and β-lactam therapy continued. Once susceptibility results are available, therapy should be tailored/narrowed to match the susceptibility profile.

Even when microbiology results are negative, the absence of positive cultures can be informative to deescalation. For example, empirical vancomycin coverage is often included for patients with sepsis of unknown etiology or nosocomial pneumonia to cover for methicillin-resistant *S. aureus*. *S. aureus* grows very well in standard cultures. If after 24 to 48 hours there is no growth of gram-positive cocci in clusters, empirical vancomycin typically can be discontinued.

The other factor that must be considered is the clinical response to therapy. Any time antibacterial therapy is initiated, the treating clinician should know a priori which parameters to follow to determine if the therapy is effective. This is a particularly important principle when therapy is empirical and the etiologic pathogen(s) is not known or its sensitivities are not known. Antibacterial therapy active against a target pathogen is remarkably effective. Signs, symptoms, and biomarkers of most types of infections should improve dramatically within 1 to 2 days after the initiation of active therapy. The key is to determine which signs, symptoms, or biomarkers of infection are present at baseline, and to use changes in those biomarkers to guide subsequent therapeutic decisions.

Fever and leukocytosis are among the oldest clinical biomarkers used by clinicians to determine response to antimicrobial therapy. Both are frequently used, and resolution of both can be extremely informative that the infection is being adequately treated with the selected regimen. A more recently studied biomarker for enabling early cessation (or avoidance entirely) of antibacterial therapy is procalcitonin. Dozens of randomized controlled trials have investigated the impact of making the procalcitonin result available to treating providers or not. These studies have evaluated the ability of procalcitonin to help avoid antibiotic prescriptions for patients without sepsis or to help with earlier cessation of antibiotics in sicker patients with sepsis. Studies have been conducted in a variety of care settings, including the emergency department, inpatient wards, and ICUs.

Recent meta-analyses of 26 such trials found that incorporation of procalcitonin into therapeutic decision making resulted in a >25% reduction in the duration of antibiotic therapy, significant reduction in antibiotic-related adverse events, and not only no worse outcomes but actually significantly improved mortality in the face of shorter courses of antibiotic therapy.[60,61] In practice, ID experts need to help steward use of the procalcitonin, as there may be a tendency for providers to ignore negative procalcitonin results as being spurious but use a positive procalcitonin to justify antibiotic use. If stewarded properly and incorporated as an evidence-based biomarker, procalcitonin can be a useful tool for antibiotic stewardship teams to provide reassurance to clinicians that it is safe to stop antibiotics early in sick patients or potentially avoid antibiotics entirely for less sick patients.

Patients responding to initial empirical therapy with improvements in signs, symptoms, or biomarkers of infection can be continued on that therapy until microbial information becomes available to enable tailoring therapy. For patients who are responding to therapy but never have a microbial etiology identified—common for lung, abdominal, or skin infections—the empirical therapy can be continued to complete a defined course of therapy (more on duration of therapy later). Note that in this case it is still reasonable to deescalate empirical therapy by stopping agents targeting highly resistant pathogens if they have not grown in cultures (e.g., stop empirical vancomycin if methicillin-resistant *S. aureus* does not grow).

However, if the patient is not responding to therapy within 1 or 2 days, it may be necessary to consider alternative therapeutic or diagnostic interventions even in the absence of knowledge of the microbial etiology of infection.

8. If Therapy Is Not Working, Consider Source Control and Alternative Diagnoses Before Blaming Resistance and Broadening Therapy

If an antimicrobial regimen has not resulted in improvement in fever curve, white blood cell count, purulent secretions, signs of inflammation (rubor, tumor, dolor, calor), procalcitonin level, and so forth, within a day or two, the treatment decision may need to be reconsidered. Although the treating clinician's initial tendency is typically to blame "antibiotic resistance" and seek to broaden antimicrobial therapy, two alternative causes should be considered before reflexively broadening therapy.

One possibility is that adequate source control has not been obtained. Adequacy of source control, such as removing drainable pus and removing foreign materials or other niduses of infection, is the cornerstone of successful management of infections.[62–64] Failure of source control independently increases treatment failure and mortality irrespective of antimicrobial therapy.[62,65–75] Adequacy of source control must always be considered when patients do not appear to be responding to antimicrobial therapy. Investigation for source control may require imaging to establish whether drainage has been achieved or if there is a nidus of infection that is obscure externally and is responsible for treatment failure (e.g., occult subcutaneous abscess in a patient failing cellulitis therapy, new abscess formation during treatment of an intraabdominal infection, empyema in a patient with community-acquired pneumonia, failure to remove a central line in a bacteremic or fungemic patient). Broadening therapy will not result in clinical response in the absence of adequate source control.

A second explanation for lack of adequate response to therapy is incorrectly attributing the patient's clinical condition to a bacterial infection. Thus, for patients who are not responding to antimicrobial therapy, in addition to assessing source control, the differential diagnosis should be reconsidered. Common infections, such as community-acquired pneumonia and cellulitis, have signs and symptoms that can be strikingly similar to noninfectious ailments. Indeed, patients are commonly misdiagnosed with cellulitis and instead have a variety of inflammatory skin disorders ranging from venous stasis to contact dermatitis/allergic reactions to necrobiosis lipoidica diabeticorum.[76,77] Similarly, bacterial pneumonia can be clinically mimicked by pulmonary edema, pulmonary embolism, reactive airways disease, aspiration pneumonitis, viral pneumonia, and so forth.

Other frequently encountered noninfectious causes of fever not responding to antibiotics include drug fever (which may in fact be caused by the antibiotic, an unfortunate tautology that can lead to prolonged and futile antibiotic courses), malignancy, and rheumatologic disorders that mimic infections. Thus failure of therapy should prompt a reevaluation to ensure that the patient really does have a bacterial infection and not a noninfectious mimicker or a viral infection.

For patients who do not appear to be responding to antimicrobial therapy within the first day or two, a full reevaluation of source control and the differential diagnosis should precede reflexive changes to broaden therapy based on fears of antibiotic resistance or concerns for infections caused by organisms of another kingdom (e.g., fungi).

9. Distinguish New Infection From Failure of Initial Therapy

Patients may suffer from multiple, serial infections while in health care settings. Thus a patient who has evidence of clinical response to initial therapy, with resolution of signs and symptoms of infection, and who then begins to spike fevers anew, should be evaluated differently than a patient who never responds to initial therapy (the latter issue is the focus of Principle 8). New onset of infectious signs, symptoms, and biomarkers after resolution of prior infection should raise the concern of a new infection rather than persistence of the original infection.

Rarely, recrudescence of signs and symptoms may reflect emergence of antibiotic resistance on therapy from the initial pathogen—this may be seen more with specific bacterial pathogens, such as *Acinetobacter*, than with others.[78] In any event an initial apparent response to infection followed days or weeks later by new onset of infectious signs or symptoms should prompt a complete reevaluation of the patient for a new infection, including reculturing and imaging if necessary. In such patients it is generally reasonable to broaden therapy to cover highly resistant pathogens. Such patients have been treated with prior, recent courses of antibiotics, and have a higher risk of being infected by antibiotic-resistant pathogens.

When changing antibacterial therapies due to breakthrough infection or lack of response to initial therapy, it is generally advisable to change one antibiotic at a time. Such a change enables determination of which new drug is likely the cause of any improvement in signs, symptoms, and/or biomarkers of infection. Therapy then can be tailored/narrowed

TABLE 17.2 Short-Course Therapy Is Equally Effective as Longer Therapy for Many Infections

DISEASE	SHORT COURSE STUDIED (DAYS)	LONG COURSE STUDIED (DAYS)	OUTCOME
Chronic bronchitis and obstructive pulmonary disease, acute exacerbations[a]	≤5	≥7	Equivalent
Intraabdominal infection	4	10	Equivalent
Neutropenic fever	Until afebrile and stable	Until afebrile, stable, and nonneutropenic	Equivalent
Osteomyelitis, chronic	42	84	Equivalent
Pneumonia, community-acquired	3–5	7–14	Equivalent
Pneumonia, nosocomial (including ventilator-associated)	≤8	10–15	Equivalent
Pyelonephritis	5–7	10–14	Equivalent
Skin infections (cellulitis, major abscesses, wound infections)	5–6	10–14	Equivalent
Sinusitis, acute bacterial[a]	5	10	Equivalent

[a]Most patients with these diseases do not require antibiotics at all. Nevertheless, if they are prescribed, there is no advantage to prescribing longer courses.
Data from references 79 and 154.

based on that knowledge. However, in patients who are critically ill, changing multiple antibiotics at one time may be necessary, especially for patients who are hemodynamically unstable.

10. The Duration of Therapy Should Be Evidence Based and Short Course Whenever Possible

Antibiotics should be administered only for as long as necessary to optimize cure rates. Unfortunately, even in the era of evidence-based medicine, the duration of most courses of antibiotic therapy can be traced back to Constantine the Great, who decreed in 321 CE that the week would consist of 7 days.[79] This approximately 1700-year-old decree remains the primary driver for many antibiotic courses, which are proffered in 7-day increments.

Fortunately, in recent years numerous clinical trials have been conducted comparing long- to short-course antibiotic therapy, with the short courses often not based on 7-day increments. In every such trial ever conducted for patients with specific types of infections (Table 17.2), short-course therapy was found to be equally effective as longer-course therapy, and in several studies with diminished selection for antibiotic resistance.[79]

Notable exceptions to short-course therapy include otitis media in children younger than 2 years[80] and penicillin—but not cephalosporin—therapy for streptococcal pharyngitis.[81,82] In both of these cases, cure rates were lower for short-course antibiotic therapy. The reasons for the failure of the short-course therapy for these specific disease/demographics are not entirely clear. The primary purpose of antibacterial therapy in the treatment of streptococcal pharyngitis is the prevention of rheumatic fever, which requires eradication of the organism. Despite diminished rate of clinical cure at end of therapy with short-course penicillin, rheumatic fever rates were not higher in these studies. However, the rate of rheumatic fever is low, and the studies were not powered for this end point.

Shorter courses of antibiotics reduce the risk of side effects from the antibiotics and may result in decreased impact on the microbiome, avoiding superinfections and possibly other metabolic consequences of microbiome disruption that are under investigation.[83–85] Thus it is no longer necessary—and indeed no longer appropriate—to choose durations of antibiotics for many infections based on 7-day increments. Wherever possible, treatment durations should be short courses, based on trial data showing equivalent efficacy to longer courses (see Table 17.2).

CONTROVERSIAL PRINCIPLES OF ANTIBIOTIC THERAPY

The Greatly Misunderstood Dogma of Bactericidal Versus Bacteriostatic

One of the most pervasive dogmas in the field of ID has been that antimicrobial agents that are "cidal" are more effective than those that are "static." Although it seems intuitive that antibiotics that more rapidly kill bacteria should be more clinically effective, clinical data do not support this assertion. Furthermore, there are a variety of misunderstandings around the meanings of cidal and static.

Static and cidal are relative in vitro terms not based on linkage to any predictive ability of the outcome of infections in vivo. Contrary to common belief, static antibiotics *do* kill bacteria, they just require a higher concentration to achieve specific thresholds of bacterial reduction. The formal definition of a cidal antibiotic is one for which the minimum bactericidal concentration (MBC) of the drug is ≤4-fold above the MIC.[86] The MBC is the concentration of the drug that results in a 1000-fold reduction in bacterial density at 24 hours of in vitro growth in specific media, at a specific temperature, and a specific carbon dioxide concentration. The MIC is the concentration of the antibiotic that inhibits visible growth at 24 hours.

Thus antibiotics that achieve a >1000-fold reduction in bacterial density at a concentration that is 8-fold above its MIC, or 500-fold reduction in bacterial density at 4-fold above its MIC, are considered static despite clearly killing bacteria. All currently available antibiotics that are considered static do kill bacteria in vitro, just with the MBC-to-MIC ratio greater than would be for cidal agents.

Given that these definitions are based on in vitro conventions and not based on specific clinical principles, perhaps it is not surprising that there is no clinical evidence of benefit of cidal agents over static agents. A systematic literature review identified 56 randomized controlled trials published since 1985 that compared the efficacy of static versus cidal antibiotics head to head for patients with invasive bacterial infection.[87] Forty-nine of the trials found no significant difference in efficacy between static versus cidal antibiotics, including for highly lethal infections of critically ill patients, such as typhoid fever, severe pneumonia, severe sepsis, and so forth.

The exceptions that did find a clinical efficacy difference between static and cidal agents disprove the rule that cidal agents are intrinsically more effective. In fact, six trials found the static agent linezolid to be superior in efficacy to cidal agents (vancomycin or cephalosporins).[87] In contrast, only one trial found a cidal antibiotic superior in efficacy to a static agent. That trial compared tigecycline versus imipenem for the treatment of ventilator-associated pneumonia and found that tigecycline was inferior.[88] However, subsequent pharmacologic analysis determined that the tigecycline dose used in the trial was too low, resulting in inadequate drug levels compared with the susceptibility of bacteria causing the infections.[89] When a subsequent trial was conducted with double the dose of tigecycline, tigecycline was similar in efficacy to imipenem for the same disease.[90]

Similar conclusions are drawn from studies in which rapidly cidal antimicrobial strategies have been compared with agents that are more slowly cidal. Daptomycin is one of the most rapidly bactericidal agents known, yet it was not superior in efficacy to the slowly cidal agent vancomycin for the treatment of staphylococcal bacteremia or right-sided endocarditis.[91]

Similarly, addition of aminoglycosides to β-lactam therapy results in a synergistic, marked improvement in the rapidity of kill of staphylococci and more rapid clearance of bacteria from the blood in patients.[92–94] However, meta-analysis of several studies comparing β-lactam monotherapy with combination therapy with β-lactam and aminoglycosides found no difference in clinical cure or mortality, whereas nephrotoxicity was worse.[94] Thus there is no clear, overall clinical benefit apparent from the increased rate of bacterial kill. Nevertheless, addition of aminoglycosides for several days—keeping the aminoglycoside duration short to minimize nephrotoxicity—may be a rational approach for patients with refractory staphylococcal bacteremia.

The last frontier of the static versus cidal debate lies in the territory of bacterial endocarditis. In the 1950s Finland found that static agents, including a variety of tetracyclines and macrolides, resulted in poor outcomes when used to treat endocarditis.[95,96] Similarly, a rabbit study and several case series suggested excess treatment failure rates when clindamycin was used to treat aortic valve staphylococcal endocarditis.[97] These studies led to the belief that static agents generally are inferior as therapeutic agents for endocarditis.

However, there are a number of problems with such assertions. Tetracyclines and macrolides achieve very low blood concentrations. Thus, based just on pharmacologic principles (low blood concentrations), having nothing to do with how rapidly they kill microbes, these agents would not be anticipated to be desirable agents to use to treat high-grade bloodstream infections. Furthermore, clindamycin is static for anaerobic bacteria but is actually cidal for staphylococci.[98] Thus, if clindamycin performs poorly in treating staphylococcal endocarditis, it is actually a negative mark against a cidal agent, not a static agent.

In contrast, although never studied in a randomized controlled trial, the static agent linezolid, which has favorable bloodstream pharmacokinetics, has been found to result in high cure rates for bacterial endocarditis in a number of published case series.[99–101] Finally, the rapidly cidal antibiotic daptomycin has been described to result in worrisome failure rates in some patients with left-sided endocarditis.[102]

Overall, there is no evidence that cidal antibiotics are intrinsically more clinically effective than static antibiotics. The majority of studies comparing static and cidal agents head to head for the treatment of infections have found no difference in clinical outcomes or mortality. When differences have been found in such head-to-head studies, they have usually favored the static agent as being clinically superior to the cidal agent. When static agents have been found inferior, the explanation appears to be more likely inadequate dosing and/or achievable levels at the site of infection, and not rapidity of kill of the microbe. Thus pharmacokinetic-pharmacodynamic principles and achievement of adequate levels of the drug at the site of infection appear to be more important parameters than static versus cidal properties for predicting clinical antimicrobial efficacy.

Combination Therapy: The Good, the Bad, and the Ugly

Whether or not combination antibiotic therapy is appropriate is highly dependent on the intended purpose. There are general circumstances where combination therapy, if judiciously applied, is rational and appropriate (the "good"). There are also settings in which combination therapy is wasteful or toxic (the "bad"). Finally, there are settings in which there may be theoretical but unproven advantages of combination therapy, leaving the clinician in a quandary as to whether combination therapy should be used or not (the "ugly").

The Good

There are three general settings, elaborated as follows, in which combination antimicrobial therapy is known to be advantageous. The first is in the empirical setting, when treating an infection for which the potential etiologic microbial species are sufficiently broad that one agent is unlikely to cover all of them. The second is for preventing the emergence of resistance in specific clinical settings. The third is a rare circumstance in which two active agents are known to result in superior clinical outcomes compared with a single active agent against a susceptible organism.

The Good #1: Ensuring Adequate Empirical Coverage

Many types of serious infections may require more than one antimicrobial agent to be started empirically to ensure coverage of the potential etiologic pathogens. For example, when treating community-acquired pneumonia, a second agent (macrolide or doxycycline) is added to cover atypical organisms that the backbone β-lactam antibiotic will not cover. If a microbial etiology is identified, the empirical therapy can then be narrowed to targeted, definitive therapy.

Intraabdominal infections are polymicrobial in nature, caused by both aerobic and anaerobic bacteria in the gut. Agents that are superior for killing the aerobes and facultative anaerobes (e.g., cephalosporins) may not have adequate activity to cover the obligate anaerobes as well as metronidazole. Thus combination therapy is appropriate for the treatment of intraabdominal infections, although there are some single agents that when used are broad enough to provide adequate coverage as monotherapy (e.g., piperacillin-tazobactam, carbapenems).

For empirical therapy, the breadth of coverage warranted depends on the local resistance patterns among the likely pathogens. For routine nosocomial pneumonia coverage in a patient not exposed to prior antibiotics, monotherapy with a third- or fourth-generation cephalosporin, piperacillin-tazobactam, or meropenem is likely to cover most gram-negative bacterial pathogens encountered. Use of more than one agent should not be necessary in most situations. In contrast, in a specific ICU or ward where infections caused by organisms resistant to these β-lactam agents are regularly encountered, it may be reasonable to start two empirical antibiotics to try to ensure at least one active agent is being administered. This practice may be referred to colloquially as the use of "double gram-negative coverage." Dual-agent empirical therapy also may be reasonable to start in a patient with a history of recent (e.g., within 90 days) exposure to broad-spectrum antibiotics, or known colonization by resistant pathogens.

If empirical multiple-agent therapy is warranted for the possibility of encountering resistant gram-negative bacilli, it is important to consider the potential overlap in the coverage. An often used pairing is a cephalosporin (e.g., ceftazidime or cefepime) plus a fluoroquinolone. Unfortunately, bacterial strains resistant to a third- or fourth-generation cephalosporins are often resistant to fluoroquinolones as well. Thus very little may be gained by adding a fluoroquinolone to an advanced-generation cephalosporin. When using empirical double coverage for the purpose of trying to assure that at least one agent is active, the local antibiogram should guide selection of the second agent. Generally speaking, an aminoglycoside may offer less overlapping coverage to β-lactams than fluoroquinolones and hence increase the likelihood that at least one active agent is being administered.

When multiple empirical agents are initiated, the goal should be to deescalate therapy by stopping all but one of the empirical agents once a microbial etiology is known. Unfortunately, in practice clinicians often continue multiple-agent empirical therapy even after a microbe is identified and therapy could be deescalated to target that pathogen. This continuation may occur because clinicians forget that multiple antibiotics are being administered or because they do not wish to "rock the boat" if a patient is improving on the initial therapy. In either case the role of the ID expert is to prompt deescalation. Continuation of multiple agents in this setting exposes the patient to side effects and both the patient and society to selection for antibiotic resistance from the unneeded additional agents.

One specific scenario in which the treating clinician may make a conscious choice to continue two active agents after an organism is identified is in the treatment of *Pseudomonas aeruginosa*. As discussed further, this practice is generally unnecessary and inappropriate.

The Good #2: Preventing Resistance Emergence

TB is an unusual infection. The etiologic organism has no environmental reservoir, is found only in mammals (and especially humans), grows exceedingly slowly, and spends much of its time in a nonreplicating persister state in vivo in the infected host, making it invisible to most antibiotics. It also can achieve very high bacterial densities (particularly in cavitary disease) such that spontaneous mutations can lead to emergence of resistance on therapy. Thus combination therapy is required for

active TB, not only to ensure initial therapy is adequate but also to ensure resistance does not emerge on therapy. Indeed, active TB is the classic bacterial example for which use of combination therapy prevents the emergence of resistance on therapy, which would result in clinical failure.

The other major category of disease for which combination therapy is required to prevent the emergence of resistance on therapy is viral infections. Infections caused by the human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are the classic paradigms for combination therapy to prevent emergence of resistance. Resistance is catastrophic in these settings, as they convert a treatable (HIV) and curable (HCV) infection into potentially fatal illnesses.

However, as will be discussed further, whether or not combination therapy prevents the emergence of resistance among typical, pyogenic bacterial infections remains unclear. TB, HIV, and HCV are examples of infections for which the pathogens are not normal microbiota, have no environmental reservoir, and have very high rates of emergence of resistance while on therapy. These factors fundamentally distinguish TB, HIV, and HCV from typical pyogenic bacterial infections. As will be discussed further, it is not safe to extrapolate the appropriate use of combination chemotherapy for these three infections as a model for the use of combination regimens to prevention of emergence of antibiotic resistance among typical bacterial pathogens.

The Good #3: Improving Cure

There are a select few bacterial infections for which use of combination therapy with more than one active agent against susceptible bacteria is likely to result in superior clinical cure rates than use of a single active agent. For bacteria these diseases can be separated into one of two categories: slow-growing infections often caused by intracellular pathogens or rapidly growing infections associated with exotoxin-mediated tissue destruction. Combination therapy is more commonly needed to optimize cure for complex eukaryotic pathogens, particularly those with multiple phases of their life cycle.

Slow-growing infections/nonreplicating persisters. One type of bacterial infections for which combination therapy may result in superior outcomes are those in which nonreplicating persister organisms are fundamental to the pathogenesis of the infections. Nonreplicating persister bacteria shut down metabolic pathways, making them invisible to most antibiotics because they are not expressing the metabolic targets of the antibiotics.[103–105] TB is the classic example. Rifampin and pyrazinamide are capable of killing nonreplicating persister bacteria more effectively than other agents. As a result, the addition of rifampin and pyrazinamide allowed shortening courses of therapies from years to 6 months for active pulmonary TB.[106,107] Thus combination therapy for TB illustrates all three potential advantages: (1) ensuring initial empirical therapy is active, (2) preventing the emergence of resistance, and (3) improving clinical cure with a shorter duration of therapy.

Similarly, chronic osteomyelitis has a relatively high rate of relapse after an initial course of therapy. Multiple randomized controlled clinical trials and observational studies have found that addition of rifampin to backbone antibiotic therapy decreases the rate of late relapses of bone and prosthetic joint infections.[41,108]

Another example where combination therapy results in superior outcomes is the treatment of endocarditis caused by refractory, slow-growing bacteria, including *Enterococcus* spp. or penicillin-resistant streptococci. Addition to penicillin/ampicillin of gentamicin, or more recently ceftriaxone, results in superior cure and survival rates for such infections.[109]

Combination therapy also results in superior cure rates for the slow-growing, intracellular pathogen *Brucella*, for which single therapy is not able to effectively kill the pathogen.[110] Another atypical, intracellular pathogen for which combination therapy results in superior outcomes is *Coxiella burnetii*. Addition of hydroxychloroquine to antibiotic therapy improves cure from *Coxiella* infection by a specific physiologic mechanism.[111] Hydroxychloroquine alkalinizes the phagolysosome that *Coxiella* hides within, making the organism more susceptible to antibacterial therapy that it would otherwise resist. Without this adjunctive therapy, the pH in the phagolysosomal becomes highly acidic, which neutralizes the activity of the antibiotics that would otherwise be effective to kill the pathogen.

Exotoxin-mediated necrotizing infections. The opposite spectrum of bacterial diseases for which combination therapy may improve cure are exotoxin-mediated diseases that are extremely rapid, destructive, and aggressive. Necrotizing fasciitis caused by exotoxin-producing streptococci, or *Clostridium* tissue necrosis, are examples. The theoretical advantage of adding clindamycin or linezolid as a second agent for treating such infections is that these antibiotics act by terminating protein synthesis inside the bacteria. As such, they can decrease the production of the exotoxin causing tissue degeneration, even while the bacteria are being killed. Adding protein synthesis inhibitors (clindamycin or linezolid) to backbone antibacterial therapy has indeed been shown to result in marked improvement in survival in preclinical models of necrotizing fasciitis and in several retrospective studies in patients as well.[112–115]

However, neither infections caused by slow-growing atypical bacteria or exotoxin-producing bacteria are reflective of the pathogenesis of most typical bacterial infections. As discussed later, it is not necessarily safe to extrapolate the salutary effects of combination therapy for these diseases to typical, pyogenic bacterial infections.

