996; Hadi et al., 1994; Lackey et al., 1996). Again, conflicting information concerning volume of distribution was generated from the different studies, with the largest study reporting volumes of distribution at steady state that differ up to 150% between animals. Because aminoglycoside antibiotics generally have poor lipid solubility and move into the extracellular space slowly, these differences in volume of distribution could relate to differences in gastric fill and body fat in the individual camelids. The studies agree on prolonged elimination half-lives of around 3 hours.

As with other species, once-daily dosing of camelids with aminoglycosides has become more popular than more frequent dosing. The rationale for this is to allow trough concentrations to drop below 2.0 μg/ml to prevent nephrotoxicity, while at the same time maintaining efficacy due to the agent's post-antibiotic effect. Because of camelids' slow elimination of aminoglycosides, this strategy appears to be especially valid. Dosing camelids at 2.5 mg/kg intravenously maintains concentrations above the toxic threshold for at least 6 hours after each dose in many camelids (Lackey et al., 1996), which would represent a high risk for toxicosis with 3-times-a-day dosing. Dosing at 4.0–5.0 mg/kg maintains concentrations above the toxic threshold for about 12 hours (Dowling et al., 1996; Lackey et al., 1996), and also provides peak concentrations necessary for antimicrobial activity.

Nephrotoxicoses in camelids administered aminoglycosides both once a day and more frequently have been reported in the literature (Hutchison et al., 1993) and anecdotally. The slow elimination, spare urine production, and extreme variability in volumes of distribution potentially make camelids very susceptible to relative overdose, especially when they are dehydrated or drink insufficient water. Such problems have not been reported (scientifically or anecdotally to the authors) in camelids administered aminoglycosides and concurrent intravenous fluids. Thus, it is especially important to ascertain hydration status before administering aminoglycosides to camelids and during the course of treatment.

Ceftiofur sodium and gentamicin appear to have similar enough pharmacokinetic properties when given intravenously or intramuscularly, that the same dosage and dosing frequency may be used for either route. Additionally, recent evidence from other species suggests that many antibiotics have comparable absorption from the subcutaneous route. The subcutaneous route has become very popular in camelids for all antibiotics formerly administered intramuscularly due to lack of large muscle masses and ease of administration. Unless a very fast effect is desired or the particular antibiotic is known to cause adverse reactions when given subcutaneously, this route is considered acceptable.

Intravenous sulfa antibiotics and trimethoprim-sulfa combinations have been studied and used on clinical cases. The trimethoprim studies show poor agreement. In alpacas, trimethoprim has a large volume of distribution and rapid clearance, similar to the rat, and rapidly drops to subtherapeutic concentrations (Chakwenya et al., 2002). Trimethoprim (3 mg/kg) in llamas acts more similarly to the horse, resulting in plasma concentrations > 1 μg/ml for up to 12 hours; its reported volume of distribution is smaller than many other species (Christensen et al., 1996). Thus, trimethoprim appears to offer little advantage in the alpaca, but may be useful in llamas against sensitive microorganisms.

Sulfamethoxazole acts more similarly in llamas and alpacas (Christensen et al., 1996; Chakwenya et al., 2002), but with a smaller volume of distribution and faster clearance in alpacas. Camelids are fairly similar to sheep and cattle in these regards, with considerably faster clearance than horses or people. Active secretion into the renal tubules and trapping of the excreted agent in alkaline urine may contribute to this rapid clearance. It may also reflect a significant difference between New World camelids and camels, which are reported to have acidic urine and slow sulfa clearance (Kumar et al., 1998). Injectable sulfamethoxazole may have some value in treating sensitive infections, especially in llamas.

At metabolically scaled doses of 55–62 mg/kg, sulfadimethoxine in llamas has a higher volume of distribution at steady state and a shorter half-life than in cattle, and also may reach only subtherapeutic blood concentrations (Junkins et al., 2003; Boxenbaum et al., 1977). Similar volumes of distribution and peak concentrations and even faster clearance have been described in dromedary camels (Chatfield et al., 2000). No

evaluation of higher doses has been described, but a metabolically scaled dose of 69 mg/kg was suggested for camels, and a higher, and possibly unsafe, dose might be necessary to reach desirable concentrations in New World camelids. The role of protein-binding of sulfadimethoxine on clearance has been investigated in other species, and shown to be essential to the long half-life (Bevill et al., 1982). This has not been investigated in camelids, but the likelihood that hypoproteinemia, a common finding in sick camelids, will alter drug excretion must be considered. For these reasons, intravenous sulfadimethoxine appears unsuitable for most antimicrobial applications in camelids.

Florfenicol use has become more popular, especially in the treatment of tooth root abscesses, and other conditions where a longer dosing interval is desirable. Recent clinical and experimental results suggest its value may be greatest against highly susceptible pathogens in focal infections rather than as a broad-spectrum treatment. This relates to its pharmacokinetic properties and the potential adverse effects.

Intravenous florfenicol had a lower volume of distribution in New World camelids than in sheep, goats, or camels, and a slightly longer half-life than in sheep or goats; a 20 mg/kg dose yields plasma concentrations > 1 μg/ml in both llamas and alpacas for about 12 hours (Ali et al., 2003; Christensen et al., 2001). When given intramuscularly at 20 mg/kg, peak plasma concentrations are achieved quickly, and are comparable between llamas and beef cattle, and higher in alpacas (Holmes et al., 2011). Elimination also appears to be similar to cattle or prolonged, but plasma concentrations generally drop below 1 μg/ml within 14–24 hours. As with other medications, the variation between individual adult camelids is considerable: volume of distribution after intravenous administration ranged from 0.25 to 2.54 L/kg in one study, and peak plasma concentrations after 20 mg/kg intramuscular injection were 4.3 ± 3 μg/ml in another. Single dose subcutaneous administration over the dorsal thorax takes slightly longer (2–3 hours) to reach a lower peak, followed by an extensive elimination phase (Holmes et al., 2011). Regardless of whether 20 or 40 mg/kg is given subcutaneously, plasma concentrations are generally below 1 μg/ml within 18–24 hours.

The long elimination half-lives (31–100 hours) following subcutaneous injection may reflect important considerations related to camelid skin. Subcutaneous injections of more than a few ml are usually given to llamas and alpacas over the dorsal thorax. In contrast, subcutaneous florfenicol in cattle is specifically supposed to be administered in the neck. Camelids' dorsal thoracic skin is seasonally covered with fiber, and plays little role in thermoregulation. It is poorly vascularized compared to axillary skin, potentially slowing the uptake of agents injected there, and yielding a long elimination half-life that is actually a reflection of slow absorption. Serial injections may increase blood flow and eventually lessen the differences between the intramuscular and subcutaneous routes (Holmes et al., 2011). Administering florfenicol, or any other medication, in the axillary region may result in faster peaks and a shorter elimination half-life, but this has not been tested.

The recent studies suggest that daily intramuscular florfenicol (20 mg/kg) may be effective against very to moderately sensitive microorganisms, which do not include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and many Gram-negative enteric bacteria. An ideal subcutaneous regimen has not been described. Lower doses (20 mg/kg, SC, q 24 h) may become adequate against sensitive pathogens, if greater absorption with serial dosing may be inferred. Higher doses (40 mg/kg, SC, q 24 h) maintain therapeutic steady-state concentrations, but are associated with evidence of toxicity, including reductions in blood proteins and cell counts, abnormal feces, and clinical disease.

Intravenous and subcutaneous enrofloxacin, and intravenous and intramuscular oxytetracycline have also been studied in llamas and alpacas. Intravenous oxytetracycline in llamas had a similar volume of distribution to camels, but a much longer half-life (Oukessou, 1992). Alpacas had a larger volume of distribution, but a similar half-life to camels. Subcutaneous administration is common in clinical practice, particularly for the treatment of *Mycoplasma haemolama* or *Anaplasma phagocytophilum* infection, but has not been evaluated scientifically, and is likely to have the same pitfalls as subcutaneous florfenicol. Preparations using a propylene glycol carrier are anecdotally associated with more local muscle irritation, shaking, and collapse than those using polyvinylpyrrolidone (povidone); reactions with either carrier are rare in New World camelids.

Enrofloxacin reaches therapeutic concentrations after intravenous or subcutaneous dosing, but conflicting information regarding its half-life is available (Christensen

et al., 2001; Gandolf et al., 2004). There is a single report of retinopathy after enrofloxacin administration to a guanaco (Harrison et al., 2006).

A variety of other parenteral antibiotics have been used in individual camelids without full knowledge of safety or efficacy. For the most part, reasonable extrapolation can be made from similar species, with caution remaining the overarching principle. As an example of this, tilmicosin, which is labeled for use in cattle and sheep, but reported to have cardiotoxic effects in horses and goats, is also reported to have toxic effects in New World camelids (Lakritz et al., 2012).

Oral antibiotics have been studied less extensively than injectable preparations. Adult camelids should be expected to have similar problems with absorption as adult ruminants, and several studies demonstrate the flip-flop phenomenon, where apparent prolonged elimination actually reflects prolonged absorption. Trimethoprim, sulfamethoxazole, and sulfadiamethoxine antibiotics appear to have poor absorption at ruminant dosages and cannot be recommended for systemic disorders (Chakwenya et al., 2002; Junkins et al., 2003; Snook et al., 2002). Trimethoprim is virtually undetectable in the blood after oral dosing, and ion-trapping of sulfas, which is a relatively greater problem in a forage-fed camelid versus a steer on an acidifying feedlot ration, and likely to be worse in any ruminant or camelid with inappetance and relative forestomach alkalinization, may contribute to their poor absorption.

Oral tetracycline, amoxicillin-clavulanic acid, isoniazid, and chloramphenicol use have also been reported, but no pharmacokinetic studies have been performed. Oral enroflaxacin has a 29.3% bioavailability and reaches therapeutic concentrations after dosing at 10 mg/kg (Gandolf et al., 2004). Oral antibiotics might be more useful in pre-ruminant camelids, but this usage has not been investigated.

One topic that has received attention in recent years is the dosing difference between llamas and alpacas. Pharmacokinetic studies have approximately followed species popularity, with the earlier studies concerning llamas and more recent studies concerning alpacas, though the greater availability of llamas as test subjects has influenced the continued appearance of studies on llamas. Few studies have compared the two species, which recently have been declared members of separate genera.

Data from studies involving glucose indicate that adult alpacas have an extracellular (interstitial) fluid compartment that is approximately 37% larger than adult llamas (Cebra et al., 2006a). This is similar to the difference in volume of distribution for oxytetracycline found in one study (Christensen et al., 2001), whereas the volume of distribution for ceftiofur is reported to be 2.5–3 times larger in alpacas than llamas (Drew et al., 2004; Christensen et al., 1996).

A physical basis for the difference in volumes of distribution is found in the contributions of various organs to whole body weight. The full gastric viscera of llamas make up approximately 4% more of whole body weight in llamas than alpacas, meaning alpacas generally have proportionally more soft tissue and interstitial fluid (Cebra et al., 2006b). Very lipophilic compounds such as florfenicol distribute into the gastric compartments and hence have similar volumes of distribution between llamas and alpacas, whereas hydrophilic compounds do not, and are hence distribute over a proportionally larger volume in alpacas than in llamas. Dosage adjustment may be necessary, and has been demonstrated with oxytetracycline. Aminoglycoside antibiotics, which, although hydrophilic, appear to diffuse more slowly out of the vascular compartment would be less affected by this, and hence should not be dosed higher in alpacas.

The same argument can be used to adjust dosages for younger camelids. Glucose studies suggest that unweaned llama crias between 2 and 4 weeks of age have an extracellular fluid compartment that is approximately 30% larger than adult llamas (Cebra et al., 2005). Unfortunately, the importance of this difference in antimicrobial dosages has not been investigated.

Compared with many other common domestic species, much less information is available concerning the frequency and importance of bacterial isolates. A table of what has been seen at Oregon State University and what is available in the scientific literature is included (Table 32.3). Others have compiled similar findings from other institutions in unpublished formats (Dechant et al., 2012; Anderson, 2009). Sufficient data were not available to derive meaningful *in vitro* susceptibility conclusions. Since many of these bacteria are opportunists, they would likely have similar sensitivity profiles to isolates from other species. Of particular note here are the α-hemolytic streptococci, which often are resistant

Table 32.3. Bacterial isolates from camelid lesions at the Oregon State University Veterinary Diagnostic Laboratory and in selected scientific publications. Non-OSU cases are listed in parentheses.

	Wound or Superficial Lesion[a]	Tooth Root Abscess[b]	Female Repro Tract	Sepsis Adult[c]	Sepsis Cria[d]	Soft Tissue Abscess[e]	Myositis[f]
Gram-positive							
Staphylococcus spp. (coagulase –)	1	(1)	3	1			
Staphylococcus spp. (coagulase +)	5				1		
Non-hemolytic *Streptococcus*				(5)	(1)		
α-hemolytic *Streptococcus*	(2)	1		3 (5)	1		
β-hemolytic *Streptococcus*	3		1	5 (7)	3	2 (1)	2
Enterococcus spp.			5	2	1 (4)		(1)
Rhodococcus equi						(1)	
Clostridium spp.		(2)		6 (5)		(1)	1 (2)
Actinomyces spp.	1 (1)	7 (57)		1 (1)		7 (1)	
Peptostreptococcus	1	2 (5)		(1)			
Arcanobacterium pyogenes			2			1	
Listeria monocytogenes				2 (6)	(4)		
Bacillus spp.			2	(1)			
Corynebacterium pseudotuberculosis						(89)	

[a]Stone, 1993; Watt, 2000.
[b]Cebra, 1996; Coyne, 1995; Niehaus, 2007.
[c]Anderson, 1995; Bedford, 1996; Butt, 1991; Cebra, 1998; Firshman, 2008; Fowler, 1992; Hamir, 2000; Hewson, 2001; Hutchison, 1992; Jones, 2009; McLane, 2008; Middleton, 2006; Pearson, 2000; Quist, 1998; Ramos-Vara, 1998; Saulez, 2004; Seehusen, 2008; Sivasankar, 1999; Stone, 1993; Tillotson, 1997; Tyler, 1996; Underwood, 1992; van Metre, 1991.
[d]Adams, 1992; Anderson, 1995; Cebra, 2000; D'Alterio, 2003; Dolente, 2007, Frank, 1998; Parreno, 2001; Sura, 2008.
[e]Anderson, 2004; Aubry, 2000; Braga, 2006; D'Alterio, 2003; Dwan, 2008; Hong, 1995; Koenig, 2001; St. Jean, 1993; Talbot, 2007.
[f]Burkhardt, 1993; Tyler, 1996; Uzal, 2000.

to penicillin and may be the cause of some treatment failures. Also of note are the increasing published and anecdotal reports of *Salmonella* and *Streptococcus equi* subspecies *zooepidemicus* infection (Tillotson et al., 1997; Saulez et al., 2004; Middleton et al., 2006; Hewson et al., 2001; Jones et al., 2009). These last microorganisms are primary pathogens in camelids, and may affect multiple, healthy camelids on one property. With *Salmonella*, camelids may also be involved in multispecies outbreaks. *Corynebacterium pseudotuberculosis* is another primary pathogen involved in multispecies outbreaks that is being increasingly recognized as a cause of peripheral or internal lymph node abscessation in camelids. As the popularity of camelids increases, the danger of transmissible diseases and cross-species transmission also increases. This includes increasing risk of transmission to people, particularly with microorganisms such as *Salmonella*, *Listeria*, and *E. coli* O157 (Featherstone et al., 2011).

As stated above, specific localized syndromes (such as bacterial pneumonia or enteritis) are rare, so aside from the chronic, focal infections, most bacterial diseases have been grouped together as septic conditions. These animals usually present with general systemic signs including fever, inappetance, obtundation, and weakness, but may also have specific signs referable to the affected

organs. As a general conclusion, the relative equality between Gram-negative and Gram-positive isolates from wounds and camelids with sepsis supports the initial use of broad-spectrum antibiotics. Combinations of an aminoglycoside with a beta-lactam antibiotic, or of ceftiofur alone or in combination are most common. Other single medications, such as oxytetracycline, enrofloxacin, or florfenicol may be useful in some situations. Collection and culture of pertinent body fluids (blood, peritoneal fluid, pleural fluid, cerebrospinal fluid, urine, feces, aspirates, etc.) may yield information about specific pathogens and allow refinement of antibiotic selection.

Female reproductive tract infections frequently involve Gram-negative enteric bacteria and are frequently treated with gentamicin infusions, whereas tooth root abscesses and other tissue abscesses more frequently involve Gram-positive or anaerobic bacteria and are most commonly treated with long courses (20–60 days) of penicillin, ceftiofur, or florfenicol. The camelid blood parasite, *Mycoplasma haemolamae*, is most commonly treated with long-acting oxytetracycline preparations. Chapters on the individual antibiotics should be consulted for additional information concerning use and specific contraindications.

A variety of fungal diseases are reported in camelids, but only a single antifungal study has been conducted (Chan et al., 1998). Systemic mycoses include aspergillosis, cocciodiomycosis, cryptococcosis, histoplasmosis, and mucormycosis. Dermal or superficial mycoses include candidiasis, ringworm, and entomophthoramycosis.

Voriconazole given intravenously (4 mg/kg) maintains plasma concentrations > 0.1 μg/ml for at least 24 hours (Chan et al., 1998). Clearance and volume of distribution are comparable to horses but lower than in humans. Absorption after oral dosing is noted within 5 minutes, but bioavailability is less than 23%. Absorption is generally slow and unpredictable, and the need for higher doses has been postulated. Other systemic antifungal use has been largely extrapolated from ruminants. Agents used empirically include fluconazole (14 mg/kg, IV or PO as a loading doses, followed by 5 mg/kg, q 24 h), itraconazole (5 mg/kg, IV or PO, q 24 h), and amphotericin B (0.5 mg/kg diluted in 0.5–1 L of 5% dextrose IV over 1 h, q 24; after pretreatment with flunixin meglumine [0.25 mg/kg, IV]). Miconazole and clotrimazole have been used topically for fungal dermatitis. As with antibacterial use, all antifungal use in camelids is extra-label.

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33

Antimicrobial Drug Use in Swine

David G.S. Burch

Introduction

Antimicrobial drug use in swine has always been substantial and in some cases, the swine industry has been considered overreliant on their use. As farms have evolved from small back-yard operations, only 50 years ago, to today's substantially larger units, it is not surprising that antibiotics have been used to help farmers maintain their production under these major management, housing and disease pattern changes. It must be remembered that antibiotics have a cost and that farmers are unlikely to pay for them unless they can see a benefit from their use.

Swine farming has made great steps forward in management and husbandry systems to reduce antimicrobial use. Examples are the improvement of biosecurity (keeping diseases out), sourcing of high-health genetic stock and the utilization of 3-site production. The latter enables "all-in and all-out" procedures to be followed with the accompanying improvements in hygiene and infection control and the prevention of back infection from older pigs on the farm. Unlike the broiler industry, which pioneered this system and where stock is reared for only 5–6 weeks before slaughter, pigs are raised for nearly 6 months, making it not so easy to follow. In farrow-to-finish operations, often family farms, which are still the most common operation, there is still a need for antimicrobial medication to assist with health and production, in spite of the use of vaccination, as not all diseases can be controlled sufficiently that way.

The concerns regarding antimicrobial use in animals and their relation to man regarding resistance transmission via zoonotic bacteria such as *Salmonella* spp., *Campylobacter coli*, and more recently methicillin-resistant *Staphylococcus aureus* (MRSA) or more indirectly via *Escherichia coli* or *Enterococcus* spp. is under huge review at the moment and a number of changes can be anticipated over the next 5 years. In the United States there are calls to ban the use of antibiotic growth promoters and only use antimicrobials for prevention and treatment (chapter 22). The European Union (EU) banned the use of antimicrobial growth promoters in 2006, and now there are calls from the European Parliament to go a step further and stop all prophylactic use. The United States has banned the use of fluoroquinolones in poultry (drinking water use) but permitted the use of injectables in swine, but in Europe there are calls to ban the use of all fluoroquinolones and third- and fourth-generation cephalosporins in veterinary medicine. The United States has already put restrictions on the use of all cephalosporins to "on-label" use only and in the EU the cessation of use is being implemented for third-generation cephalosporins, such as ceftiofur, in the poultry industry. There was a high level of extended-spectrum beta-lactamases (ESBLs) found in *E. coli*, on average 8.5% (range 0–26.4%), from chickens (Anon., 2011). It was thought to be associated with the extensive use of injections *in ovo* and in day-old-chicks, even

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though it was not an approved use. By comparison, ESBL production in porcine *E. coli* in the EU was low at about 2.3% (range 0–3.8%; Anon., 2011). Contamination of both broiler and pig meat with ESBL-producing *E. coli* was also reported, hence the public health concern.

In the face of all this, the swine industry in conjunction with other industries is advocating the responsible use of antimicrobials via various bodies such as the UK's Responsible Use of Medicines Alliance (RUMA), the EU via EPRUMA and the United States via their National Pork Board. This is seen as the only way forward to maintain the availability of antimicrobials, which are needed in veterinary medicine to treat and prevent disease and to maintain the health and welfare of the animals under their care. At the same time, efforts are being made to reduce unnecessary use and the use of critical human antibiotics where suitable alternatives are available. In the EU antimicrobial drugs are prescription-only medicines (POMs) and used under veterinary supervision or prescription. In the United States, their use is much freer, in that many drugs can be used in feed without a prescription but their inclusion level is normally regulated by the feed mill. This may also change in the future. In some EU countries, the use of antimicrobials in feed is being stopped; for example, in the Netherlands, in an attempt to reduce overall antimicrobial usage, and it is strongly restricted in Germany and Denmark. However, neither the third- and fourth-generation cephalosporins nor fluoroquinolones are used in feed in the EU.

Responsible use calls for vets and farmers to use antimicrobials "as little as possible but as much as needed." There are a number of guidelines on how to use antimicrobials properly and it is the aim of this chapter to help with the decision making regarding the right antibiotic for the right infection, to be administered at the right dose via the appropriate route.

Antimicrobial Administration in Swine

In general, the injection of pigs, other than baby piglets, is laborious and is used primarily to treat clinically ill pigs either with acute respiratory infections such as *Actinobacillus pleuropneumoniae* or enteric infections such as swine dysentery caused by *Brachyspira hyodysenteriae*, which may be too sick to eat or drink. It is a highly effective route of administration to an individual animal, but many antibiotics require repeated injections on a daily basis. The development of long-acting formulations has improved the issue regarding compliance, to complete the course of treatment. There has been an increase in the prophylactic use of third-generation cephalosporins in baby piglets to prevent a variety of wound infections post-processing (tail docking and castration) and also early infections with *Streptococcus suis* and *Haemophilus parasuis*. This may have triggered the selection of MRSA clones (specifically CC398), which has become widespread on the continent of Europe (Anon., 2009) and also in North America (Khanna et al., 2008). It has also probably led to the very high level of resistance (41.8%) to ceftiofur that has been reported in isolates of porcine *E. coli* from clinical cases in the United States (Frana et al., 2012).

Oral administration of antibiotics is the most common route of application in swine medicine. Piglets may be treated individually by oral dosers containing antibiotics. These have proved most useful for the control of neonatal usually associated with *E. coli* and gut active and systemically active formulations of enrofloxacin, trimethoprim/sulfonamide and amoxycillin have proved very effective. Later on, when the infection is primarily in the gut, gut-active antibiotics, such as the aminoglycosides neomycin, aminocyclitol spectinomycin and the polymixin colistin, are widely used. Toltrazuril is also highly effective for the prevention and treatment of coccidiosis caused by *Isospora suis* in piglets.

Water medication is widely used and in some countries becoming more popular due to the introduction of effective automatic dosing machines. In the past it was limited to pen troughs or individual pen tanks. Larger header tanks allowed whole sheds/barns to be treated but in some cases it was difficult depending on the size of tank and frequently required 2–3 applications of a drug each day to ensure an adequate intake and duration of activity. Automatic water proportioner machines, where the antibiotic is dissolved in a concentrate, which flows into the main water system at a set target rate (approximately 1–2%, depending on the solubility of the drug) have proved most popular on larger sites. However, the antibiotic needs to be sufficiently soluble. In many cases the advantages of prompt treatment, controlled dose rates and duration of treatment has helped the replacement of medication via the feed for treatment purposes.

Feed medication in most countries is still the main route of antimicrobial administration in the swine industry. For treatment of disease it can be argued that it is not the most efficient route of administration, as it may take some days for the feed to be manufactured, delivered and work through the storage bin system to get to the pigs. For ease of administration, it is the simplest route. For the prevention of disease, for the early treatment (metaphylaxis), it is ideal, as it can be planned that the pigs receive the medicated feed on arrival or transfer from one shed/barn to another, especially when they are known to come from an infected source. The aim of metaphylaxis is to eliminate or reduce as much as possible the infectious agent, so that it does not cause disease in the next growing phase. The drug is administered at a therapeutic dose in an attempt to eliminate the infection, whether it is *B. hyodysenteriae* or *S. suis*. Low concentrations of bacteria (say 10^2) respond better to lower levels of antibiotic and are less likely to mutate than high concentrations of microorganisms (> 10^6; Drlica, 2003), which are found in clinical infections. This actually supports early treatment or preventative use rather than waiting for high levels of disease before treatment. Antibiotics are often used at lower levels to prevent infection or re-infection from a contaminated environment, especially in the case of swine dysentery. The concentrations in feed are generally lower than the treatment level but are effectively inhibitory, preventing the multiplication of the organism, so to be effective drug concentrations in the gut contents must exceed the minimum inhibitory concentration (MIC) of the bacterium. In some countries, like the United States, antibiotics can be still used for growth promotion. This is sometimes a gray area between prevention concentrations and subinhibitory concentrations, which can produce improvements in growth rate and feed conversion efficiency. Many successful growth promoters actually have a prevention of disease effect/claim, such as virginiamycin, prevents *Clostridium perfringens* infections; carbadox prevents swine dysentery (*B. hyodysenteriae*) and tylosin prevents porcine proliferative enteropathy "ileitis" (*Lawsonia intracellularis*). This possibly explains the reason why it could be relatively easy for a switch from growth promotion claims to prevention claims for some of these antimicrobials.

There are some pharmacokinetic disadvantages to administering medication via the feed, as sometimes the feed interferes with the absorption of a drug and reduces its bioavailability (Nielsen, 1997) and thereby plasma concentrations. This can have an impact particularly when treating systemic or respiratory infections (Figure 33.1).

On the other hand, oral medication whether by water or in feed is very effective for treating enteric infections, especially *E. coli, Salmonella* spp., *C. perfringens, L. intracellularis*, and *Brachyspira* spp., as effective drug concentrations in the gut contents, whether it is in

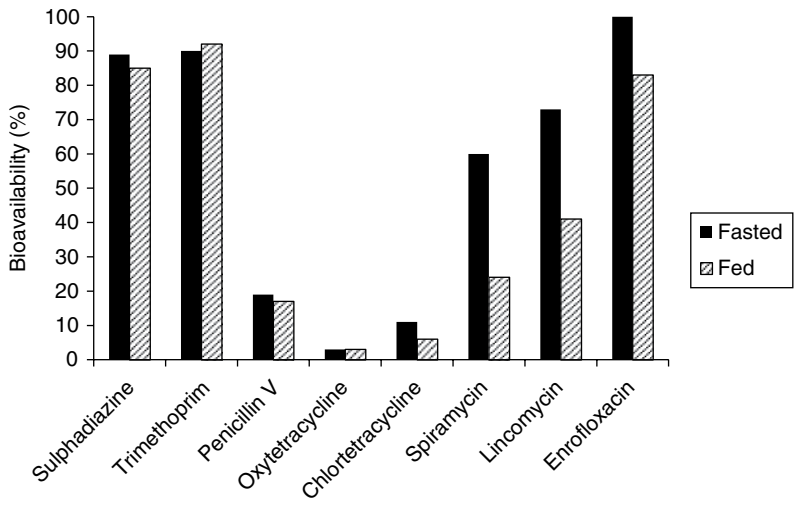

Figure 33.1. Bioavailability (%) of antimicrobials when given fasted or after feeding to pigs (Nielsen, 1997).

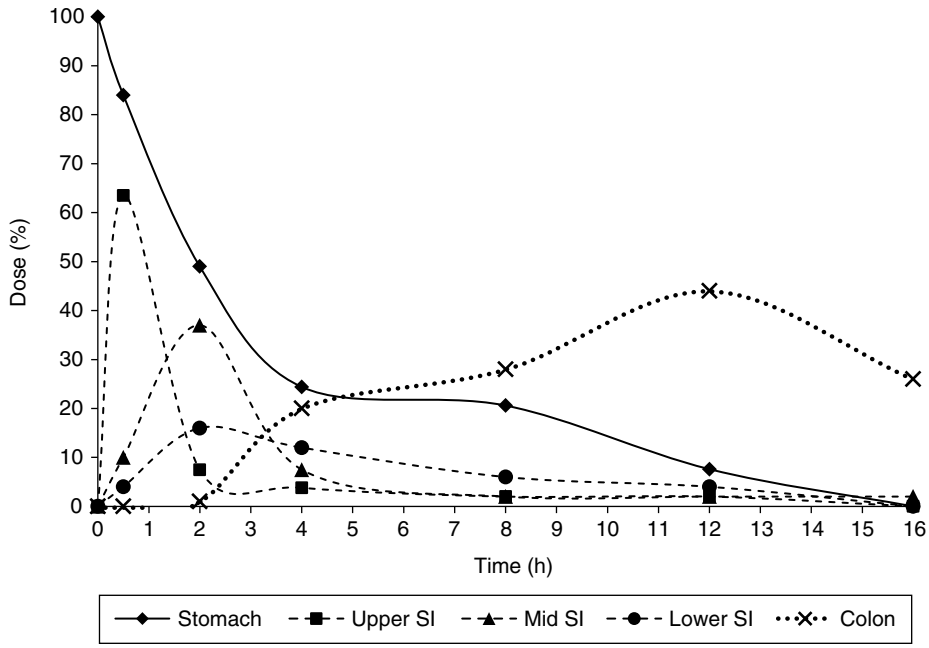

Figure 33.2. Pharmacokinetics of non-absorbed drug in the intestinal contents of swine (Burch, 2012, after Clemens et al., 1975) after a single-dose administration.

the jejunum, ileum or colon, is critical to their effect (Figure 33.2). Data following a single oral gavage of a non-absorbable substance in the middle of feeding (Burch, 2012, after Clemens et al., 1975), the drug passes out of the stomach and flows in a wave in the intestinal contents down the small intestine and accumulates in the large intestine. The waves would increase in number but decrease in height with the frequency of feeding; for example, on an ad-libitum basis, giving a more steady flow of antimicrobial concentration along the intestine to inhibit bacterial growth.

Drug dose and inclusion rate is also critical and the former depends on feed intake/kg body weight of the pig. Most pharmacokinetic and efficacy work is carried out on grower pigs and their feed intake in relation to body weight is approximately 5% (1 kg feed/20 kg body weight). In dry sows, this can fall to 1% and lactating sows to 2–2.5%. Even in finishers, on restricted feed for controlling fat deposition in castrates, it can fall to 2.5%. On this basis, it is essential to adjust the inclusion rate to achieve an effective dose rate. Where dosing is based on or limited to a standard inclusion rate in feed, it is possible to underdose in certain cases, resulting in a poor clinical response.

Water intake is frequently based on 10% of body weight. Some authors describe this as erroneous (Kyriazakis and Whittemore, 2006) and that it is more likely to be up to 15–20% based on dry feed intake. Environmental temperatures can also have a major impact on water intake. When calculating dose rates and inclusion rates in feed or water the total body weight (kg) times daily dose rate (mg/kg bwt), should be divided by the amount of food consumed or water drunk (kg or liter) in a day to give the required inclusion rate in parts per million (ppm).

For example:
In feed:
1000 kg body weight × 10 mg/kg bwt dose rate / 50 kg feed = 200 ppm (or mg drug/kg feed or g drug/ton of feed)

In water:
1000 kg body weight × 10 mg/kg bwt dose rate / 100 liters of water = 100 ppm (or mg drug/liter of water).

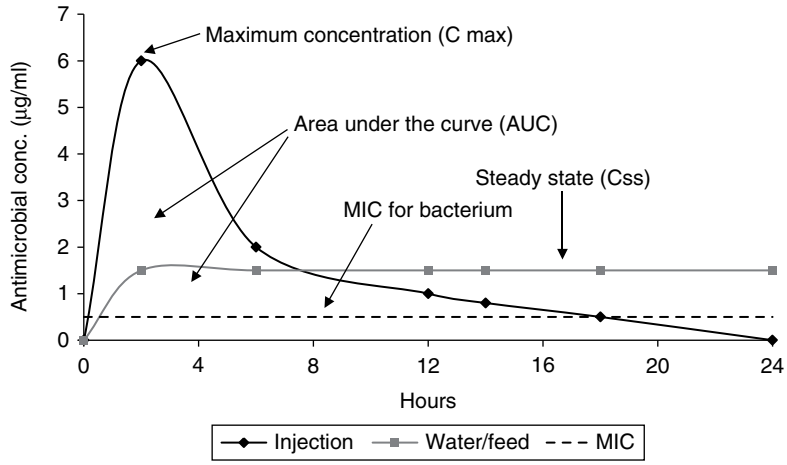

Figure 33.3. Comparative antimicrobial pharmacokinetic curves following injection or in-feed or in-water medication.

If using a water proportioner set at 1%, then the quantity of drug (1000 kg × 10 mg / 1000 = 10 g) would need to be dissolved in 1 liter of water and administered during the day.

Water and in-feed medication administered over the day gives usually a lower but flatter pharmacokinetic plasma concentration curve than following an injection or oral dose. This is still ideal for controlling systemic or respiratory bacterial infections as many of the antibiotics used are inhibitory, like the tetracyclines, and are time- and concentration-dependent in their antibacterial effect unlike the fluoroquinolones, which can exert a strong concentration-dependent bactericidal effect, especially when injected or given as a bolus dose (Figure 33.3).

Common Bacterial and Mycoplasmal Infections in Swine

The common bacterial and mycoplasmal infections in pigs are summarized in Table 33.1.

Antimicrobials Used in Swine

A wide variety of antimicrobials are available for use in swine, but the availability of certain formulations is often different between countries; for example, growth promoters are not available in the EU and trimethoprim/sulfonamide combinations are not approved for oral use in the United States, but human products may be used. The antimicrobial products used in swine are summarized in Table 33.2.

Antimicrobial Susceptibility of Porcine Isolates

The antimicrobial susceptibility of a number of key porcine bacterial pathogens is presented in this section. Susceptibility patterns are very useful to demonstrate the "wild-type" patterns and the selection of mutants and resistance following the use of antimicrobials.

Enteric Pathogens

Amoxycillin and amoxycillin + clavulanic acid (beta-lactamase inhibitor) demonstrates the way beta-lactamase enzymes exert their effect and they can be blocked or inactivated by inhibitors such as clavulanic acid (see Figure 33.4). There are high levels of resistance to tetracycline in *E. coli*, as the group is the most widely used antibiotics in pigs for both enteric and respiratory infections (Table 33.3).

In contrast, U.S. *E. coli* clinical isolates (n = 2144) from small pigs showed a comparatively high incidence of resistance to neomycin 49.5%, ceftiofur 41.8%,

Table 33.1. Common swine bacterial and mycoplasmal pathogens, diseases, and ages affected.

Bacterial spp.	Disease	Age
Enteric infections		
Escherichia coli	Neonatal scours	1–3 days
	Piglet scours	7–14 days
	Post-weaning diarrhea	5–14 days after weaning
	Mastitis, metritis, agalactia (MMA) syndrome	Sows, post-parturient
Clostridium perfringens	Type C—necrotic enteritis	1–7 days
	Type A—diarrhea	10–21 days, weaned pigs
Salmonella enterica spp.	*S.* Typhimurium—occasional diarrhea, septicemia, death	Grower pigs—from weaning onward
	S. Derby—occasional diarrhea	Grower pigs
	S. Choleraesuis—septicemia, diarrhea, death	Finishing pigs 12–16 weeks
Lawsonia intracellularis	Porcine proliferative enteropathy (ileitis)	Grower pigs
	Regional/necrotic ileitis	Grower pigs
	Porcine haemorrhagic enteropathy	Finishing pigs and young adults 16–40 weeks
Brachyspira hyodysenteriae	Swine dysentery	Growers and finishers, 6–26 weeks All ages in primary breakdown
Brachyspira pilosicoli	Intestinal spirochaetosis "colitis"	Grower pigs
Respiratory and systemic infections		
Pasteurella multocida (D)	Progressive atrophic rhinitis	1–8 weeks
Bordetella bronchiseptica		Nasal distortion lasts for life
Mycoplasma hyopneumoniae	Enzootic pneumonia	Grower and finisher pig
Pasteurella multocida	Mycoplasma-induced respiratory disease (MIRD)	Grower and finisher—secondary invader
Actinobacillus pleuropneumoniae	Pleuropneumonia	Grower and finisher—MDA can last for 10 weeks
Actinobacillus suis	Septicemia, endocarditis, arthritis, and pneumonia	1–6 weeks
Streptococcus suis	Meningitis, arthritis	2–10 weeks
Haemophilus parasuis	Glässer's disease (arthritis, polyserositis, pericarditis, peritonitis)	2–10 weeks
Mycoplasma hyosynoviae	Mycoplasmal arthritis	16 weeks plus
Mycoplasma hyorhinis	Polyserositis, arthritis, low grade pneumonia	3–10 weeks
Erysipelas rhusiopathiae	Erysipelas (dermatitis, arthritis, endocarditis)	Growers, finishers, and sows/boars
Other		
Staphylococcus hyicus	Exudative epidermitis "greasy pig disease"	1–8 weeks
Arcanobacterium pyogenes	Abscesses, often spinal	1–24 weeks

florfenicol 39.3%, gentamicin 31.1% but a low resistance to trimethoprim/sulfonamide 25.5% and enrofloxacin 1.7% (Frana et al., 2012). This is somewhat surprising but may be a reflection on the limited availability of other "front-line" drugs, such as trimethoprim/sulfonamide combinations, which are used in other countries.

With regard to enrofloxacin resistance against *E. coli*, there is an initial "wild-type" pattern, a first stage mutant pattern, which is still susceptible if situated in the gut due to its excretory pathway and a second stage, completely resistant peak at 16 μg/ml (see Figure 33.5).

The use of zinc oxide in feed at weaning and the postponement of weaning to 28 days of age in the EU have made significant contributions to the reduction of cases of post-weaning diarrhea and a reduction in the use of antibiotics to control *E. coli* and thereby, resistance levels.

The susceptibility of 197 *Salmonella enterica* serovar isolates in Indiana in the United States was reported by Huang et al. (2009). It was also reported on an individual serovar basis (Tables 33.4 and 33.5)

Generally the susceptibility patterns are similar to *E. coli* but usually they are slightly less resistant. Interestingly, there is a marked difference between sero-

Table 33.2. Antimicrobials used in swine—routes of administration, dosages (mg/kg bodyweight), and target pathogens.