Eukaryotic infections. In contrast to bacteria, there are multiple examples of infections caused by eukaryotic pathogens where combination therapy is beneficial. Cryptococcal meningitis is an example of a fungal infection for which a randomized controlled trial has demonstrated that amphotericin plus 5-fluorocytosine resulted in superior clinical outcome compared with amphotericin alone.[116,117]

Dual therapy for protozoal parasites is necessary in some cases because the two agents kill organisms in different phases of the parasitic life cycle. For example, primaquine is added to backbone therapy for *Plasmodium vivax* or *Plasmodium ovale* infection because it kills hepatic-phase hypnozoites that are not killed by other agents and cause late relapse if not treated. Similarly, acute amebic colitis can be treated with metronidazole but must be followed by a luminicidal agent (e.g., iodoquinol or paromomycin) that kills encysted organisms in the bowel lumen that are metabolically inactive and not killed by the metronidazole.

Another example of combination therapy for parasitic infections is the addition of doxycycline to antiparasitic therapy for nematode infections. Pathogenic nematodes may harbor commensal bacteria, such as *Wolbachia*, which are essential for the parasite's viability or fertility. Treatment with antibacterial agents, including tetracyclines or possibly rifampin, has been shown in some studies to enhance the ability of microfilaricidal agents (e.g., ivermectin or albendazole) to kill adult worms, reducing parasite burden.[118,119]

A final example of dual therapy for parasitic infections is neurocysticercosis. In a randomized controlled trial, combination albendazole plus praziquantel therapy resulted in superior outcomes in patients with more than two active lesions of neurocysticercosis.[120,121] The combination of albendazole and praziquantel results in dual mechanism killing of the parasite. There is also a pharmacokinetic interaction resulting in higher drug levels when the combination is used,[122] akin to use of ritonavir to boot protease inhibitor levels for the treatment of HIV. This enhancement in drug levels may improve penetration of the cysticidal agents into the CNS and the cysts within the CNS, which reflect sequestered sites within a sequestered site.

"The Bad": Redundant Definitive Therapy for Typical Bacterial Infections

In contrast to the earlier unusual infections, there are very little data in favor of using dual active agents as definitive therapy for acute, pyogenic bacterial infections. During most acute pyogenic bacterial infections, the organisms are in planktonic growth, not in multiple phases of a life cycle. There are no commensal organisms within the bacteria to kill, and pharmacology and killing activity of effective antibiotics are good. Thus there is no clear rationale for dual therapy.

The best studied organism in this regard is *P. aeruginosa*. There have been dozens of clinical investigations, including randomized controlled trials, seeking to determine if dual definitive therapy results in superior cure rates for infections caused by *P. aeruginosa* compared with monotherapy. Yet decades and dozens of studies later, there remains no

compelling evidence that dual therapy results in superior outcomes.[123] Similarly, two recent randomized controlled trials found no evidence of benefit of combination therapy for carbapenem-resistant *Acinetobacter* infections.[124,125]

Randomized controlled trials and meta-analyses of combination therapy for severe infections/sepsis have also found no advantage of dual active therapy compared with monotherapy.[123,126,127] However, dual therapy is more likely to result in drug toxicity and harm and will cause selection for resistance to two drugs instead of one in the microbiome and environment. Hence, outside of very specific infections, such as those described earlier, evidence does not currently exist to support the routine use of dual active therapy for most acute bacterial infections.

"The Ugly": Imperfect Data and Controversy

Controversy Over Superior Outcomes With Combination Therapy for Some Infections

The one acute pyogenic infection for which available data suggests dual active therapy may result in superior clinical outcomes is bacteremia due to *Klebsiella pneumoniae*. However, the data supporting this conclusion are of poor quality and are mixed. In two retrospective case series of patients with *Klebsiella* bacteremia, dual therapy (β-lactam plus aminoglycoside) resulted in far superior clinical response and survival compared with monotherapy.[128,129] Similarly, in more recent retrospective series, patients with bacteremia due to carbapenem-resistant *K. pneumoniae* (CRKP) had higher survival rates when treated with a variety of combination regimens compared with monotherapy, even when the monotherapy was active against the etiologic pathogen in vitro.[130–132] However, these data are retrospective, and the monotherapy for CRKP was colistin or tigecycline, which are known to be inferior agents. Furthermore, other case series have not found improved outcomes of *Klebsiella* bacteremia treated with dual versus monotherapy when adjusting for disease severity.[133,134] Thus the data are mixed as to the benefit of dual therapy for *Klebsiella* bacteremia, and a definitive trial would be required to settle the question.

The major impetus for combination therapy in recent years has been in the world of fungal infections, outcomes of which are traditionally worse with antimicrobial therapy than typical bacterial infections. Aside from cryptococcal meningitis, randomized controlled trials are lacking demonstrating superiority of combination therapy for invasive fungal infections. For candidemia, the combination of fluconazole plus amphotericin was compared with fluconazole alone in a randomized controlled trial.[135] The study found no significant difference in clinical cure or mortality between combination therapy or monotherapy, although patients with an intermediate severity of illness may have had benefit from combination therapy in a hypothesis-generating, post hoc analysis.[135]

Invasive aspergillosis has also been subjected to study of combination therapy. Bolstered by preclinical data and several retrospective studies suggesting benefit of combination therapy, Marr and colleagues[136] randomized patients with probable or proven invasive aspergillosis in the setting of hematologic malignancy to treatment with voriconazole plus placebo versus voriconazole plus anidulafungin.[136] The study found a trend to improved cure and survival in the combination therapy arm ($P = .08$). In a post hoc analysis of patients who had a positive galactomannan, and hence confirmed invasive aspergillosis, the survival benefit of combination therapy achieved statistical significance ($P = .04$). Doubters of combination therapy have chosen to interpret the study as negative and discouraged use of combination therapy. Supporters of combination therapy point to the near statistical miss on the primary end point, the well-diverged Kaplan-Meier survival curves, and the statistical significance in the galactomannan-positive population, which is enriched for true invasive aspergillosis. Furthermore, there was no apparent toxicity detriment of the combination regimen. Thus the study can be interpreted either way. What is clear is that any advantage of dual therapy for invasive aspergillosis is incremental rather than transformational.

Controversy Over Preventing Emergence of Resistance

Another area of confusion for combination therapy, alluded to earlier, is the potential to reduce emergence of resistance among typical, pyogenic bacterial infections. Some experts have advocated for this approach based on in vitro modeling data and preclinical animal studies, and extrapolating from infections such as TB.[137,138] It is clear that bacterial growth in the presence of two antibiotics in vitro can suppress the emergence of resistant subpopulations. Thus it may be that combination therapy can reduce the emergence of resistance among bacteria at the site of infection.

However, what remains unclear is the impact of combination therapy on the microbiome and on the environment. It is conceivable that treating with two antibiotics to prevent resistance among typical bacterial pathogens would amount to a pyrrhic victory, reducing resistance at the site of infection in the short term but resulting in selection for resistance in the microbiome and environment to two antibiotics rather than one, triggering loss of efficacy for both drugs in the long run. As mentioned, this problem does not exist for infections such as TB, HIV, or HCV because these organisms are not part of the microbiome and have no environmental reservoir. Clinical data are not available to address this issue at the current time, and the question remains controversial.

CONCLUSIONS

Antibiotics are miracle drugs that fundamentally altered the practice of medicine. Yet their incredible power to cure and heal is fleeting. The more we use them, the faster we lose them. And the transmissibility of resistance results in a shared risk across society. When any one of us uses an antibiotic, it affects the future ability of that drug to be available as an effective treatment for everyone else. No one has a right to waste this shared societal trust.

One of the founding fathers of antiinfective chemotherapy, Dr. Walsh McDermott, wrote: "It is not too much to state that the introduction of [antibiotics] has represented a force for change in the 20th century of the same general kind as James Watt's modification of the steam engine did in the 18th. The crossing of the historic watershed could be felt at the time. One day we could not save lives, or hardly any lives; on the very next day we could do so across a wide spectrum of diseases. This was an awesome acquisition of power."[15]

Experts with training in ID bear the burden of being at the front lines in this struggle to heal and cure, while preserving this awesome power and preventing its disruption of our ancestral microbiome in the process.

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18 Molecular Mechanisms of Antibiotic Resistance in Bacteria

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MOLECULAR GENETICS OF ANTIBIOTIC RESISTANCE

Genetic variability is essential for microbial evolution to occur. The fitness of a microorganism depends on its capacity to adapt to changing environmental conditions.[1] Antimicrobial agents exert strong selective pressures on bacterial populations, favoring organisms that can resist them.[1,2] Genetic variability may occur by a variety of mechanisms. Point mutations may occur at a nucleotide base pair (bp), which is referred to as *microevolutionary change.* These mutations can alter enzyme substrate specificity or the target binding site of an antimicrobial agent, interfering with its activity. Point mutations at crucial locations on "old" β-lactamase genes (e.g., genes for Temoneira-1 [TEM-1], sulfhydryl variable-1 [SHV-1]) are primarily responsible for the remarkable array of newly recognized extended-spectrum β-lactamases (ESBLs).[3,4]

A second level of genomic variability in bacteria is referred to as a *macroevolutionary change* and results in whole-scale rearrangements of multiple nucleotide sequences as a single event. These rearrangements may include inversions, duplications, insertions, deletions, or the transposition of segments of DNA from one location of a bacterial chromosome or plasmid to another location in the genome. These large-scale alterations of the bacterial genome are frequently generated by specialized genetic elements such as *integrons, transposons,* or *insertion sequences,* which have the capacity to move and insert independently throughout the bacterial genome.[2]

A third level of genetic variability in bacteria is created by the acquisition of large segments of foreign DNA carried by resistance (R) plasmids, bacteriophages, naked sequences of DNA, or specialized transposable genetic elements known as *integrative and conjugative elements* (ICE) from other bacteria.[2] These events are termed lateral or horizontal gene transfer and are now appreciated to be a frequent event, especially for naturally competent bacteria (e.g., pneumococci and gonococci) that can take up exogenous DNA from the environment. Large-scale transfer of resistance genes for different classes of antibiotics as a single event also occurs among enteric bacteria within the gut microbiota by conjugal transfer of large R plasmids. Whole-scale acquisition of many antibiotic-resistance genes at the same time is referred to as "evolution by quantum leaps,"[5] and provides a major advantage for bacteria residing in patients receiving multiple courses of different classes of antibiotics.

Inheritance of foreign DNA further contributes to bacterial genetic variability and its capacity to respond to selection pressures imposed by antimicrobial agents.[3] These mechanisms endow bacteria with the seemingly unlimited capacity to develop resistance to any antimicrobial agent (Fig. 18.1). Examples of plasmid-mediated carbapenemase-producing *Klebsiella pneumoniae*,[6] vancomycin-resistant *Staphylococcus aureus* (VRSA) and daptomycin-resistant *S. aureus,*[7,8] multidrug-resistant *Escherichia coli* plasmids now with polymyxin/colistin resistance genes,[9] and transferable quinolone resistance in enterobacteria[10] attest to the capacity of microorganisms to adapt to environmental stresses such as antibiotic exposure. When an antibiotic-resistance gene evolves, this gene can spread between bacteria by transformation, transduction, conjugation, or transposition. Favored clones of bacteria may proliferate in the microbiota of patients who receive antibiotics.[11]

Antibiotic-resistance genes existed well before the introduction of antimicrobials for treatment for human infections and can be found within bacterial genomes preserved in Arctic region permafrost samples untouched by human hands for over 30,000 years.[11] Antibiotics are often synthesized by antibiotic-producing bacteria when they are about to enter the late static growth phase and are about to become dormant or sporulate. Antibiotic-producing bacteria are resistant to the antibiotics they produce. Therefore, antibiotics left in the microenvironment by dormant bacteria are avoided as potentially toxic to competitors yet serve as a ready source of carbon (food) for the next generation of the antibiotic-producing bacterial strain once the growth phase begins again. Environmental levels of multiple classes of antimicrobial agents are now so common in soil and water samples that multiple bacterial genera have strains that subsist entirely on antibiotics as their sole carbon source.[12] These bacteria express remarkably high levels of multiple antibiotic resistance to a wide array of antibiotic classes. Aquatic environments are particularly rich with bacterial populations replete with antibiotic-resistance genes.[12] Such environmental bacterial strains likely provide potential human pathogens with a source of novel antibiotic-resistance genes.[12,13] Selection pressures placed on microbial populations by antibiotics favor the expansion of strains that have the capacity to resist the inhibitory effects of antibiotics.

These resistant populations proliferate and spread antibiotic-resistance genes vertically to subsequent generations and horizontally to susceptible strains of related bacteria, or even between species or different genera.[14] Although some antibiotic-resistance genes place a metabolic "burden" on bacteria, many microorganisms evolved strategies to limit this cost by repressing gene expression when not needed or by phase variation. This allows favorable, but sometimes "costly," antibiotic-resistance genes to be held in reserve in the absence of antibiotic selection pressure yet express their resistance potential on reexposure to antibiotics.[15]

Continuous exposure to foreign DNA within microbial communities is so commonplace that many bacteria have evolved systems to defend their genomes from exogenous DNA, phages, and plasmid insertions. This is accomplished through at least two mechanisms: (1) species- and strain-specific DNA modifying enzymes (e.g., methylation of selected sequences of host DNA into specific patterns) and restriction enzymes that survey cellular host DNA and degrade foreign DNA that lacks appropriate DNA modification sequences; and (2) a type of adaptive defense system against foreign DNA known as CRISPR (clustered regularly interspaced short palindromic repeats).[16] CRISPRs are detectable in nearly 50% of all bacterial genomes, and this genetic element protects their genomes from attack by foreign DNA during transformation, phage invasion, or plasmid insertion. This same CRISPR-associated caspase 9 system in bacteria has become an indispensable genetic tool for gene insertion and editing by molecular biologists doing animal and human genetic studies.[17]

The mechanism of protection in bacteria is mediated by insertion of small sequences of the mobilized invading DNA between palindromic repeats within the CRISPR. On reexposure to similar DNA sequences from phage or invading bacteria, the existing sequence within the CRISPR is transcribed into a small RNA (known as crRNA) that associates with CRISPR-associated nucleases and prevents integration of the targeted foreign DNA.

Maintaining the fidelity of the host genome, while permitting limited variation by microevolutionary and macroevolutionary changes, allows pathogens to strike a balance between genomic stability and plasticity in rapidly changing microenvironments. Recent evidence in the enterococci indicates that deletion of CRISPR elements is inversely related to multiple antibiotic-resistance development. CRISPR-deficient strains have been selected for, and are particularly well-adapted for,

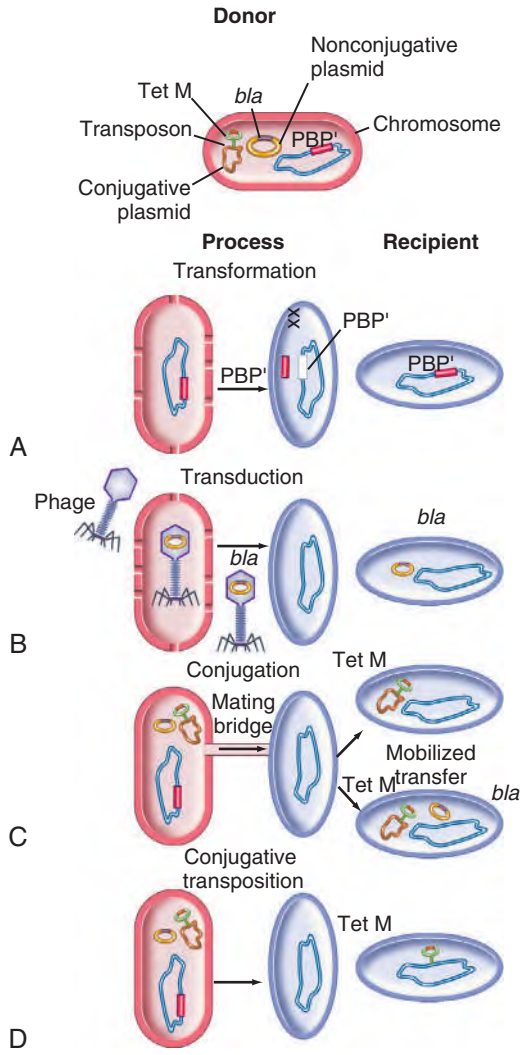

FIG. 18.1 Examples of recombination events and molecular spread of antibiotic-resistance genes. The donor organism depicted here has three antibiotic-resistance genes: the first on the chromosome, designated as *PBP,* a low-affinity penicillin-binding protein; the second (a β-lactamase gene labeled *bla*) on a small nonconjugative plasmid; and the third (*Tet M,* a tetracycline resistance determinant) on a transposon residing on a large self-conjugative plasmid. (A) Genetic exchange may occur by transformation (naked DNA transfer for dying bacteria to a competent recipient). This generally results in transfer of homologous genes located on the chromosome by recombination enzymes (RecA). (B) Transduction also may transfer antibiotic-resistance genes (usually from small plasmids) by imprecise packaging of nucleic acids by transducing bacteriophages. (C) Conjugation is an efficient method of gene transfer, requiring physical contact between donor and recipient. Self-transferable plasmids mediate direct contact by forming a mating bridge between cells. Smaller nonconjugative plasmids might be mobilized in this mating process and be transported into the recipient. (D) Transposons are specialized sequences of DNA that possess their own recombination enzymes (transposases), allowing transposition ("hopping") from one location to another, independent of the recombination enzymes of the host (RecA independent). They may transpose to nonhomologous sequences of DNA and spread antibiotic-resistance genes to multiple plasmids or genomic locations throughout the host. Some transposons possess the ability to move directly from a donor to a recipient, independent of other gene transfer events (conjugative transposons or integrative and conjugative elements).

health care–associated infections. These strains have significantly larger genomes because of insertion of large sequences of DNA, including genes that mediate multiple antibiotic resistance.[18]

Plasmids

Extrachromosomal elements were present in bacteria before the advent of antibiotics.[11,14] The introduction of antibiotics into clinical medicine in the 20th century created selection pressures, however, that favor the dissemination of resistance genes via mobile genetic elements.[2,3,13] Plasmids are particularly well adapted to serve as agents of genetic exchange and resistance-gene dissemination.[1,3] Plasmids are autonomously replicating genetic elements that generally consist of covalently closed, circular, double-stranded DNA molecules ranging from less than 10 kilobase pairs (kbp) to more than 400 kb. They are extremely common in bacteria.[14] Although multiple copies of a specific plasmid, or multiple different plasmids, or both may be found in a single bacterial cell, closely related plasmids often cannot coexist in the same cell. This observation led to a classification scheme of plasmids based on incompatibility (Inc) groups.[1,4]

Plasmids may determine a wide range of functions besides antibiotic resistance, including virulence and metabolic capacities. All plasmids possess an origin of replication for DNA polymerase to bind and replicate plasmid DNA. Plasmids must also retain a set of genes that facilitate their stable maintenance in host bacteria. The transfer of plasmid DNA between bacterial species is a complex process, and the genes needed for transfer (*tra* genes) make conjugative plasmids larger than nonconjugative ones. Some small plasmids may be able to transfer to other bacteria via the use of the conjugation apparatus provided by coresident conjugative plasmids or even conjugative transposons. Many plasmid-encoded functions enable bacterial strains to persist in the environment by resisting noxious agents, such as heavy metals. Mercury released from dental fillings may increase the number of antibiotic-resistant bacteria in the mouth.[19] Compounds such as hexachlorophene and quaternary ammonium compounds are used as topical bacteriostatic agents, and plasmid-mediated resistance to these agents has increased significantly.[2] Recently, mathematical modeling and long-term gene transfer experiments within complex, multispecies environments such as the gut microbiome have confirmed that R plasmids serve another critical role in retention of resistance genes once antibiotic therapy is discontinued.[20] R plasmid horizontal transfer by conjugation occurs at a surprisingly high rate, allowing R plasmids to transfer and continuously "infect" a sufficient proportion of antibiotic-susceptible host bacteria to maintain the resistance genes within bacterial genomes even after the antibiotic has been discontinued. This explains the problem of lingering persistence of resistance genes in patients, even after good antibiotic stewardship programs are working to curtail unnecessary antibiotic use.[20] This chapter primarily focuses on the molecular aspects of antibiotic resistance. Readers interested in practical recommendations to limit the spread of antibiotic-resistance genes in clinical settings are referred to Chapters 14, 51, and 298.

Transposable Genetic Elements

Transposons can translocate as a unit from one area of the bacterial chromosome to another or between the chromosome and plasmid or bacteriophage DNA. Transposable genetic elements possess a specialized system of recombination that is independent of the generalized recombination system that classically permits recombination of largely homologous sequences of DNA by crossover events (the *recA* system of bacteria). The *recA*-independent recombination system ("transposase") of transposable elements usually occurs in a random fashion between nonhomologous sequences of DNA and results in whole-scale modifications of large sequences of DNA as a single event (Fig. 18.2).[2,5]

There are two types of transposable genetic elements, called *transposons* and *insertion sequences,* which have similar characteristics. Bacterial chromosomes are replete with transposable elements.[2,5] These mobile sequences probably play an important physiologic role in genetic variation and evolution in prokaryotic organisms. Transposons differ from insertion sequences in that they encode functional genes that mediate a recognizable phenotypic characteristic, such as an antibiotic-resistance marker. Either element can translocate as an independent

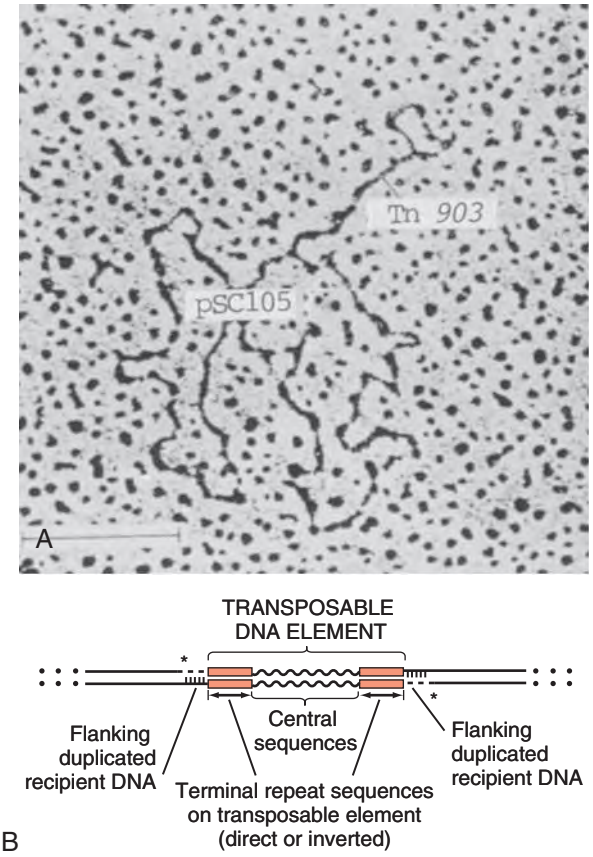

FIG. 18.2 (A) Characteristic appearance of a transposon with electron microscopy showing the stem-loop configuration. The kanamycin resistance transposon Tn*903* is inserted into a small plasmid (pSC105). After denaturation, intrastrand annealing of the complementary 1000-bp, inverted repeat, terminal sequences of the transposon form the stem structure. The kanamycin-resistance gene and the genes necessary for transposition are located in the central loop structure. (B) Structure of a transposable element inserted into a recipient DNA sequence. The transposon (*rectangles* and *wavy lines*) consists of a central sequence containing the phenotypic marker gene(s) (antibiotic-resistance gene) and the "transposase" genes. The terminal-repeat sequences of the transposon flank the central sequences on both sides. Insertion of the transposon results in single-strand, staggered cuts in the recipient DNA *(asterisks).* Subsequent gap-filling DNA synthesis and ligation results in duplication of a short sequence of recipient DNA at either end of the transposon.

unit. Both elements are usually flanked on either end by short identical sequences of DNA in reverse order *(inverted repeats).* These inverted-repeat DNA termini are essential to the transposition process. Transposons (Tn) and insertion sequences (IS) are incapable of autonomous self-replication and must exist on a replicon, such as the chromosome, bacteriophage, or plasmid, to be replicated and maintained in a bacterial population. Some transposons have the capability to move from one bacterium to another without being fixed within a plasmid or bacteriophage. These elements are referred to as *conjugative* transposons, or integrative and conjugative elements (ICE). The ubiquitous transposable element Tn*916* and its derivatives are examples of conjugative transposons and have been found primarily in aerobic and anaerobic gram-positive organisms, although they can also exist in gram-negative bacteria.[2,21]

Transposition usually results in the localized replication of the transposable element from the original donor sequence of DNA and the insertion of a copy of the transposable element into the recipient sequence of DNA (replicative transposition).[1,2] Transposition, like point mutation, is a continuous, ongoing process in bacterial populations. An example of this phenomenon is the spread of a tetracycline-resistance transposon among *Neisseria gonorrhoeae, Mycoplasma hominis,* and *Ureaplasma urealyticum.*[22,23]

An important variant of transposition is "one-ended" transposition, wherein only one end of the transposon is responsible for asymmetrical replication. This type of element is highly efficient in mobilizing resistance genes adjacent to its insertion site. These elements play a prominent role, along with ICE and integrons, in the evolution of large regions of the chromosome where multiple resistance genes accumulate into one set of resistance gene cassettes known as resistance genomic islands.[2] Some of these genomic islands are enormous in size and scope. For example, the AbaR1 genomic island of *Acinetobacter baumannii* is 86 kb long and contains 46 different antimicrobial resistance genes to a wide swath of antimicrobials, antiseptics, and heavy metals.[24] Once genomic resistance islands form, they often persist and gain new genes over time. These islands serve as convenient depots for insertions of new DNA without the risk of insertion into a critical metabolic or structural gene of the bacterial genome.

The "fitness cost" of acquiring new resistance genes is minimized by deposition of genes in genomic resistance islands.[2] However, the metabolic cost of retaining all these accessory, antibiotic-resistance genes by host bacteria remains. The extra metabolic burden is worth it only in the presence of repeated environmental antibiotic exposures, as might exist in hospitals and special care areas, such as the critical care unit, where antibiotic selection pressures favor multidrug-resistant organisms. R plasmids containing antibiotic-resistance genes can be shed by bacteria replicating for prolonged periods in antibiotic-free environments, despite persistence mechanisms employed by many types of R plasmids.[20]

Transposons contribute to the evolution of R plasmids that contain multiple antibiotic-resistance determinants.[21] The spread of carbapenem resistance from metallo-β-lactamases (MBLs) among the Enterobacteriaceae in some European hospitals was facilitated by transposition of resistance genes on integrons to separate plasmids from different bacterial genera of gram-negative bacilli.[25] High-level vancomycin resistance *(vanA)* in enterococci is mediated by a composite transposon that encodes a series of genes needed to express vancomycin resistance.[26] Single transposons may also encode multiple antibiotic-resistance determinants within their inverted-repeat termini.[2]

Although lateral gene transfer is usually more frequent between closely related bacterial strains, genetic exchange of antibiotic-resistance genes does occur between bacteria of widely disparate species and even different genera.[27] Identical aminoglycoside-resistance genes occur in streptococci and *Campylobacter*,[28] and enterococci apparently have acquired aminoglycoside[29] and β-lactam[30] resistance from staphylococci. Given the highly variable environmental selection pressures created by the clinical, agricultural, and industrial use of antibiotics and the plasticity of bacterial genomes, the ongoing evolution of multiresistant species seems inevitable.[31,32]

DNA Integration Elements

The structural genes that mediate antibiotic resistance often are closely linked and may exist in tandem along the bacterial chromosome or plasmid. Genetic analysis of sequences of DNA adjacent to antibiotic-resistance genes has revealed that unique integration units often exist near promoter sites.[32]

These integration elements, called *integrons,*[33] function as recombination "hot spots" for site-specific recombination events between largely nonhomologous sequences of DNA. Integrons facilitate the lateral transfer and integration of antibiotic-resistance genes from mobile gene cassettes. The integron provides its own unique integrase function[33] that facilitates *recA*-independent recombination and a specialized attachment and integration site consisting of a variable length (57–141 bp) but often a 59-bp spacer sequence of highly conserved DNA. This 59-bp element is preserved at the 3′ end of inserted antibiotic-resistance genes.[34,35]

Although these integration elements differ structurally and functionally from transposons,[34] they seem to be widespread in bacterial populations and play an important role in the dissemination of antibiotic-resistance genes.[2] Integrons do not transpose independently as a specific unit structure from one sequence of DNA to another. This capability of autonomous movement of large sequences of DNA is reserved primarily for transposons, insertion sequence elements, and bacteriophages. Integrons can become flanked, however, by transposable elements and become mobilized as an integrated structure into an existing transposon.

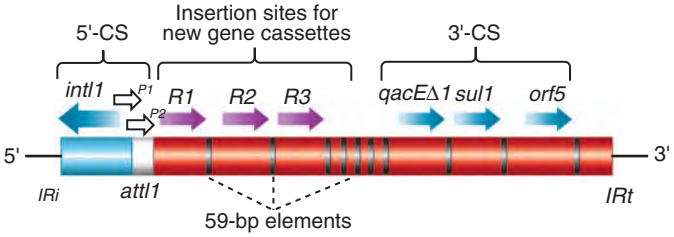

FIG. 18.3 Organization of a hypothetical class I integron. The 5′ conserved sequence (5′-CS) contains a site-specific integrase *(intI1)*; an attachment site *(attI1),* which functions as a receptor for new gene cassettes; and two potential promoter sites (*P1* and *P2*). The promoter is the initiation site for the transcription of the multiple, potential, antibiotic gene cassettes (labeled *R1, R2, R3*) that are inserted downstream from the promoter. Repeated, variable-length, but usually 59-bp, elements flank the central antibiotic-resistance gene cassettes. The conserved 3′ end of the integron (3′-CS) usually consists of a gene for resistance to quaternary ammonium compounds, a sulfonamide-resistance gene, and another open reading frame *(orf5).* The outer boundaries of the integron structure are flanked by a 25-bp inverted repeat sequence (noted as *IRi* and *IRt*).

The principal role of integrons is to provide a convenient insertion site for antibiotic-resistance genes from foreign DNA sources.

Five classes of integrons that encode antibiotic-resistance genes have been recognized, with type 1 integrons being the most common in pathogenic microorganisms.[36] A schematic representation of a class 1 integron is shown in Fig. 18.3. Integrons also serve as expression cassettes for antibiotic-resistance genes in that an efficient promoter site is provided in close proximity to the 5′ end of the newly inserted DNA sequence. The frequency of transcription of integrated cassettes of antibiotic-resistance genes depends on the proximity of the gene to the promoter at the 5′ upstream end of the integron. The level of expression of a resistance gene diminishes as the distance between the promoter and the specific antibiotic-resistance gene cassette increases.[31,32] Numerous clusters of different antibiotic-resistance genes have been identified that have evolved through specific insertions into common integrons. Integrons have been found to possess five or more antibiotic-resistance genes lined up in a tandem sequence along a single, functioning integron.[36]

Complex integrons have recently been described that are typified by a common open reading frame *(orf 513)* linked to the 3′ end of a typical integron, followed by a series of inserted genes (often ESBL) after a duplication of the 3′ end of the integron. This common 3′ end of type 1 integrons encodes genes for resistance to quaternary ammonium compounds *(qacE1),* sulfonamide resistance *(sul1),* and an open reading frame of unknown function *(orf5).* These complex integrons can be mobilized and spread by adjacent insertion sequences to disseminate among bacterial populations.[35,37,38]

MOLECULAR MECHANISMS OF ANTIBIOTIC RESISTANCE

At least eight distinctive mechanisms of antibiotic resistance have been described in bacteria (Table 18.1). Examples of each of these mechanisms are described in the following paragraphs.

Enzymatic Inhibition of Antimicrobial Activity

β-Lactamases

Resistance to β-lactam antibiotics occurs primarily through production of β-lactamases, enzymes that inactivate these antibiotics by splitting the amide bond of the β-lactam ring. They have likely coevolved with bacteria as mechanisms of resistance against natural antibiotics over time.[39,40] The selective pressure exerted by the widespread use of antimicrobial therapy in modern medicine may have accelerated their development and spread.[41–43]

β-Lactamases are encoded either by chromosomal genes or by transferable genes located on plasmids and transposons. In addition, β-lactamase genes *(bla)* frequently reside on integrons, which often carry multiple resistance determinants. If mobilized by transposable

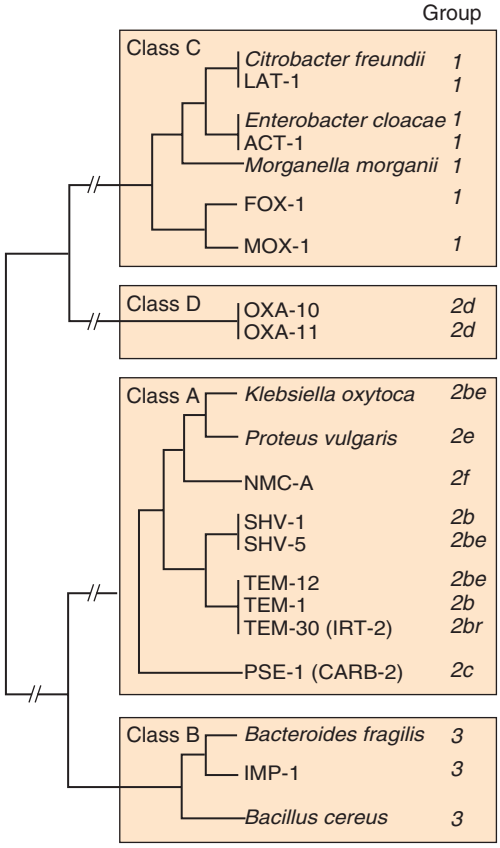

FIG. 18.4 Correlation between amino-acid sequences (Ambler classes) and functional properties of β-lactamases (Bush-Jacoby-Medeiros groups). *ACT-1,* AmpC type-1; *CARB-2,* carbenicillin-2; *FOX-1,* cefoxitin-1; *IMP-1,* active on imipenem; *IRT-2,* inhibitor-resistant TEM-2; *LAT-1,* latamoxef; *MOX-1,* moxicillin-1; *NMC-A,* not metalloenzyme carbapenemase-A; *OXA,* oxacillin; *PSE-1, Pseudomonas*-specific enzyme; *SHV,* sulfhydryl variable; *TEM,* Temoneira. *(Modified from Philippon A, Dusart J, Doris B, Frère JM. The diversity, structure and regulation of* β*-lactamases.* Cell Mol Life Sci. *1998; 54:341-346.)*

elements, integrons can facilitate further dissemination of multidrug resistance among different bacterial species.[38]

β-Lactamases can be classified according to their amino-acid structure into four molecular classes, A through D (Table 18.2), as first suggested by Ambler and later modified and expanded in the Bush-Jacoby-Medeiros classification system (Table 18.3).[39,40] Class A, C, and D β-lactamases hydrolyze the β-lactam ring through a serine residue at their active site, whereas class B enzymes are MBLs that use zinc $(Zn)^{2+}$ to break the amide bond (Fig. 18.4).

The first β-lactamase was described as a "penicillinase" capable of hydrolyzing penicillin in *E. coli* in 1940, about the same time as the first clinical use of penicillin was reported in the literature.[41] The next years witnessed the rapid spread of plasmid-encoded penicillin resistance among the majority of *S. aureus* clinical isolates.[42] Among gram-negative organisms, the rise in ampicillin resistance in the 1960s was ascribed to the emergence of TEM-1, a plasmid-encoded β-lactamase named after a Greek patient, Temoniera, in whom the first isolate was recovered.[43] The family of TEM β-lactamases was disseminated worldwide through various Enterobacteriaceae, in addition to *Pseudomonas aeruginosa, Haemophilus influenzae,* and *N. gonorrhoeae*.[44] Similarly, both chromosomally encoded and plasmid-mediated SHV-type β-lactamases, with a molecular structure related to TEM enzymes, became widely prevalent among *E. coli* and *K. pneumoniae* isolates.

Extended-Spectrum β-Lactamases

The pharmaceutical industry's development of third-generation cephalosporins, initially stable to the action of TEM- and SHV-type

TABLE 18.1 Eight Major Mechanisms of Resistance by Antimicrobial Class

	β-LACTAM	AMINO-GLYCOSIDE	CHLORAM-PHENICOL	MACROLIDE	SULFONAMIDE	TETRACYCLINE	TRIMETHOPRIM	QUINOLONE	GLYCOPEPTIDE	LINOSAMIDE; STREPTOGRAMIN	RIFAMPIN
Enzymatic inactivation	+++	+++	+++	+ (gram-negative)	–	–	–	+	–	–	–
Decreased permeability	+ (gram-negative)	+ (gram-negative)	+ (gram-negative)	++ (gram-negative)	–	+ (gram-negative)	+ (gram-negative)	+ (gram-negative)	++ (gram-negative)	+ (gram-negative)	–
Efflux	+	+	+	++	–	+++	–	+	–	–	–
Alteration of target site	++	++	–	+++	++	+ *(Helicobacter pylori)*	+++	+++	+++	+++	+++
Protection of target site		–	–	–	–	++	–	+	–	–	–
Overproduction of target	–	–	–	–	++	–	++	–	+	–	–
Bypass of inhibited process	–	–	–	–	+	–	+	–	–	–	–
Bind up antibiotic	–	–	–	–	–	–	–	–	++	–	–

+++, Most common mechanism; ++, common; + less common.

TABLE 18.2 Ambler Classification of β-Lactamases

CLASS	ACTIVE SITE	ENZYME TYPE	SUBSTRATES	EXAMPLE
A	Serine	Penicillinases:		
		Broad-spectrum	Benzylpenicillin, aminopenicillins, carboxypenicillins, ureidopenicillins, narrow-spectrum cephalosporins	PC1 in *Staphylococcus aureus* TEM-1, SHV-1 in *Escherichia coli, Klebsiella pneumoniae,* other gram-negative bacteria
		Extended-spectrum (β-lactamase)	Substrates of broad-spectrum plus oxymino-β-lactams (cefotaxime, ceftazidime, ceftriaxone) and aztreonam	In Enterobacteriaceae: TEM-derived, SHV-derived, CTX-M–derived; PER-1, VEB-1, VEB-2, GES-1, GES-2, IBC-2 in *Pseudomonas aeruginosa*
		Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems	KPC-1, KPC-2, KPC-3 in *K. pneumoniae;* NMC/IMI, SME family
B	Metallo-β-lactamases (Zn^{2+})	Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems	NDM-1 in Enterobacteriaceae, IMP, VIM, GIM, SPM, SIM lineages in *P. aeruginosa, Acinetobacter* spp.
C	Serine	Cephalosporinases	Substrates of extended-spectrum plus cephamycins	AmpC-type enzymes in Enterobacteriaceae, *Acinetobacter* spp.
D	Serine	Oxacillinases:		
		Broad-spectrum	Aminopenicillins, ureidopenicillin, cloxacillin, methicillin, oxacillin, and some narrow-spectrum cephalosporins	OXA-family in *P. aeruginosa*
		Extended-spectrum	Substrates of broad-spectrum plus oxymino-β-lactams and monobactams	OXA-derived in *P. aeruginosa*
		Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems	OXA-derived in *Acinetobacter* spp.

AmpC, Ampicillin C; *CTX-M,* cefotaxime-M; *GES,* Guyana extended-spectrum β-lactamase; *GIM,* German imipenemase; *IBC,* integron-born cephalosporinase; *IMI,* imipenem hydrolyzing; *IMP,* imipenem; *KPC, K. pneumoniae* carbapenemase; *NDM,* New Delhi metallo-β-lactamase; *NMC,* not metalloenzyme carbapenemase; *OXA,* oxacillin; *PC1,* penicillin 1; PER, *Pseudomonas* extended resistance; *SHV,* sulfhydryl variable; *SIM,* Seoul imipenemase; *SME, Serratia marcescens* extended-spectrum β-lactamase; *SPM,* Sao Paulo metallo-β-lactamase; *TEM,* Temoneira; *VEB,* Vietnam extended-spectrum β-lactamase; *VIM,* Verona integron-encoded metallo-β-lactamase.

TABLE 18.3 Bush-Jacoby-Medeiros Functional Classification Scheme for β-Lactamases

GROUP	ENZYME TYPE	INHIBITION BY CLAVULANATE	MOLECULAR CLASS	NO. OF ENZYMES	EXAMPLES[a]
1	Cephalosporinase	No	C	57	*Enterobacter cloacae* P99 (C), MIR-1 (P)
2a	Penicillinase	Yes	A	20	*Bacillus cereus* I, *Staphylococcus aureus* (B)
2b	Broad-spectrum	Yes	A	16	SHV-1 (B), TEM-1 (P)
2be	Extended-spectrum	Yes	A	81	*Klebsiella oxytoca* K1 (C), TEM-3 (P), SHV-2 (P)
2br[b]	Inhibitor-resistant	Diminished	A	13	TEM-30 (IRT-2) (P)
2c	Carbenicillinase	Yes	A	15	AER-1 (C), PSE-1 (P), CARB-3 (P)
2d	Cloxacillinase	Yes	D or A	21	*Streptomyces cacaoi* (C), OXA-1 (P)
2e	Cephalosporinase	Yes	A	19	*Proteus vulgaris* (C), FEC-1 (P)
2f[b]	Carbapenemase	Yes	A	3	IMI-1 (C), NMC-A (C), KPC (P), Sme-1 (C)
3	Carbapenemase	No	B	15	*Stenotrophomonas maltophilia* L1 (C), NDM-1 (P), IMP-1 (P)
4	Penicillinase	No		7	*Burkholderia cepacia* (C), SAR-2 (P)

[a]*B,* Both; *C,* chromosomal; *P,* plasmid.
[b]New groups; derived from Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother.* 1995; 39:1211–1233.
AER, Aeromonas; *CARB,* carbenicillin; *FEC,* isolated from feces; *IMI,* imipenem hydrolyzing; *IMP,* imipenem; *IRT,* inhibitor-resistant TEM; *KPC, K. pneumoniae* carbapenemase; *MIR,* Miriam Hospital; *NDM,* New Delhi metallo-β-lactamase; *NMC-A,* not metalloenzyme carbapenemase-A; *OXA,* oxacillin; *PSE, Pseudomonas*-specific enzyme; *SAR,* southern Africa–related enzyme; *SHV,* sulfhydryl variable; *TEM,* Temoneira.

β-lactamases, was soon followed by the emergence and global spread of ESBL, capable of hydrolyzing monobactam and broad-spectrum cephalosporins.[3,43–45] In addition, increasing reports of carbapenemase emergence and spread have raised concern over the currently limited antimicrobial arsenal against infections with multidrug-resistant gram-negative bacteria.[46,47]

TEM-derived β-lactamases. TEM-1 is the most common β-lactamase in gram-negative bacteria, and it can hydrolyze penicillins and narrow-spectrum cephalosporins in Enterobacteriaceae, *N. gonorrhoeae,* and *H. influenzae.*[3] The extended-spectrum of activity of TEM-derived ESBLs occurs through changes in a single or a few amino acids that change the configuration of the enzyme at its active site, making it more accessible to the bulky R1 oxymino side chains of third-generation cephalosporins (cefotaxime, cefpodoxime, ceftazidime, ceftriaxone), and monobactam (aztreonam).[3,45,47] The first TEM-derived ESBL, TEM-3, was reported in 1988.[48] There are now more than 220 TEM-derived ESBLs (see Lahey Clinic [Burlington, MA] website, www.lahey.org/Studies/temtable.asp). These enzymes are found primarily in *E. coli* and *K. pneumoniae* isolates but also in other Enterobacteriaceae, such as *Enterobacter aerogenes, Morganella morganii, Proteus* spp., and *Salmonella* spp. The majority of TEM-derived ESBLs remain susceptible to inhibition by clavulanic acid, although inhibitor-resistant TEM variants have also been described.[49]

SHV-derived β-lactamases. The SHV-1 β-lactamase has a biochemical structure similar to that of TEM-1 (68% of amino acids are shared[48–50]), and its ESBL derivatives are also produced by point mutations (one or more amino-acid substitutions) at its active site. SHV-type β-lactamases are found primarily in *K. pneumoniae* strains. A three-dimensional image of TEM-type and SHV-type ESBLs is provided in Fig. 18.5.

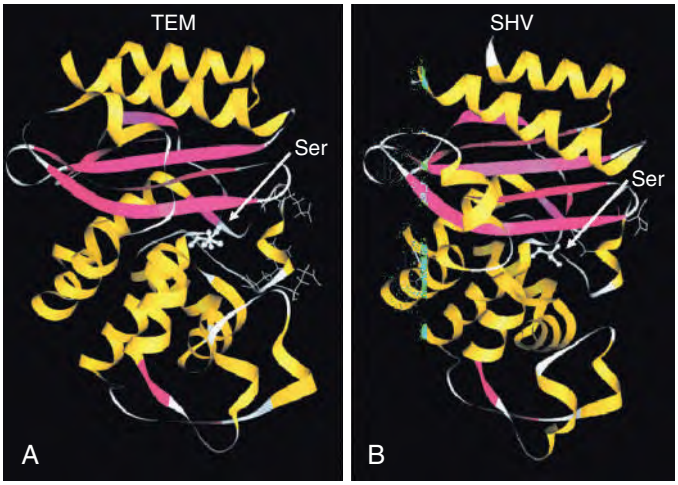

FIG. 18.5 Ribbon diagrams of TEM β-lactamases (A), and SHV β-lactamases (B). α-Helices are shown in yellow, β-strands in pink, and turns in gray. The *(Ser)* serine residue (marked by *white arrows*) involved in the hydrolysis of the β-lactam antibiotic ring is shown in ball-and-stick mode in the active site at the center of each molecule. The surrounding atoms, shown in stick mode, represent various sites of amino-acid substitutions (point mutations) that yield an extended-spectrum β-lactamase phenotype. *SHV,* Sulfhydryl variable; *TEM,* Temoneira. *(Modified from Jacoby GA, Munoz-Price SL. The new beta-lactamases.* N Engl J Med. *2005; 352:380–391.)*

CTX-M–derived extended spectrum β-lactamases. Cefotaxime-M (CTX-M) β-lactamases are not evolutionarily related to SHV and TEM families because they are thought to have been acquired by plasmids from the chromosomal ampicillin C (AmpC) enzymes of *Kluyvera* spp., environmental gram-negative rods of low pathogenic potential.[51] In general, the CTX-M family hydrolyzes cefotaxime and ceftriaxone better than ceftazidime, and they are inhibited more by tazobactam than by clavulanic acid,[50] although point mutations leading to increased activity against ceftazidime can occur. CTX-M enzymes have disseminated rapidly and are now among the most prevalent ESBLs worldwide. Recent reports of community-acquired bloodstream infections with multiresistant, CTX-M *E. coli* isolates from Spain and Israel have raised significant public health concerns.[52,53] The ST131 (O25:H4) clone associated with the CTX-M-15 enzymes has emerged as an important multidrug-resistant pathogen and may have been responsible for the majority of infections with multidrug-resistant *E. coli* infections in Europe and the United States since 2007.[54]

OXA-derived β-lactamases. Oxacillin (OXA)-type β-lactamases are also plasmid derived and hydrolyze oxacillin and its derivatives very effectively; they are poorly inhibited by clavulanic acid.[42,47] OXA-derived ESBLs have been described mainly in *P. aeruginosa,* in which they confer high-level resistance to oxymino-β-lactams.[47]

AmpC β-lactamase enzymes. AmpC β-lactamases are primarily chromosomal enzymes that confer resistance to penicillins, narrow-spectrum cephalosporins, oxymino-β-lactams, and cephamycins and are not susceptible to β-lactamase inhibitors such as clavulanic acid (molecular class C, functional group 1).[55] Cefepime and aztreonam are usually poor substrates, although modulation by point mutations at the R2 loop of the active site have been responsible for variants with increased ability to hydrolyze cefepime. AmpC production in gram-negative bacilli is normally repressed. However, a transient increase in production (10- to 100-fold) can occur in the presence of β-lactam antibiotics in the following species that possess inducible AmpC enzymes: *Enterobacter, Citrobacter freundii, Serratia, M. morganii, Providencia,* and *P. aeruginosa.*[55] AmpC β-lactamase production returns to low levels again after antibiotic exposure is discontinued, unless spontaneous mutations occur in the *ampD* locus of the gene, leading to permanent hyperproduction (derepression) in these species. Third-generation cephalosporin use in *Enterobacter* spp. infections can therefore select for the overgrowth of these stably derepressed mutants, leading to the emergence of antibiotic resistance during treatment.[56]

More than 20 plasmid-mediated AmpC enzymes, derived from chromosomally encoded genes in Enterobacteriaceae or *Aeromonas* spp., have been described in *E. coli, K. pneumoniae, Salmonella enterica,* and *Proteus mirabilis.* β-Lactam resistance attributable to this system appears to be increasing and confers a resistance phenotype similar to that of *Enterobacter* spp.[44]

Carbapenemases. Carbapenemases confer the largest antibiotic-resistance spectrum because they can hydrolyze not only carbapenems but also broad-spectrum penicillins, oxymino-cephalosporins, and cephamycins. The *K. pneumoniae* carbapenemase (KPC) enzymes are currently the most important class A serine carbapenemases. Since initially reported from *K. pneumoniae* isolates in several northeastern US outbreaks,[57] KPCs have been found worldwide in multiple other gram-negative species, such as *E. coli, Citrobacter, Enterobacter, Salmonella, Serratia*, and *P. aeruginosa.*[6]

Class B MBLs use a Zn^{2+} cation for hydrolysis of the β-lactam ring; are susceptible to ion chelators, such as ethylenediaminetetraacetic acid (EDTA); and are resistant to clavulanic acid, tazobactam, and sulbactam. They confer resistance to all β-lactam antibiotics except monobactams. Chromosomally encoded MBLs are primarily found in environmental isolates of *Aeromonas, Chryseobacterium,* and *Stenotrophomonas* spp. and are of usually low pathogenic potential.[58] Most clinically important MBLs belong to five different families (imipenem [IMP], Verona integron-encoded metallo-β-lactamase [VIM], Sao Paulo metallo-β-lactamase [SPM], German imipenemase [GIM], and Seoul imipenemase [SIM]), typically transmitted by mobile gene elements inserted into integrons and spread through *P. aeruginosa, Acinetobacter,* other gram-negative nonfermenters, and enteric bacterial pathogens.[24]

The New Delhi metallo-β-lactamase–1 (NDM-1) has received the most attention recently. Originally described in a *K. pneumoniae* isolate from India in 2008, NDM-1 enzymes have since been reported in the United States, the United Kingdom, and a number of other countries, primarily in connection with travel to India or Pakistan.[59,60] These enzymes confer resistance to all β-lactams except aztreonam. However, most MBLs reside on mobile gene cassettes inserted into integrons that harbor additional antibiotic-resistance genes to other antimicrobial classes; this multidrug resistance can be transferred to other species via transposons and plasmids, severely limiting therapeutic options in serious infections.[61] Recent meta-genomic analyses have indicated that the majority of MBLs probably originate from *Shewanella* spp. and that the genes for many more, as yet uncharacterized, metallo-enzymes are present in environmental and commensal bacterial species.[62]

Finally, class D carbapenemases have been described among four subfamilies of OXA-type β-lactamases (OXA-23, OXA-24, OXA-58, and OXA-146), primarily in *A. baumannii.* The intrinsically weaker carbapenemase activity is augmented by coupling β-lactamase production with an additional resistance mechanism, such as decreased membrane permeability or increased active efflux.[63]

Gram-Positive Bacterial β-Lactamases

Among gram-positive bacteria, staphylococci are the major pathogens that produce β-lactamase. Staphylococcal β-lactamases preferentially hydrolyze penicillins. Most are inducible and are excreted extracellularly.[3] The genes that determine staphylococcal β-lactamases usually are carried on small plasmids or transposons. Larger plasmids encoding β-lactamase and other resistances also exist and can transfer by conjugation, not only between strains of *S. aureus* but also between *S. aureus* and *Staphylococcus epidermidis.*[64]

Enterococci produce a plasmid-determined β-lactamase that seems to be of staphylococcal origin.[65] Since the appearance of the first strain in Texas in 1981, β-lactamase–producing enterococci have been found throughout the United States and in South America.[66] The genes often coexist with genes that determine high-level resistance to gentamicin and may occur on transposons and on plasmids. These transposons are like staphylococcal β-lactamase transposons and may have been derived from them.[67]

Anaerobic Bacteria

β-Lactamases also contribute to the resistance of anaerobic bacteria to β-lactam antibiotics.[68,69] The β-lactamases of *Fusobacteria* and *Clostridia*

spp. are principally penicillinases.[70] The β-lactamases produced by *Bacteroides fragilis* are predominantly cephalosporinases, some of which have been found to hydrolyze cefoxitin and imipenem and may be transferable.[71] Most of the cephalosporinases are inhibited by clavulanate, sulbactam, or tazobactam. Some isolates of *Bacteroides* spp. produce carbapenemases, metalloenzymes inhibited by EDTA but not clavulanate, which confer resistance to imipenem.