Family/Antimicrobial	Route of Administration and Dosage (mg/kg)			Use/Indication
	Injection	Water	Feed	
Tetracyclines:				*M. hyopneumoniae*
Oxytetracycline	10 (LA 20)	10–30	20	*P. multocida*
Chlortetracycline		20	10–20	*A. pleuropneumoniae*
Tetracycline		20–40		*H. parasuis*
Doxycycline	4–6	5	5	*L. intracellularis*
				E. coli (R*)
				Salmonella spp. (R*)
Diaminopyrimidine/Sulfonamide				*P. multocida*
Trimethoprim /sulfadiazine	15 (2.5 + 12.5)	30 (5 + 25)	15 (2.5 + 12.5)	*B. bronchiseptica*
				A. pleuropneumoniae
				S. suis
				S. hyicus
				H. parasuis
				L. intracellularis
				E. coli
				Salmonella spp.
Penicillins:				*S. suis*
Penicillin G	10 (LA 20)	–	–	*P. multocida*
Penicillin V	–	10	10	*H. parasuis*
				A. pleuropneumoniae
				A. pyogenes
				C. perfringens
				E. rhusiopathiae
Synthetic penicillins:				*S. suis*
Amoxycillin	7 (LA 15)	20	15–20	*P. multocida*
Ampicillin	7.5	–	–	*H. parasuis*
Plus clavulanic acid	+1.75	5	–	*A. pleuropneumoniae*
(beta-lactamase inhibitor)				*A. pyogenes*
				C. perfringens
				E. rhusiopathiae
				E. coli
				Salmonella spp.
Cephalosporins:				*S. suis*
Cephalexin (1G)	7	–	–	*P. multocida*
Ceftiofur (3G)	3 (LA 5)	–	–	*H. parasuis*
Cefquinome (4G)	1–2	–	–	*A. pleuropneumoniae*
				A. pyogenes
				C. perfringens
				E. rhusiopathiae
				E. coli
				Salmonella spp.
Fluoroquinolones:				*M. hyopneumoniae*
Enrofloxacin	2.5	–	–	*P. multocida*
Danofloxacin	1.25	–	–	*A. pleuropneumoniae*
Marbofloxacin	2	–	–	*H. parasuis*
				E. coli
				Salmonella spp.

(*continued*)

Table 33.2. Antimicrobials used in swine—routes of administration, dosages (mg/kg bodyweight), and target pathogens. (*continued*)

	Route of Administration and Dosage (mg/kg)			
Family/Antimicrobial	Injection	Water	Feed	Use/Indication
Thiamphenicols:				*P. multocida*
Thiamphenicol	10–30	–	10	*A. pleuropneumoniae*
Florfenicol	15 (LA 30)	15	15	*H. parasuis*
				S. suis
				B. bronchiseptica
Aminoglycosides:				Injection
Streptomycin	25	–	–	*S. aureus*
Neomycin	– (NA)	11	11	*P. multocida*
Apramycin	–	7.5–12.5	4–8	*E. coli*
Gentamicin	– (NA)			*Salmonella* spp.
Amikacin	– (NA)			Orally
Aminocyclitol:				*E. coli*
Spectinomycin	– (NA)	10–50	2.2 (+lincomycin)	*Salmonella* spp.
Polymixin:				*E. coli*
Colistin	–	50,000iu	50,000iu	*Salmonella* spp.
Macrolides:				*M. hyopneumoniae*
Tylosin	2–10	25	3–6 (T)	*L. intracellularis*
			1.2–2.4 (P)	*B. hyodysenteriae* (R*)
Tylvalosin	–	2.125–4.25	2.125–4.25	*B. pilosicoli* (R*)
Tilmicosin	–	15–20+	8–16+	+Plus *A. pleuropneumoniae*
Tildipirosin	4+	–	–	*H. parasuis*
Triamilide:				*P. multocida*
Tulathromycin	2.5+	–	–	*S. suis (R*)*
Lincosamides:				*M. hyopneumoniae*
Lincomycin	10	4.5	5.5–11 (T)	*M. hyosynoviae*
			2.2 (P)	*L. intracellularis*
			1.1–2.2	*B. hyodysenteriae*
			(+spectinomycin)	*B. pilosicoli*
Pleuromutilins:				*M. hyopneumoniae*
Valnemulin	–	–	3.75–10 (T)	*M. hyosynoviae*
Tiamulin			1.0–1.5 (P)	*L. intracellularis*
	10–15+	8.8–20+	5–11 (T)	*B. hyodysenteriae*
			1.5–2 (P)	*B. pilosicoli*
				+Plus *A. pleuropneumoniae*
Anticoccidials:				*Isospora suis*
Toltrazuril			20	
Miscellaneous:			Inclusion rate	Claim
Growth promoters (not EU):				
Virginiamycin			5.5–110 ppm	GP + Swine dysentery(*B. hyo*)
Bacitracin MD			4.4–220 ppm	GP + *B. hyo; C. perfringens*
Zinc bacitracin			11–55 ppm	GP only
Flavophospholipol (Bambermycin)			2.2–4.4 ppm	GP only
Avilamycin			10–40 ppm	GP only
			10–50 ppm	GP + Swine dysentery;
Carbadox				S. Choleraesuis
Salinomycin			15–60 ppm	GP only
Metals				*E. coli*
Zinc oxide			3500 ppm	(Postweaning diarrhea)

LA = long-acting formulation; NA = not approved; R* = resistance problems; T = treatment; P = prevention; GP = growth promotion; + = plus additional claims; *B. hyo* = *B. hyodysenteriae*.

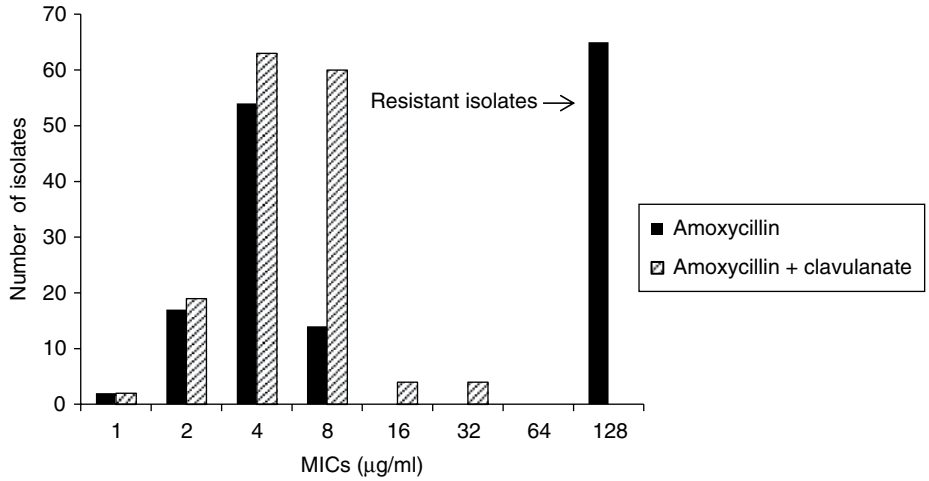

Figure 33.4. Susceptibility patterns demonstrated by *E. coli* against amoxycillin and amoxycillin + clavulanic acid (Klein et al., 2012).

Table 33.3. Antimicrobial susceptibility of 152 isolates of *E. coli* from the EU (Klein et al., 2012).

Antimicrobial	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	MIC range (µg/ml)	Resistance (%)
Amoxycillin	8.0	> 128	1.0–>128	43
Amoxycillin + clavulanic acid	4.0	8.0	1.0–32	0 (enteric)
Streptomycin	32	> 128	4.0–>128	44
Neomycin	1.0	32	0.25–>128	5
Apramycin	4.0	16	1.0–32	0 (enteric)
Gentamicin	0.5	2.0	0.25–>128	9
Enrofloxacin	0.03	1.0	0.008–16	20 (systemic) 7 (enteric)
Ciprofloxacin	0.015	0.5	0.008–16	20 (systemic) 7 (enteric)
Colistin	0.25	0.25	0.12–8.0	0
Trimethoprim + sulfonamide	0.25	> 16	0.015–>64	45
Tetracycline	> 128	> 128	14–>128	80

vars with *S*. Typhimurium showing a higher resistance pattern than *S*. Derby and *S*. Choleraesuis.

Brachyspira spp. seem to develop resistance more slowly than *E. coli* presumably because it is a slow growing microorganism, however, most isolates are now resistant to tylosin but many are still susceptible to the pleuromutilins, tiamulin, and valnemulin. There are intermediate levels of resistance to lincomycin, tylvalosin, and doxycycline (Table 33.6). In the United States, carbadox and salinomycin were also shown to be active against *B. hyodysenteriae* as well as a number of other *Brachyspira* spp. (Clothier et al., 2011; Table 33.7).

Antimicrobial susceptibility of *B. pilosicoli* and *B. intermedia* were generally better than for *B. hyodysenteriae* (Clothier et al., 2011) possibly, as they are less frequently used to treat infections with these bacteria, as they tend to be milder. This was confirmed by Williamson et al., (2010; Table 33.8).

Lawsonia intracellularis, the cause of ileitis, is a more difficult microorganism to work with, as it requires cell

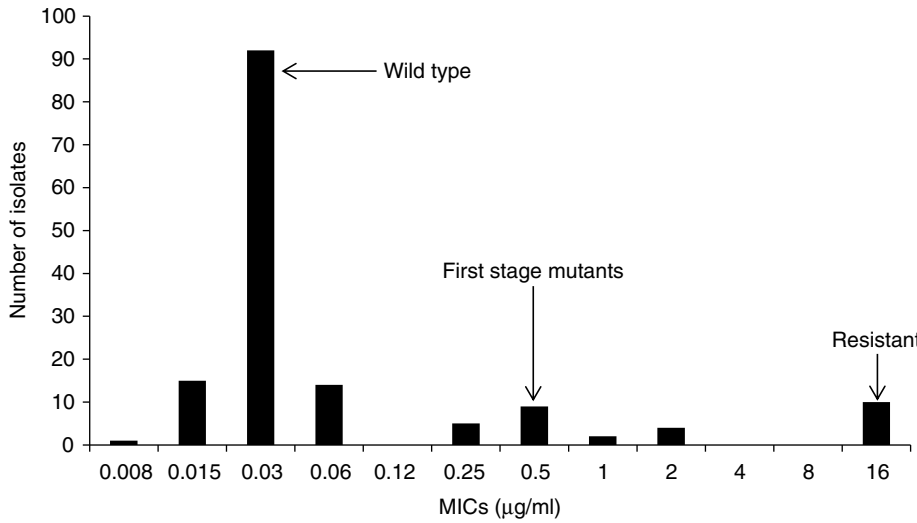

Figure 33.5. Susceptibility pattern demonstrated by *E. coli* against enrofloxacin (Klein et al., 2012).

Table 33.4. Susceptibility of 197 U.S. isolates of *Salmonella* spp. (Huang et al., 2009).

Antibiotic	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	Resistance (%)
Ampicillin	> 32	> 32	0.25–>64	55.8
Amoxicillin/ Clavulanic acid	8.0	> 32	1.0–>64	21.8
Cephalothin	4.0	> 32	1.0–>64	20.8
Ceftiofur	1	> 4	0.06–>8	19.3
Enrofloxacin	0.06	0.12	≤ 0.03–0.25	0
Florfenicol	4.0	> 8.0	0.5–>16	41.1
Gentamicin	0.5	8	0.12–>4.1	6.6
Spectinomycin	32	> 128	16–>128	42.6
Tetracycline	> 16	> 16	0.5–>32	83.8
Trimethoprim/ Sulfonamide	≤ 0.5	≤ 0.5	≤ 0.5–8.0	8.6

Table 33.5. Resistance (%) of different U.S. *Salmonella* spp. (Huang et al., 2009).

	Resistance (%)			
Antibiotic	Overall (n = 197)	*S.* Typhimurium Var. Copenhagen (n = 39)	*S.* Derby (n = 30)	*S.* Choleraesuis Var. Kunzendorf (n = 27)
Ampicillin	55.8	84.6	6.7	81.5
Amoxicillin/ Clavulanic acid	21.8	53.9	6.7	0
Cephalothin	20.8	10.3	6.7	0
Ceftiofur	19.3	7.7	6.7	0
Enrofloxacin	0	0	0	0
Florfenicol	41.1	82.1	20	0
Gentamicin	6.6	10.3	0	0
Spectinomycin	42.6	92.3	46.7	7.4
Tetracycline	83.8	97.4	89	92.6
Trimethoprim/ Sulfonamide	8.6	7.7	6.7	0

cultures to grow the bacterium. The most comprehensive intracellular MIC work was reported by Wattanaphansak et al. (2009) where he tested 10 isolates of *L. intracellularis* from EU and U.S. sources and the test was repeated 2 times, with slightly different results (Table 33.9). The iMIC appears to be the most useful for comparison with therapeutic concentrations of antimicrobials in the ileal contents (Burch, 2005) and as such demonstrates there might be some resistance associated with lincomycin and chlortetracycline but not with the other compounds.

Clostridium spp. are also a cause of increasing interest especially in young pigs. In some countries like the United States, both *C. perfringens* Type C and A, and *C. difficile* are associated with severe clinical problems. It is interesting that most of the growth promoters, except

Table 33.6. Antimicrobial susceptibility of *B. hyodysenteriae* in 70 UK isolates (Williamson et al., 2010).

Antibiotic	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)
Tiamulin	0.125	2.0	≤ 0.06–>8.0
Valnemulin	≤ 0.03	4.0	≤ 0.03–>4.0
Lincomycin	> 32	> 32	0.5–>32
Tylosin	> 128	> 128	2.0–>128
Tylvalosin	> 32	> 32	0.5–>32
Doxycycline	1.0	16	0.5–>16

Table 33.7. Antimicrobial susceptibility of *B. hyodysenteriae* in 24 U.S. isolates (Clothier et al., 2011).

Antibiotic	Median MIC (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)
Tiamulin	0.125	0.5	0.125–4.0
Valnemulin	0.125	0.5	0.125–2.0
Lincomycin	32	64	1.0–64
Salinomycin	0.25	0.5	0.25–0.5
Carbadox	0.015	0.03	0.008–0.06

Table 33.8. Antimicrobial susceptibility of *B. pilosicoli* in 55 UK isolates (Williamson et al., 2010).

Antibiotic	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)
Tiamulin	0.125	0.5	≤ 0.06–>8.0
Valnemulin	≤ 0.03	0.5	≤ 0.03–>4.0
Lincomycin	0.5	32	≤ 0.25–>32
Tylosin	8.0	> 128	2.0–>128
Tylvalosin	1.0	> 32	≤ 0.25–>32
Doxycycline	0.25	4.0	0.5–8.0

flavomycin, have a strong activity against *C. perfringens* (Table 33.10).

Respiratory and Systemic Pathogens

One of the major respiratory pathogens with potential to develop antimicrobial resistance is *A. pleuropneumoniae* but generally resistance is much lower than for enteric bacteria such as *E. coli* (Tables 33.11 and 33.12). The tetracyclines would normally be considered a frontline therapy for *A. pleuropneumoniae*. However, in some countries such as Italy, a surprisingly high level of resistance has been reported (Vanni et al., 2012), except to ceftiofur, amoxycillin/clavulanic acid, the fluoroquinolones, and florfenicol.

Slightly better susceptibility results were achieved against *Pasteurella multocida*, except for tetracyclines, which are again considered the first line of therapy (Table 33.13). *Streptococcus suis*, the cause of streptococcal meningitis, still remains remarkably susceptible to the penicillins but shows poor susceptibility to the tetracyclines and tilmicosin (Table 33.14).

A comparative study looking at 30 UK and 30 Spanish isolates of *H. parasuis* (Martin de la Fuente et al., 2007) highlights the difference in susceptibility patterns in

Table 33.9. Estimated intracellular MIC (iMIC) for a number of antimicrobials of 20 results (10 isolates × 2 tests; (Wattanaphansak et al., 2009) against *L. intracellularis*.

Antimicrobial	$iMIC_{50}$ (μg/ml)	$iMIC_{90}$ (μg/ml)	Range (μg/ml)
Tiamulin	0.125	0.125	0.125–0.5
Valnemulin	0.125	0.125	0.125
Tylosin	2.0	8.0	0.25–32
Lincomycin	64	> 128	8.0–> 128
Chlortetracycline	8.0	64	0.125–64
Carbadox	0.125	0.25	0.125–0.25

Table 33.10. Susceptibility of *Clostridium* spp. to antibiotics.

Antimicrobial	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)
Clostridium perfringens—Dutte and Devriese, 1980—58 Belgian isolates			
Bacitracin	0.06	0.12	0.03–0.12
Carbadox	0.03	4.0	0.007–16
Flavomycin	≤ 128	≤ 128	≤ 128
Virginiamycin	0.25	0.5	0.25–2.0
Lincomycin	2.0	256	0.12–≥512
Penicillin G	0.12	0.5	0.06–1.0
Tetracycline	16	32	0.06–≥64
Tiamulin	–	–	0.25–4.0
Clostridium perfringens—Devriese et al., 1993—95 Belgian isolates			
Tylosin	0.012	0.012	0.012–≥64
Clostridium perfringens—Agnoletti et al., 2010—30 Italian and 38 Danish isolates			
Tiamulin (Italian)	4.0	64	0.125–128
Tiamulin (Danish)	2.0	4.0	0.25–8.0
Valnemulin (Italian)	0.125	8.0	0.063–32
Valnemulin (Danish)	0.063	0.125	0.016–0.25
Clostridium difficile—Post and Songer, 2002—80 U.S. isolates			
Bacitracin	> 256	> 256	–
Virginiamycin	0.25	2.0	–
Tylosin	0.25	64	–
Tilmicosin	0.5	> 256	–
Tetracycline	8	32	–
Tiamulin	4	8	–
Ceftiofur	256	> 256	–
Clostridium perfringens—Agnoletti et al., 2010—15 Italian and Danish isolates			
Tiamulin	8	16	0.125–16
Valnemulin	0.5	1.0	0.063–1.0

different countries, showing it is important to develop local farm and national data (Table 33.15).

Mycoplasma hyopneumoniae, the cause of enzootic pneumonia, is also a slow growing organism and generally antibiotic resistance is low (Table 33.16). It is the precursor of many cases of complicated secondary bacterial pneumonia, associated with *P. multocida*, and also plays a key role in the porcine respiratory

Table 33.11. Antimicrobial susceptibility of *A. pleuropneumoniae* in 129 EU isolates (Klein et al., 2012).

Antimicrobial	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	Resistance (%)
Amoxycillin	0.5	0.5	0.25–32	5
Amoxycillin + clavulanic acid	0.25	0.5	0.06–1.0	0
Cephalexin	2.0	2.0	0.12–4.0	0
Ceftiofur	0.015	0.03	0.008–0.06	0
Enrofloxacin	0.03	0.06	0.008–2.0	1
Florfenicol	0.25	0.5	0.12–0.5	0
Trimethoprim+ sulfonamide	0.06	0.25	0.008–16	5
Tetracycline	1.0	16	0.25–32	15
Tilmicosin	8.0	16	4.0–16	0
Tiamulin	8.0	16	0.25–16	0

Table 33.12. Antimicrobial resistance of Italian *A. pleuropneumoniae* isolates from 2009 (Vanni et al., 2012).

Antimicrobial	Resistance (%)	Antimicrobial	Resistance (%)
Penicillin G	72.7	Enrofloxacin	9.6
Amoxycillin	82.6	Marbofloxacin	2
Amoxycillin + Clavulanate	8.9	Trimethoprim+ Sulfonamide	32.7
Cephalexin	21.7	Tilmicosin	51.3
Ceftiofur	7.7	Tulathromycin	66.7
Tetracycline	58.8	Tiamulin	13.5
Doxycycline	25	Streptomycin	100
Florfenicol	7.7	Gentamicin	63.6

Table 33.13. Antimicrobial susceptibility of *P. multocida* in 135 EU isolates (Klein et al., 2012).

Antimicrobial	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	Resistance (%)
Amoxycillin	0.25	0.25	0.06–128	1
Amoxycillin + clavulanic acid	0.25	0.25	0.12–0.25	0
Cephalexin	2.0	4.0	1.0–8.0	0
Ceftiofur	0.004	0.03	0.002–0.5	0
Enrofloxacin	0.015	0.03	0.008–0.25	0
Florfenicol	0.5	0.5	0.25–1.0	0
Trimethoprim+ sulfonamide	0.06	0.5	0.008–16	3
Tetracycline	0.5	2.0	0.25–32	22
Tilmicosin	8.0	16	1.0–16	0

disease complex (PRDC), where viruses are also involved. Some antibiotic resistance was demonstrated against lincomycin, tylosin and tilmicosin (1 isolate and enrofloxacin (5 isolates). Acquired resistance to these antimicrobials had not been described in *M. hyopneumoniae* field isolates previously.

Table 33.14. Antimicrobial susceptibility of *S. suis* in 110 EU isolates (Klein et al., 2012).

Antimicrobial	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)	Resistance (%)
Amoxycillin	≤ 0.03	≤ 0.03	0.03–0.25	0
Amoxycillin + clavulanic acid	≤ 0.06	≤ 0.06	0.06–0.25	0
Cephalexin	0.12	0.5	0.06–4.0	0
Ceftiofur	0.12	0.5	0.06–2.0	0
Enrofloxacin	0.5	0.5	0.12–8.0	1
Florfenicol	0.5	0.5	0.25–1.0	0
Trimethoprim + sulfonamide	0.06	1.0	0.008–16	7
Tetracycline	32	32	0.25–32	82
Tilmicosin	> 128	> 128	4.0–>128	54

Table 33.15. Antimicrobial susceptibility of *H. parasuis* isolates (30 from UK and 30 from Spain; Martin de la Fuente et al., 2007).

	UK			Spain		
Antibiotic	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Resistance (%)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Resistance (%)
Penicillin	≤ 0.12	0.5	0	8.0	> 8.0	60
Ampicillin	≤ 0.25	2.0	6.7	16	> 16	56.7
Ceftiofur	≤ 0.5	1.0	0	≤ 0.5	4.0	6.7
Gentamicin	1.0	8.0	10	8.0	> 8.0	26.7
Oxytetracycline	0.5	4.0	6.7	4.0	> 8.0	40
Tilmicosin	< 4	8	0	16	> 32	40
Enrofloxacin	≤ 0.12	0.25	0	0.25	> 2.0	20
Florfenicol	≤ 0.25	1.0	0	0.5	1.0	0
Tiamulin	≤ 4.0	16	3.3	16	> 32	40
Trimethoprim+ Sulfonamide	≤ 0.5/9.5	2/38	10	> 2/38	> 2/38	53.3

Table 33.16. Antimicrobial susceptibility of *M. hyopneumoniae* in 21 Belgian field isolates (Maes et al., 2007)—final MICs, 14 days after inoculation.

Antimicrobial	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC range (μg/ml)
Enrofloxacin	0.06	0.5	0.03->1.0
Oxytetracycline	0.5	2.0	0.12->2.0
Doxycycline	0.5	1.0	0.12–2.0
Lincomycin	≤ 0.06	0.12	≤ 0.06->8.0
Spectinomycin	0.5	1.0	≤ 0.12–1.0
Gentamicin	0.5	1.0	≤ 0.12–1.0
Florfenicol	0.25	0.5	≤ 0.12–1.0
Tiamulin	0.03	0.12	≤ 0.015–0.12
Tylosin	0.06	0.12	≤ 0.015->1.0
Tilmicosin	0.5	0.5	≤ 0.25->16

Conclusion

Apart from some microorganisms such as *E. coli, B. hyodysenteriae* and *A. pleuropneumoniae*, where on occasions severe antimicrobial resistance has been determined, the antimicrobial resistance situation is generally not that extensive. However, the difference does vary from country to country. In most cases, an infection can be treated using existing approved antimicrobials, providing that their availability can be maintained for use in pigs. It is thought that it is unlikely there will be many new antibiotics in the near future.

Care should be taken to not overuse antimicrobials and veterinarians should consider reduction of use where possible and practical, and address management and housing issues at the same time. Vaccine alternatives should be considered, where appropriate. Susceptibility testing should become routine. If antibiotics are used responsibly and sensibly, there is no major reason why our current armoury should not be sufficient for the foreseeable future. It must be remembered that most of the antimicrobials used in pigs are already over thirty years old and that the majority of them are still working. Even the more modern antibiotics such as third- and fourth-generation cephalosporins, if used carefully and not for widespread prophylaxis, will maintain their efficacy

Overall, antimicrobials are extremely useful and helpful tools but one of the major challenges in swine medicine is to overcome the management and production issues that have often resulted in the requirement to use antibiotics in the first place.

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Antimicrobial Drug Use in Poultry

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Whether fully integrated or not, the commercial poultry industry is a very intensive animal agriculture system. One poultry house or barn can contain as many as 100,000 commercial layers or commercial broilers. At the hatchery level, depending on the type of equipment in place, one incubator can contain more than 120,000 eggs or developing embryos. This ultimately means that disease prevention, on all levels of the poultry production continuum, is the major focus for a poultry veterinarian. Antimicrobials are critically important in the prevention and treatment of diseases in the poultry industry. Under circumstances when husbandry and biosecurity procedures fail to prevent the introduction of a disease agent, appropriate antimicrobial therapy can become necessary to prevent pain and suffering in these birds as well as economic losses to the producer. When the poultry veterinarian makes a diagnosis and decides that birds need to be treated with an antimicrobial drug, they must then determine the appropriate drug formulation and route of administration.

Categories of Antimicrobial Drug Use in the Poultry Industry

Antimicrobial drug use in poultry can be divided into three categories of use: therapeutic, preventative/prophylactic and growth promoting. Antimicrobial drugs in the therapeutic category are used to treat or cure a clinically detectable disease. Because sick birds may be off feed, therapeutic antimicrobials are typically administered via the drinking water; however, certain circumstances or disease conditions may dictate administration in feed instead or concomitantly with water. The preventative or prophylactic category includes the antimicrobial drugs that are used to prevent disease. Prophylactic antimicrobials are administered prior to the appearance of clinical signs of disease in a flock. The route of antimicrobial administration may depend on the timing or age of bird when the treatment is applied. In poultry production, population health begins at the hatchery where eggs from various flocks are comingled and the disease and microbiological status of individual eggs can impact all the other birds hatching at that time. When increased bacterial contamination has been identified in association with eggs coming from a particular breeder flock, birds from that flock may be treated preventatively using *in ovo* (eggs) or subcutaneous (day-old chicks or poults) injection of an antimicrobial until the underlying cause for such contamination is identified and corrected. Other routes for administration of prophylactic antimicrobials include oral administration via drinking water or feed. The last category of antimicrobial use, growth promotion, is the most controversial. Antimicrobials in the growth promotion category were and are only

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administered in feed. Many antimicrobials were first approved for poultry based on their observed growth-promoting effects: improved feed efficiency and growth rates. The improved production results in an economic benefit that is greater than the cost of the antimicrobial drug. Due to increasing concerns that growth-promoting use of antimicrobials in poultry has a negative impact on human health due to antimicrobial resistance, there has been mandated and voluntary removal of growth promotors from poultry production in many jurisdictions. With the bans on such use, it has become evident that much of the growth promotion effect is due to the control and prevention of subclinical enteric disease. In some cases these antimicrobials may be the same as those approved for therapeutic use; however, the dose level for growth promotion is generally less than the therapeutic dose. In countries such as the United States and Canada, where products have both therapeutic and growth-promoting label claims, these products are rarely used for growth promotion. For example, penicillin and tetracyclines are rarely used as growth promotants even though there are regulatory approvals for this.

In considering these broad categories of antimicrobial use in poultry, the distinction between therapeutic, preventative and growth-promoting categories is not always clear. The poultry veterinarian is faced with making a decision regarding treatment of a population; individual treatment is often not possible or practical. As not all birds in that population will be clinically ill, the antimicrobial treatment will be therapeutic for a portion of the population and preventative for another portion of the population. To further complicate the matter, growth-promoting antimicrobials are known to kill or inhibit the growth of disease-causing agents, including bacteria or coccidia. These products are particularly effective at prevention of necrotic enteritis, a condition triggered by enteric overgrowth of *Clostridium perfringens* (Grave, 2006; Smith, 2011). While the exact mode of action for growth promotion associated with the use of antimicrobials is debatable (Dibner and Richards, 2005; Neiwald 2007), growth promotion is clearly a "side effect" of disease prevention.

Antimicrobial Drug Use in the Poultry Industries of Canada, The United States, and Europe

Because of issues involving antimicrobial resistance, not all of the categories of antimicrobial use are permitted in the different poultry producing countries throughout the world.

Approved, Prohibited for Extra-Label Use, or Banned for Use

Antimicrobial use, in animals or humans and in any of the previously described categories, has the potential to select for bacterial strains that are resistant to the antimicrobial used (O'Brien, 2002). For this reason, antimicrobial use in food-producing animals, particularly with respect to use for growth promotion or disease prevention (as the line between the two is not clear), has been and still is a focus of scientific, political and consumer debate (Casewell et al., 2003; Phillips et al., 2004; Kelly et al., 2004; Cox and Popken, 2004; Cox, 2005; Phillips, 2007). The association of antimicrobial use in food animals with antimicrobial resistance of concern in human health has resulted in different approaches to the control of antimicrobial use in Canada, the United States and Europe. The use of antimicrobials for growth promotion and/or disease prevention in poultry is currently permitted in the United States and Canada. Such use has been banned in the countries of the European Union (EU); a process that started with banning a few products in the 1970s but now includes all antimicrobial growth promoters (Dibner and Richards, 2005; Castanon, 2007).

Subsequent to the EU bans, a number of reviews and risk assessments on the use of in feed antimicrobials have been conducted (Cox and Popken, 2004; Kelly et al., 2004; Phillips et. al, 2004; Cox, 2005. Thus far no bans have been implemented in North America. Therapeutic antimicrobial uses have not yet been banned in Europe, but have been targeted in the United States. Enrofloxacin and sarfloxcin were approved as therapeutic antimicrobials for the control of colibacillosis in poultry in the United States. In 2005, these fluoroquinolones were banned from use in poultry. The primary reason for this ban was to allay concerns regarding rising fluoroquinolone resistance rates in human

cases of camplylobacteriosis (FDA, 2005). A more recent prohibition of use in the United States occurred in January 2012 with a ban on the extra-label use of cephalosporins. This ban particularly targeted the extra-label use of ceftiofur, when administered *in ovo* for metaphylaxis in cases of known or anticipated *E. coli* challenge (FDA, 2012). Such use was associated with *E. coli* isolations from poultry carcasses containing genes that rendered them resistant to human third-generation cephalosporins. So while extra-label use is prohibited, ceftiofur remains approved for subcutaneous administration to day-old chicks and turkey poults.

In Canada, there have been no bans on antimicrobials in any of the categories of use. Extra-label drug use in Canada is not codified like the United States; Canadian veterinarians have the privilege of extra-label drug use. The definition of extra-label drug use (ELDU) as defined by Health Canada is "the use or intended use of a drug approved by Health Canada in an animal in a manner not in accordance with the label or package insert" (Health Canada, 2011). While not recommended by Health Canada, it is possible for Canadian veterinarians to use the cattle injectable enrofloxacin formulation in poultry, and to use the injectable ceftiofur product (approved for subcutaneous injection in day-old turkey poults) in chicks and administered either *in ovo* or subcutaneously.

Consequences of Antimicrobial Bans

The consequences of the ban on enrofloxacin and sarafloxacin use in poultry in the United States are being extensively studied. Anecdotally it appears that the rates of fluoroquinolone and cephalosporin resistance in bacteria of importance to human health are not changing; however, peer-reviewed publications are not yet available. The bans have removed effective treatments of bacterial disease from the poultry veterinarian's armamentarium. Veterinarians have valid concerns that antimicrobial bans can cause significant animal welfare concerns in the face of an untreatable disease outbreak. In a somewhat extreme example, the potentially difficult decision regarding early slaughter of entire flocks may need to be considered should no approved therapy exist.

The outcomes or concerns relating to bans on antimicrobial use for growth promotion and/or prevention are better documented and understood. The EU bans on growth-promoting antibiotics initially led to significant animal and human health concerns. In the poultry industry, necrotic enteritis is the disease of primary concern and is a challenge to manage in the absence of the antimicrobial growth promoters (Wierup, 2001; Casewell et al., 2003; Dibner and Richards 2005; Grave et al., 2006). Similar observations have been made in the United States when poultry producing companies voluntarily removed in-feed antimicrobials in order to produce an "antibiotic-free" product for specific markets (Smith, 2011). In the EU, control of coccidiosis will become a major health issue in poultry production, as the ionophore antibiotics are scheduled to be banned in 2013 (Castanon, 2007).

Of concern for human health in the EU was the increased use of therapeutic antimicrobials in poultry to treat clinical disease; primarily necrotic enteritis but also other forms of infectious enteritis (Casewell et al., 2003; Grave et al., 2006). Unlike the majority of the in-feed antimicrobials approved for growth promotion, many of the antimicrobials used for therapy are related to or the same as those used in human medicine (Casewell et al., 2003; Phillips et al., 2004; Phillips, 2007). Another unintended consequence for human health that was overlooked is the importance of the antimicrobial growth promoters in modulating and promoting good intestinal health. Intestinal integrity in a poultry flock is extremely important, especially in the slaughter and processing of birds; the normal poultry intestinal tract contains a plethora of bacterial organisms, many of which are nonpathogenic to the bird but pathogenic for people. Inflammation and disease of the intestinal tract weakens the gut wall and increases the risk of intestinal breakage and the potential for greater contamination of the final product (Russell, 2003). While meat is not sterile, good intestinal health vital in reducing the bacterial load on poultry products provided to the consumer.

The remaining use of antimicrobials in poultry for growth promotion in countries such as the United States and Canada, and the use of antimicrobials considered critically important in human medicine for therapy of food animals will continue to be scrutinized. The benefits of these products for health, both human and animal, however, also need to be considered. Consumer and retailer pressure in some regions has resulted in removal of these antimicrobials from broiler feeds. Producers

supplying export markets with poultry products may also be required to discontinue use of antimicrobial growth promoters if they wish to continue to supply certain markets where bans are in place, or where consumers demand that antimicrobial use is discontinued (Dibner and Richards, 2005). Overall, the general trend for the future is reduced antimicrobial use. This ultimately means that when the question of whether to treat or not to treat a flock is raised for the poultry veterinarian, there are more factors than ever to consider in the decision making process; effectiveness against the disease agent, pharmacokinetics and pharmacodynamics of the medication, withdrawal times, pathology and physiology, economics/cost-benefit, animal welfare, impact on foodborne pathogens, and impact on the ability to market the final product.

Factors Influencing Antimicrobial Administration in the Poultry Industry

Husbandry and Economics

Under current husbandry conditions in the poultry industry, segregation and medication of individual sick birds is not feasible. The low economic value of the individual bird makes it is cost-prohibitive to individually dose each bird in a house, which eliminates parenteral administration of drugs such as aminoglycosides and cephalosporins. An additional argument against parental administration is that the stress on birds when individually handled can result in a more rapid progression of the disease. Since sick birds continue to drink, therapeutic antimicrobials labeled for use in drinking water are most often used.

Antimicrobial interventions must be administered early in the course of disease. Bacterial infections in birds tend to progress rapidly, and there is frequently a very short time from initial infection to death. In addition, birds are adept at producing inflammatory responses, but poor at resolving the products of such responses. As prey species, poultry tend to hide clinical signs of disease. Spotting the prodromal, subtle signs of infection in individuals in a flock of 10,000–100,000 birds is both important and problematic. Treatment of all individuals in contact and at high risk of exposure (i.e., the entire flock) is the only practical approach to disease outbreaks in large flocks. Thus the decision to treat a "sick flock" of birds means veterinarians will be administering antimicrobials not only to the sick birds, but also to all birds in that flock that have been or will be exposed to the disease agent. In making this decision to treat the "sick flock," the poultry veterinarian must also decide, based on clinical judgment, whether the "flock" to be treated includes the entire farm or only the house containing the most clinically affected birds. A rapidly spreading disease may necessitate prophylactic treatment of all houses on the farm.

When considering treatment in the drinking water or the feed, the poultry veterinarian must take into account lighting schedules and feed programs, which can also strongly influence both feed and water consumption. Laying hens begin to eat when the lights are turned on and then consume water after eating. Broiler chickens and turkeys that have continuous light eat and drink intermittently at 3- to 4-hour intervals. The majority of water intake in replacement breeders under feed restriction occurs for only a few hours after feeding.

Production Type/Bird Type

Within the poultry industry, integrated or not, there is a continuum or flow of birds and bird types. For example, in the chain of production of a commercial broiler (meat-type chicken), the parents of that bird are hatched at a hatchery, reared and brought into egg production. Eggs collected from that flock will return to a hatchery for incubation, to hatch into broiler chickens that will be grown and ultimately slaughtered for meat. Prevention of disease at all levels within this continuum is extremely important; there can be serious downstream consequences if not prevented. The consequences are also impacted by the type of bird and the point in this chain of production at which the disease occurs. For example, disease in a flock producing hatching eggs can not only have a severe impact on overall health and productivity of that flock, but some bacterial diseases such as *Mycoplasma* can be vertically transmitted to offspring, and if not treated, the spread of disease is amplified (Bradbury 2005). Conversely, treatment of flocks in egg production can impact the production or quality of the eggs depending on the antimicrobial used. The use of tetracyclines in flocks in egg production can adversely affect the amount of calcium available to the hen for eggshell formation as these medications are known to chelate with divalent cations. Poor shell quality in the

eggs in turn increases the risk of bacterial contamination of the egg, which when placed in a single incubator with up to 120,000 other eggs, increases the risk of bacterial disease for all other embryos as well. Disease in a broiler flock, where there is only a short time between hatching and slaughter, can be challenging from the perspective of medication withdrawal times. When flocks approach market age, the number of antimicrobial treatment options available for different diseases diminish because their administration would result in violative antimicrobial residues present at the time of slaughter. Antimicrobial residues could in turn result in condemnation of slaughtered birds, or could mean postponing slaughter, which is accompanied by other challenges such as limited space in the barn as the birds will continue to grow. Additionally, postponing slaughter can result in oversized birds. It is critically important that birds meet slaughter specifications, as oversize birds are a challenge for processing equipment, creating a welfare concern for humane slaughter and a challenge to physically process the slaughtered bird, resulting in carcass damage and potentially increased risk of microbial contamination at processing.

Feed and Water Consumption

Flock treatment is the method of choice, with drinking water and feed the primary means of delivering antimicrobials to commercial poultry (Vermeulen et al., 2002). When birds become sick, there is a significant reduction in consumption of both feed and drinking water. The decline in drinking water consumption is usually less than that of feed. Therefore, the route of choice for administering antimicrobials in the early stages of a disease is usually by the birds' drinking water. If therapy lasts more than 5–7 days, then the veterinarian may choose to have the antimicrobial drug added to the birds' feed, if an approved feed-grade product is available. This change to feed can be based upon the flock beginning to recover and eating more. In general, feed-grade antimicrobials are also less expensive than water soluble ones, and are preferred when a suitable drug with a clinically effective inclusion rate is available.