Contribution of β-Lactamases to β-Lactam Antibiotic Resistance

The level of antibiotic resistance mediated by a particular β-lactamase in a population of bacteria is determined by at least five variables. The efficiency of the β-lactamase in hydrolyzing an antibiotic depends on (1) its rate of hydrolysis and (2) its affinity for the antibiotic. Other variables are (3) the amount of β-lactamase produced by the bacterial cell, (4) the susceptibility of the target protein (penicillin-binding protein [PBP]) to the antibiotic, and (5) the rate of diffusion of the antibiotic into the periplasm of the cell.

Within the bacterial cell, β-lactamases contribute to antibiotic resistance in several ways. The simplest model is that of penicillinase-producing staphylococci, in which the bacteria, on exposure to penicillin, begin to produce β-lactamase, which they excrete extracellularly. Two events then take place concurrently: (1) penicillin lyses bacteria and (2) β-lactamase hydrolyzes penicillin. If viable bacterial cells remain after the level of penicillin has declined to less than the minimal inhibitory concentration (MIC), regrowth of bacteria occurs.[40]

Another model is exemplified by gram-negative bacteria, which (1) produce a β-lactamase that remains trapped in the periplasmic space and (2) have no barrier to antibiotic penetration. An example is *H. influenzae* strains that produce the TEM-1 β-lactamase.[72] In this model and the first one discussed, a marked inoculum effect occurs in that the MIC for a large inoculum (10^6 colony-forming units [CFUs] per milliliter) may be 1000-fold greater than that for a small inoculum (10^2 CFUs/mL). Lysis of the organism by ampicillin releases the trapped β-lactamase into the microenvironment, providing partial protection to adjacent bacteria residing in the same location. If a high inoculum exists before ampicillin exposure occurs, the release of all the periplasmic β-lactamase enzymes in a confined space might be sufficient to protect some of the remaining viable bacteria of the original population of microorganisms. However, the low level of resistance of single cells makes it possible for ampicillin to cure some infections caused by β-lactamase–producing strains of *H. influenzae* when the initial inoculum of infecting bacteria is low.

Another model is exemplified by ampicillin resistance of *E. coli* strains that produce the TEM-1 β-lactamase. These bacteria have a barrier to entry of β-lactam molecules (the outer membrane), and they produce a β-lactamase that remains localized to the periplasmic space. In this model, the kinetics are more complicated. The enzyme is situated strategically between the barrier to antibiotic penetration (outer membrane) and the antibiotic targets (PBPs on the cytoplasmic membrane). In this position, the enzyme can destroy antibiotic molecules sequentially as they make their way through the barrier, analogous to a sharpshooter with abundant ammunition who aims at targets passing through a single entry point. Consequently, high levels of resistance occur with single bacterial cells, in contrast to the previous example.[40]

Variations on this model occur when the amount of β-lactamase produced increases with exposure to a β-lactam (induction), as occurs in *Enterobacter* and *Pseudomonas* spp. High levels of β-lactamase are produced only after a period of exposure to the inducing antibiotic, and resistance may be expressed late. When *Enterobacter* strains are exposed to two β-lactam antibiotics, one of which is a potent inducer (e.g., cefamandole), antagonism between the two antibiotics may result.[70]

Table 18.4 lists mechanisms of resistance to β-lactam antibiotics. Often these mechanisms work in concert and may accumulate in a single patient. An example is a 19-month-old child with aplastic anemia who over 3 months had nine blood isolates of *E. coli,* all derived from a common ancestor, despite multiple courses of antibiotics, including ceftazidime.[72] The first isolate produced a TEM-1 β-lactamase but was susceptible to ceftazidime (MIC, 0.25 μg/mL). A subsequent isolate became resistant (MIC of ceftazidime, 32 μg/mL) by acquiring a new plasmid-determined β-lactamase (SHV-1) linked to an efficient promoter and turning off production of an outer membrane porin. An even higher level of resistance (MIC of ceftazidime ≥128 μg/mL) occurred when the SHV-1 β-lactamase mutated to form the ESBL SHV-8, which hydrolyzes ceftazidime much more rapidly. By turning off porin production to slow the rate of entry of ceftazidime into the periplasmic space and producing an extended-spectrum, ceftazidime-inactivating β-lactamase, the infecting *E. coli* used two mechanisms synergistically to achieve a high level of resistance to ceftazidime.[73]

Aminoglycoside Resistance–Modifying Enzymes

Among aerobic bacteria, aminoglycoside resistance is most commonly due to enzymatic inactivation through aminoglycoside-modifying enzymes. These may be coded by genes on plasmids or chromosomes. Several aminoglycoside-modifying enzymes have been shown to be carried on transposons.[74]

Aminoglycoside-modifying enzymes confer antibiotic resistance through three general reactions: *N*-acetylation, *O*-nucleotidylation, and *O*-phosphorylation. For each of these general reactions, there are several different enzymes that attack a specific amino or hydroxyl group. The nomenclature for these enzymes lists the molecular site where the modification occurs after the type of enzymatic activity. An aminoglycoside acetyltransferase (AAC) that acts at the 3′ site is designated AAC(3′) (Table 18.5). There may be more than one enzyme that catalyzes the same reaction, however, and Roman numerals may be necessary (e.g., AAC[3′]-IV).

Enzymatic aminoglycoside resistance is achieved by modification of the antibiotic in the process of transport across the cytoplasmic membrane.[74] Resistance to a particular aminoglycoside is a function of two different rates—that of drug uptake versus that of drug inactivation. An important factor in determining the level of resistance is the affinity of the modifying enzyme for the antibiotic. If an enzyme has a high affinity for the specific aminoglycoside, drug inactivation can occur at very low concentrations of the enzyme.

The differences in the worldwide distribution of aminoglycoside-modifying enzymes may be partially a function of antibiotic selection pressures and may have had profound implications on the choice of antibiotics used at specific medical centers. Aminoglycoside phosphotransferase (APH)(3′) and APH(3″) are distributed widely among gram-positive and gram-negative species worldwide and have led to decreased use of kanamycin and streptomycin. The gene for aminoglycoside nucleotidyltransferase (ANT)(2″) has been associated with multiple nosocomial outbreaks in the 1990s across the United States. The gene

TABLE 18.4 Mechanisms of Resistance to β-Lactam Antibiotics

I. Alter target site (PBP)
  A. Decrease affinity of PBP for β-lactam antibiotic
    1. Modify existing PBP
      a. Create mosaic PBP
        Insert nucleotides obtained from neighboring bacteria (e.g., penicillin-resistant *Streptococcus pneumoniae*)
        Mutate structural gene of PBP(s) (e.g., ampicillin-resistant β-lactamase–negative *Haemophilus influenzae*)
    2. Import new PBP, e.g., mecA in methicillin-resistant *Staphylococcus aureus*
II. Destroy β-lactam antibiotic
  A. Increase production of β-lactamase
    1. Acquire more efficient promoter
      a. Mutate existing promoter
      b. Import new one
    2. Deregulate control of β-lactamase production
      a. Mutate regulator genes (e.g., *ampD* in "stably derepressed" *Enterobacter cloacae*)
  B. Modify structure of resident β-lactamase
    1. Mutate its structural gene (e.g., extended-spectrum β-lactamases in *Klebsiella pneumoniae*)
  C. Import new β-lactamase(s) with different spectrum of activity
III. Decrease concentration of β-lactam antibiotic inside cell
  A. Restrict its entry (loss of porins)
  B. Pump it out (efflux mechanisms)

PBP, Penicillin-binding protein.

TABLE 18.5 Aminoglycoside-Modifying Enzymes

ENZYMES	USUAL ANTIBIOTICS MODIFIED	COMMON GENERA
Phosphorylation		
APH(2″)	K, T, G	SA, SR
APH(3′)-I	K	E, PS, SA, SR
APH(3′)-III	K, ±A	E, PS, SA, SR
Acetylation		
AAC(2′)	G	PR
AAC(3′)-I	±T, G	E, PS
AAC(3′)-III, -IV, or -V	K, T, G	E, PS
AAC(6′)	K, T, (A)	E, PS, SA
Adenylation		
ANT(2″)	K, T, G	E, PS
ANT(4′)	K, T, A	SA
Bifunctional Enzymes		
AAC(6′)APH(2″)	G, Ar	SA, Ent
AAC(6′)-Ib cr	G, K, T, FQ*	E

A, Amikacin; *AAC,* aminoglycoside acetyltransferase; *ANT,* aminoglycoside nucleotidyltransferase; *APH,* aminoglycoside phosphotransferase; *Ar,* arbekacin; *cr,* ciprofloxacin resistance; *E,* Enterobacteriaceae; *Ent,* enterococci, **FQ,* fluoroquinolone (acetylates the piperazine ring in some fluoroquinolones), *G,* gentamicin; *K,* kanamycin; *PR, Providencia-Proteus*; *PS,* pseudomonads; *SA,* staphylococci; *SR,* streptococci; *T,* tobramycin.

TABLE 18.6 Mechanisms of Tetracycline Resistance

RESISTANCE MECHANISM	COMMON TET DETERMINANTS	COMMON BACTERIAL SPECIES
Drug efflux	Tet A-L, P[a], V, Y, Z, *otrB, tcr*(3), Tet 30	Enterobacteriaceae, *Pseudomonas, Streptomyces, Staphylococcus, Streptococcus* spp.
Ribosomal protection	Tet M, O, P[a], Q, S, T, W, *otrA*	Gram-positive and gram-negative anaerobes, *Neisseria, Haemophilus, Enterococcus. Staphylococcus, Streptococcus* spp.
Enzymatic inactivation	Tet X, Tet 34, Tet 37	*Bacteroides, Vibrio* spp.
Unknown mechanism	Tet U, *otrC*	*Mycobacterium, Enterococcus* spp.
Altered ribosomal target	—	*Helicobacter pylori*

[a]Tet P has two different genes mediating different mechanisms of resistance.

for aminoglycoside acetyltransferase AAC(6′)-I has been found to be more prevalent in enteric bacteria and in staphylococci in East Asia.[75] The AAC(3′) group of enzymes have been responsible for outbreaks of antibiotic resistance in South America, Western Europe, and the United States. Although each outbreak of aminoglycoside-resistant Enterobacteriaceae has its own pattern, the most typical manner of spread has been the appearance of a plasmid-carrying, aminoglycoside-resistant strain of *K. pneumoniae,* usually carrying the *ANT(2″)* gene, with subsequent dissemination to other strains of the species and further spread later to other species and genera of Enterobacteriaceae.[76]

Major increases in plasmid-mediated aminoglycoside resistance have been noted among enterococci,[77] initially in the developing world, but increasingly in the United States and Europe. Their clinical impact is exacerbated by the frequent cotransmission of β-lactamases, resulting in a loss of synergy when combination therapy is used for serious enterococcal infections. *S. aureus* and *S. epidermidis* have become increasingly resistant to aminoglycosides because of the interspecies and intraspecies dissemination of plasmid-mediated, aminoglycoside-modifying enzymes.[77]

The two most interesting developments in aminoglycoside-modifying enzymes have been the discovery of bifunctional enzymes. The first example is the AAC(6′) APH(2″) enzyme, which has two functioning active sites, one for acetylation and the other for phosphorylation of aminoglycosides. This bifunctional enzyme probably arose from a fusion event of the genes for these two enzymes. This enzyme is now widespread in staphylococci and enterococci, frequently residing on a common transposon Tn*4001* found on the chromosome and on transferable plasmids. The gene *aac(6′) aph (2″)* accounts for most of the high-level gentamicin and arbekacin resistance observed in methicillin-resistant *S. aureus* (MRSA) and enterococcal isolates in many countries worldwide.[78,79]

Recently, another major discovery with regard to aminoglycoside-modifying enzymes involves evidence of variant enzymes that can modify the structure of an entirely different class of antimicrobial agent. The first bifunctional enzyme that can modify aminoglycosides and a fluoroquinolone (ciprofloxacin) was described in 2006.[80] This enzyme, designated as AAC(6′)-Ib-cr, not only acetylates kanamycin, gentamicin, and tobramycin, but also acetylates the piperazinyl side group of ciprofloxacin. This acetylated quinolone is fourfold less active than the parent compound and may lead to clinically significant resistance in enteric bacteria, particularly in strains that possess other mechanisms for diminished quinolone activity. This enzyme has two important mutations in the gene for the basic AAC(6′) enzyme (W102R and D179Y) and 12 unique base pairs at its 5′ end that are essential to alter substrate specificity and allow ciprofloxacin acetylation function for this enzyme. The widespread use of ciprofloxacin and other fluoroquinolones over the past 2 decades has changed the selection pressures on bacterial populations, promoting development of quinolone resistance.[80]

Chloramphenicol Acetyltransferase

Resistance to chloramphenicol in gram-positive and gram-negative organisms is mediated primarily by the inactivating enzyme *chloramphenicol acetyltransferase.* This is an intracellular enzyme that inactivates the drug by 3-*O*-acetylation[81] and is encoded by plasmid-borne or chromosomal genes. Despite homology at the active site of this enzyme, there is considerable diversity between chloramphenicol acetyltransferase enzymes isolated from gram-positive and gram-negative organisms.[82]

Macrolide-, Lincosamide-, Streptogramin-Inactivating Enzymes

Although resistance to erythromycin and other macrolides is frequently the result of alteration in the ribosomal target site or efflux pumps, several substrate-inactivating enzymes have been characterized.[83] Erythromycin esterases have been isolated from *E. coli* that hydrolyze the lactone ring of the antibiotic and result in its inactivation. This is a plasmid-mediated resistance determinant that is constitutively produced and results in high-level resistance to erythromycin (MIC >2000 μg/mL).[84] This type of high-level macrolide-resistance gene is often linked with the carbapenemase-producing, NDM-1–bearing plasmids.[59] These resistance genes limit the usefulness of oral erythromycin or other macrolides in reducing the aerobic gram-negative flora of the intestinal tract before gastrointestinal surgical procedures. Other plasmid-mediated resistance genes generate specific inactivating enzymes in *Staphylococcus haemolyticus* and *S. aureus* that adenylate[85] lincosamides or acetylate[86] or hydrolyze[87] streptogramins.

Tetracycline Inactivation

A tetracycline-inactivating enzyme called TetX has been described rarely in *Bacteroides* spp.[88] Tetracycline resistance is principally mediated by other mechanisms, including efflux and ribosomal protection (Table 18.6). However, environmental bacteria frequently express enzymes

called tetracycline destructases, and it is possible that such enzymes might find their way into clinically relevant pathogens in the future.[89]

Decreased Permeability of Bacterial Membranes

Outer Membrane Permeability

Alexander Fleming recognized early in the history of antibiotic development that penicillin is effective against gram-positive bacteria but not against gram-negative bacilli.[90] This difference in susceptibility to penicillin is due in large part to the presence of the outer membrane in gram-negative bacilli, a thick lipopolysaccharide layer that acts as a barrier to the penetration of many antibiotics into the cell.[91] Situated outside the peptidoglycan cell wall of gram-negative bacteria, this outer membrane is absent in gram-positive bacteria. The lipopolysaccharide is made up of tightly bound hydrocarbon molecules that impede the entry of hydrophobic antibiotics, such as nafcillin or erythromycin. Agents that disrupt the integrity of the lipopolysaccharide layer, such as polymyxin, or mutations that lead to the production of defective lipopolysaccharides, result in increased permeability of hydrophobic antibiotics.[92]

The passage of hydrophilic antibiotics through this outer membrane is facilitated by the presence of porins, proteins that are arranged to form water-filled diffusion channels through which antibiotics may traverse.[93] Bacteria usually produce many porins; approximately 10^5 porin molecules are present in a single cell of *E. coli*. Bacteria can regulate the relative number of different porins in response to the osmolarity of the surrounding media. In hyperosmolar media, *E. coli* may repress production of the larger porins (OmpF) while continuing to express smaller ones (OmpC).[94]

The rate of diffusion of antibiotics through this outer membrane is a function not only of the numbers and properties of the porin channels but also of the physicochemical characteristics of the antibiotic. In general, the larger the antibiotic molecule, the more negatively charged, and the greater the degree of hydrophobicity, the less likely that it will penetrate through the outer membrane.[95] Small hydrophilic molecules with a zwitterionic charge, such as imipenem, are highly permeable. Conversely, larger highly charged molecules, such as carbenicillin, are much less permeable.

Mutations resulting in the loss of specific porins can occur in clinical isolates and determine increased resistance to β-lactam antibiotics. Resistance to aminoglycosides and carbapenems emerging during therapy has been associated with a lack of production of outer membrane proteins. For example, emergence of imipenem resistance during therapy, observed in up to 25% of *P. aeruginosa* infections, has been ascribed to mutational loss of its OprD protein (also known as the D2 porin).[96]

Resistance to nalidixic acid and other quinolones has been associated with alterations of outer membrane proteins in *Serratia marcescens* and *P. aeruginosa*.[97,98,99] Single-step, high-level mutational resistance to nalidixic acid by aerobic gram-negative bacilli occurs with a 10^{-7} frequency, whereas only low-level resistance to the newer quinolones (<10 × MIC) usually is obtained with a single-step selection of less than 10^{-9}.[100] Plasmid-mediated chloramphenicol resistance resulting from decreased cell membrane permeability has been shown in *E. coli*.[101]

Inner Membrane Permeability

The rate of entry of aminoglycoside molecules into bacterial cells is a function of their binding to a usually nonsaturable anionic transporter, whereupon they retain their positive charge and subsequently are "pulled" across the cytoplasmic membrane by the internal negative charge of the cell.[102] This process requires energy and a threshold minimal level of internal negative charge of the cell that has to be present before significant transport occurs *(proton motive force)*.[103] The level of the internal charge that is required may depend on the actual aminoglycoside concentration at a given time. The energy generation or the proton motive force that is required for substrate transport into the cell may be altered in mutants resistant to aminoglycosides.

These aminoglycoside-resistant isolates can develop during the course of long-term aminoglycoside therapy.[101] These isolates usually have a "small colony" phenotype resulting from their reduced rate of growth. They may be unstable and revert to a sensitive phenotype in the absence of selective aminoglycoside pressure. The clinical significance of these isolates is not clear. They may retain some virulence[104] and rarely may cause fatal bacteremia.[105] Because oxidative metabolism is essential for aminoglycoside uptake action and cell growth and development, *Pseudomonas* mutants have been found that have been deficient in specific cytochromes. Resistant mutants with defective electron transport systems have been described in *E. coli, S. aureus,* and *Salmonella* spp.

Promotion of Antibiotic Efflux

Tetracyclines

Active efflux of antimicrobial agents is recognized increasingly as a common mechanism of resistance in many clinically relevant pathogens. Some strains of *E. coli, Shigella* spp., and other enteric organisms express a membrane transporter system that leads to multidrug resistance by drug efflux.[106] Many of these are multicomponent, regulated, energy-dependent transporter systems that promote the active efflux of multiple classes of antibiotics. Specific efflux pumps also exist that promote the egress of single classes of antimicrobial agents.

The major mechanism of resistance to tetracyclines found in enteric gram-negative organisms results from the decreased accumulation of tetracycline (see Table 18.6). This reduced uptake is an energy-dependent process that is related to the generation of an inner membrane protein produced by the tetracycline-resistance determinant (designated as Tet) or resistance gene products for oxytetracycline derivatives (Otr). The primary mechanism for the decreased accumulation of tetracycline is through the active efflux of the antibiotic across the cell membrane.[107] Decreased uptake of tetracycline from the extracellular environment also accounts for decreased accumulation of tetracycline inside resistant cells. These resistance determinants may be found on the chromosome or plasmids and frequently are found on transposable genetic elements. Tetracycline-resistance genes are generally inducible by subinhibitory concentrations of tetracycline. There are now over 40 recognized tetracycline-resistance determinants, most of which mediate drug efflux.[107,108] The determinants have been designated in the past by letters (e.g., Tet A, Tet B). Because there are now more determinants than letters in the English alphabet, new *tet* genes now are designated by numbers.[106] Tetracycline efflux pumps primarily affect first-generation tetracyclines, but have little activity against newer glycylcyclines (tigecycline) or the aminomethylcyclines such as omadacycline.[109]

Macrolides and Streptogramins

In some strains of *Streptococcus pneumoniae, Streptococcus pyogenes, S. aureus,* and *S. epidermidis,* an active efflux mechanism causes resistance to macrolides, streptogramins, and azalides.[110] This efflux mechanism is mediated by the *mef* (for *m*acrolide *ef*flux) genes in streptococci and *msr* (for *m*acrolide *s*treptogramin *r*esistance) genes in staphylococci.[111] A similar efflux system, encoded by a gene called *mreA* (for *m*acrolide *r*esistance *e*fflux), has been described in group B streptococci.[112] This mechanism of resistance is prevalent in community-acquired infections,[113] and dissemination of these resistance genes among important bacterial pathogens is a considerable threat to the usefulness of macrolide antibiotics (Table 18.7).

β-Lactams

Active efflux mechanisms also may contribute to the full expression of β-lactam resistance in *P. aeruginosa*. Multidrug efflux pumps in the inner and outer membrane of *P. aeruginosa* act in concert with periplasmic β-lactamases and membrane permeability blockers to protect the bacterium from β-lactam agents.[114]

Fluoroquinolones

Active efflux of fluoroquinolones has been detected in enteric bacteria[115] and staphylococci.[113] This efflux may be related to a multiple antibiotic-resistance transporter[113] (i.e., NorA) or a specific quinolone efflux pump (i.e., EmrAB, AcrAB, or plasmid-mediated *qepA* gene efflux system).[116] This mechanism of limiting access of high levels of fluoroquinolones works in concert with other mechanisms (point mutations of DNA

TABLE 18.7 Resistance Mechanisms Against the Macrolides, Lincosamides, and Streptogramins

BACTERIAL SPECIES	GENE DESIGNATION	PHENOTYPE	RESISTANCE MECHANISM	RESISTANCE PATTERN: 14- OR 15-MEMBERED RING	RESISTANCE PATTERN: 16-MEMBERED RING	RESISTANCE PATTERN: CLINDAMYCIN	RESISTANCE PATTERN: STREPTOGRAMIN B
Streptococci, Enterococci	*erm (A, B)*	MLS_B—inducible	Ribosomal methylation	(s) I or R	(s) I or R	(s) I or R	(s) I or R
	erm (A, B)	MLS_B—constitutive	Ribosomal methylation	R	R	R	R
	mef (A or E)	M	Efflux	I or R	S	S	S
	L4/L22 mut	M	Ribosomal mutation	R	R	S	S
	inu (B)	L	Inactivation	S	S	S-I	S
Staphylococci	*erm (A, C)*	MLS_B—inducible	Ribosomal methylation	R	(s)	(s)	(s)
	erm (A, C)	MLS_B—constitutive	Ribosomal methylation	R	R	R	R
	msr (A or B)	MS_B	Efflux	R	S	S	R
	vgb, vgbB	S_B	Inactivation	S	S	S	R
	ere (A or B)	M	Inactivation	R	R	S	S
	inu (A)	L	Inactivation	S	S	S-I	S

Fourteen- or 15-membered ring structures: erythromycin, clarithromycin, azithromycin. Sixteen-membered ring structures: spiramycin.
I, Intermediate susceptibility; *L,* lincosamides; *M,* macrolides; *MLS_B,* macrolides, lincosamides, and streptogramin B; *R,* resistant; *(s),* appears susceptible in vitro but may select resistant clones in vivo; *S,* sensitive.

gyrases, gyrase protection, permeability barriers, and acetylation) for full expression of quinolone resistance.[117]

Altered Target Sites

Alteration of Ribosomal Target Sites

Macrolides, Lincosamides, Streptogramins

Resistance to a wide variety of antimicrobial agents, including tetracyclines, macrolides, lincosamides, streptogramins, and the aminoglycosides, may result from alteration of ribosomal binding sites. Failure of the antibiotic to bind to its target site or sites on the ribosome disrupts its ability to inhibit protein synthesis and cell growth. For macrolides, lincosamides, and streptogramin B (MLS_B), this is the principal mechanism of multiple-agent resistance among aerobic and anaerobic gram-positive organisms.[111] Resistance is mediated by the products of the *erm* (erythromycin ribosome methylation) gene, the variety of methylase enzymes (MLS_B, determinant) that dimethylate adenine residues on the 23S ribosomal RNA (rRNA) of the 50S subunit of the prokaryotic ribosome, disrupting the binding of MLS_B to the ribosome (see Table 18.7). Different classes of this resistance determinant may be located on plasmids or on the bacterial chromosome.

MLS_B resistance resulting from ribosomal methylation has been described in many species, including *S. aureus, Streptococcus sanguinis, B. fragilis,* and *Clostridium perfringens.* MLS_B resistance may be constitutive or inducible by either older macrolides (e.g., erythromycin) or newer azalides. In *S. pneumoniae*, resistance is encoded by the *erm(B)* gene, which is responsible for the methylation of loop V of the 23S ribosomal subunit.[118] In addition, point mutations in ribosomal proteins L4 and L22 of the 50S subunit have also been described that render *S. pneumoniae* resistant to macrolides.[119]

Inducible resistance in streptococci is generated by a variety of lincosamides and macrolides, resulting in cross-resistance to the MLS_B antibiotics. In staphylococci, only 14 or 15 numbered macrolides induce MLS_B methylation, and the organisms express resistance to macrolides only.

Tetracyclines

Tetracycline resistance may be mediated by a variety of mechanisms, the most common of which are efflux mechanisms and ribosomal protection mechanisms (see Table 18.6).[120] An additional, unusual mechanism of tetracycline resistance by altering the target site of action has been found in *Helicobacter pylori.*[121] This organism can possess a mutation in its 16S rRNA that limits tetracycline binding to its target site at the 30S subunit of the bacterial ribosome.

Aminoglycosides

Resistance to aminoglycosides is also mediated at the ribosomal level. In Enterobacteriaceae and nonfermenting gram-negative bacteria, methylation of the 16S rRNA (the site where aminoglycosides bind and inhibit protein synthesis) by enzymes usually carried on plasmids is mediated by at least seven different genes (*armA, rmtA, rmtB, rmtC, rmtD, rmtE,* and *npmA*).[122] This is now recognized as a major mechanism of resistance to all parenteral aminoglycosides, which appears to be spreading globally.[123,124] Mutations of the S12 protein of the 30S subunit have been shown to interfere with binding streptomycin to the ribosome. Ribosomal resistance to streptomycin may be a significant cause of streptomycin resistance among enterococcal isolates.[125] Ribosomal resistance to the 2-deoxystreptamine aminoglycosides (gentamicin, tobramycin, and amikacin) seems to be uncommon and may require multiple mutations in that these aminoglycosides seem to bind to several sites on the 30S and 50S subunits of the prokaryotic ribosome. Ribosomal resistance often is associated with decreased intracellular accumulation of the drug.[126]

Ketolides

Ketolides have seen limited use clinically, and yet clinical isolates of *S. pneumoniae* resistant to telithromycin have already been reported. Resistance is due to constitutive expression of the *erm* gene, mutations in domains II and V of the 23S rRNA binding sites, and mutations within ribosomal proteins L4 and L22.[127]

Oxazolidinones

Resistance to linezolid, the main oxazolidinone currently in clinical use, has been described in gram-positive isolates (*S. aureus, Enterococcus faecium, Enterococcus faecalis, S. epidermidis*) as a result of point mutations within the genes encoding the 23S rRNA of the 50S ribosomal subunit, the antibiotic's main binding site.[128] Specifically, translocation of peptidyl–transfer RNA (tRNA) from the A site to the P site during translation from bacterial ribosomes is inhibited through guanine (G) to uridine (U) substitutions at position 2576 or similar binding sites in the peptidyl transferase region of 23S rRNA.[129] This remains the most common mechanism of linezolid resistance among clinical isolates. Resistance development has been observed during prolonged linezolid therapy in vancomycin-resistant enterococci (VRE) bacteremia,[130] and a number of other pathogens, including coagulase-negative staphylococci and *S. aureus.* Another mechanism of resistance, mediated by the *cfr* gene, is the result of methylation of adenosine at position 2503 of 23S rRNA by a methyl transferase reaction.[131]

Alteration of Cell Wall Precursor Targets: Glycopeptides

Enterococci

Glycopeptide antibiotics (vancomycin, teicoplanin) bind to D-alanyl-D-alanine dipeptide at the termini of peptidoglycan precursor stem peptides, preventing their incorporation into the cell wall and stopping further peptidoglycan synthesis. In enterococci, high-level acquired vancomycin resistance develops by alteration of these cell wall precursor targets through an amino-acid substitution that changes their end to a D-alanyl-D-lactate depsipeptide. The gene responsible for this change, *vanA,* encodes a protein that synthesizes these modified peptidoglycan precursors, greatly reducing vancomycin binding affinity.[132,133]

Resistance of enterococci to vancomycin has been classified as phenotypes A through N based on genotype, type of target site alterations, and levels of resistance to vancomycin or teicoplanin (Table 18.8).[134,135] Strains of *E. faecium* and *E. faecalis* with high-level resistance to vancomycin and teicoplanin have class A resistance, which can transfer by conjugation from *E. faecium* to other gram-positive bacteria,[136] including *E. faecalis, S. pyogenes, Streptococcus sanguis,* and *Listeria monocytogenes*. Strains of *E. faecium* and *E. faecalis* with class B resistance have levels of resistance to vancomycin that range from high (MIC, 1024 µg/mL) to low (MIC, 4 µg/mL) and are susceptible to teicoplanin. The genes determining the VanB phenotype are self-transferable by conjugation to other *Enterococcus* strains[137,138] and have also been observed in anaerobic species, presumably through genetic transfer within the human bowel.

All isolates of *Enterococcus gallinarum, Enterococcus casseliflavus,* and *Enterococcus flavescens* possess intrinsic low-level resistance to vancomycin and are susceptible to teicoplanin (class C phenotype). The resistance is mediated by chromosomal genes known as $vanC_1$, $vanC_2$, or $vanC_3$, which encode the synthesis of alternative dipeptides, D-alanine-D-serine, where vancomycin and other glycopeptide antibiotics, such as teicoplanin, bind to D-alanine-D-alanine (D-ala-D-ala) dipeptide, which is present at the termini of the stem peptide in peptidoglycan precursors.

Strains of *E. faecium* and *E. faecalis* with class B resistance have levels of resistance to vancomycin that range from high (MIC, 1024 µg/mL) to low (MIC, 4 µg/mL) and are susceptible to teicoplanin. Vancomycin, but not teicoplanin, can induce resistance to vancomycin and teicoplanin in these strains. The genes determining the VanB phenotype are self-transferable by conjugation to other *Enterococcus* strains.[137,138]

All isolates of *E. gallinarum, E. casseliflavus,* and *E. flavescens* possess low-level resistance to vancomycin and are susceptible to teicoplanin (class C phenotype). The resistance is mediated by chromosomal genes known as $vanC_1$, $vanC_2$, or $vanC_3$. The *vanC* gene complex gives rise to resistance to vancomycin by synthesis of an alternative dipeptide, D-alanine-D-serine, in which a serine replaces the terminal alanine. Other variant genes known as *vanE* and *vanG* have been found in enterococcal species that also mediate various levels of glycopeptide resistance[134] (see Table 18.8).

Staphylococci

Since 1987, reports from the United States and Japan have documented outbreaks of vancomycin-intermediate *S. epidermidis,*[139] *S. haemolyticus,*[140] and *S. aureus* (VISA).[141] The first high-level VRSA isolate in the United States was recovered in 2002 from a diabetic patient on chronic hemodialysis harboring both *S. aureus* and VRE in a foot ulcer.[142] DNA sequencing revealed identical *vanA* genes in both isolates, and further molecular analysis revealed plasmid-mediated transfer of resistance through the Tn*1546* genetic element encoding the *vanA* gene from the *enterococcus* (VRE) strain into the vancomycin-susceptible MRSA strain, rendering it vancomycin resistant (VRSA).[143]

VISA arose likely during prolonged exposure to vancomycin, often at suboptimal concentrations, and are the result of multiple mutations in the genes controlling cell wall biosynthesis. Phenotypically, VISA clones express unusually thick peptidoglycan cell walls that are not completely cross-linked.[144] The cell wall in some strains of VISA contains nonamidated glutamine precursors that provide an increased number of false binding sites to vancomycin.[145–147] The vancomycin molecules are absorbed to these excess binding sites, preventing the antibiotic from reaching its target, and allowing peptidoglycan synthesis in the cytoplasmic membrane to continue uninhibited.[143]

Alteration of Target Enzymes

β-Lactams

β-Lactam antibiotics inhibit bacteria by binding covalently to PBPs in the cytoplasmic membrane. These target proteins catalyze the synthesis of the peptidoglycan that forms the cell wall of bacteria.[148] Alterations of PBPs can lead to β-lactam antibiotic resistance.

In gram-positive bacteria, resistance to β-lactam antibiotics may be associated either with a decrease in the affinity of the PBP for the antibiotic[149] or with a change in the amount of PBP produced by the bacterium.[150] Multiple mechanisms seem to be present in some clinical isolates. Penicillin-resistant strains of *S. pneumoniae* isolated in South Africa have shown several changes in PBPs (i.e., decreased affinity of some PBPs, loss of others, and appearance of PBPs not present in the more susceptible cells).[150] The genes that encode these PBPs are mosaics, composed of segments from susceptible pneumococci and segments from resistant commensal streptococci.[151] In *S. aureus*[152,153] and *E. faecium,*[154] additional PBPs may be inducible (i.e., their production is stimulated by exposure of the microorganism to the β-lactam antibiotic). These inducible PBPs have a lower affinity for β-lactam antibiotics, making them less susceptible to inhibition by low concentrations of the drug.

MRSA Resistance

In *S. aureus*, methicillin resistance is conferred by the expression of the *mecA* gene, which encodes PBP2A, a protein with low affinity for β-lactam antibiotics, conferring resistance to methicillin, nafcillin, oxacillin, and cephalosporins. The *mecA* gene is the structural component of the *mec* gene cassette and is inserted into the larger staphylococcal cassette

TABLE 18.8 Vancomycin Resistance in Enterococci and Staphylococci

	A	B	C	D	E	G	L	M	N
Vanco (µg/mL) (MIC)	64 to >500	4 to >500	2–32	64–128	16	12–16	8	>256	8
Teico (µg/mL) (MIC)	16 to >500	0.5–2	0.5–2	4–64	0.5	0.5	0.5	64 to >256	0.5
Expression	Inducible	Inducible	Constitutive, inducible	Constitutive	Inducible	Inducible	Inducible	Inducible	Inducible
Genetic location	P, C	P, C	C	C	C	C	C	P, C	P, C
Target alteration	D-ala-D-lac	D-ala-D-lac	D-ala-D-ser	D-ala-D-lac	D-ala-D-ser	D-ala-D-ser	D-ala-D-ser	D-ala-D-lac	D-ala-D-ser
Common species	*Enterococcus faecalis, Enterococcus faecium, Staphylococcus aureus*	*E. faecalis, E. faecium*	*Enterococcus gallinarum* (C-1) *Enterococcus casseliflavus* (C-2) *Enterococcus flavescens* (C-3)	*E. faecium*	*E. faecalis*	*E. faecalis*	*E faecalis*	*E faecium*	*E faecium*

C, Chromosome; *D-ala,* D-alanine; *D-lac,* D-lactate; *D-ser,* D-serine; *MIC,* minimal inhibitory concentration; *P,* plasmid; *Teico,* teicoplanin; *Vanco,* vancomycin.

chromosome mec (SCCmec), which appears to have been acquired through horizontal transfer from a coagulase-negative *Staphylococcus* species.[154,155] At least five different SCCmec types of various genetic sequences and size have been described: types I to III, associated with health care–associated MRSA strains, tend to be larger and multidrug resistant; types IV and V, associated with community-acquired MRSA strains, tend to be smaller in size and more susceptible to antibiotics other than β-lactams.

Expression of the methicillin-resistance gene is controlled by two regulatory components of the *mec* gene: *mecR1-mecI,* and the β-lactamase genes *blaI, blaRI,* and *blaZ,* which can downregulate *mecA* transcription. Although the *mecA* gene is present in all MRSA isolates, the phenotypic expression of methicillin resistance is more variable. For example, *S. aureus* isolates grown at 32°C, rather than at 37°C, are more likely to express methicillin resistance.[155] In addition, the expression of methicillin resistance seems to be modified also by auxiliary genes, such as *fem* and *aux,* which are present in the staphylococcal chromosome and affect various steps in peptidoglycan synthesis.[156]

The PBPs of β-lactamase–negative, penicillin-resistant strains of *N. gonorrhoeae, Neisseria meningitidis*, and *H. influenzae* have shown reduced penicillin-binding affinity.[157–159] Their PBPs seem to be encoded by hybrid genes containing segments of DNA scavenged from resistant strains of related species, similar to penicillin-resistant pneumococci. Mutations leading to a loss of outer membrane proteins also may be associated with the acquisition of penicillin resistance in non–penicillinase-producing strains of *N. meningitidis*, suggesting that altered permeability also contributes to resistance.[160] Progressive loss of β-lactam activity through multiple mechanisms in *N. gonorrhoeae,* coupled with its remarkable capacity to acquire other resistance genes by transformation and plasmid transfer, can give rise to pan-resistant, untreatable gonorrhea.[161]

Permeability changes and decreased affinity of PBPs are mechanisms found jointly in clinical isolates of *P. aeruginosa*[162] and in non–β-lactamase-producing strains of *H. influenzae.*[163] Multiple mutations may be necessary to develop this type of resistance.

Quinolones

DNA gyrase (also called *bacterial topoisomerase II*) is necessary for the supercoiling of chromosomal DNA in bacteria to have efficient cell division.[164] Another related enzyme, topoisomerase IV, also is required for segregation of bacterial genomes into two daughter cells during cell division. These enzymes consist of two A subunits encoded by the *gyrA* gene and two B subunits encoded by the *gyrB* gene (or *parC* and *parE* for topoisomerase IV). Although spontaneous mutation in the *gyrA* locus is the most common cause of resistance to multiple fluoroquinolones in enteric bacteria, B-subunit alterations also may affect resistance to these drugs. Quinolone resistance may also occur from a combination of decreased cell wall permeability, efflux, or enzyme protection mechanisms.[10,113]

DNA gyrase is the primary site of action in gram-negative bacteria, whereas topoisomerase IV is the principal target of quinolones in gram-positive bacteria, including *S. aureus*. Mutations in a variety of chromosomal loci have been described that resulted in altered DNA gyrases resistant to nalidixic acid and the newer fluoroquinolones in Enterobacteriaceae and *P. aeruginosa.*[165] Many of these mutations involve the substitution of single amino acids at the quinolone-resistance determining region (QRDR, located between amino acids 67 and 106 in the gyrase A subunit) that is involved in the generation of the DNA gyrase–bacterial DNA complex.[166] Clinical isolates of *C. freundii* in Japan have been found to be highly resistant to the newer quinolones via alterations in the DNA gyrase.[167]

Plasmid-mediated quinolone resistance has been found in various Enterobacteriaceae and is conferred by *qnr*-encoded proteins that bind to the DNA gyrase antibiotic target and protect it from quinolone action. Although fluoroquinolone resistance associated with plasmid-borne *qnr* genes is low-level resistance, these genes are usually linked to other antibiotic-resistance determinants carried on the same mobile element and have been associated with clinical phenotypes of multidrug resistance.[9,113,168,169] Another plasmid-derived quinolone-resistance determinant, encoded by the *aac(6′)-Ib-cr* gene and derived by mutation of a plasmid-contained aminoglycoside-modifying enzyme, appears widely disseminated among *E. coli* isolates in the United States, mediating low-level ciprofloxacin resistance.[78,113]

Sulfonamides

There are two common genes that mediate resistance to sulfa drugs in pathogenic bacteria: *sul1* and *sul2.* These genes give rise to altered forms of the target enzyme for sulfonamide, dihydropteroate synthase (DHPS).[170] This enzyme is essential for folic acid synthesis in susceptible bacteria. The altered DHPS enzymes mediated by the sulfonamide-resistance genes no longer bind to sulfa, yet continue to synthesize dihydropteroate from para-aminobenzoic acid substrate. The ubiquitous *sul1* gene is part of the class 1 integron family, giving rise to widespread resistance to sulfonamides.[32,170]

Trimethoprim

Trimethoprim is a potent inhibitor of bacterial dihydrofolate reductase (DHFR). Many altered DHFR enzymes with loss of inhibition by trimethoprim have been described from genes found primarily on R plasmids. These altered DHFR genes are widespread in gram-negative bacteria and are found in staphylococci (*dfrA* gene).[171,172]

Protection of Target Site

Tetracyclines

Tetracycline resistance also may occur through a mechanism that interferes with the ability of tetracycline to bind to the ribosome. Resistance genes such as *tetM* and others (see Table 18.6) protect the ribosome from tetracycline action. The Tet M determinant is dispersed widely in gram-positive organisms in addition to *Mycoplasma, Ureaplasma, Campylobacter,* and *Neisseria* spp.[22,23] The *tetM* gene generates a protein with elongation factor–like activity that stabilizes ribosomal-transfer RNA interactions in the presence of tetracycline molecules.

Fluoroquinolones

The newly recognized plasmid-mediated, antibiotic-resistance gene mediating quinolone resistance seems to function as a target protection system.[10] The resistance mechanism seems to protect DNA gyrase from binding to quinolones, allowing the bacterium to resist quinolone inhibitory effects. When this low-level resistance determinant is expressed in concert with other quinolone-resistance genes, such as DNA gyrase mutations or efflux pumps, clinical failures with the use of fluoroquinolones can result.[113]

Overproduction of Target

Sulfonamides and Trimethoprim

Sulfonamides compete with para-aminobenzoic acid to bind the enzyme DHPS and halt the generation of pteridines and nucleic acids. Sulfonamide resistance may be mediated in some bacteria by the overproduction of the synthetic enzyme DHPS. The gene responsible for DHPS is *folP,* and strains of bacteria that produce excess DHPS can overwhelm sulfa inhibition.[170] Trimethoprim resistance may occur in a similar fashion, by making excess amounts of DHFR from the bacterial chromosomal gene *folA.*[172]

Bypass of Antibiotic Inhibition

Another mechanism for acquiring resistance to specific antibiotics is by the development of auxotrophs, which have growth factor requirements different from those of the wild-type strain. These mutants require substrates that normally are synthesized by the target enzymes, and if the substrates are present in the environment, the organisms can grow despite inhibition of the synthetic enzyme. Enterococci can be folate auxotrophs, requiring environmental acquisition of folic acid for growth. They become intrinsically resistant to the folic acid inhibitors (sulfa drugs or trimethoprim) in the process. In addition, bacteria with mutations in the enzyme thymidylate synthetase can retain viability but become "thymine dependent." They require exogenous supplies of thymidine to synthesize thymidylate via salvage pathways and are highly resistant to sulfa drugs and trimethoprim.[173]

A compilation of the most frequent mechanisms of resistance used by common bacterial pathogens to inhibit the actions of antibiotics is provided in Table 18.9. Multiple mechanisms are increasingly in operation

at the same time within individual bacterial cells. The problem of multiple antibiotic-resistance expression is considered in the following sections.

Resistance to Newer, Older, and Miscellaneous Agents

The major mechanisms of bacterial resistance to newer agents or older agents, such as polymyxin B and colistin, which have become the subjects of renewed interest in response to progressive antibiotic resistance, are summarized in Table 18.10.

Oxazolidinones

Linezolid resistance mechanisms are primarily related to alterations in the 23S rRNA-binding sites that inhibit linezolid actions on bacterial translation.[174] Efflux mechanisms may also contribute to reduced activity of linezolid in some bacterial species.[175]

The lipopeptide *daptomycin* is now widely used for treatment of MRSA and glycopeptide-resistant *S. aureus* (GRSA) infections. The drug induces permeability changes and loss of intracellular potassium in susceptible gram-positive bacteria. Resistance is often associated with the abnormally thick cell wall characteristic of VISA strains.[176,177] Accumulation of mutations, especially with the gene *mprF* (encoding lysylphosphatidylglycerol synthetase) indicates the alterations in potential cell membrane binding sites that account for reduced daptomycin activity.[178] In enterococci, mutations in the gene *LiaF*, involved in the bacterial cell envelope response to antibiotics and antimicrobial peptides, and the *gaped* gene, which generates an enzyme likely involved in the cell membrane phospholipid metabolism, have been suggested as mechanisms of daptomycin resistance.[179,180]

Tigecycline is a glycylcycline antibiotic with the mechanism of action of tetracyclines but with remarkable resistance to many of the standard resistance mechanisms. Evidence indicates that resistance is associated with some unusual efflux pumps expressed in some multiresistant gram-negative bacilli.[181,182] Another novel tetracycline-like agent known as omadacycline appears thus far to be resistant to the effects of multiple tetracycline resistance mechanisms.[109] It will be interesting to determine

TABLE 18.9 Resistance Mechanisms Found in Common Bacterial Pathogens

PATHOGEN	RESISTANCE PHENOTYPE		MAJOR RESISTANCE MECHANISM
Streptococcus pneumoniae	β-Lactams		Alteration of target enzymes (PBPs)
	Macrolides, lincosamides, streptogramin B		Alteration of ribosomal target sites (methylation of adenine residue in domain V of 23S rRNA—*ermB*); efflux *(mefE)*
	Tetracycline		Protection of ribosomal target site *(tetM)*
	Trimethoprim and sulfonamides		Alteration of target enzymes (dihydrofolate reductase–trimethoprim; dihydropteroate synthase—*sul1, sul2* in sulfonamines)
	Fluoroquinolones		Alteration of target enzymes (DNA gyrase—*gyrA* mutations; topoisomerase IV—*parC* mutations)
Staphylococcus aureus	β-Lactams	Penicillin	Enzymatic inhibition (penicillinase production)
		Methicillin, oxacillin, nafcillin, and cephalosporins (MRSA)	Alteration of target enzyme—PBP2A (*mecA)*
	Glycopeptide	GISA	Alteration of cell wall precursor targets (thickened cell wall binds drug, preventing it from reaching its target)
		GRSA	Alteration of cell wall precursor targets (plasmid-mediated transfer of *vanA* genes from VRE, resulting in D-ala-D-lac peptidoglycan precursors)
Enterococci	β-Lactams (ampicillin)		Alteration of target enzymes (PBP5 in *Enterococcus faecium*); enzymatic inhibition–rare (penicillinase in *Enterococcus faecalis*)
	Aminoglycosides		Altered ribosomal target site mutations, enzymatic inhibition (high-level resistance: aminoglycoside-modifying enzymes)
	Vancomycin		Alteration of cell wall precursor targets (high-level resistance: VanA, VanB, VanD phenotypes; low-level resistance: VanC, VanE, VanG phenotypes)
	Linezolid		Alteration of ribosomal target sites (G2576U mutation in domain V of 23S rRNA)
	Quinupristin-dalfopristin		Enzymatic inhibition; efflux; target modification *(E. faecium)*
Neisseria gonorrhoeae	Penicillins		PPNG: enzymatic inhibition (plasmid-acquired penicillinase); CRNG: altered target enzymes (PBPs)
	Fluoroquinolones		Alteration of target enzymes (DNA gyrase; topoisomerase IV); efflux (MtrR-CDE efflux system)
	Tetracycline		Protection of ribosomal target (*tetM* gene)
	Macrolides		Efflux; alteration in ribosomal targets (C2611T mutation in domain V of the 23S rRNA)
	MDR		Efflux (MtrR-CDE system: penicillin, tetracycline, macrolides)

Continued

TABLE 18.9 Resistance Mechanisms Found in Common Bacterial Pathogens—cont'd

PATHOGEN	RESISTANCE PHENOTYPE	MAJOR RESISTANCE MECHANISM
Pseudomonas aeruginosa	β-Lactams	Enzymatic inhibition (AmpC cephalosporinases, extended-spectrum β-lactamases, metallo-β-lactamases); active efflux (MexAB); reduced outer membrane permeability (loss of OprD channel)
	Aminoglycosides	Enzymatic inhibition (aminoglycoside-modifying enzymes); efflux (MexXY); alteration of ribosomal targets (ribosomal methylation)
	Fluoroquinolones	Efflux (MexAB, CD, EF, XY, GH, VW); alteration of target enzymes (DNA gyrase mutations—*gyrA*)
	MDR	Overexpression of the MexA-MexB-OprM active efflux system (resistance to quinolones, tetracyclines, and trimethoprim)
Acinetobacter baumannii	β-Lactams	Enzymatic inhibition (AmpC cephalosporinases, plasmid-acquired β-lactamases of the TEM, SHV, CTX-M, PER, VEB families, metallo-β-lactamases of the IMP, VIM, SIM families, and OXA-type serine carbapenemases); alteration of target enzymes (PBPs); reduced outer membrane permeability; efflux pumps
	Aminoglycosides	Enzymatic inhibition (aminoglycoside-modifying enzymes); efflux pumps
	Quinolones	Efflux pumps
	Tigecycline	Efflux pumps
Stenotrophomonas maltophilia	β-Lactams	Impermeable outer membrane Enzymatic inhibition (inducible metallo-β-lactamases L1, L2)
	TMP-SMX	Alteration in sulfonamide target enzymes (*sul1*, *sul2* genes—associated with plasmids or class 1 integrons)
	Fluoroquinolones	Alteration of target enzymes (DNA gyrase mutations); efflux pumps
	MDR	MDR efflux pump (*smeDEF* confers resistance to tetracycline, erythromycin, chloramphenicol, norfloxacin, ofloxacin)
Klebsiella pneumoniae	β-Lactams	Enzymatic inhibition (constitutive expression of penicillinases; extended-spectrum β-lactamases; KPC, NDM-1 carbapenemases); decreased outer membrane permeability
	Fluoroquinolones	Alteration of target enzymes (DNA gyrase mutations—*gyrA*); efflux; protection of target site (plasmid-mediated *qnr* genes)
	Aminoglycosides	Enzymatic inhibition (aminoglycoside-modifying enzymes); alteration of ribosomal targets (ribosomal methylation)
Bacteroides spp.	β-Lactams	Enzymatic inhibition (chromosomally encoded CepA cephalosporinases; metallo-β-lactamases); efflux (homologues of RND-pumps); alteration in drug targets (PBPs)
	Macrolides, lincosamides, streptogramin B	Alteration of ribosomal targets
	Tetracycline	Protection of ribosomal target *(tetQ)*; efflux
	Quinolones	Alteration of target enzymes (DNA gyrase mutations—*gyr A*); efflux

AmpC, Ampicillin C; *CRNG,* chromosomally resistant *N. gonorrhoeae*; *CTX-M,* cefotaxime-M; *GISA,* glycopeptide intermediate *S. aureus*; *GRSA,* glycopeptide-resistant *S. aureus*; *IMP,* imipenem; *KPC, K. pneumoniae* carbapenemase; *MDR,* multidrug resistance; *MRSA,* methicillin-resistant *S. aureus; MtrR,* multiple transferable resistance; *NDM,* New Delhi metallo-β-lactamase; *PBP,* penicillin-binding protein; *PER, Pseudomonas* extended resistance; *PPNG,* penicillinase-producing *N. gonorrhoeae*; RND, resistance-nodulation-cell division; *rRNA,* ribosomal RNA; *SHV,* sulfhydryl variable; *SIM,* Seoul imipenemase; *TEM,* Temoneira; *TMP-SMX,* trimethoprim-sulfamethoxazole; *VEB,* Vietnam extended-spectrum β-lactamase; *VIM,* Verona integron-encoded metallo-β-lactamase; *VRE,* vancomycin-resistant enterococci.