Another consideration when selecting the appropriate antimicrobial is the ambient temperature, since poultry have limited means of eliminating body heat. In large part, they cool themselves by drinking water; therefore, water consumption increases significantly as the ambient temperature increases. This affects dosage calculation and makes it possible for birds to be overdosed when a drug is administered in drinking water. This is especially important with the use of sulfonamides, as the therapeutic dose is close to the level that can result in toxic effects (Goren et al., 1984). Fortunately, bacterial diseases in general tend to be less common in hot weather.

Pathology and Disease Etiology

Escherichia coli is the leading cause of disease-related economic loss for the poultry industry throughout the world (Barnes et al., 2003). In most instances, *E. coli* infections are secondary infections following a primary viral or environmental insult (Glisson, 1998). Therefore, therapeutic antimicrobials in commercial poultry are almost always used to relieve the suffering of the sick birds, control morbidity and mortality, and minimize the financial impact of the disease on bird performance until the primary insult can be identified and controlled or eliminated. The use of therapeutic antimicrobials also decreases the public health risk associated with slaughtering birds from sick flocks. Poultry that are sick eat greater amounts of bedding material (litter), resulting in higher rates of *Salmonella* and *Campylobacter* spp. in their intestinal tracts (Corrier et al., 1999). Also, Russell (2003) found that birds from flocks having higher air sacculitis condemnation had higher levels of *E. coli* and *Campylobacter* contamination.

The choice of therapeutic antimicrobials available to treat respiratory infections caused by *E. coli* is limited (Glisson and Hofacre, 2004). The tetracyclines, enrofloxacin, and the sulfonamides are the primary drugs used to treat *E. coli* airsacculitis. It can be speculated that this limited choice of antimicrobials has, over 30 years, resulted in selection pressure on *E. coli* in the commercial poultry environment, resulting in the high levels of sulfonamide (93%) and tetracycline (87%) resistance in clinical *E. coli* isolates observed in many diagnostic laboratories (Zhao et al., 2005).

The immune status of the flock must also be taken into account when deciding which antimicrobial agent to use and the dose rate. For example, chickens experiencing an *E. coli* air sacculitis outbreak secondary to immune suppression by infectious bursal disease virus should be treated with a bacteriocidal drug such as enrofloxacin. However, a bacteriostatic drug such as oxytetracycline may be more effective in treating *E. coli*

Table 34.1. Antimicrobial treatment options in poultry.

	Antimicrobial																
Disease/Bacterial Species	Bacitracin	Bambermycins	Ceftiofur	Chlortetracycline	Enrofloxacin	Erythromycin	Gentamicin	Lincomycin	Neomycin	Novobiocin	Oxytetracycline	Penicillin	Spectinomycin	Streptomycin	Sulfonamide	Tylosin	Virginiamycin
Arthritis/ *Staphyloccus aureus*				X		X				X	X	X	X	X			
Chronic respiratory disease (CRD)/ *Mycoplasma spp.*				X	X*						X		X			X	
Colibacillosis/ *Escherichia coli*				X	X						X		X	X	X		
Erysipelas/ *Erysipelothrix rusiopathiae*												X					
Fowl cholera/ *Pasteurella multocida*				X	X*						X	X	X	X	X		
Fowl coryza/ *Haemophilus paragallinarum*				X		X					X			X	X	X	
Gangrenous dermatitis/ *Clostridium spp.*				X		X					X	X		X		X	
Necrotic enteritis/ *Clostridium perfringens*	X	X						X	X			X	X	X		X	X
Omphalitis/ *Pseudomonas spp. &/ or Enterobactericiae*			X	X			X				X		X	X	X		
Salmonellosis/ *Salmonella* spp.				X			X				X			X	X		

*Extra-label use of enrofloxacin is illegal in the United States.
Information based on published data, clinical experience; use may be extra-label.

airsacculitis that is secondary to respiratory infection by an infectious bronchitis virus.

Pharmacology

The success of antimicrobial therapy depends upon many interacting factors, including pharmacodynamics (drug interaction with the pathogen), pharmacokinetics (drug absorption, distribution, excretion) and the components of the host immune system (chapters 4 and 5). The activity of an antimicrobial agent against a particular microbe is often expressed as the minimal inhibitory concentration (MIC; chapter 2). When interpreting antimicrobial susceptibility information, the poultry veterinarian must keep in mind that this is an *in vitro* test that does not take into consideration whether the drug can reach the site of infection or whether the drug is bacteriostatic or bactericidal for the microbe. It should also be remembered that the MIC is usually performed by the laboratory on only one isolate, and as was previously stated, many infections of poultry are secondary, so a "sick flock" is often affected by multiple isolates that can have a wide range of MICs. Also, MIC breakpoint criteria in veterinary medicine are not uniform worldwide and are often based on standards for human medicine (chapter 2). Additionally, pharmacokinetic data determined in mammals are not always applicable to poultry because birds have higher body temperatures, higher metabolic rates, and shorter alimentary tracts, which often results in shorter elimination half-life times for medications. This frequently leaves the poultry veterinarian with an antimicrobial therapy decision based upon clinical judgment from previous cases rather than on the uncertain available science. The primary criterion for measuring success of treatment under poultry industry conditions is reduction of morbidity and mortality. Other important parameters include return to regular water and feed consumption, normal growth rate, and normal egg production.

Practical Antimicrobial Drug Application under Commercial Poultry Conditions

Since commercial poultry are food animals, the choice of antimicrobials to treat the most common bacterial diseases is limited (Table 34.1). The decision to treat is usually made prior to the results of culture and susceptibility testing. Oral treatment of poultry requires that the drug be stable and be uniformly distributed in either feed or water. When a feed-based antimicrobial is prescribed, the time required for the medicated feed to be manufactured, transported, and delivered through the feeding system at the farm must be taken into account.

Administering the antimicrobial in the drinking water allows for more rapid treatment. The volume of water consumed in 24 hours by the birds in the house to be treated must first be determined. Freshly medicated solutions should be prepared every day. Drinking water medication is usually administered by either a bulk tank or a water proportioner. Bulk tanks contain 500–2000 liters, and all of the medication for a given tank's volume of water is added to it. A water proportioner is a device that meters the antimicrobial from a highly concentrated stock solution into the drinking water to achieve the appropriate concentration.

It should be apparent that administering antimicrobials to poultry based solely on concentration of the active ingredient in the drinking water and ignoring the above described physiological, pathological, and husbandry conditions can lead to highly inaccurate dosing. The most accurate method is to calculate the dose based upon the total body weight of birds in the house, and then include that dose in the volume of water or feed the birds are expected to consume during each dosing interval. Dosing based on water consumption can result in a toxic overdose if the ambient temperature increases, or the amount of drug consumed may drop below the MIC of the bacteria being treated if the ambient temperature declines. Additionally, younger birds consume more water daily per unit of body weight than older birds. Dosing at a constant rate per liter of drinking water can result in overdosing of young chicks or underdosing of older birds. In addition, hens producing eggs will drink more per unit of weight than nonlaying hens or roosters. Approved daily dosages are shown in Table 34.2.

In situations where the birds' water consumption is limited, a short, intensive treatment with certain antimicrobials may be administered as a pulse dose (Charleston et al., 1998). This method should only be used with bactericidal antimicrobials and those with a wide margin of safety. Pulse dosing requires that all of

Table 34.2. Antimicrobial Treatment Options.

			Canada			United States		
Antimicrobial	Disease/ Bacterial Species	Approved for use in (bird type)	Therapeutic Dose(s)/Route = Feed mg/kg of feed (unless units defined)	Prophylactic Dose(s)/ Route = Feed mg/kg of feed (unless units defined)	Withdrawal time(s) Days (unless defined)	Therapeutic Dose(s)/ Route = Feed g/ton (U.S.) of feed (unless units defined)	Prophylactic Dose(s)/ Route = Feed g/ton (U.S.) of feed (unless units defined)	Withdrawal time(s) Days (unless defined)
Amoxicillin	Colibacillosis	Meat Chicken	8–16 mg/kg oral	–	2	15–20 mg/kg oral	–	?
Apramycin	Colibacillosis	Extra-label Use	0.25–0.5 g/l oral	–	18 CgFARAD	0.25–0.5 g/l oral	–	?
Bactitracin zinc	Necrotic enteritis	Meat Chicken Layer Chicken Turkey	–	55–110	0	100–400 g/ton	4–50	0
Bacitracin methylene disacyclate	Necrotic enteritis Ulcerative enteritis	Meat Chicken Layer Chicken (feed only)* Turkey (feed only)* Others	–	4.4–55* 27.5–158 mg/L	0	–	4–200* 100–400 mg/gal	0
Ceftiofur (subcutaneous injection of day-old birds only)	Colibacillosis Yolk sac infection	Chicken Turkey Banned from ELDU in U.S.	–	Chicken ELDU 0.17 mg/poult	21 {21}	–	0.08–0.2 mg/chick 0.17–0.5 mg/poult	0
Chlortetracycline	*Staph.* spp. CRD/Mycoplasma Colibacillosis Fowl cholera Fowl coryza	Meat Chicken Layer Chicken (feed only)* Turkey	110–220	55–110* 55–220	7	106–264.5 mg/L 100–500 g/ton	50–200	1
Enrofloxacin	CRD/Mycoplasma Colibacillosis Fowl cholera	Banned from ELDU in US	10–25 mg/kg BW	–	12–21 CgFARAD	10 mg/kg BW	–	prohib
Erythromycin	*Staph.* spp. Fowl coryza Mycoplasma	Meat Chicken Turkey	57.8–115.6 mg/L	220	1	92.5–185 g/ton 115.6–250 mg/L	92.5–185	1

Gentamicin (subcutaneous injection of day-old birds only)	Colibacillosis Yolk sac infection *Pseudomonas* spp.	Meat Chicken Turkey	–	0.2 mg/chick 1.0 mg/poult	35–63	–	0.2–1.0 mg/chick 0.2 mg/poult	35–63
Lincomycin	Necrotic enteritis	Meat Chicken	16 mg/L	3	0	2 g/ton 17 mg/L	2	0
Lincomycin + spectinomycin	Necrotic enteritis CRD Mycoplasma	Meat Chicken	833 mg/L (total activity)	–	3	50–65 mg/lb BW 530–833 mg/L	–	0
Neomycin	Necrotic enteritis	Meat Chicken Turkey*	–	9.6–19.1 mg/L	7–14	35–226 g/ton 5–10 mg/lb BW*	35–80 mg/L*	0
Neomycin + tetracycline	Bacterial enteritis CRD	Meat Chicken Turkey	140 + 200–70 + 100 mg/L		7–14	100–200 g/ton 35–40 mg/L		0–3
Nitarsone	Histomoniasis	Meat Chicken Turkey	–	187.5	5	–	170	5
Nifursol	Histomoniasis						50	
Novobiocin	*Staph.* spp. Pasteurellosis Riemerellosis	Meat Chicken Turkey* Ducks	–	385*	4* Only labeled for use in turkey in CA	200–350 g/ton 4–14 mg/lb	–	3–4
Nystatin	Candidiasis	Turkey	–	–	–	50–100 g/ton	50	?
Oxytetracycline	*Staph.* spp. CRD/Mycoplasma Colibacillosis Fowl cholera Fowl coryza	Layer Chicken* Meat Chicken Turkey	50–111* mg/L	55–220*	0–60* hours egg 7 day meat	100–500 g/ton 26.5–105.8* mg/L 6.25–200* mg/bird	50–200*	0–5
Penicillin	*Staph.* spp. Necrotic enteritis Eyrsipelas Fowl cholera Fowl coryza	Meat Chicken Turkey	297,000 IU/l	2.2	1	100 g/ton 1,500,000 IU/gal	50–100	0–1
Penicillin/Streptomycin	*Staph.* spp. Necrotic enteritis	–	–	–	–	20,000 IU + 25 mg/lb BW	–	–
Spectinomycin	*Staph.*spp. CRD/Mycoplasma Colibacillosis Necrotic enteritis Fowl cholera	Meat Chicken	–	–	ELDU CgFARAD	264–530 mg/L 2.5–10 mg/chick	132 mg/L	0–5

(*continued*)

Table 34.2. Antimicrobial Treatment Options. (*continued*)

			Canada			United States		
Antimicrobial	Disease/ Bacterial Species	Approved for use in (bird type)	Therapeutic Dose(s)/ Route = Feed mg/ kg of feed (unless units defined)	Prophylactic Dose(s)/ Route = Feed mg/kg of feed (unless units defined)	Withdrawal time(s) Days (unless defined)	Therapeutic Dose(s)/ Route = Feed g/ton (U.S.) of feed(unless units defined)	Prophylactic Dose(s)/ Route = Feed g/ton (U.S.) of feed(unless units defined)	Withdrawal time(s) Days (unless defined)
Streptomycin	*Staph.*spp. Colibacillosis Necrotic enteritis Fowl cholera Fowl coryza	Meat Chicken* Turkey Layers	85–93 mg/L	–	5 meat/egg in combination with penicillin/ vitamins	66–100* mg/L 10–15* mg/lb BW	–	4
Sulfachlorpyridazine	Coccidiosis	Meat Chicken	ELDU	ELDU	ELDU	–	0.03%	4
Sulfachlorpyridazine/ Trimethoprim	Colibacillosis Fowl cholera	Poultry	24 mg total activity/kg BW	24 mg total activity/kg BW	ELDU CgFARAD	24 mg total activity/kg BW	24 mg total activity/ kg BW	–
Sulfadiazine/Trimethoprim	Colibacillosis Fowl cholera	Meat Chicken	750 ppm	–	ELDU CgFARAD	15 mg total activity/kg BW 300 g total activity/ met ton	–	–
Sulfadimethoxine	Colibacillosis Fowl cholera	Meat Chicken Turkey	–	–	ELDU CgFARAD	250–500 mg/L	–	5
Sulfadimethoxine/ Ormetoprim	Colibacillosis Fowl cholera	Meat Chicken Turkey Ducks Partridge*	ELDU	ELDU	ELDU CgFARAD	227 + 136.2 g/ton to 454 + 272 g/ton	56.75 + 34.05 to 113.5 + 68.1	5 10 wks*
Sulfamethazine	Colibacillosis Fowl cholera Riemerellosis Coccidiosis	Meat Chicken* Turkey Ducks	1000–2500 mg/L	250 mg/L	12	1000 mg/L 110–273 mg/kg	128–187* mg/kg/day BW 110–273 mg/kg/day BW	10
Sulfaquinoxaline	Colibacillosis Fowl cholera Coccidiosis	Meat Chicken Turkey*	380 mg/L	255 mg/L	12	397 mg/L 10–45 mg/lb/day 3.5–55* mg/lb/day	3.5–60 mg/lb/day 2.5–100* mg/lb/day	10–14
Sulfaquinoxaline / Trimethoprim	Colibacillosis Fowl cholera	Meat Chicken Turkey	ELDU	ELDU	ELDU CgFARAD	30 mg total activity/kg	–	–

Sulfathiazole	Colibacillosis Fowl cholera	–	–	–	–	1000 mg/L	–	–
Sulfamethazine, sulfamerazine, sulfaquinoxaline	Colibacillosis Fowl cholera Coccidiosis	Meat Chicken Turkey	–	–	ELDU CgFARAD	160 + 160 + 80–100 + 100 + 50 mg/L	–	14
Tetracycline	*Staph.* spp. arthritis CRD/Mycoplasma Fowl cholera Fowl coryza	Meat Chicken Turkey	45–100 mg/L	45–100 mg/L	5	200–1000 mg/gal/day 25–50 mg/bird intranasal 25 mg/lb/day BW	100–200 mg/L	4–5
Tylosin	CRD/Mycoplasma Necrotic enteritis Fowl coryza	Layer Chicken* Meat Chicken Turkey	200 mg/kg 500 mg/L	11–44	0–3 not approved for use in Layers	800–1000 g/ton 530 mg/L 15–25 mg/bird intranasal 50–60 mg/lb/day	20–50* 4–50	0–5
Virginiamycin	Necrotic enteritis	Meat Chicken	–	11–22	0	–	5–20	0

Information based on published data (including Table 35.3 from previous edition of this publication), clinical experience; use may be extra-label; withdrawal times and doses must be confirmed by the reader based on product labels and government regulations. Extra-label use of enrofloxacin is illegal in United States. Banned = prohibited for use in meat- and egg-producing birds. ELDU = extra-label drug use. CgFARAD = contact the Canadian Global Food Animal Residue Avoidance Database for withdrawal information. References: CFIA, 2012; FDA, 2012; North American Compendiums, 2012.

the medication to be administered for a 24-hour period is mixed into the water the birds will consume in, for example, 6 hours.

Pharmacological Characteristics of Poultry Antimicrobials

Beta-lactams (Cephalosporins and Penicillins)

Despite years of use, penicillin G is still an effective antimicrobial for Gram-positive bacterial infections in poultry. This drug is particularly important for the therapy of clostridial infections causing necrotic enteritis (Gadbois et al., 2008). The one Gram-negative bacterium routinely treated with penicillin is *Pasteurella multocida*. Recent publications continue to indicate susceptibility of this pathogen to penicillin and support the selection of this medication in the treatment of pasteurellosis or Fowl Cholera (Huang et al., 2009; Sellyei et al., 2009). Pencillin G is formulated for both drinking water and feed administration, with water administration being the preferred initial route of administration. The broader-spectrum beta-lactams, such as ampicillin and amoxicillin, theoretically are more effective for Gram-negative infections such as *E. coli* airsacculitis; however, there is limited data published on the use and clinical efficacy of these medications in poultry species. The reportedly short half-lives of both amoxicillin and ampicillin when administered to poultry species is a desirable characteristic from the perspective of managing withdrawal times in broiler flocks, where this can be a factor limiting the options available for treatment (El-Sooud et al., 2004; Fernandez-Varon et al., 2006). One potential factor that may limit the use of these products is the reportedly poor stability of amoxicillin in aqueous solution (Jerzselle and Nagy, 2009). While there are no products currently available or approved for such use in the United States, Canada, or EU poultry industries, concerns regarding increasing bacterial resistance to amoxicillin and ampicillin have prompted some European researchers to investigate the pharmacokinetics of these antimicrobials in combination with beta-lactamase inhibitors clavulanic acid and sublactam in poultry (Fernandez-Varon et al., 2006; Jerzsele et al., 2009; Jerzsele et al., 2010).

The only cephalosporin used in poultry production is ceftiofur, a third-generation cephalosporin. Since it has poor oral absorption, ceftiofur is only approved for subcutaneous injection in day-old chicks (United States) and poults (United States and Canada). It is commonly administered along with Marek's disease vaccine to day-old chicks (Kinney and Robles, 1994), either subcutaneously or in an extra-label fashion by *in ovo* injection at approximately 18 days of incubation. The extra-label *in ovo* administration of ceftiofur in the United States has recently been banned (FDA, 2012). The need for the use of a third-generation cephalosporin should be assessed against the risk of selecting for resistance to this important group of drugs, including the danger of selection of multidrug-resistant *Salmonella* carrying the bla_{CMY2} resistance gene, since such isolates would also be resistant to ceftriaxone, a drug used to treat salmonellosis in people (chapter 8).

Polypeptides

Bacitracin is the only poultry-approved polypeptide antimicrobial. Its effect is local, as it essentially not absorbed when administered orally in poultry. Bacitracin is a very effective antimicrobial for treatment of Gram-positive enteric infections such as necrotic enteritis caused by *Clostridium perfringens* (Hofacre, 1998). It is available in both drinking water and feed additive formulations, with the feed-grade form commonly used as a preventative for necrotic enteritis.

Aminoglycosides and Aminocyclitols

Three aminoglycosides are used in poultry: gentamicin, streptomycin, and neomycin. Because aminoglycosides are poorly absorbed from the gastrointestinal tract when administered orally, their primary usage in poultry has been by subcutaneous injection. Gentamicin is the most widely used aminoglycoside, and it is used primarily as a day-old subcutaneous injection or *in ovo* injection in chickens or turkeys (McCapes, 1976; Vernimb, 1977). A dose of 5 mg/kg body weight in broiler chickens has been reported to be a suitable therapeutic dose when administered either intravenously, intramuscularly or subcutaneously. Subcutaneous administration was associated with the best absolute bioavailability (100%), while oral administration had an absolute bioavailability of zero (Abu-Basha et al., 2007a). Because gentamicin is a highly basic compound, it can damage cell-associated Marek's disease vaccine if used at too high a dose (greater than 0.2 mg/chick) or

improperly mixed with the vaccine (Kinney and Robles, 1994). Streptomycin is partially absorbed from the intestines and therefore can be considered for use to treat systemic *E. coli* infections. Neomycin is commonly used to treat enteric infections, administered either in the feed or water. Interestingly, despite poor absorption from the gastrointestinal tract there are reports that administration of neomycin has resulted in clinical efficacy in the treatment of colibacillosis in poultry, likely due to a local effect (Marrett et al, 2000).

Spectinomycin and hygromycin are poultry-approved aminocyclitols. Hygromycin is used for its anthelmintic properties rather than as an antimicrobial and is administered in the feed. Spectinomycin is a relatively safe antimicrobial in poultry that when administered once orally, at doses of 50–100 mg/kg body weight, has limited absorption from the gastrointestinal tract with absolute bioavailability reported as 11.8% and 26.4%, respectively (Abu-Basha et al., 2007b). Similar to neomycin, spectinomycin has been reported to be highly efficacious for *E. coli* infections when administered in the drinking water (Goren et al., 1988). This antimicrobial is available commercially alone or in combination with lincomycin. This combination has also been reported as efficacious in controlling early chick mortality associated with *E. coli* and *Staphylococcus aureus* when administered subcutaneously (Hamdy et al., 1979) and has been used as an alternative to gentamicin or ceftiofur for prophylaxis in some hatcheries. However, rapid development of resistance and higher cost limits the use of spectinomycin.

Apramycin is another aminocyclitol approved for use in poultry in some European countries and can be used in an extra-label fashion were permitted. Consistent with the observations of other antimicrobials in this class, oral absorption is poor (Afifi NA, Ramadan A, 1997). There are, however, reports that oral administration of apramycin for treatment of *E. coli* infections has been associated with a clinical response (reduced mortality, improved final body weight and feed conversion) and reduced intestinal colonization by *E. coli* (Cracknell et al., 1986; Leitner et al., 2001).

Macrolides and Lincosamides

The macrolides commonly used in poultry include erythromycin, tylosin, tiamulin and tilmicosin. While the use of these antimicrobials may not be permitted in all countries, all are available in formulations for administration either in the drinking water or the feed. Erythromycin is most frequently used in poultry to treat *Staphylococcus aureus* arthritis. Tylosin has been one of the most effective antimicrobials to treat mycoplasma infections in laying hens to restore egg production, reduce transovarial transmission and reduce clinical signs (Bradbury et al., 1994; Kleven, 2008). The macrolides are only bacteriostatic, which may be one reason that their use will not entirely eliminate *Mycoplasma* spp. infections from a flock and thus treatment is not considered a long-term solution. Clinical and subclinical necrotic enteritis in poultry flocks can also be successfully treated with tylosin (Brennan et al., 2001a; Collier et al., 2003; Lanckriet et al., 2010). Tiamulin, a semisynthetic macrolide available outside the United States for poultry, has excellent efficacy against *Mycoplasma* spp. infections (Laber and Schutze, 1977). Additionally, this antimicrobial has proven efficacious in the treatment of avian intestinal spirochetosis (Stephens and Hampson, 2002; Burch et al., 2006; Islam et al., 2009). It is important to note, however, that with the exception of lasalocid, tiamulin is incompatible with the ionophore anticoccidials; monensin, salinomycin, narasin, maduramicin, and semduramicin. Administration of tiamulin with these ionophores results in clinical signs consistent with ionophore toxicity, and seems to interfere with metabolism and excretion of these compounds (Islam et al., 2009). Tilmicosin, like the other antimicrobials in this family, has proven effective for control mycoplasma infections and has also been used to treat *Pasteurella multocida* and *Ornithobacterium rhinotracheale* bacterial infections (Jordan and Horrocks, 1996; Kempf et al., 1997; Jordan et al., 1999, Abu-Basha et al., 2007c; Warner et al., 2009).

The only poultry-approved lincosamide is lincomycin. Although it is absorbed with oral administration in feed or water, lincomycin is primarily used to treat enteric infections in poultry such as *Clostridium perfringens*-induced necrotic enteritis or intestinal spirochaetosis (Lanckriet et al., 2010; Stephens and Hampson, 2002). As previously described, this antimicrobial is also available in combination with spectinomycin and has been used effectively to control clinical signs and lesions associated with infections due to mycoplasma species in poultry (Hamdy et al., 1982; Hamdy et al., 1976).

Florfenicol

The potential for fatal aplastic anemia in humans resulted in the prohibition of chloramphenicol in animals grown for human consumption throughout most of the world (chapter 16). However, the closely related antimicrobial florfenicol lacks the para-nitro group associated with aplastic anemia in humans, and is available for use in food-producing animals, including poultry, for the treatment of susceptible Gram-positive and/or Gram-negative infections. There are several publications on the pharmacokinetics of florfenicol in poultry species, indicating that the oral bioavailability of this antimicrobial is relatively high; reports vary from 55.3% to 94% (Afifi and El-Sooud, 1997; Shen et al., 2002; Shen et al., 2003; Switala et al., 2007). Shen et al. (2003) suggest that some of the discrepancy in the numbers reported may relate to timing of oral administration in relation to feeding as there have been reports of variable bioavailability between fasted and fed animals. Successful clinical response to treatment with florfenicol appears to be somewhat inconsistent. The multiplication of *E. coli* and *Ornithobacterium rhinotracheale* in a dual bacterial infection model, as well as the associated clinical signs were significantly reduced in turkeys treated with 20 mg/kg body weight of florfenicol for 5 days (Marien et al., 2007). In the authors experience however, the use of florfenicol to treat *E. coli* infections in broiler chickens has not been successful. There may be several reasons for this observation including incompatibility with water administration via a proportioner when water hardness is > 275 ppm (North American Compendium, 2012). Additionally, suitable therapeutic plasma concentrations for the targeted pathogen may not be achieved as there is scant information published on the florfenicol MIC values for poultry pathogens such as *E. coli*. Several publications on the pharmacokinetics of florfenicol in poultry concur that plasma concentrations above 2 μg/ml for 11 hours can be achieved after a single dose of 30 mg/kg body weight florfenicol (Shen et al., 2003; Switala et al., 2007). As the activity of florfenicol is time-dependent, it is important that plasma concentrations can be maintained above the MIC during treatment. In the absence of MIC data for poultry pathogens, many have looked to the MIC data for bacteria isolated from other species and have extrapolated these values to conclude that florfenicol should also be effective in poultry (Anadon et al., 2008). This may not be appropriate, as there are several publications documenting florfenicol MIC_{90} data against *E. coli* to be 8 μg/ml and 16 μg/ml or higher in turkeys and chickens respectively (Salmon and Watts, 2000; Dai et al., 2008). There has been one report of severe muscle degeneration in broiler chickens treated concurrently with both lasalocid and chloramphenicol (Perelman et al., 1986); there is no information as to whether or not this may occur with concurrent use of lasalocid and florfenicol.

Tetracyclines

The tetracyclines are the most widely used antimicrobials in poultry. This is largely due to their broad spectrum of activity (*Mycoplasma*, Gram-positive and Gram-negative bacteria) and wide margin of safety. This class of antimicrobials is also one of few with label claims permitting use in egg laying breeds of chickens, at the specified dosage, with a zero day egg withdrawal. The tetracyclines are available in formulations that can be administered in feed and/or water. Since they are only slightly soluble in water at pH 7.0, concurrent use of citric acid greatly enhances their absorption from the gastrointestinal tract (Clary et al., 1981). Tetracyclines are readily chelated in the intestine by divalent cations such as calcium or magnesium, resulting in reduced absorption (chapter 15). Therefore the dosage of tetracyclines to laying hens on a high-calcium diet should be increased. After administration is complete, it is recommended to include additional calcium in the diet to improve eggshell thickness and make up for calcium lost to tetracycline binding and intestinal excretion during therapy. For this same reason, tetracyclines are also incompatible with concurrently administered oral electrolytes.

Three tetracyclines most commonly used in poultry are chlortetracycline, oxytetracycline, and tetracycline. It appears that any differences in clinical efficacy of these tetracyclines are primarily because of differences in absorption, drug distribution, or rate of excretion, and not because of differences in bacterial susceptibility, since there is complete cross-resistance (chapter 15). It should be remembered that *E. coli* air sacculitis is a secondary infection and even though the *E. coli* isolate selected for susceptibility testing demonstrates resistance to tetracyclines, therapy of a flock of poultry with a tetracycline may still be successful in reducing

the clinical signs. This may be because tetracyclines will inhibit *Mycoplasma* that predispose birds to *E. coli* infection.

Sulfonamides

The sulfonamides are broad-spectrum antimicrobials widely used to treat or prevent coccidial infections in poultry. There are a wide variety of sulfonamides available for feed and/or water administration. Sulfonamides are more soluble in an alkaline pH (chapter 17). Therefore when administering sulfonamides in acidic water, it may be necessary to raise the pH of the water with household ammonia if the drug precipitates in the bulk tank or stock solution. Conversely, if the poultry water supply is being acidified, this process should be discontinued prior to and during treatment.

The use of sulfonamides has been limited in poultry because of their narrow margin of safety and problems of tissue residues at slaughter. Toxic effects of sulfonamides include bone marrow suppression, thrombocytopenia, and depression of the lymphoid and immune function of birds (chapter 17). This is frequently manifested as pale, almost yellow colored bone marrow and petechial or ecchymotic hemorrhages on the breast, thigh, and leg muscles (Daft et al., 1989). The most frequent toxic side effect of sulfonamide therapy in laying hens is a decline in egg production and eggshell quality (loss of brown pigment). The ambient temperature must be noted when deciding to administer a sulfonamide in the drinking water because as the birds become warmer, they will increase their rate of water consumption to cool themselves. This can quickly result in sulfonamide toxicity. The combination of sulfonamides with ionophores may also predispose birds to toxic effects. The mechanism for this toxicity has not yet been elucidated; however, the effect that the drug combination has on the cytochrome P450 enzyme system has been hypothesized as one possible explanation and is being investigated (Ershov et al., 2001).

There is one potentiated sulfonamide in the United States (sulfadimethoxine/ormetoprim) approved for use in feed. In Canada, there are several products (sulfadiazine/trimethoprim) that are approved for use in salmon or horses, but are used in an extra-label manner to treat poultry. Outside of their use to treat coccidiosis in poultry, the potentiated sulfonamides are also used to treat bacterial infections caused by *E. coli* and/or *Pasteurella multocida*. The combination of these drugs allows for a therapeutic dose at a much lower level of each product, lessening the risk of overdose toxicity.

The other major "adverse effect" of administering the sulfonamides to poultry is the potential for presence of violative residues in meat or eggs. Poultry are highly coprophagic and the sulfonamides are excreted in the urine and feces; therefore, recycling by coprophagy can result in residues of the drug beyond the stated withdrawal time (Gupta and Sud, 1978). A poultry veterinarian prescribing a sulfonamide should include an additional withdrawal period to ensure adequate time for drug clearance (greater than 7–10 days) prior to harvest of meat or eggs.

Quinolones and Fluoroquinolones

Many of the quinolones, such as naladixic acid or oxolinic acid, have been used in poultry to treat primarily Gram-negative bacterial infections. However, when these compounds are used, resistance in the bacterial population in these flocks develops quickly and can eventually result in more rapid resistance developing to the fluoroquinolones (Glisson, 1997). Therefore poultry veterinarians should not recommend the use of these older quinolones in commercial poultry. While now banned for use in poultry in the United States, fluoroquinolones are available for therapeutic use in some countries and permitted for extra-label use in others.

The fluoroquinolones are some of the most effective antimicrobial compounds developed for use in poultry. These compounds are highly effective against Gram-positive, Gram-negative, and *Mycoplasma* infections. It was shown that one of the fluoroquinolones, enrofloxacin, eliminated a *Mycoplasma gallisepticum* infection in laying hens (Stanley et al., 2001). However, the fluoroquinolones are ineffective against anaerobic bacteria, such as *Clostridium perfringens*.

The fluoroquinolones have a wide margin of safety in poultry. They are rapidly absorbed from the gastrointestinal tract, reaching peak blood levels within 1–2 hours after ingestion. The long half-life of the fluoroquinolones results in a significant post-antibiotic effect. This gives the poultry veterinarian the opportunity to administer the fluoroquinolones by a "pulsed dose" method in the drinking water

(Charleston et al., 1998), which takes advantage of concentration-dependent killing to help prevent the emergence of resistance (chapter 18). Rapid development of resistance to fluoroquinolones is a significant problem (chapter 18), and has resulted in resistance increasing in *Campylobacter jejuni*. This issue is discussed in chapter 3.

The presence of multivalent cations in the intestine or in the drinking water (water hardness ≥ 1300 ppm) will adversely influence the absorption of the fluoroquinolone (Sumano et al., 2004). Therefore it is not recommended to concurrently administer electrolytes with a fluoroquinolone.

Ionophores

The primary use of ionophore antimicrobials in poultry is to prevent coccidial infections. However, they also have activity against Gram-positive bacteria, especially anaerobes such as *Clostridium perfringens* (Brennan et al., 2001b; Lanckriet et al., 2010).

Since the ionophores function by altering cell permeability of both prokaryotic and eukaryotic cells, the toxic side effects in poultry are reluctance to move and paralysis. This is caused by muscle weakness resulting from passive transport of potassium out of the cells, with calcium entering. Ionophore toxicity is more severe in adult birds and especially turkeys, even at a safe therapeutic dose for young chickens (Fulton, 2008).

Novobiocin

Novobiocin is rarely used in commercial poultry. It is primarily used to treat juvenile pullets or hens early in the laying house for *Staphylococcus aureus* arthritis. Novobiocin is poorly water soluble, and so must be administered in the feed. High cost is a major reason for its limited use.

Nitrofurans

The nitrofuran antimicrobials have been removed from systemic use in poultry in much of the world because of their carcinogenic potential. They are broad-spectrum antimicrobials that were at one time commonly added to poultry starter feed to reduce the effects of egg-transmitted *Salmonella* infections in the first 2 weeks of life. In poultry, nitrofuran toxicity results in congestive cardiomyopathy (ascites) or central nervous system signs (Zaman et al., 1995).

Responsible Use of Antimicrobials in Poultry

The responsible use of antimicrobial drugs in poultry producing meat and eggs for human consumption is based upon good professional judgment, laboratory results, medical knowledge, and information about the flock to be treated. Above all, residue avoidance is critical to ensure that people are not accidentally exposed to antimicrobial residues in poultry products. When a flock of commercial poultry begins to exhibit signs of illness, the birds should be physically examined (ante mortem and post-mortem). If possible, bacterial cultures should be performed to confirm the clinical diagnosis and to determine the susceptibility of the isolate to the chosen antimicrobial. The potential for rapid spread of disease on a poultry farm often necessitates empirical treatment prior to the results of bacterial culture and susceptibility testing. When laboratory results are available, the poultry veterinarian must use clinical judgment to decide between continuing or changing therapy. Also, a flock will usually have birds in three stages of disease development when symptoms are first noted: clinically ill, incubating with no outward signs of illness, and unaffected but susceptible. Therefore, the entire flock is treated instead of just the clinically ill birds. Such strategic medication in anticipation of major disease spread is justifiable under conditions of good husbandry practices and animal welfare. Finally, responsible therapy also allows sufficient withdrawal time for the antimicrobial to be eliminated from meat or eggs destined for human consumption. Additional information on judicious antimicrobial use is available in chapter 7 and from the American Veterinary Medical Association (http://www.avma. org/scienact/jtua/default.asp).

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35

Antimicrobial Drug Use in Companion Birds

Keven Flammer

Companion birds include members of the orders Psittaciformes (e.g., parakeets, parrots, lories, cockatoos, and macaws), Passeriformes (e.g., canaries and finches), and Columbiformes (e.g., pigeons and doves). Psittacine birds are the most common pet birds in the United States; over 50 species are commonly seen in veterinary practice. Microbial diseases are common and use of antimicrobial drugs is an important part of avian practice. Optimal treatment regimens can be developed if the principles of rational antimicrobial therapy are integrated with the unique behavioral and physiological characteristics of birds.

The general approach to selecting an avian antimicrobial treatment regimen is similar to other species. The site and cause of infection should be identified and the minimal inhibitory concentrations (MIC) of potentially effective antimicrobial drugs determined. Selection of the most appropriate drug will then depend on the severity of illness, site of infection, pharmacokinetic and pharmacodynamic properties of the selected drugs, and the routes of administration that can be accomplished by the owner or veterinary staff. Additional considerations are drug side effects, toxicity and cost.

Establishing the Cause and Site of Infection

A wide variety of primary and secondary bacterial pathogens have been identified in companion birds (Table 35.1); however, some are more common than others. In psittacine birds, Gram-negative bacterial infections are most common, especially those caused by *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas aeruginosa*. Other Gram-negative bacteria include *Bordetella* spp., *Pasteurella* spp., *Proteus* spp., *Salmonella* spp., *Serratia* spp., and *Yersinia* spp. Gram-positive bacterial pathogens include *Staphylococcus aureus* and *Enterococcus* spp. *Chlamydophila psittaci* is the most important intracellular pathogen; *Mycobacterium avium* and *M. genavense* are occasionally seen. Anaerobes are relatively uncommon, although clostridial infections of the alimentary tract do occur. Similar pathogens are found in canaries and pigeons; *Enterococcus faecalis* is an important cause of respiratory disease in canaries and there is a higher incidence of *Salmonella* spp. and *Streptococcus gallolyticus* infections in pigeons.

Mycotic infections are also important (Table 35.1). Yeasts most commonly affect the alimentary tract and common pathogens include *Candida albicans* and *Macrorhabdus ornithogaster*. Hyphal fungi are important pathogens of the respiratory tract and, occasionally, the eye and skin. *Aspergillus fumigatus* and *A. niger* are the most common isolates; *Mucor* spp., *Penicillium* spp., *Rhizopus* spp., and *Scedosporium* spp. and other opportunist moulds may rarely infect immunocompromised birds.

In companion birds, septicemia and infections of the alimentary tract, respiratory tract, and liver are the most common sites of microbial infection. It is important to note that simply culturing a potential pathogen is not an

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Table 35.1. Antimicrobial drug selection in companion avian infections.