TABLE 18.10 Resistance Mechanisms of Newer, Older, and Other Antimicrobial Agents

	POLYMYXIN	FOSFOMYCIN	DAPTOMYCIN	LINEZOLID	QUINUPRISTIN-DALFOPRISTIN	METRONIDAZOLE	TIGECYCLINE
Enzymatic inactivation	–	–	–	–	++	–	–
Decreased permeability	–	++	+ (gram-negative)	+ (gram-negative)	–	–	+
Efflux	+	-	–	+	+	–	++
Alteration of target site	+++	+	++	++	+	–	–
Protection of target site	–	-	+	–	–	–	–
Overproduction of target	–	-	–	–	–	–	–
Bypass of inhibited process	–	-	–	–	–	++	–
Bind up antibiotic	++	+	–	–	–	–	–

+++, Most common mechanism; ++, common; + less common.

the types of new resistance mechanisms that arise among pathogens in response to this aminomethylcycline agent.

A novel aminoglycoside agent, plazomicin, is now approved for urinary tract infections in 2018 and has improved activity compared with older aminoglycosides through resistance to many of the existing aminoglycoside-modifying enzymes.[183,184] This new agent has a substitution of a hydroxyl-aminobutyric acid at position 1 and a hydroxyethyl substitution at position 6′ of the aminoglycoside core structure, rendering them less susceptible to many common aminoglycoside-modifying enzymes. Some difficult-to-treat pathogens, such as highly antibiotic-resistant strains of *Acinetobacter* and *Pseudomonas* spp., might still be susceptible to plazomicin.

Quinupristin-dalfopristin is a combination of synergistic streptogramins that inhibit protein synthesis by binding to the 23S rRNA in the 50S ribosomal subunit. Resistance in gram-positive bacteria can develop through enzymatic modification (*vatD* and *vatE* genes encoding for acetyltransferases that inactivate dalfopristin), active efflux, and target alteration.[185]

Polymyxins are cationic peptide antibiotics that alter the permeability of the outer membrane of gram-negative bacteria. The use of polymyxin B or colistin (a prodrug, also called polymyxin E) has been rekindled, given the limited number of therapeutic options available to treat multidrug-resistant bacilli. Resistance has been attributable to porin mutations or binding of the agent to the polysaccharide capsule,[186] alterations in the affinity of binding to the outer membrane lipid target of polymyxin B (the *pmr* operon),[187] or increased expression of the efflux pump (the *metAB oprM* gene).[188] Recently, a new mechanism of resistance has appeared that is mediated by a plasmid-borne phosphoethanolamine transferase.[9] Addition of this phosphate moiety to the lipid A β-1,6 diglucosamine backbone of lipopolysaccharide blocks binding access of the polymyxins to their target site within the outer membrane of gram-negative bacteria. Three different mobile colistin resistance genes, designated as *mcr-1* through *mcr-3,* are now known to be spreading in enteric bacteria.[9]

Fosfomycin

Fosfomycin is a cell wall–inhibitor antibiotic that binds to an enzyme essential in the initial step in peptidoglycan biosynthesis (MurA). It has activity against both gram-positive and gram-negative bacteria. Fosfomycin resistance occurs through several mechanisms: (1) decreased permeability through reduced antibiotic uptake by impaired fosfomycin transporters; (2) modification of the antibiotic target (MurA); and (3) antibiotic modification by one of three different fosfomycin resistance proteins—FosA, FosB, or FosX—which are responsible for opening of the oxirane ring of the fosfomycin antibiotic, rendering it ineffective.[189]

Metronidazole

Metronidazole resistance is related to loss of activity of reduced nicotinamide adenine dinucleotide (NADPH) nitroreductase via mutations of the synthetic gene *rdxA*. This enzyme activity is essential to convert metronidazole to its active metabolite.[190] These mechanisms are summarized in Table 18.10.

MULTIDRUG-RESISTANCE MECHANISMS AMONG BACTERIA

Bacteria can express more than one mechanism of antibiotic resistance, leading to phenotypes of multidrug resistance, or even pan-resistance. For example, molecular analysis of *P. aeruginosa* isolates from a nosocomial outbreak in Belgium revealed the convergence of several strategies for antibiotic resistance: (1) overexpression of AmpC chromosomal β-lactamases, conferring resistance to multiple β-lactam antibiotics; (2) mutational loss of OprD porin, conferring resistance to imipenem; and (3) upregulation of the MexXY efflux system (a member of the resistance-nodulation-cell division [RND] family), which exports fluoroquinolones, tetracycline, aminoglycosides, and antipseudomonal β-lactam agents.[191]

In general, multiple antibiotic resistance in gram-negative bacteria often starts with the relatively limited outer membrane permeability to many antibiotic agents, coupled with the overexpression of multidrug-resistance efflux pumps, which can export multiple unrelated antibiotics.[192] In addition, by reducing the intracellular concentration of the antimicrobial below the MIC required for bacterial killing, efflux mechanisms may allow bacterial survival for longer periods of time, facilitating the accumulation of new antibiotic-resistance mutations (i.e., those encoding topoisomerase IV or DNA gyrase targets, rendering fluoroquinolones ineffective).[193]

The clinically important multidrug-resistance efflux pumps belong to several different families: (1) the RND family, (2) the major facilitator superfamily (MFS), (3) the staphylococcal multiresistance (SMR) family, and (4) the multidrug and toxic compound extrusion (MATE) family. Such efflux pumps are widespread among prokaryotes (Fig. 18.6) and are responsible for the export of toxic substances, allowing survival in a noxious environment, such as the biliary system for enteric bacteria.[194] They may also play a role in mediating bacterial adherence to host tissues and exporting virulence determinants, as has been described for *P. aeruginosa*.[195]

Bacteria can also acquire multidrug resistance through sequential transfer of multiple resistance determinants located on mobile genetic elements. For example, conjugative transposons such as Tn*916,* conferring resistance to tetracycline and chloramphenicol, can easily disseminate between bacterial species.[196] Transposons often coexist with other genetic

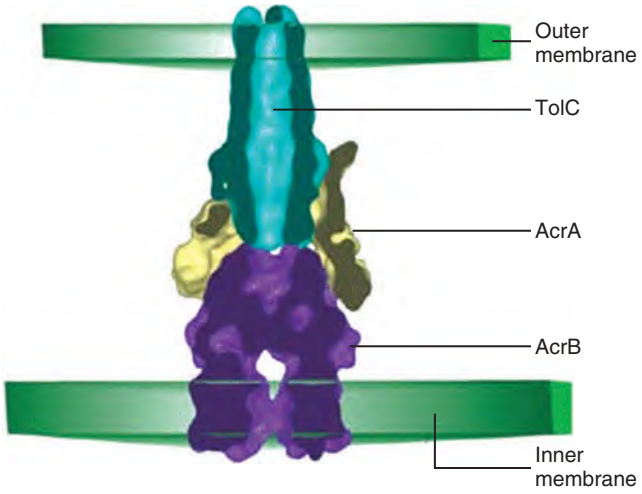

FIG. 18.6 Proposed structural model for the multidrug-resistance AcrAB-TolC efflux pump in *Escherichia coli*. The AcrAB-TolC efflux system is the most important resistance-nodulation-cell division (RND) transporter in *E. coli* and is composed of three interconnected elements: (1) the transmembrane AcrB transporter, which protrudes from the inner membrane into the periplasm; (2) the outer membrane channel TolC, which crosses from the periplasm through the outer membrane, providing the exit route for substrates into the extracellular medium; and (3) the AcrA periplasmic accessory protein, which stabilizes the complex. The pump recognizes a wide variety of substrates, including hydrophobic organic solvents and lipids, in addition to anionic, cationic, and zwitterionic antimicrobials, yielding a multidrug-resistant phenotype. *(Modified from Lomovoskaya O, Zgurskaya HI, Totrov M, et al. Waltzing transporters and "the dance macabre" between humans and bacteria.* Nat Rev Drug Discov. *2007;6:56–65.)*

elements, such as plasmids that may carry additional antibiotic-resistant determinants. For example, analysis of a plasmid encoding the *bla-CTX-15* gene responsible for resistance to extended-spectrum cephalosporins in an *E. coli* outbreak in Toronto, Canada, revealed a large multidrug-resistance region, encoding multiple transposons, and numerous other resistance genes, such as *bla(OXA-1), bla(TEM-1), tetA,* and aminoglycoside-resistance genes *aac(6′)-Ib* and *aac(3′)-II.*[195] The ability of bacteria to capture multiple antibiotic-resistance genes into existing integrons, with their seemingly endless recombination potential, is astonishing.[197,198]

CONTROL OF ANTIBIOTIC RESISTANCE

Although the emergence of antibiotic-resistant bacteria generally has been correlated with the rise and fall of specific antibiotic use in clinical practice, the chain of causality is not always clear-cut. Bacterial strains contain complex aggregations of genes that may be linked together. The use of one antibiotic may select for the emergence of resistance to another. Mobile genetic elements and rapidly evolving integron cassettes with multiple antibiotic-resistance genes endow bacteria with a remarkable capacity to resist antibiotics.[198] Although the development of antibiotic resistance may be inevitable, the rate at which it develops might be mitigated by the rational use of antibiotics and antibiotic stewardship programs.

The ability to track antibiotic-resistance genes with molecular techniques has enhanced the ability to track the spread of antibiotic resistance. With the appropriate computerized surveillance, a hospital laboratory may be able to rapidly detect the emergence of a new type of resistance or the presence of a new microbial strain within a specific unit or patient population. Techniques such as rapid microbial genomics and the increasing availability of rapid genomic sequencing can now be correlated with the phenotypic measures determined by the clinical microbiology surveillance system. Use of molecular techniques greatly augments surveillance data because large data sets may obscure subtle changes ("mini-epidemics") that may be more amenable to the institution of stringent infection-control measures.[195,197–199] Clinical methods to prevent the spread of antibiotic-resistance genes are further discussed in detail in Chapters 51 and 298.

Some bacterial strains have the ability to hypermutate in stressful environments, increasing the risk of acquisition of resistance mutations.[200,201] Because prokaryotic organisms all contribute to a common "gene pool," the total complement of favorable genes mediating antibiotic resistance (the metagenomic resistome) may disseminate among bacterial populations. Reports of the rapidly spreading NDM-type carbapenemases suggest that common, invasive, microbial pathogens may become refractory to any chemotherapeutic agent in the future.[196–198,202] Rational antibiotic-usage policies suggest the curtailment of the unnecessary use of antibiotics in situations such as animal husbandry. The causal link between the use of antibiotics for animal growth promotion and their augmentation of the resistance in human pathogens acquired from food is now reasonably well established.[203–205]

Unfortunately, the paucity of new antimicrobials in development and the rapid spread of multidrug-resistant pathogens, especially among gram-negative bacilli, have resulted in limited therapeutic options in severe infections. Resistance can develop even during therapy with seemingly adequate doses of antibacterial agents to which the pathogen appears to be susceptible with standard sensitivity testing. Three types of subclones existing within a large population of bacteria can survive a single therapeutic dose of a bactericidal antibiotic. These subpopulations include (1) bacterial persisters (antibiotic sensitive when growing but refractory when metabolically dormant); (2) rare, relatively resistant subpopulations within large populations; and (3) mutator strains (high baseline mutation rate clones), which can all be selected for, and lead to, in vivo acquisition of resistance during or after antibiotic therapy.[206] These small subpopulations are present in insignificant numbers ($<10^{-8}$) and are readily eliminated by host antimicrobial defenses under normal circumstances. Their very existence goes unnoticed during antibacterial treatment for most infectious diseases. However, these relatively resistant subpopulations can survive initial low doses of antibiotics, regrow, and become a source of in vivo development of resistance during treatment of infections with large microbial loads, infections adherent to foreign bodies or nonviable tissues, and in the absence of adequate host defenses (e.g., large populations of pathogens in undrained abscesses, infected joint prostheses, severe immunocompromised states).

Dosing strategies that follow pharmacokinetic and pharmacodynamic principles, and dosing regimens that consider mutant protection concentration (MPC) rather than just the MIC, have been suggested as another component of antibiotic stewardship programs. However, the MPC can be as much as 10- to 20-fold higher than the MIC for many classes of antibiotics.[207–210] Larger concentrations of antibiotics can eliminate resistant subpopulations that express one or two resistance mutations, allowing them to persist at concentrations just above the MIC. These surviving subpopulations are selected for during antibacterial treatment. If they can accumulate additional resistance capacity through hypermutation or acquisition of genes from neighboring bacteria, clinical failures with in vivo development of resistance might result. If the initial dose of antibiotic is high enough to eradicate even these resistant subpopulations (above the MPC), treatment can succeed and avoid this problem. Regrettably, the MPC is not easily calculated in most clinical laboratories, and the MPCs for some antibacterials might be difficult to achieve without toxicity. The higher dosing calculations could lead to greater unintended collateral damage to the patient's microbiome.[211] These pharmacokinetic and pharmacodynamic principles are best fit with fluoroquinolone use and might not extend to other antibiotic classes. They work best when resistance is mediated by stepwise increasing resistance from nonlinked, chromosomal, constitutive mutations such as point mutations to common DNA gyrase genes.[208] Mycobacterial resistance predictions using MPC as an in vitro guide might be another situation in which MPC might be predictive of in vivo resistance from nonlinked chromosomal resistance genes.[210]

Early recognition and treatment before large bacterial loads accumulate, short courses of adequate doses of antibiotics to limit resistance development, and restriction of antimicrobial agents to only patients who actually need them are all important in the prevention of antibiotic resistance. In addition, as antibiotics' deleterious effects on the microbiome become increasingly recognized, the quest for developing newer

antimicrobial therapeutic approaches that minimize both antimicrobial resistance potential and alterations in the host's normal flora is imperative.[211]

Antibiotics are a precious commodity, and we should do what we can to preserve the activity of antimicrobials to treat human infections. Dosing regimens should be chosen for both clinical efficacy and for the prevention of resistance development. Academic national and international organizations such as the United Nations, the World Health Organization, and the World Alliance Against Antibiotic Resistance (WAAAR) are now advocating for the commonsense measures to limit progressive antibiotic resistance in clinical and nonclinical use. Research priorities include (1) promotion of research into new antibiotics; (2) development of antibiotic companion agents (e.g., antibiofilm agents, immune adjuvants, antibacterial vaccines, antiexotoxin and antivirulence agents, immunotherapies); (3) limitation or termination of the nonmedical use of antibiotics for agricultural purposes; (4) consideration of the use of curing agents to delete R plasmids from bacteria[20]; and (5) introduction of nonantibiotic therapies to treat bacterial infections.[211,212,213] The best hope for the future is in the development of a greater understanding of how antimicrobial resistance spreads, intelligent use and development of improved bacterial vaccines, antibiotic stewardship, and the implementation of effective infection-control strategies.[212]

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The complete reference list is available online at Expert Consult.

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19 Pharmacokinetics and Pharmacodynamics of Antiinfective Agents

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SHORT VIEW SUMMARY

- Maximizing the safety and efficacy of antiinfective agents requires application of quantitative systems pharmacology using pharmacokinetics and pharmacodynamics (PK-PD) to ensure that the right dosage regimen is selected.
- This chapter explains the general PK principles that govern the processes by which drugs enter and leave the body through absorption, distribution, metabolism, and elimination.
- The shapes of these concentration-time profiles impact the PD activity of antiinfectives and support selection of alternative regimens (reduced frequency, longer infusions) to reduce toxicity or optimize efficacy.
- Illustrative examples of the clinical translational potential of PK-PD from in vitro to in vivo, initial clinical trial dose selection, and dose refinement once clinical knowledge is gained are provided to demonstrate the applicability of this approach to deliver precision medicine.

The right drug against the right pathogen must be administered at the right dose to be safe and effective. Pharmacokinetics (PK) and pharmacodynamics (PD) systems analyses provide the mathematical framework within pharmacology for the design of precision antiinfective dosing regimens.[1] *Pharmacology* is the knowledge base of a compound concerning its history, source, physical and chemical properties, compounding, biochemical and physiologic effects, mechanisms of action and resistance, absorption, distribution, metabolism, excretion, and therapeutic and other uses.[1]

Pharmacokinetics (PK) describes the process by which a drug enters and leaves the body based on absorption, distribution, metabolism, and excretion to define systemic exposure.[2] *Pharmacodynamics* (PD) describes the biochemical and physiologic response of the drug and its mechanism of action.[2] PK-PD analyses are integrated to define the exposure response, where the response can be a measure of safety, efficacy, or emergence of resistance. Optimal dose regimens are now defined by modeling and simulation by balancing the probability of efficacy relative to safety thresholds.[1,2] Antiinfective PK-PD is unique in pharmacology in that this relationship includes the effects of the drug on the infective pathogen and the host as well as collateral effects on the microbiome.[3,4] Because antiinfective agents affect nonmammalian target sites, the doses of these agents tend to be an order of magnitude higher than other pharmacologic classes.[5] Another challenge involves reductions in potency of antiinfective agents over time due to emergence of resistance.[5] This chapter focuses on the clinical translational potential of PK-PD from in vitro to in vivo, initial clinical trial dose selection, and dose refinement once clinical knowledge is gained. The numerous terms and corresponding abbreviations used to describe specific PK-PD parameters in this chapter are summarized in Table 19.1.

PHARMACOKINETICS

Antimicrobials are administered by the oral, intravenous, and intramuscular routes with systemic exposure quantified using serum or plasma concentrations. Measurement of tissue concentrations requires invasive or semiinvasive techniques that result in estimation using drug physiochemical properties such as protein binding. Physiology-based PK models are increasingly used in drug development to predict drug biodistribution but are difficult to validate clinically. In some instances, samples such as epithelial lining fluid, cerebrospinal fluid, and synovial fluid may be obtained in humans, but in general our understanding of tissue and body compartment drug biodistribution relies on preclinical data.[6] *Physiology-based PK models* are increasingly being used to estimate tissue drug distribution and rely on drug physiochemical properties and on species-related physiologic data (Fig. 19.1). If a drug is administered intravenously, the rate of systemic entry is defined by the rate of drug infusion (R_0). Likewise, if a drug is administered orally or by some other extravascular route, the rate of entry into systemic circulation is defined by the absorption rate constant (k_a). In this second scenario, the extent of absorption may not be complete, and so the ratio of the systemic exposure profile by oral (or extravascular) absorption to that of intravenous administration defines the bioavailability (F). The rate of drug transfer between systemic circulation to tissues and organs depends on numerous factors including (1) molecular size and charge, (2) protein binding, (3) influx and efflux transporters, and (4) cardiac output. Most drugs undergo biotransformation through various metabolic processes to support elimination primarily via the kidneys and liver into the feces. As stated, the stepwise process of drug entry and departure is regulated by complex physiologic processes that are simplified and visually represented by the plasma or serum concentration-time curve. The shape of this concentration-time curve can be modified by altering the rates of drug entry, distribution, metabolic transformation, and elimination through intrinsic or extrinsic factors.[7] As shown in Fig. 19.2, administration of a 500-mg dose of an antiinfective agent by oral and intravenous routes can lead to distinctively different profiles. In the case of intravenous administration, the rate of infusion clearly can increase or decrease the time that a concentration is above a threshold value, such as the minimal inhibitory concentration for 90% of isolates (MIC_{90}). More detailed explanations of these pharmacokinetic processes are provided as follows.

Absorption

Absorption describes the movement of drug from an extravascular space to an intravascular space.[1] For antiinfective agents, the two most common modes of drug administration that require absorption are the oral and intramuscular routes. The amount of drug that reaches the systemic circulation is expressed as a percentage of the total amount that could have been absorbed. This percentage is defined as the drug's absolute or relative *bioavailability. Absolute bioavailability* means that the amount of drug absorbed by the extravascular route of administration has been compared with the intravenous route, whereas *relative bioavailability* means that two different extravascularly administered dosage forms have been compared.[1]

A major process variable in oral drug absorption is the solubility and permeability of the compound in the gastrointestinal tract. Most drugs in development tend to be insoluble, requiring significant efforts related to formulation development. The Biopharmaceutics Classification System

TABLE 19.1 Quick Reference Pharmacologic Abbreviations and Their Definitions

TYPE OF TERM	ABBREVIATION	DEFINITION
Pharmacokinetics		
Absorption	F	Bioavailability; absolute bioavailability
	K_a	Absorption rate constant
Distribution	V_d	Volume of distribution
	V_d/F	Apparent volume of distribution
	V_{ss}	Volume of distribution at steady state
	V_{ss}/F	Apparent volume of distribution at steady state
	CL_D	Distributional clearance
	CL_D/F	Apparent distributional clearance
Metabolism	Km	Drug concentration at which the rate that an enzyme system can metabolize a drug is half of Vm (Michaelis-Menten type of metabolism [saturable metabolism])
	Vm	Maximal metabolic capacity (Michaelis-Menten type of metabolism [saturable metabolism])
	CYP	Cytochrome P-450 enzyme systems
Elimination	CL_r	Renal clearance
	CL_{nr}	Nonrenal clearance
	CL_{nr}/F	Nonrenal oral clearance
	CL_T	Total clearance
	CL_T/F	Total oral clearance
	$t_{1/2}$	Half-life
Pharmacodynamics		
	MIC_{90}	Minimal inhibitory concentration for 90% of isolates
	EC_{50}	Effective concentration for 50% of all isolates
	MPC	Mutant prevention concentration
	MSW	Mutant selection window
	IC_{50}	Inhibitory concentration for 50% of isolates
	C_{max}/MIC	Peak antimicrobial serum concentration to MIC ratio (concentration-dependent killers)
	AUC/MIC	24-h area under the serum antimicrobial concentration-time curve to MIC ratio
	AUIC	24-h area under the inhibitory curve
	t	Half-life
	T > MIC	Time that serum antimicrobial concentrations are above the organism's MIC (time-dependent killers)
	SBT	Serum bactericidal titer (concentration)
	IQ	Inhibitory quotient ratio of trough serum concentration to IC_{50}
	PAE	Postantibiotic effect

(BCS) is currently used to categorize drugs into four groups based on high solubility, low solubility, high permeability, and low permeability. Ciprofloxacin serves as a model BCS class IV compound (low solubility/low permeability) that is poorly absorbed but can be transformed to a BCS class I compound (high solubility/high permeability) through medicinal chemistry. Oral absorption can be saturable or nonsaturable with factors such as degradation in the gut by acid or proteolysis gut metabolism and first-pass liver drug metabolism by enzymes and influx and efflux transporters influencing the rate and extent of absorption. Other factors that can impair absorption and bioavailability are drug interactions with other compounds or food that may bind the drug or reduce solubility.[8] The response of certain antiinfective agents is linked to the peak concentration (C_{max}) or the total exposure (area under the concentration-time curve [AUC]), which can be adversely impacted by reduced rate of absorption even if the extent is not.[2] As shown in Fig. 19.2, a more rapid rate of intravenous administration leads to higher C_{max} values. Alternatively, the response of microbes to some antiinfective agents is linked to maintenance of concentrations above a threshold concentration such as the MIC.[3,4] In this setting the rate of infusion can be extended (see Fig. 19.2) or a controlled-release oral formulation can be used to target steady concentrations above a threshold.

Distribution

The shape of the concentration-time curve is modeled most commonly using a proportionality constant known as the *volume of distribution (V_d)* and is termed the *apparent volume of distribution (V_d/F)* when the drug is administered via the extravascular route.[9] The V_d is not a real or physiologic volume, but rather a value that relates drug concentration in the system to the amount of drug present in that system. This system can be defined as a single compartment (i.e., V_{d1}) or as multiple compartments (i.e., V_{d1}, V_{d2}, … V_{dn}) to mathematically fit the shape of the concentration-time curve. Factors that alter the physiologic distribution of drug into tissue include lipophilicity, partition coefficient of the drug between different types of tissues, blood flow to tissues, pH, and binding affinity to plasma proteins relative to tissue components.[1] However, actual measurement of concentrations in these tissues or interstitial fluids is necessary to confirm site-specific distribution and cannot be easily estimated by V_d.[9]

Drug transporters play a role in defining the net drug concentration at the site of infection through influx and efflux transporters.[10] Transporter function can be influenced by genetic and environmental factors and thus varies from person to person. Drugs binding to serum proteins have a major influence on V_d. Acidic drugs tend to bind to albumin and typically have lower V_d values because they are retained in the plasma compartment. Basic drugs tend to bind to α_1-acid glycoprotein and are retained within tissues leading to larger V_d estimates. Protein binding is an important consideration for antimicrobial agents because unbound drug is available to exert antimicrobial activity, and in vitro methods used to assess potency through the MIC evaluate unbound drug.[11] Changes in the unbound fraction of drug may be caused by

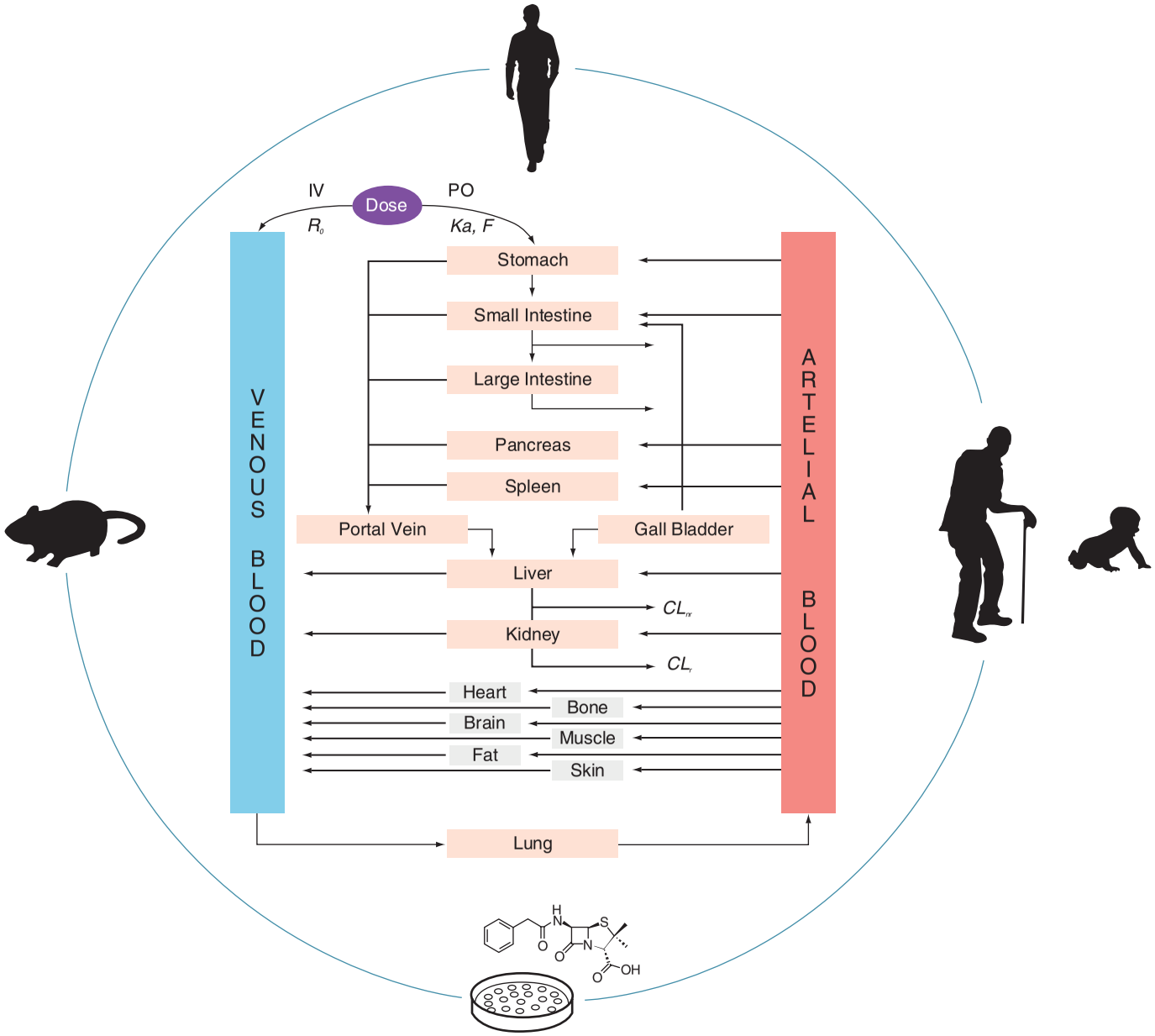

FIG. 19.1 Overview of a physiology-based pharmacokinetic model to predict plasma and tissue drug distribution in preclinical species to first in man and special populations using physiologic, microbiologic, physiochemical, and pharmacodynamics data. CL_r, Renal clearance; CL_{nr}, nonrenal clearance, *F*, bioavailability; *IV*, intravenous; K_a, absorption rate constant; *PO*, oral, R_0, rate of drug infusion.

displacement from other drugs, changes in serum protein concentrations, or accumulation of endogenous substances such as free fatty acids.[2] Although changes in protein binding may alter PK of an antimicrobial agent, substantial changes in PD would be unlikely.[12]

Metabolism and Biotransformation

Drugs undergo oxidative, reductive, and conjugation-based metabolism through enzymes primarily in the liver but in other tissues as well and by the gut microbiome. Drug metabolism is determined by genetics and environmental (external) factors.[13] Similar to transporters, some drug-metabolizing enzymes (DMEs) show genetic polymorphism, meaning that at least 1% of the population exhibits different DME activity (increased activity, decreased activity, or no activity) than the rest of the population. In some instances even for enzymes for which genetic polymorphism has not been shown, a large range in the rate and extent of drug metabolism can be found.[14] Environmental factors are many but include concurrent drugs, underlying diseases (both infectious and noninfectious), nutrients, herbal preparations and supplements, nutritional status, pregnancy, and sex (although for antiinfective agents sex differences have not been reported in general).

Drug metabolism reactions are classified as either phase I or phase II reactions.[2,8] *Phase I reactions* can transform a substrate into an active or inactive metabolite and in some cases into a more toxic substrate. Phase I reactions generally are under the control of the *cytochrome P-450 (CYP) system.* CYP enzymes are heme-containing proteins that are located in the endoplasmic reticulum of a variety of cell types, most abundantly in the liver. CYP enzymes are controlled by a superfamily of genes that are classified into families according to their amino acid sequences. Each family is divided further into subfamilies. The term *CYP3A4* designates a mammalian enzyme (CYP) family 3, subfamily A, gene 4. To date, many drugs that are metabolized by phase I enzymes have been shown to be metabolized by five primary CYP enzymes. In decreasing order of importance for drug metabolism, they are CYP3A, CYP2D6, CYP2C, CYP1A2, and CYP2E1. Although a complete discussion of the CYP system is beyond the scope of this chapter, many of the newer antiinfective agents, particularly antiretroviral agents, can induce,

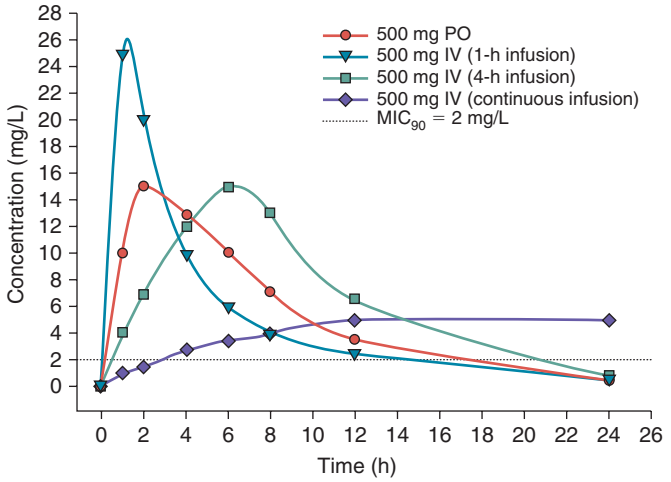

FIG. 19.2 Simulated serum concentration-time profile based on administration of a single antiinfective dose by oral *(PO)* and intravenous *(IV)* routes, with infusion at varying rates. *MIC_{90},* Minimal inhibitory concentration for 90% of isolates.

activate, or inhibit CYP enzymes, and in many cases they are substrates for CYP enzymes and are affected by changes in CYP activity.

CYP enzymes are affected by many factors that stimulate or inhibit their ability to metabolize drugs. Genetic factors have been shown to result in a phenomenon called *polymorphism.* Simply put, polymorphism means that individuals vary in their genetically determined ability to metabolize CYP substrate. For some CYP enzymes, such as CYP2D6, distinct poor, intermediate, extensive, and ultrarapid metabolic patterns exist in a population; in a white population, 4% to 6% are poor metabolizers, and the rest fall into the other metabolic groups, with the majority being extensive metabolizers. CYPs such as CYP2C9, CYP2C19, CYP2A6, and CYP2B6 also show genetic polymorphism. These CYPs are important in drug metabolism. For other CYPs, such as CYP3A, genetic polymorphism has been described; however, the significance of this remains confusing. These phenomena have important implications for antiinfective agents, for which efficacy against infecting organisms and toxicity to the host are determined by the PK of the agent and its resultant PD effect.

Clinically, drug, food, disease, and herbal effects on the CYP system may translate into inhibition, activation, or induction of metabolism. Inhibition of CYP activity occurs through reduction of enzyme production, inactivation, or competition for CYP substrate. Generally individuals with increased enzyme activity exhibit a greater inhibition of the CYP system with an inhibiting agent than individuals with less activity. Enzyme inhibition may result in increased PD effect, with the potential not only for greater efficacy but also for greater toxicity. This inhibitory process may be used in the clinical setting advantageously. Ritonavir has been used to decrease the activity of CYP3A isozymes in the gut, allowing greater absorption of other protease inhibitors (PIs) such as tipranavir and darunavir and reducing the overall cost of therapy. Cobicistat, an analogue of ritonavir, was specifically developed as a PK enhancer to boost oral absorption of antiretrovirals.[15] Induction of CYP increases production of the DME and a resultant increase in the ability to metabolize specific compounds. An example is the induction by rifampin of CYP3A with a subsequent increase in the metabolism of PIs. Many inducers of CYP enzymes also induce phase II conjugation reactions and transporters. Activation increases DME activity but to a much lesser extent (approximately 65% less) than enzyme induction.

Phase II reactions, which also show genetic polymorphism, involve conjugation of the parent compound with larger molecules, which increases the polarity of the parent molecule and permits excretion. Although phase II reactions generally lead to inactivation of the parent compound, occasionally conjugation increases the potency of the parent compound or results in the formation of another biologically active compound. When the conjugated compounds are secreted into the intestine, enzymatic cleavage may occur with release and reabsorption of the active parent compound, a phenomenon called *enterohepatic recirculation.*[1] As mentioned, the liver is not the only place in the body where metabolism occurs. Metabolism and detoxification of foreign substances can occur in most other organ systems.

Elimination

The AUC over a specific time period is proportional to the dose administered (for drugs that follow linear PK) and inversely related to *total drug clearance (CL_t).*[7] CL_t reflects the unit volume of a system that is cleared of drug per unit time (e.g., L/h). The physiologic drug clearance process is driven by elimination of the biotransformed or unchanged drug. This elimination of drugs is further categorized as renal and nonrenal clearance. *Renal clearance (CL_r)* describes the volume per unit time that the body eliminates a substance via the kidneys, through various mechanisms including glomerular filtration, tubular secretion (an energy-dependent process), and tubular reabsorption. It is important to realize that almost all xenobiotics are freely filtered through the glomerulus, but in most instances they undergo tubular reabsorption. Tubular secretion is a transporter-mediated process, and dose-dependent PK can be shown for substances that undergo tubular secretion as their primary route of elimination (e.g., piperacillin-tazobactam). *Nonrenal clearance (CL_{nr} or CL_{nr}/F)* describes the sum of clearance pathways that do not involve the kidneys.[2] These mechanisms may involve the biliary tree (e.g., ceftriaxone) or the intestine (e.g., azithromycin). Furthermore, the composition and enzymatic activity of the intestinal microbiota affect whether deconjugation occurs that impacts the rates of excretion and reabsorption. Other, uncommon mechanisms can be used such as elimination of alcohol through the skin and lungs (respiration) and ionization, DNA chelation, and inactivation of aminoglycosides by the sputum in patients with cystic fibrosis with elimination through expectoration.[16] Extracorporeal elimination through procedures such as dialysis (hemodialysis or peritoneal dialysis) also can be construed as a form of nonrenal elimination.[17] As expected, significant interindividual variability exists in the PK of drugs. *Population PK analyses* are used to identify and quantify sources of interindividual variability of PK parameters to better define doses in the broad population as well as special populations.

PHARMACODYNAMICS

Antiinfective PD is a science that is used to integrate PK information and in vitro measures of drug potency with effect.[4] This effect can be measured in vitro and in vivo (animal models) as the rate and extent of microbial death/growth inhibition or emergence of resistance.[18] Alternatively, this effect can be defined clinically by a measure of biologic response such as survival, time to clinical response, probability of clinical response, and so on.[7] As expected, PK-PD systems analysis currently represents a stepwise hierarchical process that integrates in vitro and in vivo data followed by clinical validation.[19]

Antimicrobial Potency

An antiinfective agent may inhibit growth and replication ("-static") or cause bacterial cell death ("-cidal"). A factor that affects whether a drug is bacteriostatic or bactericidal is the concentration at the site of action, but this may not imply differences in clinical efficacy.[20] Antimicrobial agents may be bacteriostatic at low concentrations but bactericidal at high concentrations. These bacteriostatic and bactericidal concentrations have been used to quantitate the activity of an agent against an organism. Approaches to measure this activity have broadly included use of agar-based dilution and broth macrodilution and microdilution systems.[21] Agar-based dilution systems lead to measurement of activity as a zone of inhibition. Broth dilution systems lead to measurement of an MIC that is based on a doubling-dilution ($\log_2$) scale (e.g., 0.5, 1, 2, 4 mg/L).[21] Agar-based methods can also incorporate susceptibility testing using an E-Test strip that creates antimicrobial gradients to quantify the MIC on an arithmetic scale (e.g., 0.5, 0.75, 1, 1.5, 2 mg/L). The *MIC for 90% of all surveyed isolates* of a bacterial species (MIC_{90}) and the *inhibitory or effective concentration for 50% of all surveyed isolates* of a strain of virus (IC_{50} or EC_{50}) are conventionally used to describe drug activity against pathogens. The minimal bactericidal concentration

provides information on the lowest concentration at or above the MIC required to kill a microorganism. Although these in vitro parameters are helpful epidemiologically, they represent fixed values that do not reflect the dynamic in vivo process such as (1) the time course of activity or the potential for persistent antiinfective effect after the concentration at the site has decreased below the MIC or minimal bactericidal concentration, (2) the interaction of the immune system with the drug, and (3) exposures necessary to prevent the development of resistance or organism mutation. Importantly, these parameters reflect specific drug-organism in vitro measurements that cannot reflect the combination drug use profile as empirical therapy and for documented polymicrobial infections.[21]

Although antiinfective agents can be used individually, in many instances they are used together. *Synergism* is defined as activity of two or more antiinfective agents given together that is greater than the sum of activity had the agents been given separately. *Additivity* (also known as *indifference*) is defined as activity of two or more agents together that equals the sum of activity of each agent. *Antagonism* is defined as activity of two or more antiinfective agents given together that is lower than the activity of the most active agent given separately. Combinations of agents are used to enhance efficacy and rate and extent of organism killing or to reduce the development of resistance but can have conflicting results.[22]

Pharmacodynamics Indices

PD combines PK parameters and microbiology parameters to describe drug effect in relation to some measure of exposure. These PK measures of exposure include the maximal concentration (C_{max}; peak), minimal concentration (C_{min}; trough) or AUC integrated over a specified time period (AUC_{0-t}) or infinity (AUC_{0-inf}). Alternatively the duration of time that the concentration exceeds a threshold value can be correlated to an event related to efficacy, safety, or emergence of resistance. In the case of antiinfective agents, three PK-PD indices based on serum or plasma concentrations have been associated with efficacy and include C_{max}/MIC, AUC/MIC, or T > MIC.[19] As shown in Fig. 19.3, measurement and classification of the relevant C_{max} are not straightforward. For an intravenously administered agent, C_{max} occurs at the end of the infusion, whereas this is more variable for an orally administered agent but typically between 1 and 4 hours. The rise and fall in drug concentrations are often evaluated through blood sampling and most often have two phases. The first phase follows a more rapid initial decline referred to as the *distribution phase,* followed by a reduction in the slope referred to as the *elimination phase.* This second phase in the concentration-time profile often coincides with the concentration profile expected in the interstitial space of tissues with the scale of this profile dependent on the degree of plasma protein binding. From this perspective, C_{max} and C_{min} are single point estimates but are rarely measured at the exact time of their occurrence, which requires a monoexponential or biexponential function to translate.[23] This information can also be modeled and integrated over time to generate an overall exposure or AUC that is easier to translate between serum and other tissue compartments.[7] Thus the effect of many antimicrobial agents often correlates to the AUC/MIC index because AUC (mg · h/L) represents both concentration (mg/L) and time-dependent (h) components.[24] Antimicrobial agents deemed to be concentration dependent with a good correlation to C_{max}/MIC also have some degree of correlation to AUC/MIC.[24] The same phenomenon is true for antimicrobial agents deemed to manifest time-dependent PK-PD.[24] For antimicrobial agents that are deemed to be time dependent (good correlation to T > MIC), a good correlation with AUC/MIC is also observed if this agent has persistent sub-MIC effects.[18] For antiinfective agents, such as antiviral and some antiretroviral agents that are activated intracellularly, serum and plasma PK are often not reflective of the site of cellular and subcellular activity. These agents are also retained intracellularly for longer periods of time than reflected by the intravascular concentration-time profile.

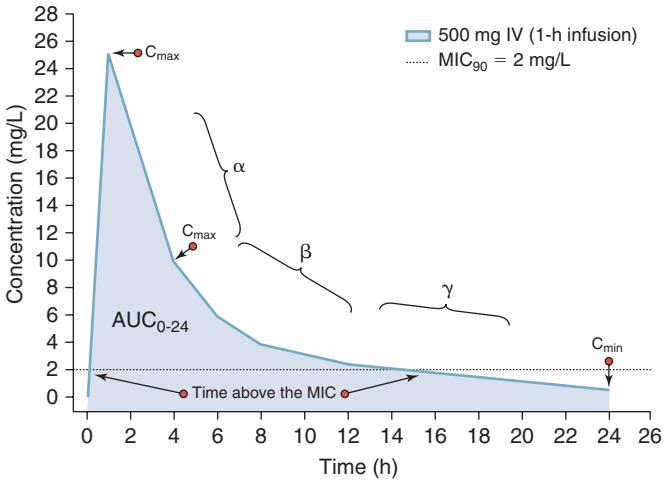

FIG. 19.3 Common antibiotic pharmacokinetics and minimal inhibitory concentration *(MIC)* pharmacodynamics relationships based on an antiinfective agent (e.g., an aminoglycoside) with a triphasic serum concentration-time profile. *AUC_{0-24},* Area under the curve for first 24 hours after time 0; *C_{max},* maximum concentration; *MIC_{90},* minimum inhibitory concentration for 90% of isolates.

Methodology for Study of Pharmacodynamics Effects of Antiinfective Agents

In Vitro Models

The most widely accepted model used to study in vitro PK-PD effects of antiinfective agents is the "hollow fiber model" system.[25,26] The system uses a cartridge that is composed of thousands of hollow porous fibers that are sealed at each end so that growth media that enter one end go through the inside of the fibers to the opposite end. Microorganisms or virally infected cells are inoculated on the outside of the fibers and multiply in the space between the fibers known as the extracapillary space. In this system, antiinfective agents, nutrients, and metabolic waste can cross the fibers but the larger microorganisms cannot cross through the pores. As a result, the microorganisms can be exposed to predetermined dynamic or static concentrations of antiinfective agents under conditions that can simulate the expected PK in humans.[25,26] The extracapillary compartment can be sampled via a port to quantify the microorganism load and drug concentrations. Although these models offer control over bacterial inoculum and drug concentration-time profiles that mimic clinical cases, they do not currently assess the effects of the immune system on organism killing or growth inhibition. They assess the relationship of free drug concentrations to effect, assisting in the development of relationships of protein-bound drug in humans. However, these in vitro models result in relatively high organism loads and lack host immune function and so have the theoretical potential of predicting higher effective doses than would be defined by immune intact animal models. More recent applications have included the evaluation of antiinfective combinations against multidrug-resistant gram-negative pathogens. This approach streamlines experimental designs and aids evaluation in clinical trials in a more efficient manner. This is a valuable approach to improve on existing agents given the limited number of available antiinfective agents against multidrug-resistant pathogens[26] and the important collateral effects of antibiotic overdosing.

Animal Models

Animal models have used a variety of species, often with the animals rendered neutropenic before infection. Craig and others[27–29] showed that the presence of neutrophils may affect antibacterial activity with fluoroquinolones, penicillin, clindamycin, and doxycycline. Animal infectious disease models have been developed to mimic human infections. Animal models allow for frequent sampling of blood and tissue and allow a broad dosage range to be investigated along with a wide range of organism inocula, allowing investigators to study the effects of variation in a single parameter at a time. Problems with animal models include a lack of standardization of inocula size (often large inocula are required to produce infection). The faster rate of drug elimination in small mammals compared with humans often leads to the use of dosing regimens that may match human AUC values but

may not replicate human concentration-time profiles. Immunocompetent animals have been used to attempt to develop more realistic guidelines for PK-PD targets in infected patients, many of whom are not neutropenic. Despite these limitations, animal models can yield necessary data to aid dose translation from preclinical to early stage clinical studies.