Site or Type of Infection	Diagnosis	Common Organisms	Suggested Drugs	Comments
Sick bird—severe illness, cause unknown	Septicemia, multiple organ infection	Aerobic bacteria, especially *E. coli* and *Klebsiella*. *Salmonella* in pigeons	Enrofloxacin; piperacillin; cefotaxime	Use IV, IM, or SQ route.
		Pseudomonas aeruginosa	Ceftazidime or piperacillin ± amikacin; meropenem	Maintain hydration to avoid toxicity. Limited studies in birds.
		Chlamydophila psittaci	Doxycycline	Use IV route if severely ill, oral or IM if stable.
		Aspergillus	Amphotericin B	Use IV route.
Sick bird—mild illness, cause unknown	Septicemia, multiple organ infection	Aerobic bacteria, especially *E. coli* and *Klebsiella*	Enrofloxacin; trimethoprim-sulfamethoxazole; ampicillin-clavulanic acid	
		Pseudomonas aeruginosa	Ceftazidime or piperacillin ± amikacin; meropenem	Maintain hydration to avoid toxicity. Limited studies in birds.
		Chlamydophila psittaci	Doxycycline	Use oral, medicated food, or medicated water routes.
		Aspergillus	Itraconazole;	
			terbinafine;	Dose and efficacy vary among species.
			voriconazole	Dose and toxicity vary among species.
Respiratory tract	Rhinitis/sinusitis	Aerobic bacteria, especially *E. coli* and *Klebsiella*	Enrofloxacin; piperacillin; cefotaxime	Gently lavage nares/sinus with saline to remove debris. Treat for at least 1 week after signs resolve. Chronic cases may require surgical debridement to remove nidus of infection.
		Pseudomonas aeruginosa	Ceftazidime or piperacillin ± amikacin; meropenem	Maintain hydration to avoid toxicity. Limited studies in birds.
		Candida albicans	Fluconazole	
		Aspergillus	Amphotericin B;	Nebulize, nasal flush.
			itraconazole	Monitor toxicity.
		Mycoplasma	Enrofloxacin; doxycycline	Role in psittacine sinusitis uncertain.
		Chlamydophila psittaci	Doxycycline	
Respiratory tract	Pnemonitis/airsacculitis	*Aspergillus*; opportunistic fungi	Amphotericin B	Nebulize 2–3x daily; IV if bird is severely debilitated.
			plus	
			itraconazole	Oral administration only. Monitor potential toxicity, especially in African grey parrots.
			or	
			terbinafine;	Treat for at least 1 month after resolution of clinical signs.
			Voriconazole	Combine with itraconazole or substitute for itraconazole.
				Dose and safety vary by species.

		Aerobic bacteria, especially *E. coli* and *Klebsiella*	Enrofloxacin; piperacillin; cefotaxime	Use IV, IM, or SQ route.
		Pseudomonas aeruginosa	Ceftazidime or piperacillin ± amikacin; meropenem	Maintain hydration to avoid toxicity. Limited studies in birds.
		Chlamydophila psittaci	Doxycycline	
		Scedosporium	Itraconazole + terbinafine	Rarely reported.
Gastrointestinal tract	Oral, gastric, intestinal candidiasis	*Candida*	Fluconazole; nystatin	Can treat oral lesions with topical amphotericin B.
	Bacterial enteritis	Opportunistic aerobic bacteria, especially *E. coli* and *Klebsiella*	Enrofloxacin; other fluoroquinolones; trimethoprim-sulfamethoxazole	Use oral route. Treat for 5–7 days.
		Campylobacter	Doxycycline	Rare in psittacines. Occasionally seen in finches.
		Spore-forming bacteria (probable *Clostridium* spp.)	Clindamycin; metronidazole	Common cause of odiferous droppings. *C. perfringens* may cause acute mortality.
	Macrorhabdus ornithogaster		Amphotericin B	Give orally. Impossible to clear infection in all affected birds.
	Bacterial enteritis	*Pseudomonas aeruginosa*	Gentamicin PO if showing mild signs. If ill, use amikacin + piperacillin or ceftriaxone; ciprofloxacin or enrofloxacin if MIC < 0.5 μg/ml; meropenem	Check husbandry for environmental sources (esp. water sources, contaminated food, etc.)
	Cloacitis	Opportunistic aerobic and anaerobic bacteria	Enrofloxacin or beta-lactam + clindamycin or metronidazole; topical silver sulfadiazine cream	Most common in cockatoos. Associated septicemia may cause severe debilitation.
	Pharyngitis	Spiral bacteria	Doxycycline	Reported in cockatiels.
Nervous	Bacterial meningitis/ encephalitis	Opportunistic aerobic pathogens	Cefotaxime; doxycycline; enrofloxacin	Rare. Treat aggressively. Use high end of the dosage range. Prognosis poor.
	Mycoplasma encephalitis	*Mycoplasma*	Doxycycline; enrofloxacin	Rare. Prognosis poor.
Ophthalmic	Bacterial keratitis—mild ulceration	Opportunistic bacteria	Topical bacitracin-neomycin-polymixin B combination	Topical gentamicin and topical tetracycline are other options.

(*continued*)

Table 35.1. Antimicrobial drug selection in companion avian infections. (*continued*)

Site or Type of Infection	Diagnosis	Common Organisms	Suggested Drugs	Comments
	Bacterial keratitis, severe	*Pseudomonas aeruginosa*	Topical tobramycin; topical amikacin	
	Fungal keratitis	*Aspergillus* and other opportunistic fungi	Topical miconazole; natamycin	
	Manifestation of systemic disease	*Chlamydophila psittaci*	Topical tetracycline	Also treat with oral doxycycline.
Skin	Dermatitis	Opportunistic aerobic bacteria	Enrofloxacin; trimethoprim-sulfamethoxazole; beta-lactams Topical silver sulfadiazine cream	Must also treat underlying cause. Often find multiple classes of organisms.
	Staph. dermatitis	*Staphylococcus aureus*	Cephalothin; oxacillin; trimethoprim-sulfamethoxazole	Resistant *S. aureus* uncommon in birds; MRSA occasionally seen.
		Opportunistic yeast	Fluconazole; topical amphotericin B cream	
		Opportunistic hyphal fungi	Itraconazole; topical amphotericin B cream	
Reproductive tract	Salpingitis (oviduct)	Opportunistic aerobic bacteria, especially *E. coli* and *Klebsiella*	Fluoroquinolones; beta-lactams	Check for a retained or ruptured egg. Resolution may require surgery.
Peritonitis		Mixed bacterial opportunists, especially Gram-negative bacteria	Enrofloxacin; piperacillin; cefotaxime	Consider egg yolk peritonitis if bird is female.
Multiple organs	Mycobacteriosis	*Mycobacterium avium* *Mycobacterium genavense*	Long-term multiple drug therapy	*M. genavense* may be zoonotic. Treatment is complex.
Otitis media		Gram-negative bacteria: *E. coli* and *Klebsiella*	Fluoroquinolones; beta-lactams	Most commonly reported in nestling macaws. Lavage ear and treat with topical amikacin.
		Pseudomonas aeruginosa	Ceftazidime or piperacillin; ciprofloxacin or enrofloxacin if MIC $<$ 0.5 μg/ml; meropenem	Difficult to deliver multiple injections to juvenile birds. Use oral route if an effective drug is available.

indication for antimicrobial drug treatment. It is not unusual to culture small numbers of Gram-negative bacteria or yeasts from the cloaca and choana of apparently healthy birds. Treatment may be indicated if the organism is present in large numbers and there are accompanying clinical signs. Physical exam findings, results of clinical laboratory tests, and a Gram stain of material from the suspected site of infection can help determine if a microbial infection is the cause of illness.

Choosing an Antimicrobial Regimen

To be effective, the pathogen must be susceptible to the drug at concentrations that are achievable in birds. Some microbial agents have known susceptibility (e.g., *Chlamydophila psittaci* is invariably susceptible to doxycycline), but most will require a susceptibility test to determine the most effective drugs. Susceptibility tests reporting minimal inhibitory concentrations (MIC) are quantitative and provide the most useful information to guide drug selection. Disk diffusion tests can be used, but it is important to recognize that the designations of susceptible, intermediate, and resistant may not correlate with treatment success in birds. These designations are based on the achievable drug concentrations in humans (or in a limited number of animal species) and it may be difficult to achieve similar concentrations in birds. Chapter 2 discusses susceptibility testing.

Companion birds often hide signs of disease and may present at an advanced stage of illness. If a bacterial infection is strongly suspected, it may be necessary to start empirical treatment before the results of culture and susceptibility tests are available. Table 35.1 provides a list of diseases and suggested choices for initiating antimicrobial therapy. In companion birds, Gram-negative bacterial infections are most common, especially those caused by *E. coli*, *Klebsiella* spp., and *P. aeruginosa*. Chlamydiosis most commonly occurs in birds recently obtained from commercial sources (e.g., pet stores, flea markets and breeders). *Salmonella* is common in pigeons. If these organisms are suspected, a broad-spectrum antibiotic with excellent Gram-negative spectrum is most appropriate for initiating empirical treatment; doxycycline is preferred if chlamydiosis is likely. Susceptibility data are sparse; however, one study of the MIC_{90} values for Gram-negative bacteria isolated from psittacine birds suggests that resistance to many first-generation antimicrobials (e.g., ampicillin, cephalexin, chloramphenicol, penicillin, and tetracycline) may be common in psittacines (Flammer, 1992). Because of suspicions of resistance, avian veterinarians often use fluoroquinolones and advanced-generation beta-lactams for initial treatment in severely ill birds. The treatment plan can be modified once the bird is stable and results of laboratory testing are available.

The frequency and route of administration are important considerations when choosing a dosage regimen. Most birds will need to be captured and restrained to deliver medication, so that treatment regimens with a longer dosage interval are preferred. In sick birds, a parenteral route of administration should be used to rapidly establish effective drug concentrations. Once a bird is clinically stable, it may be relinquished to the owner's care to complete antimicrobial therapy. Birds can be difficult to medicate and the procedure is often stressful for both the bird and bird owner. If oral medication is used, low-volume, palatable drug formulations can aid treatment success. Some avian veterinarians favor use of IM injection because bird restraint and drug delivery may be easier with this route. Additional pros and cons of different routes of administration are discussed below. Regardless of the treatment regimen, it is useful to check compliance and offer assistance after a few days of treatment.

Choosing the dose can be challenging because drug formularies often list a wide range of recommended dosages. This is partly because there are sparse data on the pharmacokinetics of antibiotics in many species of psittacine birds. Many dosage regimens are empirically derived or extrapolated from other species. Table 35.2 provides suggested doses for selected commonly used antimicrobial drugs. However, even doses based on pharmacokinetic studies often represent only a single-dose study in a limited number of individuals of a single species. Therefore all treated birds should be monitored carefully since safety and efficacy have not been investigated for widespread use of many of the drug dosages listed.

Basic pharmacodynamic principles should be considered when evaluating which dose to use. Drugs showing time-dependent efficacy (e.g., beta-lactams, macrolides, tetracyclines, and trimethoprim-sulfonamides) must be dosed frequently enough to

Table 35.2. Conventional dosage regimens for antimicrobial drugs in companion birds.[a]

Drugs	Dose (mg/kg)	Interval (h)	Route	Study[b]/Species	Ref[c]	Comments
Penicillins						
Ampicillin sodium	150	12–24	IM	Kin/pigeons	1	Gram-positive bacteria only.
Ampicillin trihydrate	25	12–25	PO	Kin/pigeons	1	Gram-positives only.
	125–175	12–25	PO	Kin/pigeons	1	
	100	4	IM	Kin/Amazon parrots	2	
	150–200	8–12	PO	Kin/Amazon parrots	2	
Amoxicillin sodium	50	12–24	IM	Kin/pigeons	1	Gram-positives only.
	250	12–24	IM	Kin/pigeons	1	
Amoxicillin trihydrate	20	12–24	PO	Kin/pigeons	1	Gram-positives only.
	100	12–24	PO	Kin/pigeons	1	
	150–175	4–8	PO	Empirical/psittacines		
Amoxicillin + clavulanic acid	50/10	8–12	IM	Kin/collared doves	3	Gram-positives only.
	100/25	8–12	PO	Kin/collared doves	3	
	60–120	8–12	IM	Kin/collared doves	3	
	125–250	8	PO	Kin/collared doves	3	
	125	8	PO	Kin/blue fronted Amazon parrots	4	
Piperacillin	75–100	4–8	IM	Kin/blue-fronted Amazon parrots	5	
	200	6–8	IM	Empirical/psittacines		
Ticarcillin	200	2–4	IM	Kin/blue-fronted Amazon parrots	6	
Cephalosporins						
Cephalothin	100	6	IM	Kin/pigeon	7	
Cephalexin	35–50	6	PO	Kin/pigeon	7	
Ceftiofur	10	4	IM	Kin/cockatiels	8	
	10	8	IM	Kin/orange-winged Amazon parrots	8	
Cefotaxime	75–100	4–8	IM	Kin/blue-fronted Amazon parrots	5	
Ceftazidime	50–100	4–8	IM			
Ceftriaxone	75–100	4–8	IM	Kin/blue-fronted Amazon parrots	5	
Aminoglycosides						
Amikacin	15–40	24	IM, IV	Kin/cockatiels, blue-fronted Amazon parrots, African grey parrots	9 6 10	Preferred aminoglycoside; potentially nephrotoxic.
Gentamicin	2.5–10	24	IM	Kin/cockatiels, scarlet macaws, rose breasted cockatoos.	9 11	Nephrotoxic.
Tobramycin	2.5–10	24	IM	Empirical		Empirical—based on gentamicin studies; used for *Pseudomonas aeruginosa*.

Fluoroquinolones						
Enrofloxacin	7.5–15	12–24	IM	Kin/African grey parrots	12	IM injection causes muscle irritation.
	7.5–15	12–24	SC	Kin/African grey parrots	13	Inject into subcutaneous fluid pocket containing lactated Ringer's solution. Double the dose when using q 24 h administration.
	15–30	24	PO	Kin/African grey parrots	13	High oral doses result in plasma concentrations that may be effective with once daily dosing.
	200 mg/L	24	Water	Plasma concentration/parrots	14	Achieves low plasma concentrations in psittacines.
Marbofloxacin	2.5–5	24	PO	Kin/blue and gold macaw	15	
Tetracyclines						
Oxytetracycline, long acting (LA 200, Zoetis)	50–100	48–72	IM, SC	Kin/Goffin's cockatoo	16	*Chlamydophila psittaci*; causes irritation at the site of injection.
Doxycycline	25	12	PO	Kin/pigeon	1	*Chlamydophila psittaci*; dose in birds with access to grit.
	7.5	12	PO	Kin/pigeon	1	*Chlamydophila psittaci;* dose in birds with no access to grit.
	35	24	PO	Psittacines/empirical		*Chlamydophila psittaci*.
	300 mg/kg food	24	Food	Plasma concentration/budgerigars	17	*Chlamydophila psittaci*. Diet = 1:4 mixture of hulled oat groats and hulled millet; coat seed with sunflower oil (~6 ml/kg seed).
	300–500 mg/kg food	24	Food	Plasma concentration/cockatiels	18	Diet = 60:40 mixture of hulled millet and hulled sunflower seeds; coat seed with sunflower oil (~6 ml/kg seed).
Doxycycline	300 mg/L	24	Water	Plasma concentration/cockatiels	18	May be effective for treating chlamydiosis.
Doxycycline	400 mg/L	24	Water	Plasma concentration/cockatiels	19	May be effective for treating spiral bacteria.
	400–800 mg/L	24	Water	Plasma concentration/orange-winged Amazon parrot, African grey parrot, Goffin's cockatoo	20	
Doxycycline injectable (Vibrovenös, Zoetis)	75–100	5–7 days	IM	Kin/pigeons Kin/psittacines	1 21	Use lower doses in macaws and cockatoos.
Macrolides						
Tylosin	25	6	IM	Kin/pigeons	22	
Clindamycin	25–50	8–12	PO	Empirical		Gram-positives and anaerobes.
Trimethoprim and sulfonamides						
Trimethoprim	15–20	8	PO	Kin/pigeons	1	

(*continued*)

Table 35.2. Conventional dosage regimens for antimicrobial drugs in companion birds. (*continued*)

Drugs	Dose (mg/kg)	Interval (h)	Route	Study[b]/Species	Ref[c]	Comments
Trimethoprim-sulfamethoxazole	10/50	12	PO	Kin/pigeons	1	
Trimethoprim-sulfatroxazole	10/50	24	PO	Kin/pigeons	1	
Trimethoprim-sulfamethoxazole	20/100	12	PO	Empirical		May cause regurgitation, especially in macaws.
Other						
Metronidazole	20–50	12	PO	Empirical		Anaerobes.
Antifungals						
Amphotericin B	1.5	8	IV	Empirical		*Aspergillus* and hyphal fungi.
	1.0	8–12	IT	Empirical		*Aspergillus* and hyphal fungi.
	1.0 mg/ml	8–12	Neb	Empirical		*Aspergillus* and hyphal fungi.
	100 mg/kg	12	PO	Empirical		Avian gastric yeast.
Ketoconazole	20–30	12	PO	Kin/Amazon parrots and cockatoos	23	Yeast ± *Aspergillus*.
Fluconazole	10–20	24	PO	Kin/African grey parrots, blue-fronted Amazon parrots, Goffin's cockatoos	24	Yeast. Higher dose may be toxic in African grey parrots.
Fluconazole	75–100 mg/L	24	Water	Kin/cockatiels	25	*Candida*.
Itraconazole	5–10	24	PO	Kin/blue-fronted Amazon parrot	26	*Aspergillus* and hyphal fungi.
	6	12	PO	Kin/pigeon	27	*Aspergillus* and hyphal fungi;
	2.5–5	24	PO	Empirical/African grey parrot		itraconazole may be toxic in some African grey parrots, even at the low dose indicated here.
Voriconazole	18	12	PO	Kin/African grey parrot	28	New drug, safety unknown.
Voriconazole	18	8	PO	Kin/Hispaniolan Amazon parrot	29	New drug, safety unknown.
Voriconazole	10	12	PO	Kin/Pigeon	30,31	May cause hepatic toxicity.
Nystatin	200,000–300,000 IU/kg	8–12	PO	Empirical		Yeast; not absorbed from the GI tract; must come in contact with the yeast.

[a]Adapted from Dorrestein, 2000.

[b]Kin means dose recommendations based on pharmacokinetic studies in the listed species. Empirical means studies based on anecdotal reports; no published kinetic data available for pigeons or psittacine birds.

[c]References (see bibliography): 1, Dorrestein, 1986; 2, Ensley, 1981; 3, Dorrestein et al., 1998; 4, Orosz et al., 2000; 5, Flammer, 1990; 6, Schroeder et al., 2001; 7, Bush et al., 1981; 8, Tell et al. 1998; 9, Ramsay et al., 1993; 10, Gronwall et al., 1989; 11, Flammer et al., 1990a; 12, Flammer et al., 1991; 13, Flammer, 2005; 14, Flammer et al., 2002; 15, Carpenter et al., 2003; 16, Flammer et al., 1990b; 17, Flammer et al., 2003; 18, Powers et al., 2000; 19, Evans et al., 2008; 20, Flammer et al., 2001; 21, Jakoby and Gylstorff, 1983; 22, Bush et. al., 1982; 23, Kollias et al., 1986; 24, Flammer, 1996; 25, Ratzlaff et al., 2011; 26, Orosz et al., 1996; 27, Lumeij et al., 1995; 28, Flammer, et al., 2008; 29, Sanchez-Migallon et al., 2010; 30, Beernaert et al., 2009a; 31, Beernaert et al., 2009b.

Disclaimer: As noted in the text, safety and efficacy data for widespread use of drugs in birds is lacking; none of these drug doses are warranted to be either safe or effective.

maintain plasma concentrations above the target MIC for most of the dosing interval. Birds rapidly excrete most beta-lactam drugs, so penicillins and cephalosporins should be dosed at least 3–4 times daily unless pharmacokinetic data demonstrates less frequent administration is adequate. Cephalosporins that show prolonged activity in other species (e.g., cefovecin in dogs) may have short activity in birds (Thuesen et al., 2009). Concentration-dependent antibiotics (e.g., fluoroquinolones and aminoglycosides) can probably be dosed once daily if high peak concentrations and large area under the curve values are achieved. Since these values may depend on the route of administration, parenteral routes may be required to achieve the desired concentration for resistant organisms.

Controlled studies involving large numbers of different avian species are lacking, so that veterinarians should monitor treatment efficacy and potential toxicity. This is especially important when using drugs with a narrow therapeutic range or treating an unfamiliar species. Relevant chapters in this book should be consulted on specific antimicrobial drugs and their potential side effects and contraindications.

Using broad-spectrum antimicrobials may impact normal intestinal microflora. Psittacine birds have predominately Gram-positive gut flora, and reduction of this flora after treatment can render the birds more susceptible to secondary infections by yeasts and Gram-negative opportunist bacteria. This is especially common when treating nestling birds or when using prolonged antimicrobial therapy in adults such as treatment for chlamydiosis (Flammer, 1994). The incidence of secondary infections can be reduced by maximizing husbandry during treatment. In addition, birds that have sustained long-term treatment should be cultured to identify potential opportunistic superinfections.

Anatomical and Physiological Considerations

Differences in anatomy and physiology may alter drug pharmacology in birds as compared to mammals. For example, granuloma formation is a common avian response to infection by many microbial agents. Granuloma formation can inhibit drug penetration so that surgical debridement, use of lipophilic drugs, and prolonged treatment may be needed to improve the success of treatment.

In mammals, gastric emptying and drug dissolution are often the rate limiting steps for oral drug absorption. Companion birds have a crop, and passage of ingesta from the crop may delay oral drug absorption. For example, a lag phase of 20–40 minutes was observed in studies investigating the pharmacology of oral suspensions of doxycycline in fasted birds (Flammer, unpublished observation, 2005). There is little absorption from the crop, and its neutral pH may precipitate some drugs that are solubilized in acid or base (e.g., chlortetracycline), further delaying absorption (Dorrestein, 1986).

Alimentary tract motility in birds also differs from mammals (Denbo, 2000). Birds have a two-part stomach composed of the proventriculus and ventriculus. Grit is retained in the ventriculus and may expose orally administered drugs to high concentrations of calcium and magnesium. This can reduce the absorption of tetracyclines and fluoroquinolones. There is also both normograde and retrograde movement of ingesta through the proventriculus, ventriculus, and small intestine, which might expose acid-sensitive drugs to greater degradation by gastric acids. Companion birds also have a short intestinal tract that may limit drug absorption, especially when food is present and competes for absorption.

The lower respiratory system of birds consists of the lungs and air sacs (Powell, 2000). The air sacs are poorly vascularized and topical drug delivery via nebulization may be needed to augment systemic drug administration. At rest, birds may ventilate only a small portion of their total air sac volume, so that nebulization may be enhanced by gently stimulating the bird to increase respiration and promote greater drug penetration.

The renal system of birds differs considerably from mammals (Goldstein, 2000). Avian kidneys contain both mammalian and reptilian nephrons and may excrete drugs differently than expected from mammalian physiology. Uric acid is the major end product of avian nitrogen metabolism and is produced in the liver. Sulphonamide drugs may be excreted via some of the same metabolic pathways as uric acid, so that caution should be used if sulfa drugs are given to uricemic birds (Quesenberry, 1988). Birds lack a bladder, and waste from the kidney is transported directly to the cloaca.

Cloacal contents can be refluxed into the colon to promote additional water absorption. As a consequence, avian water balance may be independent of the glomerular filtration rate and renally excreted drugs may face reabsorption in the colon. As a final consideration, birds have a renal portal system. Theoretically, renally excreted drugs could face a first-pass effect before reaching systemic circulation if injected into the leg muscles.

Routes of Administration

The route of administration will depend on the drug, available drug formulation, condition of the bird, and ability of the owner and/or veterinary staff to deliver the drug. Severely ill birds should be treated using parenteral routes to quickly establish effective drug concentrations. Achievable plasma concentrations are often route-dependent. As a guideline, concentrations follow the following pattern: IV > IM ≥ SC > PO > medicated food or water (Flammer, 1994).

- Intravenous (IV) route: It is difficult to deliver IV drugs in birds so this method is usually reserved for one-time administration of antimicrobials or emergency drugs. Birds can be catheterized, but it is more difficult to maintain IV catheters in birds than in other small animals. The right jugular and right and left brachial veins are the most accessible in psittacines. The medial metatarsal vein is accessible in pigeons.
- Intraosseous (IO) route: Fluids given via the intraosseus route quickly reach systemic circulation (Aguilar et al., 1993). Intraosseus catheters can be installed in the distal ulna or tibiotarsus. This route is most often used to administer fluids; however, it is an acceptable route for IV antimicrobial drug formulations. Care should be taken to flush fluid through the IO catheter and bone to avoid leaving concentrated drug in the IO site.
- Intramuscular (IM) route: The pectoral muscles are the most accessible sites for IM administration in parrots and passerines; the leg muscles are sometimes used in racing pigeons. Small needle size (25–30 gauge) and small volume of injection are necessary. The author prefers to use injection volumes that are less than 1 ml/kg. Irritating drugs (e.g., enrofloxacin and tetracyclines) should be avoided unless there is a compelling reason to use this route.
- Subcutaneous (SC) route: Medications can be given subcutaneously in the groin, axilla and the dorsal region between the shoulders. Non-irritating drugs are preferred. Injectable tetracyclines (e.g., oxytetracycline) have been used, but can cause skin sloughs (Flammer et al., 1990b). Enrofloxacin can be injected into a SC pocket of lactated Ringer's solution and achieve plasma concentrations comparable to IM injection, without causing severe irritation (Flammer, 2005).
- Oral (PO) route: Liquid solutions and suspensions are often used. Capsules can be given to pigeons but are difficult to administer to parrots and small passerines. Drugs that are unpalatable or require large volumes are more difficult to administer. Only non-irritating drugs should be used, as birds may aspirate drug into the trachea or pass it rostrally into the choanal slit. It can be surprisingly difficult to medicate psittacines via the oral route, so owner compliance should be verified if this route is chosen. As an alternative, drugs can be administered via a crop tube; however, this method is technically difficult and is usually performed in a veterinary hospital setting.
- Medicated food: Medications can be added to palatable food vehicles such as mash diets and treat foods. It is difficult to monitor food (and therefore drug) consumption, so this route should be reserved for treatment of clinically stable birds with proven dosage regimens. Lower plasma drug concentrations are usually achieved than with other routes, so this method is used only to treat highly susceptible bacteria. It is important to use the same diet as is used in published methods, since food consumption is largely based on the energy content of the diet (Flammer, 1994). Medicated food recipes for treating chlamydiosis are available for some species.
- Medicated water: Delivering medication via this route usually establishes low plasma drug concentrations. This route should be avoided unless there is data proving therapeutic plasma drug concentrations can be achieved. For example, water medicated with enrofloxacin at 200 mg/L achieves low, sustained plasma concentrations of 0.05–0.2 μg/ml (Flammer et al., 2002). Doxycycline medicated water has been shown to achieve plasma drug concentrations that are greater than 1 μg/ml and should be effective for treating chlamydiosis and spiral bacteria in cockatiels treated with 300–400 mg/L (Powers et al., 2000; Evans et al.,

2008) and cockatoos and grey parrots treated with 400–800 mg/L (Flammer et al., 2001). Water medicated with fluconazole at 100 mg/L achieved plasma drug concentrations that should be effective for treating candidasis in cockatiels (Ratzlaff et al., 2011).

- Topical: Topical drugs can be applied to the skin or eye. A minimal amount of topical cream or ointment should be used, as birds may ingest or spread medications into their feathers when preening. Where possible, water-soluble formulations are preferred, as they are easier to wash off if the bird spreads them into the feathers. Silver sulfadiazine cream is a popular choice for treating avian skin infections because it has broad-spectrum activity and is easy to clean up. Topical products containing corticosteroids should be avoided since birds may be more susceptible to the immunosuppressive effects.
- Antimicrobials are occasionally injected directly into the site of infection. Intratracheal injection can be used to deliver topical amphotericin B (~1 ml/kg) to treat fungal infections of the trachea. Amphotericin B and clotrimazole have been used to topically treat fungal lesions on the air sacs. Topical antibiotics are sometimes used to treat upper respiratory infections via injection into the nares (nasal flush) or periorbital sinus (sinus flush).
- Nebulization: Nebulization can be used to deliver topical medication to portions of the air sacs and lungs. It is most often used when treating respiratory fungal infections. A nebulizer that produces particles less than 3 μm in diameter should be used. Birds ventilate only a small portion of their respiratory tract at rest, so that stimulation or mild exercise during nebulization might increase drug penetration. In studies investigating tylosin and oxytetracycline, nebulization achieved therapeutic local concentrations for approximately 4–6 hours, but did not establish therapeutic plasma concentrations (Locke et al., 1984; Dyer et al., 1987).

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36

Antimicrobial Drug Use in Rabbits, Rodents, and Ferrets

Colette L. Wheler

Introduction

Veterinary practitioners who care for small mammal pets, such as rabbits, rodents, and ferrets, face several challenges when using antimicrobial medications in these species. Some antimicrobials are known to be toxic to rabbits and some rodents, so careful selection of the most appropriate drug is critical. In Canada and the United States, there are very few antimicrobials specifically approved for treatment of these patients, necessitating use of drugs extra-label. An alternative source or formulation of drug may be needed, which may involve compounding or importing medications from other countries (following strict federal regulations) or the use of human drug formulations. Many antimicrobials must be reconstituted prior to administration, and subsequently have a fairly short shelf-life, even if refrigerated. Very little of the drug is usually needed to treat the patient, so the remainder is often frozen in aliquots for economic reasons and to avoid wastage. However, information on the stability of these frozen, reconstituted products is often unavailable or difficult to find.

Drug dosages are generally based on extrapolation from other species and/or clinical experience, and many pharmacokinetic studies performed in these animals are actually models for human trials. In addition, most drugs are not manufactured in a form that is convenient for administration to small, easily stressed patients, so unique treatment methods must be developed to ensure owner compliance. The number of animals being treated and their intended use must also be taken into consideration, since the treatment of one patient kept as a companion animal will differ significantly from that of hundreds being bred for the pet trade, used as laboratory animals, being farmed for fur, or, in the case of rabbits, being raised for meat.

Lastly, many conditions requiring antimicrobial therapy are actually secondary to inadequate nutrition or husbandry, so these issues must also be addressed for a positive therapeutic outcome.

The following sections discuss these many challenges in more detail, and conclude with a series of tables listing some reported dosages of antimicrobials, and common conditions in small mammal pets. Some information is also included for hedgehogs and sugar gliders, since their popularity as pets is increasing in North America, and this information can be difficult to find.

Antimicrobial Toxicity

Most veterinary practitioners are aware that some antimicrobials are toxic to rabbits and some rodents, especially when given orally. Disruption of the normal population of intestinal flora occurs, and this dysbiosis allows proliferation of clostridial or coliform bacteria, and subsequent release of toxins. Hind-gut fermenters, such as rabbits, guinea pigs, chinchillas, and hamsters, are particularly susceptible to this condition, and narrow-spectrum antibiotics, such as beta-lactams,

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macrolides, and lincosamides are most responsible. Diarrhea usually appears within 24–48 hours following administration of the drug, and most cases are fatal. Pathogenic conditions and sudden alterations in diet may also predispose the animal to dysbiosis, and even antimicrobials that are considered safe can sometimes cause problems. Rats, mice, gerbils, and ferrets are less vulnerable to this condition.

Other forms of antimicrobial toxicity can also occur in small mammals. Neuromuscular blockade of skeletal muscle may occur with high dosages of aminoglycosides, resulting in an ascending flaccid paralysis, respiratory arrest, and coma. Anesthesia may be a predisposing factor to this condition. As in other species, these drugs are also potentially nephrotoxic and ototoxic to small mammal species. Streptomycin has been reported to be toxic in gerbils.

Although normally safe in rabbits, rodents, and ferrets, fluoroquinolone antimicrobials (e.g., enrofloxacin) may cause arthropathies in young animals. Chloramphenicol is generally safe to use in small mammals, and many bacteria infecting these animals are highly susceptible to this drug. However, chloramphenicol has occasionally been associated with irreversible aplastic anemia in humans, so appropriate directions for prevention of exposure, such as wearing gloves and hand washing, must be given when this antibiotic is prescribed. In addition, chloramphenicol is prohibited for use in food-producing animals, such as meat rabbits.

Potential toxicities must always be kept in mind when selecting an antimicrobial based on culture and sensitivity results, as the most appropriate choice may result in dysbiosis or other problems in a particular species. Supportive ancillary therapies, such as administration of fluids along with aminoglycosides, and good nursing care, as well as provision of adequate nutrition and a comfortable, stress-free environment will also aid in successful treatment.

Extra-Label Use, Compounding, and Importation

In Canada and the United States, there are a limited number of drugs labeled for use in rabbits, rodents, and ferrets, and very few of these are antimicrobials. Some antimicrobials are approved for use in mink, and dosages for mink would likely be valid in ferrets, since they are closely related species. In Canada, antimicrobials labeled for use in rabbits and mink include: procaine penicillin G (IM use only) for the treatment of rabbits and mink, chlortetracycline feed premix for the treatment of mink, and neomycin/oxytetracycline water soluble powder for the treatment of mink. In order to provide appropriate care for small mammal patients, veterinarians are required to use many drugs extra-label.

In Canada and the United States, extra-label drug use refers to the use of a federally approved drug in a manner that is not in accordance with the label or package insert. It is the responsibility of the veterinarian to be aware of, and follow, the rules and regulations in their particular jurisdiction. In the United States, further clarification of extra-label drug use was made in 1994 with the introduction of the Animal Medicinal Drug Use Clarification Act (AMDUCA). This act clearly explains legitimate extra-label drug use by veterinarians, and outlines the specific conditions that must be followed for acceptable extra-label drug use (see chapter 26).

In the United States, extra-label use of medicated feeds was initially excluded from the AMDUCA; however, this oversight was rectified when the Food and Drug Administration Center for Veterinary Medicine issued a Compliance Policy Guideline on Extra-label Use of Medicated Feeds for Minor Species in 2001 (www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074659.htm).

Extra-label use of human antimicrobial formulations is also fairly common for treatment of small mammal patients. Many of these products are single dose vials that have a fairly short shelf-life once reconstituted. Treatment of the small mammal patient may occur for a longer period than the shelf-life of the drug, or the total amount needed may be very small. Rather than discard the remainder, veterinarians often freeze small aliquots of the product for future use. The stability of these reconstituted products after freezing is often not easy to find; however, some information can be found in the *Handbook of Injectable Drugs* by Lawrence Trissel, which is available in hardcopy and electronic format, and *Plumb's Veterinary Drug Handbook* by Donald Plumb, as well as in some package inserts.

An alternative source of drug sometimes needs to be explored by veterinarians for the treatment of small

mammal patients, such as compounding or importing medications from other countries. Compounding is a type of extra-label drug use whereby the original drug dosage form is manipulated by a veterinarian or pharmacist, or an entirely new product is manufactured by a compounding pharmacy, to create a customized medication to meet a specific need. This could involve anything from altering the concentration of a drug by diluting it other than according to the package instructions, or mixing a crushed tablet into a liquid, to the custom creation of a medicated tablet or liquid that is particularly palatable to the intended species.

Importation of a more suitable drug or drug formulation from another country is another option for veterinarians. For example, a suspension of metronidazole is available in some countries that is much more accurate for dosing small patients than the tablet form available here. Mechanisms exist in both Canada and the United States for legal importation of drugs (www.hc-sc.gc.ca/dhp-mps/vet/edr-dmu/index-eng.php and www.fda.gov/AnimalVeterinary/Products/ImportExports/ucm050077.htm).

Drug Dosages

Although there are many antimicrobial dosages published for rabbits, rodents, and ferrets, very few pharmacokinetic studies or clinical trials have been performed specifically for these animals; rather they are carried out primarily to establish information for future human trials. Because of this, antimicrobial dosages for these patients are generally based on extrapolations from other species and/or clinical experience. Lack of scientifically derived dosages, combined with the extra-label use of most antimicrobials, are daily challenges of veterinarians who care for small mammal patients. Clients should be informed of this, and give written consent for treatment of their animals were appropriate.

Extrapolation of drug dosages from one species to another can be done in several ways. Straightforward linear extrapolations based on body weight alone tend to result in overdosing of larger animals and underdosing of smaller ones. This method is only appropriate with drugs that have large margins of safety and wide therapeutic margins, or if the two animals are similar in taxonomy, body size, and physiology.

Metabolic scaling is a method popular in zoological medicine, and uses a formula based on body weight; a constant based on the energy group of the animal; and the known pharmacokinetic data of the drug in one species, to calculate the dosage of the drug in other species.

Allometric scaling uses mathematical equations to analyze differences in anatomy, physiology, biochemistry, and pharmacokinetics in animals of different sizes. Known pharmacokinetic parameters in several species are used in the equations to estimate the pharmacokinetic parameter in an unknown species, and thus predict drug dosage. Allometric scaling is commonly used in the pharmaceutical industry to determine the first dosage in human trials. There are several reports in the literature validating the use of allometric scaling to predict pharamacokinetic parameters in small mammal species for several drugs, including some fluoroquinolone antimicrobials. Tables 36.1–36.3 present drug dosages for the treatment of common microbial diseases. Tables 36.4–36.11 present clinical signs and suggested drugs for common bacterial diseases.

Drug Administration

Rabbits and rodents are prey species, and are generally less tolerant of handling and other manipulations than predator species such as ferrets, dogs, and cats, especially when debilitated. Administration of antimicrobials in these prey species must be performed in a way that allows for the entire dose to be given without unduly stressing the patient. The method of administration must also be achievable for the client, otherwise frustration and non-compliance may result. Available antimicrobial formulations are often too large and/or too concentrated for small mammals and need to be split up or diluted for accurate dosing.

Routes of antimicrobial administration in rabbits, rodents, and ferrets include oral (liquid, pill, or capsule); subcutaneous (usually in the loose skin over the shoulders); intraperitoneal (generally reserved for very small rodents); intramuscular (generally avoided in very small animals); topical; and less commonly, via intravenous or intraosseous catheter; nebulization; gavage; nasoesophageal or esophagostomy tube (rabbits, ferrets); or antimicrobial-impregnated implants. Injections are more

Table 36.1. Reported antimicrobial drug dosages in rabbits, guinea pigs, and chinchillas. Caution: Most uses and dosages are extra-label.

Drug	Rabbit*	Guinea Pig	Chinchilla
Amikacin	2–5 mg/kg q 8–12 h; SC, IM	2–5 mg/kg q 8–12 h; SC, IM	2–5 mg/kg q 8–12 h; SC, IM, IV
Azithromycin	5 mg/kg q 48 h; IM OR 15–30 mg/kg q 24 h; PO	15–30 mg/kg q 12–24 h; PO	15–30 mg/kg q 24 h; PO
Captan powder	–	–	5 ml/475 ml bathing dust
Cephalexin	11–22 mg/kg q 8–12 h; SC	50 mg/kg q 24 h; IM	–
Chloramphenicol	30 mg/kg q 8–12 h; PO, SC, IM, IV**	20–50 mg/kg q 6–12 h; PO, SC, IM, IV	30–50 mg/kg q 12 h; PO,SC,IM, IV
Chlortetracycline	50 mg/kg q 24 h; PO	–	50 mg/kg q 12 h; PO
Ciprofloxacin	5–20 mg/kg q 12 h; PO	5–20 mg/kg q 12 h; PO	5–20 mg/kg q 12 h; PO
Clindamycin	Do not use	7.5 mg/kg q 12 h; SC; Do not use PO	7.5 mg/kg q 12 h; SC; Do not use PO
Doxycycline	2.5 mg/kg q 12 h; PO	2.5 mg/kg q 12 h; PO	2.5 mg/kg q 12 h; PO
Enrofloxacin	5–10 mg/kg q 12 h; PO, SC, IM OR 200 mg/L dw q 24 h	0.05–0.2 mg/mL dw q 24 h OR 5–15 mg/kg q 12 h; PO, SC, IM	5–15 mg/kg q 12 h; PO, SC, IM
Fenbendazole			20–50 mg/kg q 24 h; PO
Fluconazole	38 mg/kg q 12 h; PO	16-20 mg/kg q 24 h × 14 d; PO	16 mg/kg q 24 h × 14 d; PO
Gentamicin	1.5–2.5 mg/kg q 8 h; SC, IM, IV	2–4 mg/kg q 8–12 h; SC, IM	2 mg/kg q 12 h; SC, IM, IV
Griseofulvin (avoid in pregnant animals)	25 mg/kg q 24 h × 30–45d; PO	25–50 mg/kg q 12 h × 14–60d; PO OR 1.5% in DMSO for 5–7 d; topically	25 mg/kg q 24 h × 30–60d; PO
Itraconazole	20–40 mg/kg q 24 h; PO	5–10 mg/kg q 24 h; PO	5 mg/kg q 24 h; PO
Ketoconazole	10–40 mg/kg q 24 h; PO	10–40 mg/kg q 24 h; PO	10–40 mg/kg q 24 h; PO
Lime sulfur dip	Dilute 1:40 with water, dip q 7d for 4–6 wk	Dilute 1:40 with water, dip q 7d for 4–6 wk	Dilute 1:40 with water, dip q 7d for 4–6 wk
Marbofloxacin	2 mg/kg q 24 h; IM, IV OR 5 mg/kg q 24 h; PO	4 mg/kg q 24 h; PO, SC	4 mg/kg q 2 h4; PO, SC
Metronidazole	20 mg/kg q 12 h; PO	25 mg/kg q 12 h; PO	10–20 mg/kg q 12 h; PO; use with caution
Oxytetracycline	50 mg/kg q 12 h; PO OR 1 mg/mL dw	–	50 mg/kg q 12 h; PO
Penicillin G, benzathine	42,000–60,000 IU/kg q 48 h; SC, IM	Toxic	Avoid
Penicillin G, procaine	42,000–84,000 IU/kg q 24 h; SC, IM	Toxic	Avoid
Sulfadimethoxine	10–15 mg/kg q 12 h × 10d; PO	10–15 mg/kg q 12 h; PO	25–50 mg/kg q 24 h × 10–14d; PO
Sulfamethazine	1 mg/mL dw	1 mg/mL dw	1 mg/mL dw
Sulfaquinoxaline	1 mg/mL dw	1 mg/mL dw	–
Terbinafine	100 mg/kg q 12–24 h; PO	10–40 mg/kg q 24 h × 4–6 wk; PO	10–30 mg/kg q 24 h × 4–6 wk; PO
Tetracycline	50 mg/kg q 8–12 h; PO OR 250–1000 mg/L dw q 24 h	10–40 mg/kg q 24 h; PO	0.3–2 mg/mL dw q 24 h OR 10–20 mg/kg q 8–12 h; PO
Trimethoprim-sulfa	30 mg/kg q 12–24 h; PO, SC, IM	15–30 mg/kg q 12 h; PO, SC	15–30 mg/kg q 12 h; PO, SC
Tylosin	10 mg/kg q 12 h; PO, SC	10 mg/kg q 24 h; PO, SC; use with caution	10 mg/kg q 24 h; PO, SC

*Observe correct withdrawal time in meat rabbits.
**Do not use in meat rabbits.
PO, per os; SC, subcutaneous; IM, intramuscular; IV, intravenous; dw, drinking water.

commonly used in clinic than at home; however, some clients are willing to master the procedure, particularly if the pet objects excessively to being medicated orally, or if it has a sore mouth, or tends to nip.

Self-administration, where the animal willingly takes the entire dose on its own, preferably with minimal or no restraint, is the best and least-stressful method of medication (for both the animal and the administrator). Flavored antimicrobial preparations, such as trimethoprim/sulfa or chloramphenicol palmitate suspensions, are willingly consumed by some of these patients. Crushed pills, liquids, or capsule contents can be mixed with small amounts of

Table 36.2. Reported Antimicrobial Dosages in Hamsters, Gerbils, Rats, and Mice. Caution: Most uses and dosages are extra-label.

Drug	Hamster	Gerbil	Rat	Mouse
Amikacin	2–5 mg/kg q 8–12 h; SC	2–5 mg/kg q 8–12 h; SC	10 mg/kg q 12 h; SC	10 mg/kg q 8–12 h; SC
Ampicillin	Toxic	6–30 mg/kg q 8 h; PO	20–100 mg/kg q 12 h; PO, SC	20–100 mg/kg q 12 h; PO, SC OR 500 mg/L dw
Azithromycin			30 mg/kg q 24 h; PO	
Cephalexin	–	25 mg/kg q 24 h; SC	15 mg/kg q 12 h; SC	60 mg/kg q 12 h; PO
Cephaloridine	10–25 mg/kg q 24 h; SC	30 mg/kg q 12 h; IM	10–25 mg/kg q 24 h; SC	10–25 mg/kg q 24 h; SC
Cephalosporin	–	–	–	30 mg/kg q 12 h; SC
Chloramphenicol palmitate	50–200 mg/kg q 8 h; PO	50–200 mg/kg q 8 h; PO	50–200 mg/kg q 8 h; PO	0.5 mg/mL dw OR 50–200 mg/kg q 8 h; PO
Chloramphenicol succinate	20–50 mg/kg q 12 h; SC	20–50 mg/kg q 12 h; SC	30–50 mg/kg q 12 h; SC	30–50 mg/kg q 12 h; SC
Chlortetracycline	20 mg/kg q 12 h; PO, SC	–	–	25 mg/kg q 12 h; PO, SC
Ciprofloxacin	7–20 mg/kg q 12 h; PO	7–20 mg/kg q 12 h; PO	7–20 mg/kg q 12 h; PO	7–20 mg/kg q 12 h; PO
Doxycycline	2.5–5 mg/kg q 12 h; PO; do not use in young or pregnant animals	2.5–5 mg/kg q 12 h; PO; do not use in young or pregnant animals	5 mg/kg q 12 h; PO	2.5 –5 mg/kg q 12 h; PO
Enrofloxacin	0.05–0.2 mg/mL dw×14d OR 5–10 mg/kg q 12 h; PO, SC	0.05–0.2 mg/mL dw×14d OR 5–10 mg/kg q 12 h; PO, SC	0.05–0.2 mg/mL dw×14d OR 5–10 mg/kg q 12 h; PO, SC	0.05–0.2 mg/mL dw×14d OR 5–10 mg/kg q 12 h; PO, SC
Erythromycin	–	–	20 mg/kg q 12 h; PO	20 mg/kg q 12 h; PO
Gentamicin	5 mg/kg q 24 h; SC	2–4 mg/kg q 8 h; SC	5–10 mg/kg divided q 8–12 h; SC	2–4 mg/kg q 8–12 h; SC
Griseofulvin (avoid in pregnant animals)	25–50 mg/kg q 12 h×14–60d; PO OR 1.5% in DMSO for 5–7 d; topically	25–50 mg/kg q 12 h×14–60d; PO OR 1.5% in DMSO for 5–7 d; topically	25–50 mg/kg q 12 h×14–60d; PO OR 1.5% in DMSO for 5–7 d; topically	25–50 mg/kg q 12 h×14–60d; PO OR 1.5% in DMSO for 5–7 d; topically
Ketoconazole	10–40 mg/kg q 24 h×14d; PO	10–40 mg/kg q 24 h×14d; PO	10–40 mg/kg q 24 h×14d; PO	10–40 mg/kg q 24 h×14d; PO
Metronidazole	7.5 mg/70–90gm animal q 8 h	7.5 mg/70–90gm animal q 8 h	10–40 mg/kg q 24 h; PO	2.5 mg/mL dw × 5 d OR 20–60 mg/kg q 8–12 h; PO
Neomycin	0.5 mg/mL dw OR 100 mg/kg q 24 h; PO	2 g/L dw OR 100 mg/kg q 24 h; PO	2 g/L dw OR 25 mg/kg q 12 h; PO	2 g/L dw
Oxytetracycline	0.25–1 mg/mL dw or 16 mg/kg q 24 h; SC	0.8 mg/mL dw or 10 mg/kg q 8 h; PO or 20 mg/kg q 24 h; SC	500 mg/L dw or 10–20 mg/kg q 8 h; PO	500 mg/L dw OR 10–20 mg/kg q 8 h; PO
Sulfadimethoxine	10–15 mg/kg q 12 h; PO	10–15 mg/kg q 12 h; PO	10–15 mg/kg q 12 h; PO	10–15 mg/kg q 12 h; PO
Sulfamerazine	1 mg/mL dw q 24 h	0.8 mg/mL dw q 24 h	1 mg/mL dw	1 mg/mL dw OR 500 mg/L dw
Sulfamethazine	1 mg/mL dw q 24 h	0.8 mg/mL dw q 24 h	1 mg/mL dw	1 mg/mL dw
Sulfaquinoxaline	1 mg/ml dw q 24 h	1 mg/ml dw q 24 h		
Tetracycline	0.4 mg/mL dw q 24 h OR 10–20 mg/kg q 8–12 h; PO	2–5 mg/mL dw q 24 h OR 10–20 mg/kg q 8–12 h; PO	2–5 mg/mL dw OR 10–20 mg/kg q 8 h; PO	2–5 mg/mL dw OR 10–20 mg/kg q 8 h; PO
Trimethoprim/sulfa	15–30 mg/kg q 12–24 h; PO, SC	30 mg/kg q 12–24 h; PO, SC	15–30 mg/kg q 12 h; PO, SC	30 mg/kg q 12 h; PO, SC
Tylosin	2–8 mg/kg q 12 h; SC, PO OR 500 mg/mL dw	0.5 mg/mL dw q 24 h OR 10 mg/kg q 24 h; PO, SC	0.5 mg/mL dw OR 10 mg/kg q 24 h; PO, SC	0.5 mg/mL dw OR 10 mg/kg q 24 h; PO, SC

PO, per os; SC, subcutaneous; IM, intramuscular; dw, drinking water.

Table 36.3. Reported antimicrobial dosages in ferrets, hedgehogs, and sugar gliders. Caution: Most uses and dosages are extra-label.

Drug	Ferrets	Hedgehogs	Sugar Gliders
Amikacin	10–15 mg/kg q 12 h; SC, IM	2–5 mg/kg q 8–12 h; SC, IM	10 mg/kg q 12 h; IM
Amoxicillin	20–30 mg/kg q 8–12 h; PO	15 mg/kg q 12 h; PO, SC	30 mg/kg divided q 12–24 h; PO, SC
Amoxicillin/ clavulanate	12.5–25 mg/kg q 8–12 h; PO	12.5 mg/kg q 12 h; PO	12.5 mg/kg divided q 12–24 h; PO, SC
Ampicillin	5–30 mg/kg q 8–12 h; SC, IM, IV	–	–
Azithromycin	5 mg/kg q 24 h; PO	–	–
Ceftiofur	–	20 mg/kg q 12–24 h; SC	–
Cephalexin	15–30 mg/kg q 8–12 h; PO	25 mg/kg q 12 h; PO	30 mg/kg divided q 12–24 h; PO, SC
Chloramphenicol	25–50 mg/kg q 12 h; PO, SC, IM	30–50 mg/kg q 6–12 h; PO, SC, IV	–
Chlortetracycline	–	5–20 mg/kg q 12 h; PO	–
Ciprofloxacin	5–15 mg/kg q 12 h; PO	5–20 mg/kg q 12 h; PO	10 mg/kg q 12 h; PO
Clarithromycin	12.5–25 mg/kg q 12 h; PO	5.5 mg/kg q 12 h; PO	–
Clindamycin	5–10 mg/kg q 12 h; PO	5.5–10 mg/kg q 12 h; PO	–
Doxycycline	–	2.5–10 mg/kg q 12 h; PO, SC	–
Enrofloxacin*	10–20 mg/kg q 12–24 h; PO, SC, IM	5 mg/kg q 12 h; PO, SC	2.5–5 mg/kg q 12–24 h; PO, SC, IM
Erythromycin	10 mg/kg q 6 h; PO	10 mg/kg q 12 h; PO	20 q 12 h; PO
Fluconazole	50 mg/kg q 12 h; PO	–	–
Griseofulvin (avoid in pregnant animals)	25 mg/kg q 12–24 h; PO	50 mg/kg q 24 h; PO	20 mg/kg q 24 h; PO
Itraconazole	15 mg/kg q 24 h; PO	5–10 mg/kg q 12–24 h; PO	5–10 mg/kg q 12 h; PO
Ketoconazole	10–30 mg/kg q 12–24 h; PO	10 mg/kg q 12–24 h; PO	–
Lincomycin	–	–	30 mg/kg divided q 12–24 h; PO, IM
Metronidazole	20 mg/kg q 12 h; PO	20 mg/kg q 12 h; PO	25 mg/kg q 24 h; PO
Neomycin	10–20 mg/kg q 6 h; PO	–	–
Nystatin	–	30,000 IU/kg q 8–24 h; PO, topical	5,000 IU/kg q 8 h x 3d; PO
Oxytetracycline	–	25–50 mg/kg q 24 h; PO, in food	–
Penicillin G procaine	40,000 IU/kg q 24 h; SC	40,000 IU/kg q 24 h; SC, IM	22,000–25,000 IU/kg q 12–24 h; SC, IM
Piperacillin	–	10 mg/kg q 8–12 h; SC	–
Sulfadimethoxine	–	2–20 mg/kg q 24 h; PO, SC	5–10 mg/kg q 12–24 h; PO, SC
Tetracycline	25 mg/kg q 12 h; PO	–	–
Trimethoprim/sulfa	15–30 mg/kg q 12 h; PO, SC, IM	30 mg/kg q 12 h; PO, SC	15 mg/kg q 12 h; PO
Tylosin	10 mg/kg q 8–12 h; PO, SC	10 mg/kg q 12 h; PO, SC	–

*Dilute if giving by subcutaneous or intramuscular route to avoid tissue necrosis at injection site.
PO, per os; SC, subcutaneous; IM, intramuscular; dw, drinking water.

palatable liquids, gels, or food to encourage consumption. Small rodents, such as rats and mice, willingly take vanilla-flavored human nutritional supplements, such as Boost or Ensure, directly from a syringe or small dish. Hamsters favor rice-based baby cereal, rabbits like bananas, chinchillas are partial to raisins, and ferrets enjoy malt-flavored cat laxatives or pet nutritional supplements such as Nutri-Cal. The internet abounds with suggestions from clients and veterinarians alike, including Cool Whip, maple syrup, V.A.L. syrup, canned pumpkin, cooked sweet potato, coconut milk, raspberry-flavored gelatin, etc. The availability of a suitable vehicle, compatible with both the antimicrobial and the patient, is limited only by the imagination of the veterinarian.

Manual administration of pills, capsules, and liquids to rabbits and rodents is made challenging by their

Table 36.4. Antimicrobial treatment in mice. Caution: Most uses and dosages are extra-label.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Integument	Scabbing over dorsum and perineum; dermatitis; abscesses	*Staphylococcus aureus, Proteus* spp., *Streptococcus* spp.	Secondary to fighting/bite wounds or self-trauma due to acariasis. Trim toenails.	Ampicillin, chloramphenicol, tetracyclines
	Mastitis	*S. aureus*	Lance and drain in addition to antibiotics.	Ampicillin, chloramphenicol, fluoroquinolones
	Pruritis, weight loss, hyperkeratosis, alopecia	*Corynebacterium bovis*	Affects immunocompromised mice. Low mortality. Treatment not curative.	Ampicillin, penicillin
	Alopecia, erythema, crusting on face, head, neck, tail	*Trichophyton mentagrophytes, Microsporum gypseum* (less frequently)	Uncommon, zoonotic.	Griseofulvin (avoid in pregnant animals)
Respiratory	Rhinitis, dyspnea, otitis media, upper respiratory tract disease, pneumonia	*Mycoplasma pulmonis, Staphylococcus aureus, Streptococcus* spp.	Often concurrent with Sendai virus or CAR bacillus; decrease intracage ammonia levels.	Tylosin, fluoroquinolones, tetracyclines; enrofloxacin PLUS doxycycline for its immunomodulating effect
	Dacryoadenitis, sneezing, dyspnea, pneumonia	*Pasteurella pneumotropica, Klebsiella pneumonia, Bordetella bronchiseptica*	Often concurrent with Sendai virus or CAR bacillus; decrease intracage ammonia levels.	Chloramphenicol, fluoroquinolones, tylosin, aminoglycosides
	Pneumonia	CAR bacillus	Primary or opportunist with other respiratory pathogens.	Sulfamerazine, ampicillin, trimethoprim-sulfa
Gastrointestinal	Stunted growth, diarrhea, rectal prolapse, death; transmissible murine colonic hyperplasia	*Citrobacter rodentium, Clostridium piliforme*	Genotype, age, and diet influence course and severity of disease.	Tetracyclines, neomycin, metronidazole
	Liver disease, death, chronic active hepatitis, rectal prolapse	*Helicobacter hepaticus*		Amoxicillin (1.5–3 mg/30 g/d) PLUS metronidazole (0.69 mg/30 g/d) PLUS bismuth subsalicylate (0.185 mg/30 g/d), combined PO
	Anorexia, dehydration, diarrhea, death (Tyzzer's disease)	*Clostridium piliforme*	Concurrent fluid therapy essential.	Tetracyclines
	Anorexia, weight loss, lethargy, dull coat	*Salmonella enteriditis, S. typhimurium*	Zoonotic; recommend culling infected animals.	Treatment not recommended
Urogenital	Oophoritis, salpingitis, metritis, infertility, abortions	*Mycoplasma pulmonis, Pasteurella pneumotropica, Klebsiella oxytoca*		Tylosin, fluoroquinolones, tetracyclines

(*continued*)

Table 36.4. Antimicrobial treatment in mice. Caution: Most uses and dosages are extra-label (*continued*).

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
	Urethral gland obstruction, preputial gland abscesses	*Pasteurella pneumotropica, Staphylococcus aureus*		Chloramphenicol, fluoroquinolones, aminoglycosides
CNS	Head tilt, torticollis	*Mycoplasma pulmonis, Streptococcus* spp.		Chloramphenicol, tylosin, fluoroquinolones
	Eye abscesses, conjunctivitis, panophthalmitis	*Pasteurella pneumotropica*		Tetracyclines, aminoglycosides
General	Septicemia, death; mice that survive acute infection may have chronic arthritis, limb deformity, limb amputation; streptobacillosis	*Streptobacillus moniliformis*	Zoonotic potential.	Ampicillin, tetracycline
	Rough hair coat, hunched posture, inappetence, nasal and ocular discharge, arthritis	*Corynebacterium kutscheri*	Antibiotic treatment not curative.	

Table 36.5. Antimicrobial treatment in hamsters. Caution: Most uses and dosages are extra-label.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Integument	Cheek pouch abscesses, bite wound abscesses	*Staphylococcus aureus, Streptococcus* spp., *Pasteurella pneumotropica, Actinomyces* spp.	Drain and flush; complete excision of abscess beneficial.	Chloramphenicol, tetracyclines, fluoroquinolones
	Swollen lymph nodes, lymphadenitis	*Staphylococcus aureus, Streptococcus* spp.		Chloramphenicol, tetracyclines, fluoroquinolones
	Mastitis	Beta-hemolytic streptococci	Glands warm and swollen. Supportive treatment; self-limiting infection.	
	Alopecia, dry skin, yellow flaky seborrhea	*Trichophyton mentagrophytes*	Zoonotic. Sometimes pruritic; improve cage ventilation.	Griseofulvin (avoid in pregnant animals)
Respiratory	Sneezing, dyspnea, upper respiratory tract disease, pneumonia	*Pasteurella pneumotropica, Streptococcus pneumonia; Streptococcus* spp.	Secondary to poor nutrition and husbandry.	Chloramphenicol, tetracyclines, fluoroquinolones
		CAR bacillus	Opportunist with other respiratory pathogens.	Sulfamerazine, sulfonamides
Gastrointestinal	Diarrhea, stained perineum, lethargy, anorexia, rectal prolapsed, proliferative ileitis ("wet tail")	*Lawsonia intracellularis*	Especially in 3-to 10-week-olds; difficult to treat successfully; concurrent fluid therapy, analgesics, supportive care.	Chloramphenicol, tetracyclines, fluoroquinolones, trimethoprim-sulfa. Start with double the recommended dose for 1 day
	Enteritis	*E. coli, Clostridium difficile*	Concurrent fluid therapy, analgesics, supportive care essential.	Fluoroquinolones, metronidazole, tetracyclines,
	Anorexia, dehydration, diarrhea, death, Tyzzer's disease	*Clostridium piliforme*	Concurrent fluid therapy, analgesics, supportive care essential.	Tetracyclines
	Catarrhal enteritis in weanlings	*Giardia muris*		Metronidazole
CNS	Squinting, rubbing eye, corneal ulceration	*Pasteurella* spp., *Streptococcus* spp.	Topical treatment. Prone to proptosis.	Chloramphenicol, tetracyclines

Table 36.6. Antimicrobial treatment in gerbils. Caution: Most uses and dosages are extra-label.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Integument	Red, crusty nares, staining on forepaws, nasal dermatitis ("sore nose" or "red nose")	*Staphylococcus aureus, Staphylococcus* spp., *Streptococcus* spp.	Secondary to irritation due to Harderian gland secretions.	Chloramphenicol, trimethoprim-sulfa, fluoroquinolones
	Mid-ventral marking gland infection, dermatitis	*Staphylococcus aureus, Streptococcus* spp.		Chloramphenicol, trimethoprim-sulfa, fluoroquinolones
	Alopecia, hyperkeratosis	*Trichophyton mentagrophytes, Microsporum gypseum*	Zoonotic.	Griseofulvin (avoid in pregnant animals)
Respiratory	Sneezing, dyspnea, weight loss	Beta-hemolytic streptococci, *Pasteurella pnuemotropica*	Rare; concurrent therapy with oxygen, mucolytics, bronchodilators may help.	Fluoroquinolones, oxytetracycline, sulfonamides
Gastrointestinal	Lethargy, anorexia, diarrhea, death, Tyzzer's disease	*Clostridium piliforme*	Highly susceptible.	Tetracyclines
	Diarrhea, salmonellosis symptoms, death	*Salmonella enteriditis, S. typhimurium*	Zoonotic; recommend culling affected animals. Fluid therapy is essential if treatment attempted.	Chloramphenicol, fluoroquinolones
	Enteritis, diarrhea, dehydration	*E. coli*		Chloramphenicol, fluoroquinolones

relatively long, narrow oral cavity, large tongue, and small gape. Ferrets have the wider gape characteristic of most carnivores, but generally dislike having their mouths pried open, and may bite. In ferrets, rabbits, and some larger rodents, administration of pills and capsules can be accomplished with careful use of a pilling-device designed for cats. Guinea pigs and chinchillas have fleshy cheek invaginations just behind the incisors that act as "one-way valves." Small pills or pill pieces can be pushed past theses invaginations with the fingers (through the diastema). The presence of the substance in the mouth stimulates a chewing response that aids in intake, especially if the medication is somewhat palatable. In rabbits and rodents, liquids can be given using a small syringe inserted partway into the mouth to avoid dribbling and stimulate a swallowing response. Gentle restraint and careful cleaning of the face and chin will help minimize stress and prevent skin irritation.

Distribution of antimicrobial medication in the food or water is generally reserved for treatment of large numbers of animals, such as in research facilities, rabbitries, chinchilla farms, and pet-breeding operations, where individual dosing would be time-consuming and impractical. Problems inherent with mass-medication include variable intake by sick animals, reduced palatability of the food or water, uneven distribution of the drug, and possible water-quality effects on the chemical composition of the compound.

Injectable antimicrobials are most often administered to small mammals subcutaneously in the loose skin over the shoulders. The procedure is quick and minimally stressful when performed correctly. Concurrent fluid therapy can also be given in this large space, provided the two compounds are compatible. Small rodents can be restrained with one hand and injected with the other. The rodent is firmly grasped by the scruff, and either left standing, or partially lifted off the exam table, while the injection is administered. Positive re-inforcement following the procedure facilitates repeat treatments. Larger rodents and rabbits can be wrapped in a towel or restrained by an assistant to facilitate injection. Ferrets should be securely grasped by the scruff, or around the neck in a "turtle-neck" hold, to prevent excessive wriggling. Careful restraint is particularly necessary for rabbits, to prevent thrashing and spinal fractures; for chinchillas, to prevent damage to the fur ("furslip"); for gerbils, to prevent degloving of the tail; and for ferrets and hamsters, to minimize the risk of bites.

Table 36.8. Antimicrobial treatment in guinea pigs. Caution: Most uses and dosages are extra-label.

Site	Disease/Clinical Signs	Common Infecting Organisms	Comments	Suggested Drugs
Integument	Enlarged lymph nodes; cervical lymphadenitis	*Streptococcus zooepidemicus;* also *Streptococcus moniliformis* and *Yersinia pseudotuberculosis*	May cause septicemia; complete surgical excision of infected lymph nodes beneficial.	Chloramphenicol, fluoroquinolones
	Abscesses	*Staphylococcus aureus, Streptococcus* spp., *Pseudomonas aeruginosa, Pasteurella multocida, Corynebacterium pyogenes*	Secondary to bite wounds (especially males), trauma, dental disease.	Fluoroquinolones, trimethoprim-sulfa, chloramphenicol, azithromycin, metronidazole
	Mastitis	*Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Pasteurella* spp., *E. coli, Proteus* spp.	Hot compresses; milk out infected glands.	Chloramphenicol, trimethoprim-sulfa
	Swollen, ulcerated foot; ulcerative pododermatitis; osteomyelitis	*Staphylococcus aureus, Actinomyces* spp.	Secondary to inappropriate bedding, hypovitaminosis C, trauma.	Chloramphenicol, fluoroquinolones, trimethoprim-sulfa, azithromycin, metronidazole
	Circular areas of alopecia, crusts; pruritis	*Trichophyton mentagrophytes, Microsporum canis*	Zoonotic.	Fluconazole, itraconazole, ketoconazole, terbinafine, griseofulvin (avoid in pregnant animals)
Respiratory	Rhinitis, tracheitis, otitis media, oculonasal discharge, upper respiratory tract disease and/or pneumonia	*Bordetella bronchiseptica, Streptococcus zooepidemicus, Streptococcus pneumoniae, Streptococcus zooepidemicus*	*Bordetella bronchiseptica* commonly carried by dogs and rabbits; some success with Bordetella bacterins.	Amikacin, fluoroquinolones, chloramphenicol
		S. pneumoniae, S. zooepidemicus, K. pneumoniae		Amikacin, fluoroquinolones, chloramphenicol
Gastrointestinal	Anorexia, diarrhea, enteritis, death	*Clostridium difficile, Salmonella typhimurium, Salmonella enteriditis, E. coli, Yersinia pseudotuberculosis, Pseudomonas aeruginosa, Listeria monocytogenes*	Concurrent fluid therapy essential; amikacin best for *P. aeruginosa*.	Chloramphenicol, systemic amikacin or gentamicin
	Diarrhea, dehydration, bloating, death	*Clostridium difficile, E. coli*	Spontaneous, or following administration of antibiotics.	Metronidazole, chloramphenicol, control pain
	Anorexia, ascites, diarrhea, death; Tyzzer's disease	*Clostridium piliforme*	Recent weanlings, predisposed by crowding, poor sanitation.	Tetracyclines
	Diarrhea; coccidiosis	*Eimeria caviae*	Most common in juveniles.	Sulfonamides
	Failure to gain, weight loss, diarrhea, death; cryptosporidiosis	*Cryptosporidium wrairi*	In humans, newer macrolides such as roxithromycin and azithromycin have shown some efficacy.	Sulfonamides

Table 36.7. Antimicrobial treatment in rats. Caution: Most uses and dosages are extra-label.

Site	Clinical Signs/Diagnosis	Common Infecting Organisms	Comments	Suggested Drugs
Integument	Abrasions/ulcerations over shoulders and back; ulcerative dermatitis	*Staphylococcus aureus*	Secondary to primary wounds caused by self-trauma. Trim toenails.	Ampicillin, chloramphenicol,
	Abscesses, furunculosis	*P. pneumotropica*, *K. pneumoniae*	Secondary opportunist; drain and flush abscesses.	Chloramphenicol, fluoroquinolones, aminoglycosides
	Mastitis	*P. pneumotropica*, *Staphylococcus aureus*	Hot compress, drainage.	Chloramphenicol, fluoroquinolones, aminoglycosides
	Alopecia, pruritis	*Microsporum* spp.	Zoonotic.	Griseofulvin (avoid in pregnant animals)
Respiratory	Snuffling, sneezing, dyspnea, vestibular disease, depression chromodacryorrhea, upper respiratory tract disease and/or pneumonia; murine respiratory mycoplasmosis (MRM)	*Mycoplasma pulmonis*	Common; improve nutrition, husbandry; decrease intracage ammonia levels.	Combination enrofloxacin 10 mg/kg & doxycyline 5 mg/kg beneficial; tetracycline, tylosin
		CAR bacillus	Often concurrent with mycoplasmas or viruses.	Sulfamerazine, ampicillin, chloramphenicol, enrofloxacin
	Serosanguinous to mucopurulent nasal discharge, rhinitis, conjunctivitis, otitis media	*Streptococcus pneumoniae*	Immunocompromised animals at greatest risk.	Oxytetracycline
	Rough coat, hunched, oculonasal discharge, dyspnea, granulomatous pneumonia; pseudotuberculosis	*Corynebacterium kutscheri*	Immunocompromised animals at greatest risk; antibiotics will not eliminate infection.	Ampicillin, chloramphenicol, tetracyclines
	Conjunctivits, panophthalmitis, oculonasal discharge, dyspnea, head tilt	*Pasteurella pneumotropica*	Immunocompromised animals at greatest risk.	Enrofloxacin
Gastrointestinal	Diarrhea, dehydration, anorexia, death; Tyzzer's disease	*Clostridium piliforme*		Tetracyclines
	Diarrhea, dry coat, unthriftiness, death	*Salmonella enteriditis*	Zoonotic; recommend culling affected animals.	
Urogenital	Infertility, oophoritis, salpingitis, metritis, pyometra	*Mycoplasma pulmonis*		Tylosin, fluoroquinolones, tetracyclines
	Preputial gland abscess	*P. pneumotropica*, *Staphylococcus aureus*		Chloramphenicol, fluoroquinolones
CNS	Head tilt, circling, torticollis, otitis interna	*Mycoplasma pulmonis* ± secondary bacterial invaders		Fluoroquinolones, chlormaphenicol, tylosin

Urogenital	Metritis, pyometra, abortions, stillbirths	*Bordetella bronchiseptica, Streptococcus* spp., *Corynebacterium pyogenes, Staphyloccus* spp., *E. coli*	Ovariohysterectomy recommended in non-breeding sows.	Fluoroquinolones, trimethoprim-sulfa, chloramphenicol
	Orchitis, epididymitis	*Bordetella bronchiseptica, Streptococcus* spp.		Chloramphenicol, systemic amikacin or gentamicin
	Cystitis	*Staphylococcus pyogenes, Staphylococcus* spp., fecal coliforms	Urinary calculi often present.	Trimethoprim-sulfa, fluoroquinolones
Eye	Ocular discharge; conjunctivitis	*Chlamydophila caviae, Bordetella bronchiseptica, Streptococcus pneumoniae*	Topical treatment; often secondary to hypovitaminosis C.	Tetracyclines, fluoroquinolones, chloramphenicol
Ear	Head tilt, otitis media/interna	*Streptococcus pneumoniae, Streptococcus zooepidemicus, Bordetella bronchiseptica, Staphylococcus aureus*		Fluoroquinolones, trimethoprim-sulfa, chloramphenicol, metronidazole
General	Anorexia, soft stools, dyspnea, hepatitis, lymphadenitis, septicemia, death	*Salmonella typhimurium, Salmonella enteriditis*	Zoonotic; recommend culling infected animals.	Treatment not recommended

Table 36.9 Antimicrobial treatment in ferrets. Caution: Most uses and dosages are extra-label.

Site	Disease/Clinical Signs	Common Infecting Organisms	Comments	Suggested Drugs
Integument	Dermatitis, abscesses	*Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., *Pasteurella* spp., *Actinomyces* spp., *E. coli*	Secondary to bite wounds; debride and flush.	Ampicillin, chloramphenicol, fluoroquinolones
	Cervical masses with sinus tracts containing thick yellow-green pus, actinomycoses	*Actinomyces* spp.	Debride and flush.	Clavulanic acid–amoxicillin, chloramphenicol
	Skin black, dam ill, dehydrated; acute gangrenous mastitis	*Staphylococcus* spp., coliforms	Immediate surgical excision of infected gland; contagious between dams.	Clavulanic acid–amoxicillin, chloramphenicol
	Glands firm, scarred, not painful or discolored; chronic mastitis	*Staphylococcus* spp., *E. coli*	Contagious between dams; appears insidiously when kits 3 weeks old.	Treatment generally ineffective
	Alopecia, crusts, hyperkeratosis, broken hair shafts	*Trichophyton mentagrophytes, Microsporum canis*	Zoonotic.	Itraconazole, griseofulvin (avoid in pregnant animals)
Respiratory	Dyspnea, cyanosis, upper respiratory tract disease and/or pneumonia	*Streptococcus zooepidemicus, Streptococcus pneumoniae, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bordetella bronchiseptica, Listeria monocytogenes*	Secondary to influenza virus, respiratory syncytial virus, canine distemper virus.	Ampicillin, tetracyclines, fluoquinolones
		Pneumocystis jiroveci		Trimethoprim-sulfamethoxazole
		S. pneumoniae, S. zooepidemicus, K. pneumoniae		Amikacin, fluoroquinolones, chloramphenicol
Gastrointestinal	Dental tartar, gingivitis, periodontal disease	Multiple etiologies	Improve diet; dentistry.	Metronidazole
	Inappetance, vomiting, bruxism, diarrhea, melena, hypersalivation, anemia, gastritis, gastric/duodenal ulceration; *Helicobacter mustelae* gastritis	*Helicobacter mustelae*	Rule out foreign body, lymphoma, Aleutian disease, coronavirus.	Amoxicillin 10 mg/kg PLUS metronidazole 20 mg/kg PLUS bismuth subsalicylate 17 mg/kg (1 ml/kg) combined and given PO, q 12 h for 14–21 days OR Clarithromycin 25 mg/kg PLUS omeprazole 1 mg/kg PO, q 24 h OR enrofloxacin 4 mg/kg PLUS colloidal bismuth subcitrate 6 mg/kg PO, q 12 h

	Diarrhea, wasting, tenesmus, prolapsed rectum; proliferative bowel disease	*Lawsonia intracellularis*		Chloramphenicol, tylosin
	Acute gastric distension, dyspnea, cyanosis, sudden death; gastric bloat	*Clostridium perfringens*	Treat as for bloat in canine patients.	Metronidazole
	Fever, bloody diarrhea, lethargy	*Salmonella newport, S. typhimurium, S. choleraesuis*	Zoonotic; recommend culling infected animals.	Treatment not recommended
	Weight loss, diarrhea, vomiting, granulomatous inflammation; mycobacteriosis	*Mycobacterium* spp.	Zoonotic potential—consider culling.	
	Diarrhea; coccidiosis	*Coccidia* spp.		Sulfonamides
	Diarrhea; giardiasis	*Giardia* spp.		Metronidazole
Urogenital	Straining, hematuria, cystitis	*Staphylococcus* spp., *Proteus* spp.	Urolithiasis often present.	Fluoroquinolones, ampicillin, sulfonamides

Table 36.10. Antimicrobial treatment in chinchillas. Caution: Most uses and dosages are extra-label.

Site	Disease/Clinical Signs	Common Infecting Organisms	Comments	Suggested Drugs
Integument	Abscesses	*Staphylococcus aureus, Streptococcus* spp., *Pseudomonas* spp.	Secondary to wounds; complete surgical excision beneficial.	Chloramphenicol, tetracyclines, fluoroquinolones
	Patches of alopecia, scales on nose, ears, and feet	*Trichophyton mentagrophytes*	Zoonotic.	Griseofulvin (avoid in pregnant animals), itraconazole, fluconazole
Respiratory	Anorexia, upper respiratory tract disease, dyspena and/or pneumonia	*Pasteurella multocida, Bordetella* spp., *Streptococcus. Pneumoniae, Pseudomonas aeruginosa*	Overcrowding, high humidity, poor ventilation are presdisposing factors. Amikacin best for *Pseudomonas aeruginosa*.	Fluoroquinolones, trimethoprim-sulfa, chloramphenicol, amikacin
Gastrointestinal	Anorexia, decreased fecal output, diarrhea, enteritis, sudden death	*Yersinia enterocolitica, Clostridium perfringens, E. coli, Proteus* spp., *Salmonella typhimurium, Salmonella enteriditis, Pseudomonas aeruginosa, Listeria monocytogenes, Corynebacterium* spp.	Concurrent fluid therapy essential; sulfonamides best for *Listeria monocytogenes*.	Chloramphenicol, trimethoprim-sulfa, fluoroquinolones, metronidazole (use with caution)
	Diarrhea, dehydration, bloating, death	*Clostridium* spp., *E. coli*	Spontaneous, or following administration of antibiotics.	Metronidazole (use with caution), chloramphenicol, trimethoprim-sulfa
	Diarrhea ± rectal prolapse; giardiasis	*Giardia* spp.		Fenbendazole, metronidazole (use with caution)
Urogenital	Depression, abortions	*Listeria monocytogenes*	Highly susceptible.	Sulfonamides, tetracyclines
	Metritis, fever, purulent vaginal discharge	*E. coli, Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp.		Aminoglycosides, fluoroquinolones
Otic	Vestibular signs, head tilt, anorexia; otitis media and/or interna	*Pseudomonas aeruginosa, Listeria monocytogenes*, anaerobes		Fluoroquinolones, trimethoprim-sulfa, chloramphenicol
CNS	Depression, ataxia, convulsions, sudden death	*Listeria monocytogenes*	Highly susceptible.	Trimethoprim-sulfa, tetracyclines
General	Septicemia, death	*Streptococcus* spp., *Enterococcus* spp., *Pasteurella multocida, Klebsiella pneumoniae, Actinomyces* spp., *Fusobacterium necrophorum*	Zoonotic; recommend culling infected animals.	Chloramphenicol, fluoroquinolones

Table 36.11. Antimicrobial treatment in rabbits. Caution: Most uses and dosages are extra-label.

Site	Disease/Clinical Signs	Common Infecting Organisms	Comments	Suggested Drugs
Integument	Abscesses	*Pasteurella multocida, Staphylococcus aureus, Pseudomonas* spp., *Streptococcus* spp., *Bacteroides* spp.	Can be located anywhere on body; complete surgical excision beneficial.	Chloramphenicol, tetracyclines, fluoroquinolones
	Ulcerative podocermatitis; "sorehock"	*Staphylococcus aureus, Pasteurella multocida,*	Often secondary to inappropriate substrate.	Chloramphenicol, tetracyclines, fluoroquinolones
	Dermatitis	*Staphylococcus aureus*	Usually secondary to poor husbandry.	Chloramphenicol, tetracyclines, fluoroquinolones
	Ulceraton/necrosis of face, feet; dental and internal abscesses (Schmorl's disease)	*Fusobacterium necrophorum*	Associated with poor hygiene and husbandry.	Cephalosporins, chloramphenicol, tetracyclines, metronidazole
	Wet chin, dewlap ("slobbers") or urine scald ("hutch burn"); exudative dermatitis	*Pseudomonas aeruginosa, Streptococcus* spp., *Staphylococcus* spp.	Secondary to moist skin. *P. aeruginosa* may turn fur green. Correct underlying cause (dental disease, obesity, inappropriate waterers).	Fluoroquinolones, amikacin, gentamicin
	Mastitis	*Staphylococcus aureus, Pasteurella* spp., *Streptococcus* spp.	Hot compresses; milk out affected glands often.	Amikacin, fluoroquinolones, chloramphenicol, tetracyclines
	Alopecia, scaling, crusting on eyelids, at base of ears, and muzzle	*Trichopyton* spp., *Microsporum* spp.	Zoonotic.	Griseofulvin (avoid in pregnant animals), itraconazole, ketoconazole, terbinafine
	Crusty lesions on nose and lips ± concurrent genital lesions	*Treponema cuniculi*		Parenteral penicillin, cephalexin, tetracyclines, chloramphenicol
Respiratory	Snuffling, oculonasal discharge, conjunctivitis, upper respiratory tract disease and/or pneumonia	*Pasteurella multocida*	Very common; treatment rarely curative.	Parenteral penicillin, fluoroquinolones, tetracyclines, amikacin, gentamicin
		Bordetella bronchiseptica, Staphylococcus aureus, Pseudomonas aeruginosa	Usually secondary to *Pasteurella multocida*.	Amikacin, fluoroquinolones, tetracyclines

(continued)

Table 36.11. Antimicrobial treatment in rabbits. Caution: Most uses and dosages are extra-label *(continued)*

Site	Disease/Clinical Signs	Common Infecting Organisms	Comments	Suggested Drugs
Gastrointestinal[a]	Diarrhea, death; iota-enterotoxemia	*Clostridium spiroforme*	Spontaneous, or following administration of antibiotics.	Metronidazole, chloramphenicol
	Diarrhea; coccidiosis	*Eimeria* spp.	Hepatic or intestinal; improve sanitation.	Sulfonamides
	Diarrhea, death; colibacillosis	*E. coli*	Especially neonates 1–14 days old and weanlings.	Sulfonamides, fluoroquinolones, amikacin
	Diarrhea, death	*Salmonella* spp., *Pseudomonas* spp.	Concurrent fluid therapy essential.	Chloramphenicol, fluoroquinolones
	Diarrhea, death; Tyzzer's disease	*Clostridium piliforme*		Tetracyclines
Urogenital	Reddening, edema to dry, scaly, slightly raised areas of external genitalia; veneral spirochetosis ("rabbit syphilis")	*Treponema paraluiscuniculi*		Parenteral penicillin, tetracyclines, chloramphenicol
	Abortion	*Listeria monocytogenes, Pasteurella multocida*		Trimethoprim-sulfa, chloramphenicol, tetracyclines
	Cystitis	*E. coli, Pseudomonas* spp.		Trimethoprim-sulfa, fluoroquinolones
	Orchitis, metritis, uterine abscesses	*Pasteurella multocida, Staphylococcus aureus*		Chloramphenicol, tetracycline, gentamicin
	Polydypsia, polyuria, depression, anorexia, renal failure	*Leptospira* spp.	Contact with wild rodents; diagnosis by serology.	Parenteral penicillin
Ocular	Clear to white discharge from one or both eyes, conjunctivitis	*Pasteurella multocida, Staphylococcus aureus*	Treat topically; flush tear ducts.	Chloramphenicol, tetracycline, aminoglycosides
CNS	Head tilt, nystagmus, torticollis; "wry neck"	*Pasteurella multocida*	Usually due to otitis media.	Chloramphenicol, fluoroquinolones
	Ataxia, torticollis, tremors, convulsions	*Encephalitozoon cuniculi*	Diagnosis by clinical signs and serology.	Fenbendazole, albendazole, tetracyclines
General	Lethargy, anorexia, pyrexia, septicemia	*Pasteurella multocida, Listeria monocytogenes*		Fluoroquinolones, aminoglycosides, tetracyclines, chloramphenicol

[a]Where applicable, provide aggressive supportive care, including fluids (SC, IV, intraosseous), analgesics, high-fiber diet (via syringe or naso-gastric tube if necessary), cisapride or metaclopramide, excellent nursing care; cholestyramine at 2 g per 20 ml water q 24 h by gavage may bind bacterial toxins.

Intraperitoneal injection is suitable for smaller rodents, and is a common route of drug administration in laboratory animals. The procedure is quick and easy to perform, which minimizes patient discomfort. The rodent is firmly scruffed and turned upside down to expose the abdomen. Injections are given in the mid- to lower-right quadrant, to avoid puncturing the cecum. Intraperitoneal injection in animals with voluminous intestines, such as guinea pigs and rabbits, is not recommended.

Small rodents generally lack sufficient muscle mass to accommodate intramuscular injections. Soft tissue trauma and irritation, leading to self-mutilation, may occur, and drug uptake can be unreliable. In larger rodents, rabbits, and ferrets, intramuscular injections can be given in the lumbar, gluteal, or quadriceps muscles, taking care not to penetrate the bone or sciatic nerve. Alternate routes of drug administration are generally easier and safer, and therefore preferable.

Topical antimicrobial preparations, especially those containing corticosteroids, should be used sparingly and cautiously in rabbits, and rodents. Due to their fastidious grooming habits, ingestion of amounts of drug sufficient to cause undesirable systemic effects may result. In addition, use of oil-based products should be avoided if possible, especially in chinchillas and gerbils, which require dust-baths to keep their fur healthy.

Ophthalmic preparations are less concentrated than other topical medications, and are useful not only for the eyes, but for other parts of the body too. These preparations can also be injected into the naso-lacrimal ducts of rabbits following flushing, or instilled into the nares.

The intravenous route of antimicrobial administration is not used routinely and is usually reserved for initial treatment of critically ill patients. In ferrets and rabbits, a catheter can be placed in the cephalic vein for administration of fluids and other drugs, and the ear vein is sometimes catheterized in rabbits, but may result in subsequent ear necrosis. Injections can be attempted directly into the ear, cephalic, lateral saphenous, medial saphenous, femoral, or tail vein of some small mammals, but this requires a large amount of skill and often anesthesia of the patient. In severely debilitated patients in which venous access is not possible, placement of an intraosseous catheter in the tibia or femur may be indicated.

Nebulization of antimicrobial drugs is sometimes used to treat upper and lower airway disease in small mammal pets. A mask may be tolerated by some animals, or a small chamber, such as an anesthesia induction chamber, can be used. Patients should be supervised at all times during confinement to detect undue stress, overheating, or other problems.

Gavage is used primarily in experimental studies, where accurate administration of the drug is critical. In rabbits and ferrets, soft plastic feeding tubes or catheters can be introduced into the esophagus through a speculum. The barrel of a 3 cc syringe with the end cut off works well in rabbits. In rodents, curved, ball-ended metal or plastic feeding needles are commercially available for oral dosing. Correct restraint and gentle insertion, allowing gravity and the swallowing reflex to pass the tube into the esophagus, are critical for success, but once the technique is mastered, it is quick and relatively stress-free for the animal.

Administration of oral antimicrobials in very debilitated patients can also be accomplished through placement of a nasogastric tube (in rabbits) or esophagostomy tube (in rabbits, larger rodents, and ferrets), especially if repeated administrations are necessary and the animal becomes unduly stressed by oral manipulations, or in animals that also require nutritional supplementation. Nasogastric tube placement in rabbits is not technically difficult to perform and several descriptions of the procedure are available in the literature. Esophagostomy tube placement requires general anesthesia; however, post-operative animal comfort is greater than with nasogastric tubes, breathing is not compromised, and tubes are rarely pulled out.

Antimicrobial-impregnated implants are used primarily for treatment of facial and tooth-root abscesses in rabbits. In biomedical research, small mammal models have been used to study the elution kinetics and other effects on bone formation and implant integration of various antimicrobial-coated orthopedic implants for use in people.

Animal Numbers and Use

The number of animals requiring treatment, and their intended use must always be kept in mind when prescribing antimicrobial medications in rabbits,

rodents, and ferrets. The method of treating a single small mammal patient will often differ significantly from that of treating hundreds of animals being bred for the pet trade, used as research subjects, being farmed for fur, or, in the case of rabbits, being raised for meat. The cost and feasibility of treatment, the effect of the drug on the animal, the deleterious effects that handling may have on the animal, and the possibility of the animal being consumed by humans are some of the factors that will affect the choice of antimicrobial, its formulation, and the method of administration.

Veterinary treatment of animal colonies used for biomedical research must be compatible with the intended scientific use of the animals, so as not to render them useless. A colony-treatment approach, rather than an individual animal approach, is commonly implemented and medications are generally incorporated into feed or water. Particular attention must be paid to treatment of genetically modified animals; not only are they usually very valuable and often irreplaceable, they sometimes do not metabolize drugs in a predictable way due to their altered genetic background. With particularly valuable animals, treatment of a small number and observation for deleterious side effects may be indicated, prior to treating the larger population. Several companies specialize in incorporating test compounds or medical therapies into palatable diets, treats, or feed for a wide variety of animals used in biomedical research.

A number of antimicrobials are prohibited for use in food-producing animals. One of these is chloramphenicol, a drug that is frequently used in rabbits and rodents due to its effectiveness and relative safety, but has been associated with irreversible aplastic anemia in humans. Strategic and tactful questioning must sometimes take place to determine whether the patient might eventually be used as a food source for humans.

Extra-label medications are occasionally used in meat rabbits, for example, antimicrobial mixtures labeled for prevention of necrotic enteritis in chickens are sometimes added to commercial rabbit feed to manage enteritis problems. Both the producer and the veterinarian are responsible for ensuring no drug residues are present in meat produced for human consumption; however, withdrawal times are not available for these drugs when used extra-label. In these situations specific withdrawal recommendations can be obtained from a food animal residue avoidance databank (in Canada: www.cgfarad.usask.ca; in the United States: www.farad.org).

Enhancing Therapeutic Success

Treatment of small mammal patients usually involves more than just choosing the correct antimicrobial agent. Many infections are secondary to a compromised immune system caused by stress or inadequate nutrition and/or husbandry. A thorough history is necessary to detect preexisting problems that may be unknown to the client. For example, guinea pigs are unable to synthesize vitamin C and require supplementation of 10–25 mg daily. Undersupplementation is very common in these animals, leading to subclinical hypovitaminosis C, altered immune function, and secondary bacterial infections. Although ascorbic acid is present in guinea pig food, the stability varies, and the actual amount available depends on the milling date and storage conditions. Owners may not be aware that most chows should be used within 90–180 days of being milled. Other options for provisions of vitamin C include water supplementation, flavored tablets, or daily feeding of small amounts of vitamin C-rich fruit or vegetables, such as kale, parsley, beet greens, kiwi fruit, broccoli, orange, or cabbage. Oversupplementation of this water-soluble vitamin is generally not a concern since excess amounts are eliminated from the body.

Altered immune function can also occur due to stress. Prey species, such as rabbits and rodents, are particularly susceptible to stressors and the effects can be long-lasting. Studies in laboratory animals have shown that transport, separation from cage mates, and confinement in an unfamiliar container can affect the cardiovascular, endocrine, immune, central nervous and reproductive systems, and it can take at least 2 days for rabbits to acclimate to a new environment after travel. Noise and odors are also stressful to rabbits and rodents, and their heart and respiratory rates can increase rapidly in response to catecholamine release. Prey species are particularly sensitive to predator odors so an attempt should be made to minimize exposure of rabbits and rodents to dogs, cats, and ferrets, and their vocalizations and smells, while in the clinic.

Prey species instinctively mask any signs of weakness or illness. In addition, their quiet nature, secretive habits,

and confinement to a cage can allow them to become debilitated before the average owner notices a problem. Being handled by unfamiliar people with unfamiliar voices and scents, having samples collected, and the unfamiliar hospital environment will add to the overall stress load. Rodents, especially guinea pigs, seem to lack a strong will to live, so good nursing and supportive care is necessary both for physical and psychological health. Due to their small size, sick rodents can quickly become hypothermic and debilitated from not eating or drinking. Administration of warmed fluids subcutaneously, provision of supplemental heat (taking care not to overheat the animal), and hospitalization in a safe, quiet environment is almost always indicated. Ample amounts of soft, comfortable bedding and placement of food and water stations within easy reach is also important. If the animal is not eating, gentle handling and syringe-feeding a palatable diet is indicated, taking care to administer the food slowly to prevent aspiration. Pain behaviours can be difficult to detect in rodents, but include anorexia, unkempt fur, piloerection, immobility or restlessness, lethargy, pressing of the abdomen to the floor or table, bruxism, hunched posture, half-closed eyes, isolation from a group, unusual aggression, and guarding of specific areas of the body. Analgesics should be used as necessary. Ensuring the animal stays clean and well groomed is also important. During hospitalization, assessment of the animal's condition, especially body weight, should be performed 2–3 times a day to monitor progress.

Facilities housing large groups of animals, such as rabbitries, fur farms, and pet-breeding farms, must have sufficient environmental controls in place to maintain the animals at the appropriate temperature, and to ensure adequate ventilation. Heat or cold stress can compromise the animals, and excessive build-up of ammonia can cause irritation to the mucous membranes, creating a portal for entrance of bacteria.

It is important to recognize that, in addition to prescribing antimicrobial therapy in small mammal patients, and demonstrating the correct method of administration to ensure compliance, veterinarians must also advise clients on correct management, nutrition, and husbandry practices. A good understanding of the normal anatomy, physiology, and behavior of these vulnerable patients will help both the veterinarian and the client provide them with the best care possible.

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37

Antimicrobial Drug Use in Reptiles

Ramiro Isaza and Elliott R. Jacobson

Antimicrobial therapy is an important component in clinical management of reptiles affected with bacterial or mycotic disease. Selecting the appropriate antimicrobial agent for reptiles is based on similar principles and considerations common to antimicrobial selection in domestic species. However, this process is more complicated in reptiles because of the number and diversity of species, their unique anatomic and physiological features, the diversity of infectious agents, and even behavioral characteristics that make safety an important factor in drug and route considerations. Once a candidate antimicrobial is selected, the process is further complicated by the relatively few pharmacokinetic studies performed in reptiles. This chapter will focus on the process of antimicrobial selection in reptiles while highlighting the unique differences and challenges associated with selecting antimicrobial agents for these species.

Reptile Infectious Agents

Bacterial and fungal infections are important causes of morbidity and mortality in captive reptiles (Austwick and Keymer, 1981; Clark and Lunger, 1981; Cooper, 1981; Hoff et al., 1984; Jacobson, 1999; Jacobson, 2007). From the literature, it appears that Gram-negative bacterial infections are common in captive reptiles (Paré, et al., 2006). Although not as well documented, Gram-negative bacterial infectious diseases are also reported in wild populations of reptiles. For instance, die-offs of American alligators (*Alligator mississippiensis*) have been associated with *Aeromonas hydrophila* infections (Shotts et al., 1972).

In addition to Gram-negative bacteria, a wide variety of other bacteria are becoming recognized as either primary or secondary pathogens of reptiles (Jacobson, 2007). Stewart (1990) found a variety of anaerobic bacteria in a series of reptile cultures. In other studies, bacterial pathogens such as mycoplasma, *Chlamydophila*, and mycobacteria were reported in reptiles (Homer et al., 1994; Jacobson and Telford, 1990; Jacobson et al., 1989; Jacobson et al., 2002; Jacobson, 2007; Soldati et al., 2004). It is apparent that as methods of detection are improved and applied in reptile samples, the range of bacterial pathogens will continue to expand.

Mycotic infections are also common in captive reptiles (Paré et al., 2006). Mycotic infections of the integumentary and respiratory systems are particularly common (Austwick and Keymer, 1981; Migaki et al., 1984). For example, the *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) is an important fungal pathogen of reptiles (Paré et al., 1997; Paré et al., 2003; Bertelsen et al., 2005).

Other pathogens, including protozoal, helminth, and viral agents have been described in reptiles and subsequently well reviewed in the literature (Jacobson, 2007).

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Studies evaluating the pharmacokinetics of antimicrobial drugs active against these pathogens are currently limited (Gaio et al, 2007; Allender et al., 2012). Due to the lack of pharmacokinetic studies, this chapter will not discuss these pathogens further, instead focusing on bacterial and mycotic pathogens.

With the increasing numbers of studies describing reptile pathogens, some species and groups of reptiles are becoming associated with specific bacterial and mycotic pathogens. Tables 37.1–37.3 provide a partial listing of these disease associations and provide the clinician with a preliminary guide for selecting antimicrobials. Examples of these species associations include: *Neisseria iguana* isolated from both the normal oral cavity and bite wounds of captive green iguanas (*Iguana iguana;* Plowman et al., 1987; Barrett et al., 1994); *Mycoplasma agassizii* associated with chronic upper respiratory disease in both the desert tortoise (*Gopherus agassizii*) and the gopher tortoise (*Gopherus polyphemus;* Jacobson et al., 1991; Brown et al., 1995; Brown et al., 1999); *Mycoplasma crocodyli* as the cause of polyarthritis in Nile crocodiles (*Crocodylus niloticus*) in Zimbabwe (Kirchoff et al., 1997; Mohan et al., 1995); and *Mycoplasma alligatoris* associated with arthritis and pneumonia in the American alligator (*Alligator mississippiensis;* Brown et al., 2001; Clippinger et al., 2000).

Identifying Reptile Infectious Agents

Once a bacterial or mycotic infection is suspected in a reptile patient, the accurate identification of the primary pathogen is an essential step when choosing the most appropriate antimicrobial. If a discrete lesion is present, a biopsy specimen is ideally obtained for both cytologic and histologic examination. Concurrent with the morphological assessment, a specimen of the lesion is also submitted for culture. It is important to inform the laboratory that the culture specimen is from a reptile and may need special laboratory handling to isolate the pathogen (Origgi et al., 2007). Ideally, a portion of the biopsied sample is also retained for further molecular assessments, such as polymerase chain reaction. This aggressive diagnostic approach is recommended to accurately interpret the significance of the microorganisms cultured from reptile lesions.

Some reptile pathogens such as *Chlamydophila*, *Mycoplama*, and mycobacteria are relatively difficult to isolate from routine cultures and also often difficult to see in standard histopathological preparations. Special histological stains, immunohistochemical stains, and molecular techniques are sometimes necessary to detect their presence (Bodetti et al., 2002; Jacobson et al., 2004; Johnson et al., 2007). Soldati (2004) provides an example of this aggressive diagnostic approach using both immunoperoxidase staining and PCR amplification to detect mycobacteria and chlamydia in a retrospective study of 90 reptiles with granulomatous lesions.

In reptiles suspected of being septic, the clinician should routinely perform blood cultures. Jacobson (1992a, 2007) describes techniques for collecting percutaneous blood specimens from reptiles. The interpretation of blood culture results can be challenging, as certain bacteria such as *Clostridium* spp. have been cultured from the blood of clinically healthy reptiles (Hanel et al., 1999). Furthermore, if the clinician fails to collect the percutaneous blood sample correctly, bacteria from cutaneous contamination are commonly isolated. Collecting a truly uncontaminated ante-mortem blood sample from a reptile is far more difficult than it appears to be (Jacobson, 1992a). Thus blood culture results must be interpreted in the context of other health assessment tests and consideration of sample quality.

As in domestic species, selecting the appropriate antimicrobial agent is ideally based on the results of a quantitative susceptibility panel. Once the primary pathogen is isolated and identified, the clinician should request an antimicrobial sensitivity panel from the laboratory. The clinician may need to request a custom quantitative susceptibility panel that includes antimicrobials commonly used in reptiles.

Husbandry and Immunological Considerations

The next consideration in the process of antimicrobial selection should be an understanding that captive husbandry and the immunological status of the reptile are important. Bacterial and mycotic infections tend to become more invasive and clinically apparent in captive reptiles when husbandry conditions are suboptimal (Cooper, 1981). For example, maintaining reptiles below their optimal temperature range may induce an

Table 37.1. Antimicrobial drug selection in chelonian infections.

Site or Type	Diagnosis	Common Infecting Organisms	Suggested Drugs
Skin, shell, and subcutis	Epidermitis/Dermatitis	*Citrobacter freundii* *Serratia* *Proteus morganii* *Providencia rettgeri* *Pseudomonas aeruginosa*	Amikacin Ceftazadime Ticarcillin Enrofloxacin
		Dermatophilus chelonae	Penicillin G Ampicillin Tetracycline
		Mycobacterium chelonei	Amikacin Clarithromycin
		Mucor *Aspergillus*	Immersions in malachite green solution Fluconazole
	Subcutaneous abscesses	*Pasteurella testudinis* *Escherichia coli* *Providencia*	Amikacin Enrofloxacin
		Bacteroides *Fusobacterium*	Metronidazole Penicillin G
Oral cavity	Stomatitis	*Aeromonas hydrophila* *Pseudomonas aeruginosa* *Vibrio*	Amikacin Ceftazidime Ticarcillin Enrofloxacin Marbofloxacin
Respiratory tract	Pneumonia	*Pseudomonas aeruginosa* *Morganella morganii* *Serratia marcescens* *Acinetobacter calcoaceticus*	Amikacin Ceftazidime Ticarcillin Enrofloxacin Marbofloxacin
		Bacteroides *Fusobacterium*	Metronidazole
		Aspergillus *Geotrichum candidum* *Beauvaria* *Penicillium lilacinum* *Paecilomyces fumoso-roseus*	Ketoconazole Itraconazole Fluconazole
	Rhinitis	*Pasteurella testudinis* *Mycoplasma agassizii*	Enrofloxacin Clarithromycin
Gastrointestinal tract	Enteritis	*Salmonella* *Aeromonas hydrophila* *Flavobacterium meningosepticum*	Enrofloxacin
	Liver Abscesses	*Salmonella* *Bacteroides* *Clostridium* *Fusobacterium*	Metronidazole
	Septicemia	*Salmonella* *Aeromonas hydrophila* *Pseudomonas aeruginosa*	Amikacin Ticarcilllin Enrofloxacin Marbofloxacin

(*continued*)

Table 37.1. Antimicrobial drug selection in chelonian infections. (*continued*)

Site or Type	Diagnosis	Common Infecting Organisms	Suggested Drugs
Skeletal	Osteomyelitis/ arthritis	*Pseudomonas* *Klebsiella*	Amikacin Ceftazidime
		Mycobacterium cheloniei	Amikacin Clarithromycin
		Nocardia	Azithromycin
		Various fungi	Fluconazole Sulfonamides Doxycycline
Eye and adnexa	Conjunctivitis	*Mycoplasma agassizii*	Enrofloxacin Clarithromycin
Ear	Otitis interna	*Pseudomonas* *Escherichia coli* *Proteus* *Pasteurella testudinis*	Amikacin Ceftazadime Ticarcillin Enrofloxacin
		Bacteroides *Fusobacterium*	Metronidazole

immunocompromised condition in the patient. Furthermore, Vaughn et al. (1974) demonstrated that some lizard experimentally infected with Gram-negative voluntarily selected higher ambient temperatures. This behavior was interpreted as an induced fever, and is thought to help the lizards fight bacterial infections. Given that reptile body temperature affects immune system function, it is imperative to maintain the ill reptile under optimum environmental conditions as an important part of the therapeutic plan.

Our understanding of reptile bacterial and mycotic infections has advanced to recognize that reptiles become more susceptible to bacterial diseases when exposed to other pathogens. For example, primary viral infections, such as ophidian paramyxovirus pneumonia and herpes virus stomatitis of tortoises, are associated with severe secondary bacterial infections (Jacobson, 1992b; Origgi et al., 2004). The clinician should consider exposure to contaminated environments and a lack of proper quarantine program as important risk factors for infection with multiple pathogen. Thus the clinician's recognition of both husbandry mistakes and the proper diagnosis of co-infections are important aspects of antimicrobial selection for reptiles.

Anatomical and Physiological Considerations

The clinician needs to be aware that reptile anatomy and physiology differs significantly from domestic mammals. Reptiles have several unique features that can potentially influence the pharmacokinetics of antimicrobials and the subsequent response to treatment.

The carapace and plastron forms the characteristic shell of chelonians. This unique anatomic feature is composed of an outer keratinized epidermis overlying a base of dermal cartilage and bone. The dermal bone is highly vascularized and considered a metabolically active tissue (Jacobson, 2007). The relative metabolic activity and blood perfusion of the chelonian shell has led to the recommendation that antimicrobials should be dosed based on their entire body weight and not adjusted to subtract the weight of the turtle shell.

An anatomical feature of all snakes with eyes and some lizards is the transparent palpebral spectacle (Millichamp et al., 1983). This spectacle embryologically represents a fusion of the upper and lower eyelids that permanently covers the cornea leaving a potential subspectacular space. Infections of this subspectacular

Table 37.2. Antimicrobial drug selection in crocodilian infections.

Site or Type	Diagnosis	Common Infecting Organisms	Suggested Drugs
Oral cavity	Stomatitis	*Aeromonas hydrophila*	Tetracycline Amikacin Ceftazidime
		Candida	Nystatin
Skin	Epidermitis/ Dermatitis	*Dermatophilus*	Procaine penicillin G Tetracycline
		Morganella morganii *Pseudomonas aeruginosa* *Serratia* *Klebsiella*	Amikacin Ceftazidime
		Aspergillus *Trichophyton* *Trichosporon*	Ketoconazole Itraconazole Fluconazole
Respiratory tract	Pneumonia	*Aeromonas hydrophila* *Citrobacter freundii* *Morganella morganii* *Providencia rettgeri* *Escherichia coli* *Salmonella*	Amikacin Ceftazidime Enrofloxacin
		Beauvaria *Fusarium* *Mucor* *Paecilomyces*	Ketoconazole Itraconazole Fluconazole
		Mycoplasma alligatoris	Enrofloxacin Oxytetracyline
Yolk infection	Omphalitis	*Aeromonas hydrophila*	Tetracycline Amikacin
Liver	Hepatitis	*Escherichia coli* *Salmonella* *Aeromonas hydrophila*	Amikacin Ceftazidime Enrofloxacin
		Aeromonas hydrophila	Oxytetracycline Enrofloxacin
Eye	Uveitis	*Aeromonas hydrophila*	Amikacin Ceftazidime Tetracycline
Cardiovascular	Septicemia	*Salmonella* *Aeromonas hydrophila*	Amikacin Ceftazidime Enrofloxacin
Serosa/joints	Polyserositis/arthritis	*Mycoplasma alligatoris*	Enrofloxacin Oxytetracyline

Table 37.3. Antimicrobial drug selection for infections in snakes and lizards.

Site or Type	Diagnosis	Common Infecting Organisms	Suggested Drugs
Oral cavity	Stomatitis	*Pseudomonas aeruginosa* *Aeromonas hydrophila*	Amikacin Ceftazidime Piperacillin Enrofloxacin Marbofloxacin
Skin and subcutis	Abscesses	*Proteus* *Providencia* *Pseudomonas* *Salmonella* *Serratia* *Clostridium* *Pseudomonas aeruginosa*	Amikacin Ceftazidime Piperacillin Azithromycin Enrofloxacin
		Fusobacterium *Bacteriodes*	Metronidazole
	Bacterial dermatitis	*Citrobacter* *Klebsiella* *Pseudomonas* *Neisseria*	Amikacin Ceftazidime Enrofloxacin
	Mycotic dermatitis	*Geotrichum* *Fusarium* *Chrysosporium*	Ketoconazole Itraconazole Fluconazole
Respiratory tract	Pneumonia	*Pseudomonas aeruginosa* *Pseudomonas maltophilia* *Providencia rettgeri* *Aeromonas hydrophila* *Morganella morganii*	Amikacin Ceftazidime Ceftiofur Piperacillin Enrofloxacin Marbofloxacin Azithromycin
Gastrointestinal tract	Enteritis	*Pseudomonas aeruginosa* *Aeromonas hydrophila* *Escherichia coli* *Salmonella*	Trimethoprim/sulfadiazine Ciprofloxacin Metronidazole
	Hepatitis	*Pseudomonas aeruginosa* *Morganella morganii* *Providencia rettgeri* *Aeromonas hydrophila* *Escherichia coli* *Salmonella*	Amikacin Ceftazidime Cefoperazone Enrofloxacin
		Clostridium	Metronidazole
Skeletal	Osteomyelitis	*Salmonella* *Escherichia coli* *Pseudomonas aeruginosa*	Amikacin Ceftazidime Piperacillin Enrofloxacin Marbofloxacin
Eye	Subspectacle infections	*Pseudomonas aeruginosa* *Providencia rettgeri* *Proteus*	Ophthalmic gentamicin
	Uveitis	*Pseudomonas aeruginosa* *Serratia* *Klebsiella*	Amikacin Ceftazadime Piperacillin
	Conjunctivitis	*Pseudomonas aeruginosa*	Amikacin Enrofloxacin

space have been reported and are difficult to treat with topically applied antimicrobial agents that do not appear to penetrate this barrier (Millichamp et al., 1983). In treating reptiles with subspectacular infections, a wedge is carefully excised from the lower half of the spectacle and the appropriate antimicrobial drug applied directly through the wedge shape hole onto the surface of the cornea.

Most species of reptiles have a renal portal system that can shunt blood from the caudal half of the body through the kidneys before reaching the systemic circulation. This blood flow pattern can potentially alter the pharmacokinetics of drugs and is the basis for recommendations that intramuscular and subcutaneous injections be given in the cranial half of the reptile body. However, few studies have tested this hypothesis. Holz (1997a) reported that in red-eared sliders (*Trachemys scripta elegans*) the blood from the caudal region of the body did not necessarily flow through the kidney via the renal portal system. Instead, the blood draining the caudal portion of the body perfused both the liver and the kidneys, indicating that the renal portal shunt was only partially functional. In a related study, Holz (1997b) also found that red-eared sliders receiving gentamicin either in a forelimb or hind limb had no significant differences in the pharmacokinetic parameters, indicating a minimal pharmacokinetic effect from the renal portal system. In contrast, the same study noted that red-eared sliders receiving carbenicillin in the hind limb had significantly lower blood concentrations for the first 12 hours post-injection than those that received the same dose in a forelimb. Despite this finding for carbenicillin, the authors concluded that this difference was not clinically important and questioned the necessity of forelimb injections (Holz et al., 1997b). Because, the renal portal system varies in development, anatomy, and function between various groups of reptiles and the pharmacokinetic evidence is conflicting, many clinicians still recommend injecting potentially nephrotoxic drugs and drugs eliminated primarily through the renal system in the cranial half of the body.

In contrast to mammalian pus, reptiles infected with bacterial and mycotic pathogens tend to develop solid exudates within discrete granulomatous lesions (Montali, 1988, Jacobson, 2007). These pathogens are located within the necrotic center of heterophilic granulomas, within histiocytes (macrophages) in histiocytic granulomas, or near the capsule of the chronic granulomas. Granulomas can limit the penetration of many antimicrobial agents into the sites of infection. When possible, surgical removal of the granulomatous masses prior to antimicrobial therapy can improve the chances of a positive therapeutic outcome.

Physiological and husbandry factors can also influence drug pharmacokinetics and therefore drug selection in reptiles. The ambient temperature of the reptile enclosure directly affects the pharmacokinetics of antimicrobials. Mader (1985) studied gopher snakes (*Pituophis melanoleucus catenifer*) given amikacin and housed at ambient temperatures of either 25°C or 37°C. When housed at 37°C, the apparent volume of distribution was larger and body clearance of amikacin was faster. In another study of gopher tortoises (*Gopherus polyphemus*), the mean residence time of amikacin was significantly shorter in tortoises acclimated to 30°C than those kept at 20°C, and clearance at 30°C was approximately twice that in the tortoises kept at 20°C (Caligiuri et al., 1990). In contrast, Johnson et al. (1997) found no significant pharmacokinetic differences among the snakes given amikacin and housed at 25°C and 37°C. No explanation for this discrepancy was offered, suggesting that the effect of temperature on drug pharmacokinetics is either species-specific or requires further evaluation.

Behavioral and Safety Considerations

The size and temperament of a reptile can influence antimicrobial drug selection and the route of administration. Some reptiles are extremely timid and nervous, and may not be suitable for repeated handling and intramuscular injections. In such cases the antimicrobial must be administered orally, preferably in food if the animal is still eating. Most species of reptiles weigh less than 100 g and many lizards are under 30 g as adults. The clinician may be limited to those antimicrobials that can easily be diluted to a concentration that can be precisely and safely injected. At the other end of the spectrum, some reptiles are quite large in size and dangerous to approach. In such cases the clinician may have to choose a drug that can be administered in a relatively small volume via remote injection dart or orally in food. Venomous snakes present a similar treatment challenge, since they are dangerous to handle and manipulate for

administration of drugs. For these dangerous species drugs that can be administered every few days are preferred over drugs that must be administered each day.

Routes of Antimicrobial Administration

The authors generally reserve the use of oral antimicrobials to those cases where the primary infection is in the gastrointestinal tract, the species is not tolerant of injections, the selected antimicrobial is only available in an oral formulation, or when safety considerations make injections dangerous. An additional indication for oral medication is when large numbers of reptiles are infected and must be treated simultaneously. In these situations the individual administration of drugs is not practical and the usage of medicated food may be warranted.

Several problems exist with oral medication of reptiles. First, very few pharmacokinetic studies have been performed on drugs administered orally to reptiles. Thus, for the vast majority of antimicrobials the dose selected will not be based on existing literature. Secondly, the gastrointestinal transit time varies greatly among the various reptile species. Transit time is usually slowest in the large herbivorous reptiles. For example, the transit time in large tortoises may be as much as 21 days. Even in some carnivorous reptiles, the transit time may be quite prolonged. Carnivorous reptiles, such as pythons, are adapted to infrequent meals and increase their gastric and intestinal mucosa in response to feeding (Secor, 2008). This massive change in gastrointestinal metabolism is likely to influence antimicrobial absorption and treatment frequency. Thus, in reptiles it may be difficult to achieve optimum and consistent therapeutic concentrations of antimicrobials in blood following oral administration. Martelli (2009) published a pharmacokinetic study of enrofloxacin in estuarine crocodiles (*Crocodylus porosus*) where delayed absorption and subtherapeutic drug concentrations were measured with the oral route. In contrast, repeated twice-weekly oral doses of clarithromycin in desert tortoises (*Gopherus agassizii*) attained target drug concentrations (Wimsatt et al., 2008). Clearly, oral absorption in reptiles is species- and drug-specific and requires further investigation.

While clinicians can often administer oral antimicrobials in the food of reptiles actively feeding, orally medicating reptiles not feeding is often difficult. In giant tortoises, extracting the head beyond the shell margins and then forcing the mouth open is usually impossible. Furthermore, these overzealous efforts to force the mouth open can injure the keratinized epidermal hard parts over the mandibles and dentary bones. In general, giant tortoises must be anesthetized and a pharyngostomy tube inserted for oral medication. Pharyngostomy tubes are easy to insert and routinely used in tortoises and other chelonians (Norton et al., 1989).

As a generalization, non-venomous snakes are the easiest group of reptiles to medicate orally. The mouth of most snakes is simple to open and the glottis is easy to see and avoid. In these snakes a lubricated French catheter or nasogastric tube is passed down the esophagus with minimal resistance. Since the cranial esophagus is extremely thin in most snake species, the end of the catheter should be round and smooth. The use of excessively rigid catheters should be avoided as they may penetrate the esophageal mucosa. While the stomach of most snakes is located from one-third to half the distance from the head to the cloaca, it is not necessary to pass a catheter this far. In most situations, passing the catheter halfway between the stomach and oral cavity is satisfactory.

Most of the injectable antimicrobials commonly used in reptiles are injected intramuscularly, subcutaneously and occasionally intraceolomically. The problem with intravenous administration in reptiles is that peripheral vessels are difficult to catheterize (Jacobson et al., 1992a). While blood can be collected from a number of vascular sites in different species of reptiles, most of this sampling is "blind" and is not suitable for repetitive intravenous infusions (Olson et al., 1975; Samour et al., 1984).

The intramuscular and subcutaneous injections are practical and provide the most predicable drug absorption. Snakes and lizards are the easiest reptiles to inject intramuscularly because of the large epaxial dorsal muscles of the body associated with the ribs and vertebrae. In lizards, the forelimb muscle masses are usually small limiting injection volumes. The best site for intramuscular injections in chelonians is the pectoralis musculature located, medial and caudal to the base of the forelimbs just within the cranial margins of the shell.

Despite the ease of intramuscular and subcutaneous drug administration, the authors tend to avoid placing

Table 37.4. Conventional dosage regimens for antimicrobial drugs in reptiles.

Drugs	Species	Route of Administration	Dose	Dose Interval	References
Amikacin	American Alligator	IM	2.25 mg/kg	96 h	Jacobson, 1988
	Gopher Tortoise	IM	5 mg/kg	48 h	Caligiuri, 1990
	Snakes	IM	5 mg/kg; 2.5 mg/kg	1st loading dose; thereafter 72 h	Mader, 1985
	Ball python	IM	3.5 mg/kg	No given	Johnson, 1997
Azithromycin	Ball python	PO	10 mg/kg	2 to 7 days	Coke, 2003
Carbenicillin	Snakes	IM	400 mg/kg	24 h	Lawrence, 1984a
	Tortoises	IM	400 mg/kg	48 h	Lawrence, 1986
Ceftazidime	Snakes	IM	20 mg/kg	72 h	Lawrence, 1984b
	Loggerhead Sea Turtle	IM; IV	20 mg/kg	72 h	Stamper, 1999
Ceftiofur	Snakes	IM	15 mg/kg	120 h	Adkesson, 2011
Chloramphenicol	Snakes	SQ	50 mg/kg	12–72 h depending on species	Clark, 1985
Clarithromycin	Desert Tortoise	Oral	15 mg/kg	48–72 h	Wimsatt, 1999
		Oral gavage	15 mg/kg	84 h	Wimsatt, 2008
Enrofloxacin	Gopher Tortoise	IM	5 mg/kg	24–48 h	Prezant, 1994
	Star Tortoise	IM	5 mg/kg	12–24 h	Raphael, 1994
	Loggerhead Sea Turtle	PO	20 mg/kg	Not given	Jacobson, 2005
	Red-eared slider	IM	5 mg/kg	Not given	James, 2003
		PO	10 mg/kg	Not given	
	American Alligator	IV	5 mg/kg	36 h	Helmick, 2004
	Estuarine Crocodile	PO, IM, IV	5 mg/kg	Not given	Martelli, 2009
	Green Iguana	IM	5 mg/kg	24 h	Maxwell, 2007
	Burmese Python	IM	10 mg/kg	48 h	Young, 1997
Fluconazole	Loggerhead Sea Turtle	SQ	21 mg/kg; 10 mg/kg	1st dose; thereafter 5 days	Mallo, 2002
Itraconazole	Kemp's Ridley Sea Turtle	PO	15 mg/kg	72 h	Manire, 2003
			5 mg/kg	24 h	
	Spiny Lizard	PO	23.5 mg/kg	Daily	Gamble, 1997
Ketoconazole	Tortoise	PO	15–30 mg/kg	24 h	Page, 1991
Marbofloxacin	Loggerhead Sea Turtle	IM, IV	2 mg/kg	24 h	Lai, 2009
		PO		Not given	Martin, 2009
	Ball Python		10 mg/kg	48 h	Coke, 2006
Metronidazole	Green Iguana	PO	20 mg/kg	48 h	Kolmstetter, 1998
	Yellow Rat Snake	PO	20 mg/kg	48 h	Kolmstetter, 2001
	Red Rat Snake	PO	50 mg/kg	48 h	Bodri, 2006
	Red-eared Slider Turtle	IC	20 mg/kg	48 h	Innis, 2007
Oxytetracycline	American Alligator	IV	10 mg/kg	5 days	Helmick, 2004
	Loggerhead Sea Turtle	IM	41 mg/kg; 21 mg/kg	1st dose; thereafter 72 h	Harms, 2004
Piperacillin	Snakes	IM	100 mg/kg	24 h	Hilf, 1991
Ticarcillin	Loggerhead Sea Turtle	IM	50 mg/kg	24 h	Manire, 2005
			100 mg/kg	48 h	

large volumes of irritating drugs such as enrofloxacin into reptile muscles. The authors have had several snakes develop necrotizing skin lesions following injection of more than 1 ml of enrofloxacin at a single site. The authors have also seen severe necrosis of pectoralis musculature in sea turtles injected with enrofloxacin. In one case, a gopher tortoise that received an intramuscular injection of enrofloxacin in a forelimb eventually had to

have the necrotic limb surgically amputated. Because of this, the authors do not recommend enrofloxacin be administered by injection to reptiles.

Injectable drugs with a prolonged elimination are potentially useful in reptiles that are difficult or dangerous to handle. Adkesson (2011) reported that a long-acting formulation of ceftiofur maintained adequate plasma concentrations for 5 days in ball pythons (*Python regius*). However, in another study of subcutaneous cefovecin, a long-acting antibiotic used in dogs and cats, at a 14-day interval had an unexpectedly short half-life of only 3.9 hours in green iguanas (Thuesen et al., 2009).

The use of intraceolomic injections to administer antimicrobials is infrequently used and rarely described in pharmacokinetic studies. Innis et al. (2007) studied the pharmacokinetics of intraceolomic metronidazole in red-eared sliders (*Trachemys scripta elegans*). The potential injury from inappropriately placed or an irritating drug in the ceolomic space requires further investigation.

Antimicrobial Drug Selection

Ideally the clinician should select drugs for a reptile based on published pharmacokinetic studies conducted on that particular species, or at least a closely related species. Pharmacokinetic studies provide the clinician with a recommended drug dose and interval of drug administration that should provide therapeutic blood concentrations necessary to treat the target pathogen. Table 37.4 is a partial listing of published antimicrobial pharmacokinetic studies in various reptile species.

Of the 7,500 species of reptiles, pharmacokinetic studies have been reported for a few drugs in a small number of species commonly kept in captivity. As in most scientific literature there is a publication bias toward reporting pharmacokinetic studies that lead to dosage recommendations, versus those that fail to produce useful recommendations (Stamper et al., 2003; Thuesen et al., 2009). Studies focused on the metabolism, tissue concentrations, and potential toxicity of antimicrobials in reptiles are rare in the literature (Hunter et al., 2003). This lack of pharmacokinetic research is not surprising, in view of the relatively few researchers interested in pharmacokinetics of antimicrobials in reptiles and the lack of research support available for such studies.

Despite the lack of pharmacokinetic studies, the clinician must still select antimicrobials based on the best available evidence. Several reptile medicine textbooks, formularies, and review articles provide extensive lists of antimicrobials recommended for reptiles (Funk and Diethelm, 2006). These sources often include recommendations that have not been well evaluated for safety or efficacy, yet are commonly used empirically with apparent clinical success. An example is the trimethoprim combinations that are commonly listed in formularies for a wide variety of reptiles, but apparently lack pharmacokinetic studies in reptiles (Funk and Diethelm, 2006).

Allometric Scaling to Estimate Drug Dosages

Clinicians faced with the lack of pharmacokinetic studies and even the paucity of empirical dosage recommendations are often forced to extrapolate drug dosages from domestic mammals. In practice, clinicians use three methods to estimate proper therapeutic drug dosages (Hunter and Isaza, 2008).

The first method is to use an established drug dose derived from pharmacokinetic studies in other species. By this method, a 20 mg/kg dose of amoxicillin in dogs is applied across all reptile species regardless of size. Using a set dose results in a linear increase in the amount of drug administered as body weight increases. Although common, this method tends to overdose larger animals and underdose smaller animals.

The second method is similar to the first except that it takes the established dosage in a specific species and makes an additional assumption that links the dosage to the metabolic rates of both species. Using this method, the established drug dosage from one species is adjusted based on the ratio of the calculated metabolic rate of the patient over the calculated metabolic rate of the target species,

$$\text{Patient Dose} = \text{Set dose}_{(\text{species X})} * \text{Pmet}_{(\text{patient})} / \text{Pmet}_{(\text{species X})}$$

This method, termed metabolic scaling, is popular in zoological medicine and described for use in reptiles (Pokras et al., 1992; Mayer et al., 2006). Unfortunately,

this method of allometric scaling is controversial because formulas to estimate reptile metabolic rates are inconsistent between reptile species. For many mammals the following allometric equation is considered the best estimate of the basal metabolic rate,

$$\text{Pmet} = 70(\text{Kg})^{0.75}$$

where Pmet is the minimum energy costs (Kleiber, 1961). In contrast, a similar allometric equation

$$\text{Pmet} = 10(\text{Kg})^{0.75}$$

is suggested for use in all reptile species (Pokras et al., 1992; Mayer et al., 2006). However, when Jacobson (1996) reviewed the subject, this single reptile equation was not considered appropriate for all reptiles, since the constant varied from 1 to 5 for snakes and 6 to 10 for lizards, with no values for chelonians or crocodilians available. Additionally, he noted significant data variability in those groups where scientific studies have been performed. For instance, Bartholomew and Tucker (1964) measured the metabolic rate in lizards ranging in size from 0.002 to 4.4 kg and calculated the allometric equation to be $\text{Pmet} = 6.84(\text{Kg})^{0.62}$. This is different from findings by Bennet and Dawson (1976) for 24 species of lizards, ranging from 0.01 to 7 kg, for which the equation

$$\text{Pmet} = 7.81(\text{Kg})^{0.83}$$

was determined. Further, when one looks at studies with snakes, still different equations can be calculated (Galvao et al., 1965). In determining resting metabolic rates of 34 species of boas and pythons, the mass exponents of different species showed considerable variation (Chappell and Ellis, 1987). The problem with metabolic scaling is that reptiles represent a very heterogeneous group of vertebrates, and because of this, no single equation relating metabolic rate to body mass can be developed for calculating antimicrobial dosages. Differences in body temperature, season, reproductive status, nutrition, and overall physiology are just of few of the variables that may ultimately influence metabolic rates, making application of a single equation impossible. While at first glance metabolic scaling may appear better than extrapolation, using a single equation for all reptile species may not be valid.

In the third method, the allometric scaling of measured pharmacokinetic parameters is used for extrapolation of drug doses between species. This method is commonly used in the pharmaceutical industry to extrapolate pharmacokinetic parameters between laboratory mammal species to humans (Hunter and Isaza, 2008). Using known pharmacokinetic parameters as the basis for extrapolation has theoretical advantages over calculated metabolic rates. However, when Maxwell and Jacobson (2007) compared the pharmacokinetics of enrofloxacin over a wide range of green iguana sizes, they found that clearance and other pharmacokinetic parameters did not scale adequately allometrically. Thus this method of allometric scaling using pharmacokinetic parameters also needs further investigation in reptiles.

Conclusion

The information and discussion contained in this chapter provide initial suggestions for differential diagnosis that should always be followed with attempts to obtain a definitive diagnosis. This diagnostic process includes a culture and antimicrobial sensitivities for the isolated pathogens. Once an antimicrobial therapeutic plan is selected, the clinician needs to consider the various anatomical and practical aspects of antimicrobial usage in reptiles. Finally, the clinician needs to review the available pharmacokinetic studies in reptiles and carefully consider the most appropriate antimicrobial drug selection given the available evidence.

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Antimicrobial Drug Use in Zoological Animals

Ellen Wiedner and Robert P. Hunter

Although the scope and breadth of zoo and wildlife medicine has grown enormously over the past 2 decades, a large number of challenges remain. At the most basic level, when working with non-domestic species, determining whether an animal is actually ill, let alone affected with a potentially treatable infectious organism, can be remarkably difficult. Wild animals conceal disease extremely well, and sometimes only through necropsy will a clinician become aware that the animal had been harboring a long-standing infection.

If a diagnosis is made, determining what antimicrobials are appropriate becomes the next challenge. Pharmacological studies relevant to zoo and wildlife species continue to be scant. This is due, in part, to the technical difficulty of performing drug studies in wild animals, as well as to the perceived risks to the subject species, many of which are rare or endangered. Unfortunately, without this data, the clinician is required to extrapolate drugs and doses from studies performed in domestic animals or even in humans, which creates a new set of problems.

Finally, the technical aspects of providing a course of antimicrobial therapy to wild animals, most of whom are uncooperative and many of whom can be dangerous, even if sick, provide a final level of complexity. All of these issues are discussed below.

Antimicrobial Breakpoint Interpretation in Zoological Medicine

Global events and perceptions regarding the use of antimicrobial agents in animals have placed even more importance on the essential role of antimicrobial susceptibility testing of bacterial isolates from animals (chapter 2). However, little information is available on microorganism/antimicrobial/host interactions with zoo species.

Results of *in vitro* susceptibility tests are typically presented to the veterinarian by designating the pathogen as susceptible, intermediate, or resistant. This designation is based on breakpoints established by the Clinical and Laboratory Standards Institute (CLSI). A breakpoint is the concentration above and below which specific bacterial isolates are categorized as susceptible, intermediate, or resistant to a given antimicrobial agent. Clinical breakpoints take an antimicrobial's minimum inhibitory concentration (MIC) into consideration but are based also on additional interpretive criteria, such as concentrations of the drug that can be achieved in a given animal species (pharmacokinetics), the best presentation of the drug to the bacteria in the host (pharmacodynamics), and, when available, results of clinical trials in the target species that is the ultimate standard of efficacy.

Clinical breakpoints are species-specific and relevant only for the specific bacteria, specific drug, and specific

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body system infected only. An important limitation in interpreting the results of *in vitro* susceptibility data is that there are no breakpoints established specifically for zoological species. For all antimicrobials, the results of antimicrobial susceptibility testing from samples obtained from zoo animals are reported based on breakpoints that have been established for people or other domestic animal species. A result indicating susceptibility is unquestionably preferable to one indicating resistance. However, there are no data correlating the results to clinical efficacy and there is no guarantee that the breakpoint is valid for a given pathogen or site of infection in a given animal species.

Given the limited number of antimicrobial agents approved for use in zoological animal species, "extra-label" use of antimicrobial agents is typically practiced. The U.S. Congress, in the Animal Medicinal Drug Use Clarification Act (AMDUCA), has defined extra-label use as the "actual use or intended use of a drug in an animal in a manner that is not in accordance with the approved labeling." This includes, but is not limited to: use in species or for indications (disease or conditions) not listed in the labeling; use at a dosage level higher than those stated in the label; and use of routes of administration other than those stated in the labeling. This type of use has regulatory acceptance in many countries (chapter 26).

Intra- and Interspecies Dose Extrapolation

Species differences in drug absorption, distribution, metabolism, and excretion (ADME) for numerous pharmaceutical agents have been well documented for domestic species; however, there is limited information concerning the ADME of drugs in non-domestic species (Hunter, 2009). Lack of approved pharmaceutical agents and/or pharmacokinetic data in the literature for zoo species is a major issue for veterinarians attempting to treat these animals. Zoological medicine practitioners take approved agents (veterinary or human) and extrapolate their use to non-approved species. The range of animals a zoo veterinarian cares for varies from very small invertebrates (honeybees) to megavertebrates such as elephants and whales. The decision on dose, duration, and treatment interval is often made with limited species-specific (pharmacokinetic and/or efficacy) information. Because of the monetary value of these animals or their status as endangered species, the method of "trial and error" for antimicrobial dosage selection is inappropriate.

In zoological medicine, various methods have been used in an attempt to extrapolate or predict safe and effective dosage regimens (Hunter and Isaza, 2008). The simplest and typical method of extrapolating a dosage to a non-domestic species is to use a mg/kg dose established for another domestic species or humans. However, this calculation results in a linear increase in the amount of drug administered as body weight increases. Although common, this method tends to overdose large animals and underdose small animals. A second method is similar, except that it takes the approved dose in a specific species and makes an additional assumption that links the dosage to a physiologic function or anatomic feature. Examples are the use of basal metabolic rate or body-surface area as the basis for dosage extrapolation. Allometric scaling of pharmacokinetic parameters is the final method of dosage extrapolation between species. This is commonly used in the pharmaceutical industry to establish the first dosage in human drug investigations. Adaptation of this method for zoological medicine is believed to enhance the ability to estimate therapeutic dosages for non-domestic species. However, relatively recent data (Hunter et al., 2008; Mahmood et al., 2006; Martinez et al., 2006) question the practical use of this approach.

Allometric scaling of pharmaceuticals to predict pharmacokinetics in zoo/exotic animals has considerable benefit for zoological veterinarians. This tool, when used appropriately, can provide an estimate for designing dosage regimens. The example of differences in ketoprofen inversion across species emphasizes the need to understand and be aware of the assumptions when designing treatment regimens based on allometric scaling data. Just as mammals can range from a few grams to thousands of kilograms, reptiles and birds can also vary in body weight across a wide range. It has been suggested that it is impossible to derive a single equation correlating body mass to metabolic rate for all 6,000 species of reptiles (Funk, 2000). Without knowledge as to the extent and route of elimination of an administered pharmaceutical agent, extrapolation of dosage regimens from one class to another is difficult, if not impossible, with any certainty.

Before extrapolation of any drug dose, the veterinarian should appreciate not only the mathematical assumptions but also the limitations that are associated with allometry. Careful consideration of the available literature to understand the route of elimination and the extent of metabolism of therapeutic agents will greatly assist in determining allometric relationships of pharmacokinetic parameters. There is a continuing need to consider and apply methods for reducing the size and risk of extrapolation error, as this can affect both target animal safety and therapeutic response. Data from at least one large animal (non-human and a body weight > 70 kg) should be included to reduce potential error (Mahmood et al., 2006).

A Practical Example of Allometry and Breakpoints

An example of how the above information can be interpreted and potentially misused is the case of *Mycobacterium tuberculosis* susceptibility testing and the treatment of this bacterial disease in elephants (*Loxodonta africana* and *Elephas maximus*). Unlike cattle and other livestock, which are more apt to be infected with *M. bovis* and are euthanized if positive, in the United States, elephants are recognized for their rarity and value and are treated rather than culled. Mandatory testing and treatment of elephants with TB is overseen by the U.S. Department of Agriculture (USDA), and guidelines for drug administration in pachyderms have been derived from those established for humans (USDA, 2008). Susceptibility testing for this pathogen is described in detail, for human isolates, in the CLSI M24-A2 document. The results of *in vitro* susceptibility testing of these agents appear to correlate well with the clinical effectiveness of these agents in human patients. The interpretive criteria, or breakpoints, are provided in Table 38.1.

In elephant, the pharmacodynamics and pharmacokinetics of antituberculous drugs differ considerably compared to people. In addition, the metabolic state of *Mycobacterium tuberculosis* significantly affects its susceptibility to antimicrobials. Optimization of dosage of antituberculous drugs is necessary to achieve maximum drug exposure at the site of infection in order to maximize reduction in *M. tuberculosis* viable organisms and to minimize the emergence and selection of resistance (de Steenwinkel et al., 2010).

Table 38.1. Breakpoints for select antituberculous drugs used in elephants.

	Breakpoint Concentration (μg/mL)	
Agent	7H10 agar	7H11 agar
Isoniazid	0.2	0.2
Rifampin	1.0	1.0
Ethambutol	5.0	7.5
	10	NR
Pyrazinamide	NR	NR
Levofloxacin	1.0	ND
Moxifloxacin	0.5	0.5
Ofloxacin	2.0	2.0
Streptomycin	2.0	2.0
	10	10

NR = not recommended; ND = not determined. Where multiple values are provided, the second is when resistance has occurred and the drugs are used as "second-line therapies" (modified from M24-A2; CLSI, 2011).

There are published reports on the "population" pharmacokinetics of several antituberculous drugs in African and/or Asian elephants that were used to develop the multidrug treatment protocols for elephants published in the USDA elephant TB guidelines and were modeled after the disease in people (Peloquin et al., 2006). The issues with these types of extrapolation have been previously discussed (Hunter and Isaza, 2008). Using the human breakpoints for isoniazid established by the CLSI and the plasma concentrations reported by Maslow et al. (2005) one could conclude that the likelihood for efficacy is high with all reported concentrations > 0.2 μg/mL for the doses and routes of administration evaluated, but many concentrations were greater than 5 times, which seems excessive and could be contributing to the adverse events reported by some clinicians. Maslow et al. (2005) suggest that area under the curve (AUC) may be the driving pharmacodynamic parameter, which is not surprising given the slow growth of the target pathogen, but the target PK/PD relationship is currently unknown in elephants, and is very likely to be different than that reported for humans. This idea is further supported when the fluoroquinolones are evaluated. While in human medicine an AUC/MIC ratio of ≥ 125 for fluoroquinolones has been shown to eradicate a particular bacterial disease, this ratio cannot be directly extrapolated across species, indication, or pathogen, nor has it been determined for

antituberculous drugs. The effective AUC:MIC ratio has been reported to be different between species (Aliabadi et al., 2003). Opinions also differ within the human literature, where some report that a ratio > 25 is best, while others state that the ratio must be greater than 350 (Barger et al., 2003). This is complicated by the fact that for the fluoroquinolone ciprofloxacin, 100% of successfully treated patients had an AUC:MIC ratio > 3.6 (Barger et al., 2003). It should be remembered that the *in vivo* antimicrobial effect is the result of dynamic exposure of the pathogen to the antimicrobial and the host immune system. The comments and issues raised here also apply to rifampin (Peloquin et al., 2006) and ethambutol (Maslow et al., 2005).

Unfortunately, numerous serious adverse effects have occurred in the majority of elephants undergoing treatment. In many cases, these were severe enough that treatment needed to be discontinued, at least temporarily. Reported adverse effects include anorexia, depression, diarrhea, kidney and liver insults, blepharospasm, and death. The high incidence of severe adverse effects suggests that the doses of drugs required to achieve serum levels comparable to those targeted in people, may, in fact, be toxic to elephants (Wiedner and Schmitt, 2007).

Techniques of Administration

Administration of medications to zoo and wildlife species can be made considerably easier by training the animals to accept them. Such training has increasingly become part of the general animal care routine at many zoological institutions. A remarkable variety of species have been taught to tolerate injections, swallow tablets, and accept various other forms of drug administration. Such training requires a significant time commitment both for teaching the behaviors as well as for ongoing practice to maintain them, using placebos when the animal is healthy.

Oral Administration

Oral medication can be hidden in feed. Generally, this requires that the patient be physically separated from its social group for feeding. For some species, such as large carnivores, this is routine. For others, separation from conspecifics can cause stress. Typically, the medication is hidden in something the animal particularly enjoys such as a meatball for a carnivore or a watermelon for an elephant. Non-human primates are often more willing to take medication if it is mixed with sweet substances such as jams or juices. Compounding pharmacies that make flavored medications for children can be helpful in developing mixtures that are appealing to captive primates who require oral medication

It is unknown whether drug bioavailability is affected by its concealment in food. Another issue is that zoo animals often become very adept at identifying "doctored" food items, and will pick carefully around drugs hidden in grain balls, meat balls, and similar, leaving the medication untouched. Thus, it is important for the animals' caretakers to observe ingestion of the medication.

Pachyderms can be trained to accept oral medications using a bite block. Even using this device, however, elephants often learn to hide medications within their massive mouths for hours, only to spit them out hours later when they are unobserved (Isaza and Hunter, 2004).

Injectable Administration

Most injectable antimicrobials are given via the intramuscular route in zoo and wildlife species. Although under anesthesia, both the intravenous or subcutaneous routes are possible for a single dose, anesthetizing a sick animal repeatedly for the purpose of administering a course of antimicrobials is generally not desirable. In some situations, an intraosseous (IO) or intravenous (IV) catheter can be placed. Reptiles, birds and severely debilitated animals that will be housed in a hospital environment are best suited for this. In determining whether a particular patient is an appropriate candidate for an indwelling catheter, the clinician should assess the ease of maintaining patency and cleanliness of the catheter, and the likelihood of the animal's removing it. If these are concerns, a catheter is generally not suitable. In several groups of animals, anatomical and/or living situations make indwelling catheters inappropriate or extremely difficult. These include very small animals, such as songbirds, animals with extremely thick skins such as hippos and aquatic animals that cannot be dry-docked.

In some situations, intramuscular injections can be given via hand injection into animals trained to present body parts against the pen, chute, or cage bars (flank or thigh muscles for large carnivores; neck for hoofstock;

limbs, back, or flat palms of hands or feet for primates). A squeeze cage can be used for uncooperative animals, although ideally the animal should have received some training to enter the squeeze. In some species, manual restraint by experienced personnel is possible. Repeatedly restraining the animal, however, can be dangerous and stressful to both handler and animal, and is not recommended for long courses of antimicrobial treatment.

For untrained or uncooperative patients, intramuscular drugs can be administered using remote delivery techniques, that is, darting equipment. While a discussion of darting techniques is beyond the scope of this chapter, any veterinarian planning to work with wildlife and zoological species should have an understanding of darting equipment and techniques, and its risks, which include stress to the animal, accidental bone fractures or penetration of internal organs and equipment failure, that is, the dart fails to inject its contents either partially or entirely into the animal. The use of long-lasting depot formulations can decrease the frequency that the animal needs to be darted.

Other Routes of Administration

Great apes with air sacculitis have been successfully trained to tolerate routine nebulization with antibiotics (Gresswell and Goodman, 2011). Rectal administration of certain antimicrobials has been used successfully in elephants. Antibiotic impregnated beads, placed under anesthesia, have been used in the treatment of mandibular osteomyelitis in tammar wallabies (*Macropus eugenii;* Hartley and Sanderson, 2003). The use of an osmotic pump was tested for amikacin delivery in a corn snake (*Elaphe guttata;* Sykes et al., 2006). Although complications, such as migration of the pump, were noted with this technique, it eliminated repeated handling of an animal needing medication and its use warrants further investigation.

Treating Groups of Animals

Medicating herds or flocks of animals requires special consideration. If the animals are to be medicated in feed and water, a total dosage of medication for all animals needs to be calculated, but if the individual dose is based on the size of the smallest animal in the group for safety reasons, the largest animal will receive a subtherapeutic dose. In addition, the social behavior of the animals to be medicated must be noted. In hierarchical species such as wild equids and some ruminants, higher ranked animals will have increased access to any feed materials and will eat considerably more than those lower in rank. This, of course, means that these animals will also ingest larger amounts of medication than those lower in rank. Mass medication of zoo megavertebrates in feed and water is not done commonly, and there are scant reports of this technique.

Specific Examples of Antimicrobial Use in Zoo and Wildlife Species

Prophylactic antibiotic treatment against pneumonia in groups of free-ranging bighorn sheep (*Ovis canadensis;* Weiser et al., 2009) as well as reindeer (*Rangifer tarandus*; Pietsch et al., 1999) continues to be used in the management of these animals in North America. Following capture by various methods, the animals are physically examined, then hand-injected with oxytetracycline or florfenicol prior to release or translocation. The goal of these one-time antibiotic injections is to decrease the likelihood of stress-induced respiratory disease; however, it has been difficult to confirm the efficacy of this approach.

Tetracycline baits represent another use of antimicrobials in wildlife species. When ingested, tetracycline is incorporated into bones and teeth. Under ultraviolet light, the teeth fluoresce. The drug can also be detected in histological sections from tooth and bone in necropsy specimens. Tetracycline baits have been used for mark-capture population studies of American black bear (*Ursus americanus*: Peacock et al., 2011), polar bears (*Ursus arctos;* Taylor and Lee, 1994), and feral swine (Reidy et al., 2011), as well as for determining the use of supplemental feed in herds of white-tailed deer (*Odocoileus virginianus;* Bastoskewitz et al., 2003).

Tetracycline is also a component of oral rabies vaccines that are scattered as baits in areas inhabited by vector species such as raccoons (*Procyon lotor)* and skunks (*Mephitis mephitis*). To determine with what frequency the vaccines are being ingested by target species, the animals are captured a period of time after the vaccine baits are distributed. Under anesthesia, a tooth is removed and

analyzed for evidence of tetracycline deposition to provide an estimate of the performance of the baits and thus of vaccine efficacy (Fehlner-Gardiner et al., 2012).

An interesting area of antimicrobial research involves wildlife species that actually produce their own antimicrobial substances. The Nile hippopotamus (*Hippopotamus amphibious*) releases a red sweat from its skin that has been found to have antimicrobial properties. At low concentrations (lower than those actually occurring on the skin), one of the pigments inhibits growth of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (Saikawa et al., 2004). Antimicrobial peptides have also been found in platypus (*Ornithorhyncas anatinus*) and tammar wallaby. In the wallaby, some of these compounds are expressed in maternal milk and are hypothesized to protect the pouch-dwelling, immunologically naive and underdeveloped young. In the common seal (*Phoca vitulina*), saccharide residues are produced by apocrine skin glands and inhibit adherence of bacteria and fungi to the epidermis (Meyer et al., 2000; Meyer et al., 2003). In both common seals and northern fur seals (*Callorhinus ursinus*), lysozyme and beta-defensins are also produced by skin glands (Lynn and Bradley, 2007).

Environmental Issues and Non-Target Wildlife Species

A number of studies have documented antibiotic-resistant bacteria in wild and stranded marine mammals, raccoons, wild and captive non-human primates, seabirds, fish, rodents and wild hoofstock, and even in zoo animals. Many of these bacteria, *E. coli* in particular, demonstrate multidrug resistance. The important of these organisms as reservoirs of multidrug resistance and their ability to spread into human and domestic animal populations is unknown but under study. From the perspective of the zoo and wildlife clinician, such findings emphasize the importance of obtaining culture and sensitivity results from wild patients.

Recently, evidence of harmful antibiotic residues in wildlife has been found causing increased pathology in affected species. Studies conducted in Spain identified residues of antibiotics commonly used in livestock, specifically, enrofloxacin, ciprofloxacin, amoxicillin and oxytetracyline, in nestlings of three threatened species: Griffon vultures (*Gyps fulvus*), cinereous vultures (*Aegypius monachus*) and Egyptian vultures, *Neophron percnopterus*, avian scavengers that feed on carcasses. Affected nestlings showed liver and kidney damage as well as compromised immune systems that could be directly correlated with the antibiotic residues (Blanco et al., 2009). In another study, it was demonstrated that fluoroquinolones that could be tracked back to livestock operations were causing embryonic death in the eggs of griffon vultures and red kites (*Milvus milvus;* Lemus et al., 2009).

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Antimicrobial Drug Use in Aquaculture

Renate Reimschuessel, Ron A. Miller, and Charles M. Gieseker

As aquaculture has become a more prominent source of food fish, use of therapeutics, especially antimicrobials, to treat these animals has increased, especially as fish farming has become more intensive. Because of this, public health agencies have raised concerns worldwide about the impact of antimicrobial use in aquaculture on environmental bacteria and, potentially, on human pathogens (Serrano, 2005; Weir et al., 2012). Nevertheless, fish are vertebrates that should receive humane care, including treatment with antimicrobials when appropriate. Ideally, before an antimicrobial is appropriately prescribed as treatment the following factors should be considered: fish physiology; information regarding the pharmacokinetics of the antimicrobial in fish; the availability of an antimicrobial as an approved drug for use in fish (Food and Drug Administration [FDA], 2005; American Fisheries Society, 2011) and *in vitro* antimicrobial susceptibility testing to provide information on the likelihood of therapeutic success (Clinical and Laboratory Standards Institute [CLSI], 2006a, 2006b, 2010). In many cases, the practitioner must often interpret test results generated by a laboratory that has no standardized testing protocol as a reference, and then must prescribe a drug (either labeled or as an extra-label prescription) for which there is little or no literature detailing pharmacokinetic data in the fish species being treated.

Over the last decade, however, researchers and fish health practitioners worldwide have made considerable progress toward obtaining and making available information about therapeutic treatments in aquaculture. A number of textbooks that include formularies for doses and treatment regimens for fish have been published (Stoskopf, 1993; Noga, 1996; Carpenter, 2005). In addition, an extensive review of literature pertaining to fish pharmacokinetics (Phish-Pharm) has been published as a database, available free in a web-based journal and an on line resource (Reimschuessel et al., 2005, 2011). The information in these texts and in the database is, however, skewed toward the species and drugs that are most commonly used. What is still largely missing is information on the many exotic and ornamental fish species, as well as the "niche" food-fish species.

Treating fish with antimicrobials is somewhat more complex than treating terrestrial animals, but the basic principles for antimicrobial use are really the same (chapter 6). Five main aspects must be considered: (1) choice of an appropriate drug at an effective dose; (2) avoidance of toxicity in the animal; (3) the safety of humans administering the antimicrobial or consuming the fish; (4) avoidance of non-target species and environmental effects; and (5) legal restrictions.

Choosing the Appropriate Drug

The Pathogen

Picking an effective drug requires that the clinician make an accurate diagnosis (chapter 6). A definitive diagnosis requires isolation and identification of the

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causative organism, preferably from three to five infected fish (Hawke and Thune, 1992; Office International des Epizooties, 2003; American Fisheries Society, 2005). A list of some of the more frequently isolated aquatic bacterial pathogens from diseased fish is provided in Table 39.1. In aquaculture, especially in intensive rearing facilities, timely institution of treatment is often critical. Therefore, many practitioners rely on clinical signs and past experience when faced with a population of moribund fish. It is, however, prudent to take multiple samples for culture prior to administering the antibiotic empirically. This allows confirmation later that the appropriate drug was used or, if necessary, subsequent change of the treatment based on the organism isolated and its antimicrobial susceptibility profile. Since antimicrobial susceptibility changes with antibiotic use, it is important for the veterinarian to monitor the isolates from their aquaculture clients so that future outbreaks are not treated empirically with the incorrect drug.

Evaluating the antimicrobial susceptibility of aquatic bacterial isolates has been problematic prior to 2006, since there were no standardized methods for testing organisms that grow at lower temperatures. CLSI published guidelines (2006a, 2006b, 2010) for testing aquatic bacteria at different temperatures. Specific methods that should be used for testing aquatic bacteria may be found in these documents, along with clinical breakpoints and epidemiological cut-off values. It is essential that these standardized methods be used to ensure reliable results and allow comparisons between laboratories (chapter 2).

Table 39.1. Frequently isolated bacterial pathogens of fish.

Bacterial Pathogen	Disease
Aeromonas caviae	Motile aeromonad septicemia
Aeromonas hydrophila	
Aeromonas sobria	
Aeromonas salmonicida	Furunculosis, ulcer disease, carp erythrodermatitis
Aerococcus viridans	Gaffkemia
Edwardsiella ictaluri	Enteric septicemia of catfish
Edwardsiella tarda	Red pest disease, *Edwardsiella* septicemia
Flavobacterium branchiophilum	Bacterial gill disease
Flavobacterium columnare	Columnaris disease
Flavobacterium psychrophilum	Cold-water disease, rainbow trout fry syndrome
Francisella spp.	Francisellosis
Lactococcus garvieae	Lactococcosis, *Lactococcus* septicemia
Lactococcus piscium	
Moritella viscosa	Winter ulcer disease
Mycobacterium spp.	Mycobacteriosis
Photobacterium damselae subsp. *damselae*	Vibriosis
Photobacterium damselae subsp. *piscicida*	Photobacteriosis, fish pasteurellosis, pseudotuberculosis
Piscirickettsia salmonis	Piscirickettsiosis, salmonid piscirickettsial septicemia
Plesiomonas shigelloides	Winter disease
Pseudomonas spp.	Pseudomoniasis
Pseudomonas anguilliseptica	Red spot disease
Renibacterium salmoninarum	Bacterial kidney disease
Streptococcus agalactiae	Group B streptococcosis
Streptococcus dysgalactiae	Group C streptococcosis
Streptococcus iniae	Streptococcosis
Streptococcus phocae	Warm-water streptococcosis
Tenacibaculum maritimum	Salt-water columnaris, marine flexibacteriosis
Vagococcus salmoninarum	Cold-water streptococcosis
Vibrio salmonicida	Cold-water vibriosis, Hitra disease
Vibrio spp.	Vibriosis
Yersinia ruckeri	Enteric redmouth disease

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The Host

Choosing the correct drug depends in part on such factors as age, size, and housing of the animal. Treatment options will be different for animals that are held in net pens at sea, as opposed to those held in an indoor facility or aquarium. A treatment must also be feasible. An appropriate treatment route for aquarium fish or selected brood-stock individuals may be cost- or labor-prohibitive in commercial aquaculture. The stress associated with treatments must be balanced with the need for and expected benefits of treatment.

Drug dosage regimens also are host-dependent. Fish species reared in warm water may absorb, metabolize and excrete drugs at a different rate (often faster) than those in cold water. The salinity of the holding water also affects drug kinetics. Fish kept in saltwater drink the water whereas freshwater fish do not. Thus, antimicrobials in the gastrointestinal tract of fish species held in saltwater may bind cations that can reduce their

uptake. This is especially true for antimicrobials, such as the tetracyclines, that have low bioavailability even in freshwater fish (Elema et al., 1996). Uptake of oral difloxacin by Atlantic salmon is ten-fold less in saltwater than freshwater (100 vs. 1000 ng/ml in plasma; Elston et al., 1994). The elimination rate of oxolinic acid (oral or injected) is also faster in rainbow trout held in saltwater than freshwater (Ishida, 1992). It is therefore important to obtain information on the pharmacokinetic parameters of the drugs in the host species. This is, of course, easier said than done. An extensive review of the literature has been incorporated into the Phish-Pharm database (Reimschuessel et al., 2005, 2011), which makes much of the published data rapidly searchable. However, even with such a tool, there are many species and drugs for which there are no published studies. The veterinarian is thus often in the position of making a best guess based on data from other species held, hopefully, under similar conditions. Half-lives of drugs in fish are highly dependent on the dosage regimen, the route, and temperature. Therefore, these parameters are included in the Phish-Pharm database and should be considered when administering antimicrobials to fish.

The Dosage

Table 39.2 shows drug dosages that have been reported for fish. These dosages are compiled from a number of formularies (Stoskopf, 1993; Noga, 1996; Carpenter, 2005) and research reports. It is important to realize that the dosages listed in Table 39.2 may not have been shown to be safe or effective in all fish species. The table also lists the interval that was reported in the original citation, but it is important to remember that successful therapy often depends on maintaining adequate blood levels over a course of 7–10 days. In some cases, only the dose used for experimental purposes is listed. It is advisable to consider the half-life of the drug in that species when determining the length and frequency of treatments. In a few species (the aglomerular fish in particular), half-lives of drugs excreted by the kidney are quite prolonged (Jones et al., 1997) and must be considered when treating these animals.

Temperature is a very important factor in deciding the dose and treatment intervals. Knowledge of drug half-lives calculated from exposures at different temperatures can help the clinician choose intervals that will maximize chances for successful therapy. The half-lives listed in the Phish-Pharm database (Reimschuessel et al., 2005, 2011) may help with such decisions. Temperature as a variable in pharmacokinetics has been reported for a number of drugs. For example, Bowser et al. (1992) examined the half-life of enrofloxacin in rainbow trout at 10 and at 15°C. The half-life at 10°C was 30 hours, whereas at 15°C the half-life was 56 hours. In a study of flumequine in rainbow trout, however, researchers found that half-lives decreased with rising temperature: 3°C—569 hours, 7°C—300 hours, and 13°C—137 hours (Sohlberg et al., 1990, 1994). Bjorklund and Bylund (1991) found longer half-lives at 5 and 10 than at 16°C in rainbow trout fed oxytetracycline, whereas Chen et al. (2004) found minimal differences in a number of fish species held at different temperatures. There is considerable pharmacokinetic data in the Phish-Pharm database, and Table 39.3 summarizes half-life ranges of antimicrobial drugs in different fish species that have been extracted from the database.

The Treatment Route

Waterborne Treatments

Waterborne antimicrobial treatments will vary depending on the animal and holding conditions. Treating fish by applying the drug to the water avoids stressing the fish by handling. Three main methods are employed: (1) baths (and dips), in which the drug is added to a holding system; (2) flushes, used in flow-through systems, adding the entire dose in a short period (1–2 minutes) then allowing the system to flow, thereby diluting the dose; and (3) constant flow, also used in flow-through systems by continuously pumping in a stock solution with a chemical dosimeter.

The disadvantages of waterborne treatments include expense, waste, and potential environmental contamination. Biological filters may also be compromised due to killing the filter bacteria. A rapid rise in ammonia has been seen using therapeutic concentrations of erythromycin in a catfish recirculating system, but chloramphenicol, nifurpirinol, oxytetracycline, and sulfamerazine did not affect the filter function (Treves-Brown, 2000).

It is also important to consider the ability of a drug to be absorbed from the water. Lipophilic compounds under a molecular weight of 100 will be more likely to diffuse across the gills. Antimicrobials that are absorbed from the water include chloramines, dihydrostreptomycin, enrofloxacin, erythromycin, flumequine, furpyrinol,

Table 39.2. Antimicrobial dosages used in fish.

Drug	Dosage	Interval	Route	Comments
Amikacin	5 mg/kg	q 12 h	IM	
	5 mg/kg	q 72 h×3	IM	
Amoxicillin	25 mg/kg	q 12 h	PO	Rarely used due to few Gram-positive pathogens
	40–80 mg/kg	q 24 h 10 d	PO	
Ampicillin	10 mg/kg	q 24 h	IM	Sharks
	10 mg/kg	q 12 h 7–10 d	PO	Sharks
	50–80 mg/kg	q 24 h 10 d	PO	
Aztreonam	100 mg/kg	q 24 h 7d	IM/IP	Used by koi hobbyists
Azithromycin	30 mg/kg	q 24 h 14 d	PO	
Ceftazidime	22 mg/kg	q 72–96 h×3–5	IM/IP	
Cefquinome	5–20 mg/kg	single dose	IP	Dose used for determining PK
Chloramine-T	20 mg/L	1 h 4 d	BATH	
	2.5–20 mg/L	flush (various)	BATH	Disinfectant control bacterial gill disease and parasites
	5–10 mg/L	1 h	BATH	
Ciprofloxacin	15 mg/kg	single dose	IM/IV	Dose used for determining PK[a]
Difloxacin	10 mg/kg	single dose	PO	Dose used for determining PK[a]
Dihydrostreptomycin	0.125 mg	single dose	IM/IV	Dose used for determining PK
	10 mg	single dose	PO	Dose used for determining PK
	10 mg/kg	q 24 h	IM	Sharks
Enrofloxacin	2.5–5.0 mg/L	5 h q 24 h 5–7 d	BATH	[a]
	30–50 mg/L	4–24 h (various)	BATH	
	5–50 mg/kg	q 24 h×5–10 d	PO	[a]
	2.5–10 mg/kg	single dose	IM/IP/IV	Dose used for determining PK
Erythromycin	10–20 mg/kg	single dose	IP	For BKD before spawning
	50–100 mg/kg	q 24 h 10–21 d	PO	
	2 mg/L	1 h	BATH	For BKD in eggs
Florfenicol	5–20 mg/kg	q 24 h 10 d	PO	Salmon
	10–15 mg/kg	q 24 h 10 d	PO	Dose approved by U.S. FDA for select species
	40–50 mg/kg	q 12–24 h	PO, IM, IP	Red pacu
	25–50 mg/kg	single dose	IM	
Flumequine	10–500 mg/L	1–72 h	BATH	Increase dose in saltwater[a]
	5–50 mg/kg	q 24 h 5–10 d	PO	[a]
	30 mg/kg	single dose	IM/IP	IP (and IM) dose levels remain at effective levels for 10d[a]
	2–25 mg/kg	single dose	IV	Dose used for determining PK
Fumagillin	30–60 mg/kg	single dose	PO	Dose used for determining PK
	3–6 mg/kg	single dose	IV	Dose used for determining PK
Furpyrinol	4–32 mg/L	5 h	BATH	
Gentamicin	3 mg/kg	q 72 h	IM	Very nephrotoxic to aglomerular fish. Bath exposure does not achieve blood levels
	6 mg/kg	each week	IM	Sharks
Kanamycin	50–100 mg/L	q 72 h 5 h×3	BATH	Nephrotoxic some species. Change water 50% between treatments

	50 mg/kg	q 24 h	PO	Nephrotoxic some species
	10–20 mg/kg	q 24 h	PO	Sharks
	20 mg/kg	q 72 h×5	IP	Nephrotoxic some species
Lincomycin	40 mg/kg	q 24 h	PO	Japan
Marbofloxacin	10 mg/kg	q 24 h 1–3 d	PO	Dose used for determining PK
Miloxacin	60 mg/kg	q 24 h 6 d	PO	Japan
Nalidixic acid	13 mg/L	1–4 h	BATH	
	20 mg/kg	q 24 h	PO, IM, IV	Other doses used for PK studies
Neomycin	66 mg/L	q 3 d×3	BATH	Toxic to nitrifying bacteria in filter
	20 mg/kg	single dose	PO	Sharks to prevent bloat, poorly absorbed from gut
Norfloxacin	30–50 mg/kg	q 24 h 5 d	PO	
Oxolinic acid	25 mg/L	0.25 h q 12 h×3	BATH	
	0.15–1.5 mg/L	10 d	BATH	
	50–200 mg/L	1–72 h	BATH	
	5–25 nf.j	single dose	IV	Dose used for determining PK
	10 mg/kg	q 24 h 10 d	PO	Freshwater species
	25–75 mg/kg	q 24 h 10 d	PO	Saltwater species
Oxytetracycline	10–50 mg/L	1 h	BATH	For superficial infections
	20–50 mg/L	5–24 h q 24h 5–6 d	BATH	Change 50–75% water between treatments
	55–83 mg/kg	q 24 h 10 d	PO	Dose approved by U.S. FDA for select species
	25–50 mg/kg	q 24 h 5–7 d	IM/IP	Produces high levels for several days when given IM
	3 mg/kg	q 24 h	IV	Red pacu
Piromidic acid	10 mg/kg	q 24 h 5–10 d	PO	Japan
Sarafloxacin	10–30 mg/kg	q 24 h 10 d	PO	[a]
Sulfadiazine-Trimethoprim	30–50 mg/kg	q 24 h 7–10 d	PO	
	125 mg/kg		IP	
Sulfadimethoxine-Ormetoprim	50 mg/kg	q 24 h 5d	PO	Dose approved by U.S. FDA for select species
Sulfamerazine	220 mg/kg	q 24 h 14 d	PO	
	200 mg/kg	q 24 h 10 d	PO	
Sulfamethoxazole-Trimethoprim	20 mg/L	5–12 h q 24h 5–7 d	BATH	Change 50–75% water between treatments
	30 mg/kg	q 24 h 10–14 d	PO	
Tetracycline	80 mg/kg	single dose	PO	
Thiamphenicol	20 mg/L	1 h	BATH	
	50 mg/kg	q 24 h 7–10 d	PO	Japan
Vetoquinol	25–40 mg/kg	single dose	PO	
Virginiamycin	40 mg/kg	q 24 h 15 d	PO	

[a]Extra-label use of fluoroquinolones in food animals is prohibited by the U.S. FDA.
Data obtained from many authors.

Table 39.3. Half-lives of antibiotics in fish.

Drug	Species	$t_{1/2}$-hr	Dosage	Route	°C
Amoxicillin	Atlantic salmon	120	12.5 mg/kg sd*	IM	13
	Atlantic salmon, Seabream	14–72	40–80 mg/kg sd	IV/PO	16–22
Chloramphenicol	Carp	48–72	40 mg/kg sd	IP	9
Ciprofloxacin	Carp, Rainbow trout, African catfish	11–15	15 mg/kg sd	IM/IV	12–25
Difloxacin	Atlantic salmon	16	10 mg/kg sd	PO	11
	Carp	58, 114	20 mg/kg 3 d	PO	10, 20
Enrofloxacin	Atlantic salmon, Rainbow trout, Brown trout, Sea bass	25–105	5–10 mg/kg sd	IM/IV/PO	10–15
	Carp, Red pacu, Seabream	16–26	5–10 mg/kg sd	IM/IV/PO	25–28
Erythromycin	Chinook salmon	120	0.1 g/kg 21 d	PO	10
Florfenicol	Carp, Gourami, Tilapia	4–16	10–50 mg/kg sd	IM/PO	22–24
	Atlantic salmon	12–30	10 mg/kg sd	IV/PO	10–11
	Cod	39–43	10 mg/kg sd	IV/PO	8
Flumequine	Eel	255	9 mg/kg sd	IM	23
	Atlantic halibut, Brown trout, Corkwing wrasse, Atlantic halibut, Atlantic salmon, Cod, Goldsinny wrasse, Sea bass, Seabream, Turbot	21–96	5–25 mg/kg sd	IP/IV/PO	5–25
	Eel	208–314	10 mg/kg sd	IV/PO	23
	Rainbow trout	285–736	5 mg/kg sd	IV/PO	13 vs 3
Furazolidone	Channel catfish	1–24	1 mg/kg sd	IV/PO	24
Gentamicin	Channel catfish, Brown shark, goldfish	12–54	1–3.5 mg/kg sd	IC/IM	20–25
	Toadfish	602	3.5 mg/kg sd	IM	19
Miloxacin	Eel	35	30–60 mg/kg sd	IV/PO	27
Nalidixic acid	Rainbow trout, Amago salmon	21–46	5–40 mg/kg sd	IV/PO	14–15
Nifurstyrenate	Yellowtail	2	100 mg/kg sd	PO	23
Norfloxacin	Halibut, Japanese Seabass, Seabream	97–192	30–50 mg/kg 5d	PO	
Ormetoprim	Atlantic salmon, Channel catfish, Rainbow trout, Hyb. Striped bass	4–25	4–50 mg/kg sd	IV/PO	10–28
Oxolinic acid	Atlantic salmon, Corkwing wrasse, Channel catfish, Cod, Rainbow trout, Red seabream, Sea bass	15–87	4–20 mg/kg sd	IP/IV	8–24
	Atlantic salmon, Cod, Rainbow trout	82–146	25–75 mg/kg sd	PO	5–8
	Atlantic salmon, Gilthead seabream, Rainbow trout, Sharpsn. Seabream, Turbot	13–48	10–40 mg/kg up to 10 d	PO	9–19
Oxytetracycline	African catfish, Carp, Rainbow trout, Red pacu, Sockeye salmon	63–95	5–60 mg/kg sd	IM	12–25
	African catfish, Atlantic salmon, Ayu, Carp, Chinook salmon, Eel, Rainbow trout, Red pacu, Sea bass, Seabream, Sharpsnout seabream	6–167	5–60 mg/kg sd	IV	8–25

	Arctic charr	266–327	10–20 mg/kg sd	IV	6
	Atlantic salmon, Ayu, Black seabream, Carp, Channel catfish, Eel, Perch, Rainbow trout, Sea bass, Seabream, Hyb. Striped bass, Summer flounder, Tilapia, Walleye	43–268	10–100 mg/kg up to 10d	PO	7–27
	Arctic charr, Sockeye salmon, Chinook salmon	428–578	10–100 mg/kg sd	PO	6–11
Piromidic acid	Eel, Goldfish	24	5 mg/kg sd	PO	26
Sarafloxacin	Atlantic salmon, Cod, Eel	12–45	10–15 mg/kg sd	IV/PO	8–24
Streptozotocin	Toadfish	24	50 uCi	IV	
Sulfachlorpyridazine	Channel catfish	4–5	60 mg/kg sd	IC/PO	22
Sulfadiazine	Atlantic salmon, Carp, Rainbow trout	26–96	25–200 mg/kg sd	IV/PO	8–24
Sulfadimethoxine	Atlantic salmon, Channel catfish, Rainbow Trout, Hyb. Striped bass	7–48	25–200 mg/kg sd	IV/PO	10–27
Sulfadimidine	Carp, Rainbow trout	18–57	100–200 mg/kg sd	IV/PO	10–20
Sulfamethoxypyridazine	Rainbow trout	72	200 mg/kg sd	PO	13
Sulfamonomethoxine	Rainbow trout, Yellowtail	5–33	100–400 mg/kg sd	IV/PO	15–22
Sulfanilamide	Rainbow trout	36	200 mg/kg sd	PO	13
Sulfathiazole	Rainbow trout	60	200 mg/kg sd	PO	13
Tetracycline	Channel catfish	17, 44	4, 80 mg/kg sd	IV/PO	27
Thiamphenicol	Sea bass	21	30 mg/kg 5 d	PO	19
Tobramycin	Brown shark	48	1–2.5 sd	IM	25
Trimethoprim	Atlantic salmon, Carp, Rainbow trout	21–48	5–20 mg/kg sd	IV/PO	10–24
Vetoquinol	Cod	79	25 mg/kg sd	PO	8
	Atlantic salmon	16	40 mg/kg sd	PO	10

*sd, single dose

kanamycin, oxolinic acid, oxytetracycline, nifurpirinol, sulfadimethoxine, sulfadimidine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfisomidine and trimethoprim. Antimicrobials that are absorbed poorly or not at all include chloramphenicol and gentamicin (Treves-Brown, 2000; Reimschuessel et al., 2005).

Dipping treatments are a shorter and more controlled method of administering bath treatments. The advantages of this type of treatment are reduced waste (thus reduced expense) and less environmental contamination. The disadvantage of this type of approach is the increased stress to the animals through handling. Therefore most dip treatments are done when fish are small or in pet/aquarium cases, but commercial aquaculture producers have used tarpaulins to contain the drug (Vavarigos, 2003) and more recently, well boats to more effectively contain treatments (Burka et al., 2012). Localized topical treatment, often under light anesthesia, has been recommended for small external lesions in pet fish (Stoskopf, 1993; Noga, 1996).

Some studies have used either hyperosmotic infiltration (first high osmolarity, > 1200 mOsm/L, then lower osmolarity containing the drug) or ultrasound treatments to try to improve permeability across the gills. Drug absorption and elimination can be affected by salinity under normal conditions, and the effects of hyperosmotic treatments have not been adequately studied. Certain drugs that bind divalent cations (such as the tetracyclines) may have their bioavailability compromised by the addition of salts. Ultrasound treatments to enhance absorption may be feasible in a small aquarium setting but have not been studied extensively. Both hyperosmotic and ultrasound treatments are fairly stressful. They are mainly used for vaccination rather than antimicrobial treatment (Treves-Brown, 2000; Navot et al., 2004).

Oral Treatments

Oral treatments are the most feasible methods for large commercial aquaculture systems because they are the least stressful for the animals. However, sick fish may not eat. This was shown in a study where the concentration of oxolinic acid was examined in Atlantic salmon treated during an outbreak of winter ulcer disease (*Moritella viscosa*). Oxolinic acid was detected in plasma and tissues of healthy fish, whereas levels were below the limit of detection in moribund and dead fish (Coyne et al., 2004a). The moribund and dead fish also had no food in their gastrointestinal tracts. These results indicate that the antimicrobial appears to help actively feeding healthy fish fight off the infection, whereas fish with clinical signs are anorexic and therefore not receiving the antimicrobial. Variable intake can also occur if fish vary in size. The larger fish will probably consume more of the medicated feed than their smaller and less vigorous counterparts. Palatability, especially of the sulfonamide products, can also be a problem.

Absorption from the intestinal tract may vary from species to species. As mentioned, saltwater fish will drink and, therefore, drugs may bind cations in the water in their intestinal tracts, affecting bioavailability. The formulation of the drug may either enhance or decrease absorption.

Various methods for administering oral medication include commercial medicated feed, custom surface-coated feeds, custom feeds (e.g., gelatin diets), medicated live feeds (e.g., *Artemia* grown in or fed antimicrobials), injecting food (e.g., small fish used for food) and tube feeding (Noga, 1996; Treves-Brown, 2000). Obviously, some of these techniques are appropriate only for pet/aquarium fish.

Injectable Treatments

Treating fish with injectable antimicrobials causes handling stress and can be a massive undertaking for commercial producers. Advantages include assuring that all animals receive the drug at the desired dose. The route of administration is often intramuscular, but sometimes intraperitoneal, intravascular, or intradorsal sinus (caudal to the dorsal fin) routes are used. Intramuscular treatments are usually administered in the epaxial muscles, above the lateral line and near the caudal fin. Since there is a renal portal vascular system in fish, it is best to inject aminoglycosides cranial to the dorsal fin to avoid large doses entering the kidney.

In pet/aquarium fish, most injections are given manually. Automatic injectors, such as those used in poultry operations, can be used in commercial aquaculture. Although this seems a formidable task, vaccinations are given by injection, often manually, to many net-pen reared fish (Noga, 1996; Vavarigos, 2003).

Avoiding Toxicity in the Target Animal

Even though fish drug metabolism can be affected by environmental salinity and temperature, it is remarkably similar to mammalian drug metabolism. Both groups

have similar metabolic systems: phase 1 systems, including the heme protein monooxygenase (cytochrome P450) and the flavin monooxygenase (FMO) systems, and the phase 2 conjugation systems. The P450 systems have been identified in over 150 fish species (Whyte et al., 2000). FMO activity, however, is lacking in some fish species, e.g., channel catfish (Schlenk et al., 1995). This difference can affect the metabolism of drugs, resulting in either enhanced or reduced toxicity, depending on the chemical. For example, in the case of the herbicide aldicarb, catfish and trout take up similar amounts of the parent compound, but their metabolism of the compound differs (Perkins and Schlenk, 2000). Compared to trout, catfish are 10 times less susceptible to toxicity induced by aldicarb because they lack FMO activity. Trout (like mammals) metabolize the parent drug to a toxic sulfoxide (Montesissa et al., 1995) that is responsible for most of the toxic effects (Perkins et al., 1999). Such differences in metabolism must be considered when choosing antimicrobials. In general, however, most antimicrobials given by the oral route will not cause toxicity because fish rarely overdose by eating excessive amounts of medicated feed, and overmedicated feed is often not palatable and thus rejected.

Drugs administered via bath treatments can cause toxicity if grossly overdosed, especially if they are absorbed by the gills. In addition, saltwater fish will drink the water and thus probably get an oral dose as well. Drugs may have an effect on the water pH, which can affect the osmoregulation of the animal. High doses of tetracyclines, which are used for immersion treatments, can affect the pH of the water, inducing toxicity (Treves-Brown, 2000). Drugs can also irritate the skin or gills. Waterborne irritants can affect the gills by increasing mucus production and thus decreasing gaseous exchange.

The main antimicrobial toxicity seen in fish is that of aminoglycoside-induced nephrotoxicosis. Aminoglycosides, such as gentamicin, which cannot be excreted through filtration in aglomerular fish (including toadfish, goosefish and seahorses), can cause extensive renal necrosis in these fish at doses that are therapeutic (and non-toxic) in other fish species (Reimschuessel et al., 1996). The half-life of gentamicin in toadfish is approximately 2 weeks, compared to 2 days in goldfish. Since fish eliminate nitrogenous waste through the gills, they can survive with compromised renal function as long as the osmolarity of their environment does not change dramatically. Since their kidneys can undergo nephron-neogenesis, fish can sometimes survive such a toxic episode and regenerate their kidneys. The risks and benefits of treatment must be carefully considered before using these antimicrobials.

Renal damage has also been associated with the use of erythromycin and sulfamerazine. A number of antimicrobials, including erythromycin, nalidixic acid, and sulfonamides, can cause anorexia, especially if administered in high doses. Nalidixic acid and, to a lesser extent, oxolinic acid have induced macrocytic anemia, potentially due to their effect on DNA synthesis. Immunosuppression has been shown to occur with tetracyclines (Rijkers et al., 1980).

Ensuring Safety for Humans

Considerations for safety to humans include potential hazards associated with: (1) administration of the drug; (2) exposure of individuals from environmental contamination; and (3) consumption of the fish with respect to residues and antimicrobial resistance. Most hazards associated with administration of the drug can be managed by adequate training, specialized equipment, and personal protective clothing. Basic veterinary practices used to reduce hazards to personnel and the environment when treating terrestrial animals generally apply to treating fish.

Food safety concerns, for the most part, relate to residues of the drug used (or its metabolites) in the food product (chapter 26). To prevent harmful residues, governmental agencies establish required withdrawal periods. These periods are designed to ensure that the food product will have residue levels below the tolerance (United States) or maximum residue limit (MRL; outside the United States) established by the governing body. Tolerances and MRLs are based on the potential toxicity of the compound and an assessment of potential exposure levels, including consideration of the general risk to the consumer. The basic principles are, again, similar to those used when treating terrestrial food animals. Withdrawal periods for fish, however, can incorporate water temperature as part of the equation, sometimes in the form of "degree days" (the °C multiplied by the number of days, e.g., 50 days at 10°C = 500 degree days, as does 25 days at 20°C; Alderman, 2000; European Medicines Agency, 2005;

FDA, 2005). The European Union (EU) regulations have included the concept of degree days in their suggested generic withdrawal period of 500 degree days for compounds for which no specific withdrawal period has been set. Knowledge of the pharmacokinetics and depuration patterns of different drugs in different fish species is essential for both those establishing such periods and clinicians using the drugs. When evaluating data reporting depuration periods and residue levels, one should also consider what detection method was used. Analytical methods have changed over the years, in general becoming more sensitive. As a result, residues determined to be "below the level of detection" in the 1980s may actually be detectable using improved detection systems, and could be considered unacceptable today. It is important for clinicians prescribing aquaculture drugs to be aware of the regulations in their country to protect the safety of the consumer.

Another concern for agencies regulating food safety is the development of antimicrobial resistance among potential zoonotic bacteria in or on food-fish, as a result of antimicrobial use in aquaculture (Heuer et al., 2009). The United States Food and Drug Administration's Center for Veterinary Medicine assesses the level of risk associated with a proposed use by a qualitative antimicrobial resistance risk assessment (FDA, 2003). Recently, the U.S. FDA (2012a) issued guidance outlining their concerns regarding the development of antimicrobial resistance in human and animal bacterial pathogens when medically important antimicrobials are used in food-producing animals in an injudicious manner. Two principles guiding appropriate or judicious use of medically important antimicrobials include limitation of medically important antimicrobial drugs to uses in animals that are considered necessary for assuring animal health, and include veterinary oversight or consultation (Codex Alimentarius Commission, 2005, 2011; FDA, 2012b).

Environmental Effects and Non-target Species

Treating fish with antimicrobials, especially in large commercial systems, can affect the environment in a number of ways. These include: (1) toxicity to non-target species; (2) accumulation by non-target species; (3) accumulation in sediments; (4) presence in drinking water; and (5) alterations in the ecosystem's microbial community, including antimicrobial resistance. Local effluent discharge regulations must be considered both by the clinician and the owner of the aquaculture facility.

Toxicity to non-target species depends on the dose and the route of administration of the drug (Isidori et al., 2005). For example, furazolidone, which is usually administered by bath treatment, is extremely toxic to crustaceans (Macri et al., 1988). Bioaccumulation of antimicrobials in edible food sources can occur in non-target species, including fish, crustaceans, and plants (Samuelsen et al., 1992a; Delepee et al., 2003; Migliore et al., 2003). Accumulation in the sediment has also been documented for a number of antimicrobials, including flumequine, furazolidone, ormetoprim, oxolinic acid, and oxytetracycline (Bjorklund et al., 1991; Samuelsen et al., 1991; Capone et al., 1996; Lalumera et al., 2004). Antimicrobials and other pharmaceuticals, including those from human and terrestrial agricultural use, have been detected in receiving waters (Hirsch et al., 1999; Kümmerer, 2001; Rooklidge, 2004). Recently, researchers showed that exposure of various bacterial genera to sublethal antimicrobial concentrations led to mutant strains sensitive to the applied antimicrobial but resistant to other antimicrobials (Kohanski et al., 2010). These findings have important implications for the widespread use of antimicrobials in aquatic environments. Such changes in the antimicrobial susceptibility following antibiotic use in the aquatic setting have been reported in the past (Samuelsen et al., 1992b; Angulo, 1999; Guardabassi et al., 2000; Chelossi et al., 2003). Recent standardized methods for assessing antimicrobial susceptibility of bacteria isolated from aquatic animals should help efforts to monitor changes in susceptibility following antimicrobial exposure of pathogenic bacteria and some less fastidious environmental isolates (Miller et al., 2003, 2005; CLSI 2006a, 2006b).

Legal Considerations

Veterinarians dealing with food animals, either terrestrial or aquatic, must be familiar with the regulations regarding antimicrobial use in their country as well as in countries that may import the product (chapter 26). These regulations include: (1) prohibitions from use, for exam-

ple, chloramphenicol (local and abroad); (2) residue tolerance levels in the United States, or other regulatory levels, such as MRLs in the EU; (3) effluent and discharge regulations; and (4) general prescription regulations.

Such laws vary greatly from country to country, from almost no regulation to restrictive regulation. For example, the U.S. FDA has only approved four classical antimicrobials (florfenicol, ormetoprim/sulfadimethoxine, oxytetracycline, and sulfamerazine) for use in fish reared for food purposes. Canada has approved the first three antimicrobials, as well as sulfadiazine and trimethoprim. Approximately ten antimicrobials have received authorization for use in certain EU member states, including quinolone antimicrobials such as flumequine, oxolinic acid, and sarafloxacin. Japan has approved approximately thirty antimicrobials for use in aquaculture (Treves-Brown, 2000; Schnick, 2001; FDA, 2005). Many developing countries are beginning to formulate regulations regarding antimicrobial use in aquaculture.

Many countries are also developing provisions for using therapeutic agents that are not approved (extra- or off-label use) in minor species. Some countries, such as the United States, have established specific rules for extra-label use of approved drugs by veterinarians. In the United States, the FDA lists some substances as "low regulatory priority"; these substances are not legal for use, but it has been determined that under certain conditions no regulatory action is likely. Such substances include sodium chloride, sodium bicarbonate, and urea. Although not classical antimicrobials, these chemicals might be used in conjunction with other treatments. Also, the U.S. Minor Use and Minor Species Animal Health Act (MUMS) provides regulatory authority to the U.S. FDA to add certain drugs to an index of legally marketed but unapproved new animal drugs for use in minor species (FDA, 2004). MUMS provides more flexibility for veterinarians prescribing medicines to aquatic animals. The European Medicines Agency, which regulates antimicrobial use in the EU, is considering instituting similar policies (EMA, 2005).

In addition to prescription regulations, many countries are developing guidelines for stewardship or judicious use of antimicrobials in order to prevent antimicrobial resistance from developing in pathogenic and environmental bacteria (chapter 7). In the United States, such guidelines have been proposed by the American Veterinary Medical Association (2002) and the National Aquaculture Association (2003). They are, in general, similar to guidelines proposed for antimicrobial use in terrestrial animals. The Codex Alimentarius Commission, charged to protect the health of consumers while ensuring fair practices in the food trade, recently published Guidelines for Risk Analysis of Foodborne Antimicrobial Resistance (Codex Alimentarius Commission, 2011).

The clinician must keep abreast of recent developments in both national and international regulations regarding antimicrobial use in aquatic species. The aquaculture producer must also be conversant in these areas to assure that the therapies recommended by the clinician are appropriately implemented.

Antimicrobial Susceptibility Testing of Aquatic Bacteria

Defining conditions for antimicrobial susceptibility testing has been difficult because aquatic bacteria vary greatly in their optimal *in vitro* growth requirements. Temperature optimums of various aquatic bacteria can range from 15°C to 35°C. Some aquatic bacteria prefer or require supplementation to the basal medium, while others need a low-nutrient or diluted basal medium. Nevertheless, standardized testing protocols are essential to obtain results that are reproducible within and among laboratories (chapter 2). Such test protocols are standardized through extensive multilaboratory validation studies, and are used to establish quality control (QC) ranges to monitor performance and reproducibility (CLSI, 2008).

The CLSI has published two guidelines, M42-A and M49-A, which describe standardized methods for disk diffusion and broth dilution susceptibility testing of some bacterial isolates from aquatic animals (CLSI, 2006a, 2006b). Because of their complexity and length, full details are not given here. Specialists in the area should consult the CLSI current guidelines, and those published in the future.

The ultimate goal of any susceptibility test is to obtain a result that can be used to predict therapeutic efficacy (clinical application), detect shifts in susceptibility over time (surveillance application), or both. Currently, the only fish pathogen that has these interpretive tools available is *Aeromonas salmonicida*. These minimal inhibitory concentration (MIC) and zone diameter clinical breakpoints and epidemiological cutoff values will be

Table 39.4. Standard methods for disk diffusion susceptibility testing of aquatic bacterial pathogens

Organisms	Medium	Incubation
Group 1: Non-fastidious bacteria Enterobacteriaceae *Aeromonas salmonicida* (nonpsychrophilic strains) *Aeromonas hydrophila* and other mesophilic aeromonads *Pseudomonas* spp. *Plesiomonas shigelloides* *Shewanella* spp. Vibrionaceae and related bacteria (nonobligate halophilic strains)	MHA	22°C (24–28 h and/or 44–48 h) or 28°C (24–28 h)

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published in the next edition of CLSI's M49-A guideline for dilution susceptibility testing.

Disk Diffusion Susceptibility Testing

Since the Kirby-Bauer disk diffusion method is frequently used in aquatic animal disease diagnostics, many studies have been published using different types of basal media for testing a cornucopia of aquatic pathogens (Bauer et al., 1966; Dalsgaard, 2001). Barker and Kehoe (1995) and Dalsgaard (2001) both found Mueller-Hinton agar (MHA) to be the best medium for disk diffusion testing, based upon its consistent performance with a wide range of aquatic pathogens. An international collaborative study in 2003 conducted in accordance with existing CLSI guidelines (CLSI, 2008) standardized the disk diffusion testing method for non-fastidious aquatic isolates that grow well on MHA (Table 39.4; Miller et al., 2003). These aquatic bacteria have been labeled Group 1 isolates by the Aquaculture Working Group of the CLSI Subcommittee on Veterinary Antimicrobial Susceptibility Testing. Organisms in Group 1 prefer growth on MHA at 22°C or 28°C (CLSI, 2006a).

Disk diffusion zone diameter QC ranges were established for two control organisms, *Escherichia coli* ATCC25922 and *Aeromonas salmonicida* subsp. *salmonicida* ATCC33658, testing on MHA at both 22°C and 28°C (Table 39.5). Ranges were established for ampicillin, enrofloxacin, erythromycin, florfenicol, gentamicin, ormetoprim/sulfadimethoxine, oxolinic acid, oxytetracycline, and trimethoprim/sulfamethoxazole (Miller et al., 2003; CLSI, 2006a).

Table 39.5. Antimicrobial agents used in global aquaculture and status of quality control for disk diffusion susceptibility testing.

	Antimicrobial Agents	Suggested Disk Content	Zone Diameter QC Ranges for Testing at: 22°C	28°C	35°C
Drugs Used in Global Aquaculture with CLSI-Approved QC Ranges for Disk Diffusion Testing of Aquatic Isolates	Ampicillin	10 µg	×	×	×
	Chloramphenicol	5 µg			×
	Clindamycin	2 µg			×
	Doxycycline[a]	30 µg			×
	Enrofloxacin	5 µg	×	×	×
	Erythromycin	15 µg	×	×	×
	Florfenicol	30 µg	×	×	×
	Fosfomycin[b]	200 µg			×
	Gentamicin	10 µg	×	×	×
	Kanamycin	30 µg			×
	Minocycline[a]	30 µg			×
	Nalidixic acid	30 µg			×
	Nitrofurantoin	300 µg			×
	Ormetoprim-sulfadimethoxine[c]	1.25 µg/23.75 µg	×	×	
	Oxolinic acid	2 µg	×	×	
	Oxytetracycline[a]	30 µg	×	×	
	Penicillin	10 units			×
	Rifampin	5 µg			×
	Sulfisoxazole	250 or 300 µg			×
	Tetracycline[a]	30 µg			×
	Tiamulin	30 µg			×
	Trimethoprim-sulfamethoxazole	1.25 µg/23.75 µg	×	×	×

[a]Drugs in the tetracycline group are closely related and, with few exceptions, only oxytetracycline may need to be tested routinely.
[b]The 200 µg fosfomycin disk contains 50 µg glucose 6-phosphate.
[c]Traditionally, trimethoprim-sulfamethoxazole may be used to predict susceptibility to ormetoprim-sulfadimethoxine; however, this has not been confirmed at 22 ± 2°C or 28 ± 2°C.
Note: Laboratories may also include disks containing other antimicrobial agents. The inclusion of disks outside the recommended set can be valuable if a laboratory has data relating to its clinical significance. However, quality control data generated with disk contents other than those with quality control range established *should not be* reported as being in compliance with CLSI standards established in this guideline. Variations must be reported with results.

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Table 39.6. Potential modifications for disk diffusion susceptibility testing of aquatic bacterial pathogens.

Organisms	Medium	Incubation
Group 2: Vibrionaceae and Photobacteriaceae (obligate halophilic strains)	MHA + 1% NaCl	22°C (24–28 h and/or 44–48 h) or 28°C (24–28 h and/or 44–48 h)
Group 3: Gliding bacteria		
Flavobacterium columnare	Diluted MHA (4 g/L)	28°C (44–48 h)
Flavobacterium psychrophilum		18°C (92–96 h)
Flavobacterium branchiophilum		
Group 4: Streptococci		
Lactococcus spp., *Vagococcus salmoninarum*	MHA + 5% sheep blood	22°C (44–48 h + CO_2 if necessary for growth)
Streptococcus spp., *Carnobacterium maltaromaticum*, and other streptococci	MHA + 5% sheep blood	28°C (24–28 h and/or 44–48 h + CO_2 if necessary for growth)
Group 5: Other fastidious bacteria		
Psychrophilic *Aeromonas salmonicida* strains	MHA	15°C (44–48 h)
Vibrio salmonicida and *Moritella viscosa*	MHA + supplementation[a]	15°C (6 days)
Tenacibaculum maritimum	Diluted MHA (1:7) + inorganic ion supplementation	25°C (24–28 h)
Renibacterium salmoninarum	Unknown	15°C
Mycobacterium spp. and *Nocardia seriolae*	See CLSI standard M24	See CLSI standard M24
Erysipelothrix rhusiopathiae	Chocolate MHA	35 ± 2°C

[a]Recommended supplementation cannot be made at this time, but may include cations, horse or fetal calf serum, or NaCl.
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Aquatic pathogens in Groups 2–5 may require media other than MHA (Table 39.6). There are currently no quality control (QC) parameters in place to control their tests. In these cases, the clinician should perform the following: (1) identify the isolate; (2) determine to which "Group" the isolate belongs; (3) test the isolate on the suggested media; (4) use a QC organism under standardized conditions in parallel with the test isolate; (5) determine whether the test was within QC; (6) if a test result is not consistent by QC, determine the cause and repeat as necessary.

Clinicians should consult CLSI guideline M42-A (CLSI, 2006a), for suggested conditions to test the more fastidious aquatic bacterial genera (Groups 2–5).

Dilution Susceptibility Testing

Both broth dilution and agar dilution antimicrobial susceptibility testing methods are used in aquatic animal disease diagnostics. Results of dilution susceptibility tests provide data in the form of an MIC, which has greater clinical relevance than a zone diameter value, since it can be correlated with serum concentrations in the animal (chapter 2). Advances in automated inoculation systems for broth microdilution susceptibility testing, discussed in chapter 2, have fostered its growing popularity in many aquatic animal medicine research laboratories.

Standardized broth dilution susceptibility testing methods have been developed for non-fastidious aquatic bacteria in Group 1 at 22°C and 28°C (Miller et al., 2005; CLSI, 2006b). Group 1 bacteria are tested in undiluted cation-adjusted Mueller-Hinton broth (CAMHB).

Recently, a standardized broth dilution susceptibility testing method was developed for the gliding bacteria (Group 3), *Flavobacterium psychrophilum* and *F. columnare*, at 18°C and 28°C, respectively, in diluted CAMHB (4 g/L; Gieseker, 2011; Gieseker et al., unpublished). These gliding flavobacteria form aggregates, which must be allowed to settle out of suspension so that only the free-floating cells are tested. Laboratories should conduct preliminary cell enumerations to confirm target cell concentrations prior to working with flavobacteria. The CLSI guideline M49-A (CLSI, 2006b) will be updated with QC ranges for various antimicrobial agents in diluted (4 g/L) CAMHB.

Aquatic pathogens in Groups 2, 4, and 5 may require media other than CAMHB. There are currently no QC parameters in place to control these tests. Clinicians should consult the latest CLSI guideline M49 for suggested conditions to test other fastidious aquatic bacterial genera. In addition, the same procedures discussed above for disk diffusion apply when no QC ranges are available.

The agar dilution method is also used to determine the MICs of many aquatic pathogens. MHA is always the preferred basal medium and appears to perform well with non-fastidious aquatic isolates (Ho et al., 2000; Tang et al., 2002). Supplements may be needed to test some fastidious organisms. MHA with NaCl (Samuelsen et al., 2003; Coyne et al., 2004b), seawater (Torkildsen et al., 2000), horse serum (Michel et al., 2003) and sheep blood (McGinnis et al., 2003) have all been used. A diluted form of MHA based on a recommendation by Hawke and Thune (1992) has also been used in tests on the flavobacteria (Bruun et al., 2000; Schmidt et al., 2000).

The agar dilution method is considered the "gold" or reference standard for dilution susceptibility testing in mammals. However, because the CLSI has not published QC ranges for use in agar dilution tests conducted at temperatures less than 35°C, we recommend using broth dilution tests until standardized methods are available.

Interpreting Susceptibility Test Results for Aquatic Bacterial Pathogens

Clinicians are often expected to rely on their own experience and published data to make judgment calls on the interpretation of antimicrobial susceptibility testing data. Clinicians may also rely on their laboratory's cumulative susceptibility data, oftentimes presented in scattergram or histogram format, to make interpretations (CLSI, 2011). These species- or genus-specific distributions can be a useful tool to determine where a given clinical isolate lies within the cumulative distribution (chapter 2).

Classically, the establishment of laboratory-independent interpretive criteria begins with susceptibility data distribution comprised of geographically diverse isolates (chapter 2). For application in aquatic animal medicine, clinical breakpoints (susceptible, intermediate, or resistant) and epidemiological cut-off values (wild-type cut-offs) have only been established for *Aeromonas salmonicida* for a few antimicrobials. These criteria will be included in the next edition of CLSI's M49-A guideline (CLSI, 2006b).

Clinical breakpoints are critical values that should be specific for a particular pathogen and can be used to predict therapeutic efficacy in the host (chapter 2). Because fish, unlike terrestrial animals, are reared in heterogeneous environments that can drastically alter depuration rates and drug absorption, the pharmacokinetics/pharmacodynamics (PK/PD) for a given antimicrobial may vary greatly. This has made it difficult for researchers to include PK/PD data when attempting to set clinical breakpoints for aquatic pathogens (Coyne et al., 2004b). Most pharmacokinetic data have been obtained from studies of healthy fish in laboratory situations. It will be important to correlate these data with studies conducted under clinical conditions.

Integrated pharmacokinetic and pathogen susceptibility data can be used both in designing dosage regimens and setting clinical breakpoints. PK/PD assessments help clinicians choose the appropriate antimicrobial agent and develop new dosing regimes targeted for specific species with specific diseases (Maglio and Nicolau, 2004). There is considerable work required to define breakpoints, which will require a coordinated effort from both clinicians treating aquatic animals and the research community.

Note: The opinions and information in this chapter are those of the authors and do not represent the views and/or policies of the U.S. Food and Drug Administration

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