Clinical Trials

Preclinical and early clinical (phase I and II) PK-PD relationships are often being applied to justify dose selection for the two phase III clinical trials that are necessary to gain regulatory approval to market a drug.[18,19] This dose selection often includes testing of a single fixed dosage (e.g., 500 mg intravenously once daily) or weight-based dosage (e.g., 6 mg/kg intravenously once daily) regimen. After completion of the first phase III trial, PK-PD analyses may validate or contradict the pre–phase III study assumptions. The high cost of phase III trials limits significant modification of the study design to rectify and retest assumptions about dose selection.[19] Hence most human trials[29–38,39,40] that have defined PK-PD relationships have been based on the retrospective review of postmarketing drugs or post hoc subgroup analyses of prospectively collected data.[41–44] Little, if any, prospective data have been generated with either dosage adjustment during therapy or comparison of different dosing regimens to attain different exposures during treatment (concentration-response trials).[45] These trials have used three measures of assessment to relate to antimicrobial PK-PD: (1) clinical outcome (cure/fail or improved); (2) eradication of bacteria from the site of infection or reduction in virus concentration (viral load) in blood or other sites, or both; and (3) improvement in surrogate markers of infection such as temperature or leukocyte count. Many trials have not reported free drug PD indices. Because free (unbound) drug is considered active, correction for protein binding is important for highly bound drugs. Few human trials have focused on relationships of drug exposure to toxicity or on the development of resistance. Clinicians often fail to appreciate that exposure response is a fundamental paradigm in pharmacology, and so for every drug an opportunity exists to individualize the dosage regimen and not rely on a "one size fits all" dosage regimen.[7] However, the impracticality of this approach and limited clinical experience to generate confidence in a safety threshold has limited the application of these principles to a handful of antiinfective agents. Lack of availability of a commercial assay, limited knowledge of interlaboratory reproducibility, and clinical interpretation of systemic concentrations delay the process by which clinicians can apply these principles in practice. Despite these limitations, well-designed proof-of-concept studies are emerging in the literature and align with the desire to deliver precision antiinfective therapies.[46]

Concentration-Dependent Killing Agents

Concentration-dependent killing agents (e.g., fluoroquinolones, aminoglycosides, macrolides, azalides, ketolides, metronidazole, daptomycin, and oritavancin) exert their positive effect on bacteria when their concentrations are well above the MIC of the organism.[18] When the ratio of the concentration at the site of infection to the MIC is increased further, greater killing occurs. This concept is illustrated in Fig. 19.4 for tobramycin and ciprofloxacin against *Pseudomonas aeruginosa*.[47] In addition, some of these agents exhibit postantibiotic effect (PAE) (see "Postantibiotic Effect" later). Growth inhibition continues for a variable period after the concentration at the site of the bacteria has decreased below the MIC for the antimicrobial agent. In vivo, the C_{max}/MIC ratio has been shown to be the clinical correlate used as the PD predictor for outcome of concentration-dependent killing agents. In clinical trials, the AUC/MIC ratio also has been correlated with improved outcome.[33–44] This finding is not surprising because C_{max} and AUC increase in proportion to the administered dose and consequently are correlated.[18] For drugs such as fluoroquinolones, different goals for AUC/MIC ratios are required for gram-positive pathogens compared with gram-negative pathogens.[47–48]

Time-Dependent Killing Agents

Time-dependent killing agents include penicillins, cephalosporins, aztreonam, vancomycin (for which AUC/MIC is predictive), carbapenems, macrolides, linezolid, tigecycline, doxycycline, and clindamycin.[18] For agents active against gram-negative bacteria, the rate of kill is maximized when concentrations at the site of the bacterial infection are typically four times higher than the MIC of the organism; this is shown for ticarcillin against *P. aeruginosa* in Fig. 19.4.[3]

The amount of time that the concentration needs to be above the MIC has been the subject of debate.[19] A report using animal studies with *Streptococcus pneumoniae* in which treatment was performed with penicillins or cephalosporins showed that when T > MIC was 20% or less of the dosing interval, mortality was 100%. In contrast, a mortality rate of 0% to 10% occurred when serum concentrations were above the MIC for longer than 40% to 50% of the dosing interval.[19,49]

Evaluation of the influence of PK/PD on bacterial resistance has best been characterized with fluoroquinolones and aminoglycosides and is being explored for other agents. Blaser and colleagues[49] examined the C_{max}/MIC ratio for enoxacin and netilmicin against various gram-negative organisms. Regrowth of organisms occurred in all cultures when enoxacin or netilmicin attained ratios lower than 8. On redosing of these antibiotics after bacterial regrowth, no killing was seen because of the development of resistance. Marchbanks and associates[50] using

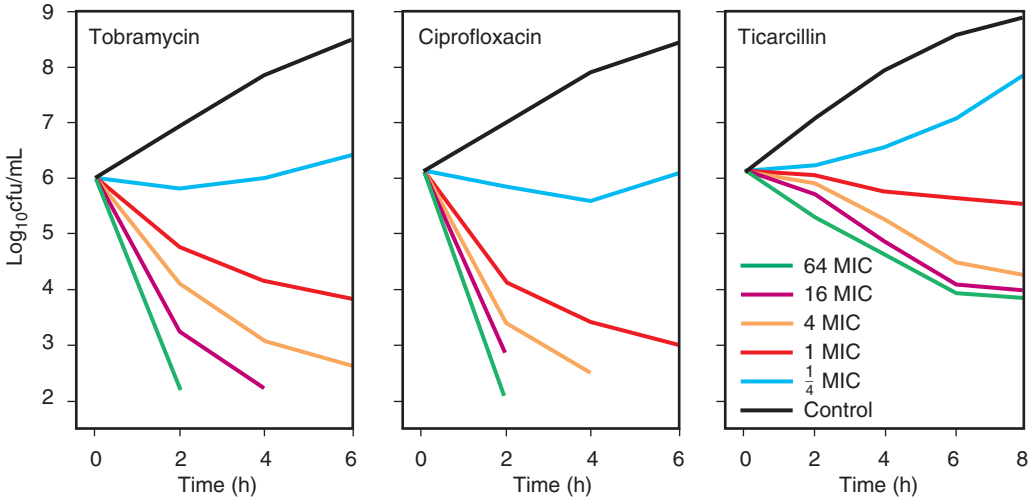

FIG. 19.4 Time-kill curves for *Pseudomonas aeruginosa* ATCC 27853 with exposure to tobramycin, ciprofloxacin, and ticarcillin at concentrations from one-fourth to 64 times the minimal inhibitory concentration *(MIC).* *(From Craig WA, Ebert SC. Killing and regrowth of bacteria in vitro: a review.* Scand J Infect Dis. *1991;74:63–70.)*

ciprofloxacin noted the development of resistant *P. aeruginosa* when the organism was exposed to a C_{max}/MIC ratio of 6 compared with no resistance when the C_{max}/MIC ratio was 12, even though both regimens showed adequate rates of bacterial killing. Additional studies have confirmed that the high initial concentrations do impact emergence of resistance when testing ciprofloxacin against *P. aeruginosa.*[51] Recent hollow-fiber model studies have also identified PK-PD–resistance emergence relationships between linezolid and *Mycobacterium tuberculosis,* which would be very difficult to characterize in vivo.[52] A disadvantage of these investigations, however, is that they do not account for the role of the immune system in "cleaning up" small numbers of resistant bacteria before they can become pathogenic.

Postantibiotic Effect

During in vitro testing of antimicrobial agents, there may be a delay before microorganisms recover and reenter a log-growth period.[47] This phenomenon is termed the *postantibiotic effect (PAE).*[3,47] The exact duration of the PAE is both species and drug dependent. Aminoglycosides and fluoroquinolones produce in vitro PAEs against gram-negative bacilli of 2 to 6 hours. β-Lactam antibiotics (except for imipenem) produce little or no PAE against gram-negative organisms under identical experimental conditions but generally induce 2-hour PAEs against gram-positive organisms.[18] Other factors that affect the in vitro PAE include combinations of antimicrobial agents, antimicrobial concentration, duration of antimicrobial exposure, and pH. Potential factors that also may affect PAE include size of inoculum, type of growth medium, and bacterial growth phase.[18]

Studies in animal models have verified that PAE is not an artifact of in vitro testing. Investigational animal models that have been studied include a neutropenic mouse thigh model, a rabbit meningitis model, a rat endocarditis model, and a guinea pig pneumonia model. These studies showed that an in vivo PAE exists against gram-negative organisms for aminoglycosides, fluoroquinolones, erythromycin, clindamycin, and tetracycline, but not for β-lactams. As in the in vitro studies, β-lactam agents produce abbreviated PAEs against gram-positive organisms.

A definitive common mechanism to explain the PAE has been elusive for years. Nonspecific binding, nonlethal damage, antibiotic persistence in the periplasmic space, and antibiotic binding kinetics all have been postulated as potential mechanisms. Srimani and colleagues[53] elegantly demonstrated that drug detoxification within individual cells after the drug is eliminated from the extracellular system can explain this phenomenon among antibiotics with variable mechanisms of action. The presence or absence of a PAE has been used to alter antimicrobial dosing schedules. Theoretically an agent with a long PAE can be dosed less frequently than an antimicrobial agent lacking a PAE. Alternatively an agent with little or no PAE may be most effective if it is given as a continuous infusion so that the serum concentration always exceeds the MIC. Dosing strategies such as these are theoretical and require clinical investigation in human studies of sufficient size before implementation into clinical practice.

Applied Clinical Pharmacokinetics and Pharmacodynamics

The exposure-response relationship predictive of effect and safety may not be complete when an antiinfective agent is first marketed. Over the past 40 years, the principles outlined earlier have been applied to improve the clinical management of patients through design of alternative drug dose regimens that take advantage of the exposure-response relationship.[18] These strategies have broadly included the use of higher-dose extended-interval dosing and continuous or extended infusions for antimicrobial agents with concentration-dependent and time-dependent PK-PD characteristics, respectively. Infected patients are in a dynamic physiologic state that is a corollary to "shooting at a moving target" with antiinfective dose selection.[19] Conceptually, use of more intensive dosing regimens at treatment initiation followed by dose titration with clinical improvement or worsening would be ideal. Testing this approach requires more complex covariate-adjusted response-adaptive designs of antiinfective agents with a companion biomarker of response to tease out true differences between regimens.[54] In the interim, examples of specific dose regimen designs that have taken advantage of the presumed PK-PD profile are provided as follows. Therapeutic drug monitoring (TDM) to improve the dosing of certain agents is also described to illustrate that the dosing of an antiinfective agent can evolve with increasing clinical experience.

Higher-Dose Extended-Interval Dosing

This dosing strategy has primarily been used to optimize the PK-PD profile of concentration-dependent antimicrobial agents such as tobramycin, levofloxacin, daptomycin, oritavancin, dalbavancin, and time-dependent antimicrobial agents such as azithromycin.[39,42,55–58] High-dose extended-interval aminoglycoside dosing serves as the model for validation of this approach. The original regulatory approved doses of gentamicin and tobramycin were 1 mg/kg three times daily, but these agents are now clinically administered as 5 to 7 mg/kg once daily in patients with good kidney function.[59] A tobramycin dose of 10 mg/kg once daily is recommended in patients with cystic fibrosis who are being managed for an acute pulmonary exacerbation secondary to *P. aeruginosa.*[59] The objective of the tobramycin regimen of 5 to 10 mg/kg once daily is to achieve a serum concentration of 16 to 20 mg/L 1.5 to 2 hours after a half-hour infusion (postdistribution phase). We seek to achieve a serum C_{max}/MIC ratio of 8 to 10, based on an MIC_{90} of tobramycin against *P. aeruginosa* of 2 mg/L because this PK-PD index has been correlated to predict clinical success.[33] The half-life of tobramycin in most patients with good kidney function is 2 to 4 hours. As a consequence, the serum tobramycin concentrations are expected to be less than 2 mg/L for 6 to 14 hours of the 24-hour dosing regimen. This sustained effect of tobramycin (despite long sub-MIC exposures) in the clinical setting is based on in vitro and animal model demonstrations of the PAE and sub-MIC effects of this agent.[53] However, aminoglycosides are rarely administered as monotherapy (except for urinary tract infections), so definitive clinical proof of this concept is unavailable. Meta-analyses also have not clearly shown this dosing concept to be superior to the individualized daily dosing approach, with the resultant dosing being one or multiple times a day depending on the individualized patient PK parameters.[58] Well-designed clinical trials in patients with cystic fibrosis have not shown a clear efficacy benefit but have suggested a lower potential for nephrotoxicity with this agent.[60] Despite this lack of clear clinical benefit, the emergence of multidrug-resistant gram-negative pathogens, convenience of this dosing strategy, and theorized benefits continue to support adoption of this aminoglycoside dosing strategy.[60]

Although the basis of this dosing strategy evolved with the aminoglycosides, the most successful clinical application of these principles has actually occurred with the use of levofloxacin, azithromycin, daptomycin, and oritavancin.[42,55,56,58] These dose regimens are also being tested with antimicrobial agents in clinical development. However, the motivation for this dosing strategy is distinct for each of these agents. The clinical outcomes associated with the use of 750 mg of levofloxacin once daily for 5 days are similar to outcomes associated with the use of 500 mg once daily for 10 days for the treatment of community-acquired pneumonia.[61] Similarly, azithromycin has been approved as a 5-day treatment course for community-acquired pneumonia based on a loading dose of 500 mg on the first day followed by 250 mg once daily for 4 days.[55] In both cases, high epithelial lining fluid and alveolar macrophage concentrations serve as the pharmacologic basis of these shorter-course regimens.[6] In the case of azithromycin, shorter 3-day courses or even single-dose administrations have been approved for certain clinical indications.[54] The prolonged intracellular retention of azithromycin and immunomodulatory effects of this agent (not predicted by in vitro studies) has been credited to support this dose design.

The application of higher doses at treatment initiation has more recently been referred to as front-loaded regimens, based on similar theories to suppress tumor growth.[62] The efficacy and safety of the new glycopeptide oritavancin with a front-loaded regimen compared with daily dose administration has been tested in a phase II study.[63] A single oritavancin dose regimen (1200 mg) was shown to have a similar safety and efficacy profile as daily administration (200 mg for 3–7 days) for complicated skin and soft tissue infections. Based on supportive phase II trial data, use of a higher dose of rifampin (15 mg/kg/day) versus the current dose of rifampin (10 mg/kg/day) was studied in patients

(≥15 years of age) with tuberculous meningitis.[64] The probability of survival was not improved with this intensified regimen.[64] Peloquin and colleagues[65] studied higher rifampin doses (20 mg/kg/day) in patients with tuberculosis and provided the pharmacologic basis to evaluate higher doses in future studies. Similarly, use of a high-dose twice-yearly combination of albendazole (800 mg) and ivermectin (400 µg/kg) was superior to standard-dose albendazole (400 mg) and ivermectin (150 µg/kg) at suppressing *Wuchereria bancrofti* microfilaremia.[66] Microfilaremia was detectable in 57% and 28% of patients with the standard dose at 12 and 24 months after treatment but was undetectable in all patients treated with the high-dose regimen. These studies suggest the potential either to maintain clinical effect with a more convenient dosing strategy or to actually improve clinical outcomes without an increase in the risk for adverse reactions.

Continuous-Infusion and Extended-Infusion Regimens

The effects of intermittent, extended, and continuous infusion on the serum concentration time profile are illustrated in Fig. 19.5 with a 3 g/day dose of an antimicrobial agent, assuming a one-compartment system with a CL = 9.3 L/h, V_{d1} = 36.3 L, and an elimination half-life of 5.4 hours. As illustrated by this simulation, concentrations above a threshold concentration of 8 mg/L are maintained for the longest period of time with the use of an initial combination of a short infusion (loading dose) followed by a continuous infusion. As a consequence, concentrations can be maintained above this threshold with a 2 g/day regimen compared with a 3 g/day regimen, that is, a 30% lower total daily dose. The concentrations in serum with a continuous infusion regimen with or without a loading dose will converge; however, use of a continuous infusion without a loading dose will lead to a delay in the time that the concentration exceeds a threshold (Fig. 19.6). Martinez and associates[18] reviewed this topic and highlighted the importance of achieving effective concentrations at treatment initiation when the organism load is expected to be at its highest.

A systematic review and meta-analysis of extended and continuous infusion of piperacillin-tazobactam and carbapenems based on non-randomized studies suggested that these dosing approaches may be associated with a lower risk for mortality compared with shorter intermittent infusions.[67] Well-designed multicenter studies have yielded varying results when evaluating the impact of longer infusion duration on the outcome of β-lactam–based therapies in the intensive care setting.[68,69] A meta-analysis of prolonged-infusion piperacillin-tazobactam studies suggested a 1.46-fold lower odds of mortality with prolonged infusions compared with intermittent infusion of this agent.[70] These data support the overwhelming in vitro and animal model data that concentrations above a threshold improve activity of β-lactams. However, surveys suggest that translation or acceptance of this dosing paradigm occurs in less than 20% of institutions in the United States and abroad.[71–73]

Dose-Refinement Considerations

Selection of a specific antiinfective dose regimen relies on the assumption that a dose-response relationship exists in support of this regimen. The dose regimen that is approved for clinical use represents the population central tendency estimates, which maximizes the probability of clinical effect. This regimen is validated internally in a subset of the general population that is evaluated through clinical trials. Hence external validity of this dose regimen for most antiinfective agents occurs after it is marketed; that is, we may study hundreds of patients for drug approval but ultimately use the drug in millions of patients. Furthermore, the optimal dose of an antiinfective agent for every patient subgroup (e.g., pregnant patients, patients receiving dialysis, pediatric patients) is not well known when it is first marketed. Therefore a system to aid dose selection or refinement in these subpopulations of patients is a common expectation and a constant clinical challenge. TDM was developed to meet this specific challenge but has not been embraced universally because it may not be apparent during drug development that a specific therapeutic range or exposure exists. Again, the evaluation of a relatively small and carefully selected patient population before market approval limits the discovery of a therapeutic exposure range.

The clearest example of this point was documented with the triazole antifungal agents, owing to high interpatient variability in drug absorption and metabolism. Itraconazole, voriconazole, and posaconazole are three triazoles that are used to manage invasive fungal infections that have now been shown to require TDM to optimize outcomes.[74] In the case of itraconazole and posaconazole, unpredictable oral absorption represents the primary reason that TDM is necessary.[74] Voriconazole is metabolized in part via cytochrome P-450 2C19 (CYP2C19) isoenzymes that are encoded by a gene that is known to be polymorphic with ethnic-based variation among individuals. Common coadministered drugs such as omeprazole can also inhibit this isoenzyme system. Therefore the dose-exposure profile of voriconazole is highly unpredictable in the clinical milieu of ethnic diversity and potential drug-drug interactions.[74] The most recent practice guidelines for the diagnosis and management of aspergillosis include clinical scenarios in which TDM of voriconazole, itraconazole, and posaconazole is justifiable.[75]

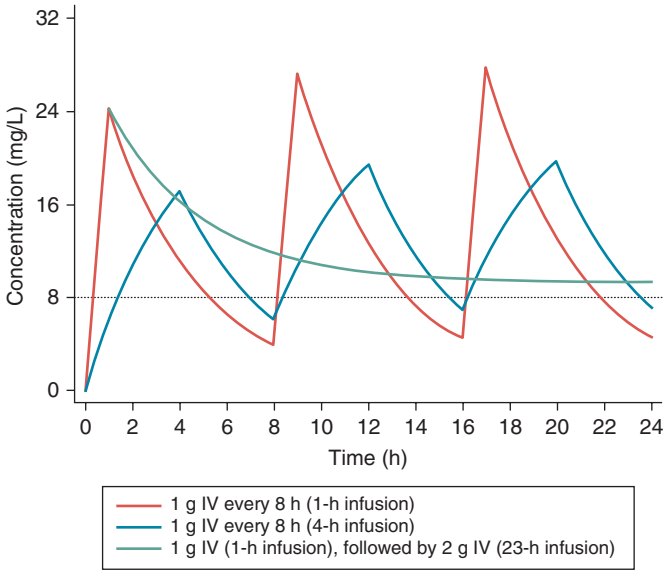

FIG. 19.5 Simulated serum concentration-time profile of an antimicrobial agent (3 g/day) administered as a 1-h, 4-h, and continuous infusion (with initial short infusion dose) with a reference concentration threshold of 8 mg/L. *IV,* Intravenous.

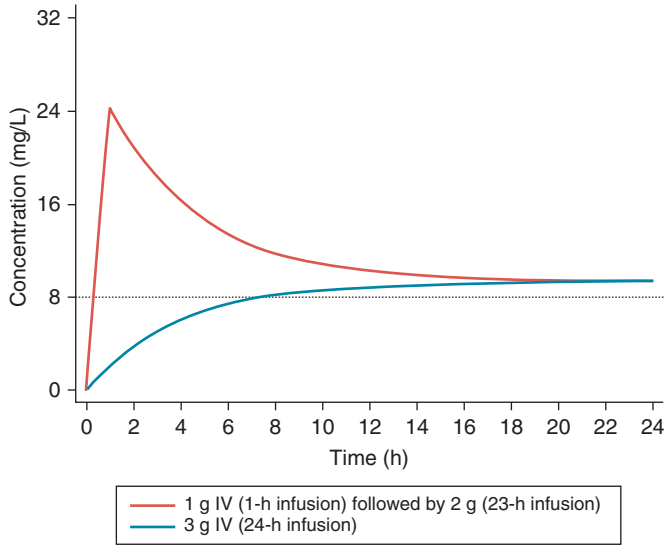

FIG. 19.6 Simulated serum concentration-time profile of an antimicrobial agent (3 g/day) administered as continuous infusion, with and without initial short infusion dose, with a reference concentration threshold of 8 mg/L. *IV,* Intravenous.

These observations with the triazole antifungal agents have also been made with antituberculosis agents.[76] Use of standard dose regimens of isoniazid may not be reasonable in all populations given the known polymorphisms in enzymes that influence its metabolism.[76] Limited access to commercially available assays prevents practical application of dose refinement that is necessary to calculate the correct dose.[77]

In practice, TDM of the aminoglycosides and vancomycin is most common, but definitions of the target exposure ranges associated with effect and toxicity have changed over time.[78,79] As with any measure, it is important to recognize that a distribution surrounds a presumed central tendency target exposure value and that this target may not be fixed or linearly translatable by MIC. With tobramycin as an example, an AUC_{24} of 75 mg · h/L and 192 mg · h/L may be sufficient for pathogens with an MIC of 0.5 to 1 mg/L and 2 to 4 mg/L based on in vitro models and mathematical simulation.[78] Let us assume that a vancomycin AUC/MIC target greater than 400 h^{-1} is associated with a higher probability of effect against a pathogen with a modal MIC_{90} of 1 mg/L. A 2-g daily dose achieves a median AUC of 400 mg · h/L in a population and so represents the average population dose. An average patient is treated with 2 g/day empirically but then is determined to be infected with methicillin-resistant *Staphylococcus aureus* (MRSA) in the bloodstream that has a vancomycin MIC of 0.5 mg/L (48 hours into the admission). Given this scenario, should we reduce the dose in half to achieve an AUC of 200 mg · h/L? The answer is most likely to be no. Alternatively if the MIC was 2 mg/L, should we double the daily dose? The answer in this scenario may be yes, but we should also expect the risk for toxicity to increase. As shown, current application of these principles at a population level is quite helpful for dose selection, but simple linear translation may be spurious, especially when factoring in uncertainty associated with MIC measurement.[80] We expect the current practice of vancomycin TDM to evolve from the simple measurement and dose adjustment that is based on trough concentrations to one that considers AUC estimation using two serum samples.[81,82] Similarly, we may discover over time that TDM is necessary with other agents used to treat MRSA such as linezolid and daptomycin that have high interpatient variability in PK in certain subpopulations such as critically ill patients. Alternatively, empirical combination antiinfective treatment as a strategy to curb antiinfective resistance against pathogens that we currently manage with monotherapy (e.g., MRSA) may be adopted over time and require our reevaluation of the necessary exposure targets and doses.

Antiretroviral Pharmacodynamics

In contrast to the above-discussed antimicrobials, antiretrovirals are unique in their site of action, which is most commonly inside or on the surface of the mammalian cell. Toxicity can result from interference with the host cell's physiology as seen with the early antiretroviral agents commonly associated with adverse events including diarrhea and mitochondrial toxicity. Yet as with other antimicrobials, failure to achieve effective concentrations can result in rapid development of drug-resistant mutations and subsequent treatment failure. Thus using dosing strategies that achieve a concentration profile between effective and toxic thresholds in each individual patient is of utmost importance. Fig. 19.7 illustrates these key concepts of the exposure-response relationship and the therapeutic window. One strategy to manage this balance is to treat with combinations of antiretrovirals that affect different points in the viral life cycle. This strategy harnesses the pharmacologic principles of additivity and synergy, where the effect of the drugs given in combination is either equal to (additive) or above (synergistic) what would be expected given the potency of each individual agent.[83] Thus combination dosing can lower the concentrations required for viral suppression thereby widening the therapeutic window.

At the present time there are more than 36 antiretrovirals approved by the US Food and Drug Administration within five therapeutic classes including six single tablet regimens consisting of three or more agents.[84] For therapeutic classes with a low genetic barrier, a single point mutation can confer resistance to most agents within that class; thus treatment failure can greatly limit subsequent treatment options. Successive antiretroviral regimens also do not perform well for as long a duration as the initial regimen, and increased mortality risk has been associated with multiple regimen failures.[85,86] Therefore optimizing the first regimen

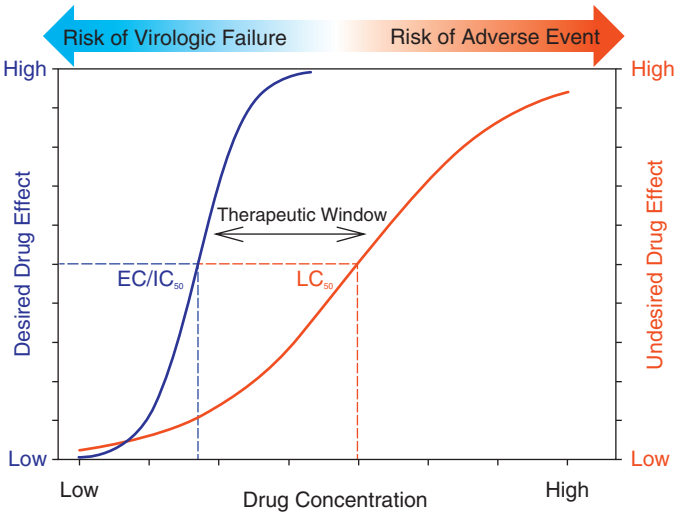

FIG. 19.7 Hypothetical exposure vs. desired (blue) and undesired (red) response curves for a drug with a narrow therapeutic window. The 50% effective concentration/inhibitory concentration *(EC/IC_{50})* is marked by blue dashed reference lines. The 50% toxic concentration *(LC_{50})* is marked by red dashed reference lines.

for success is crucial. Early-phase clinical trials frequently attempt to correlate PK parameters (i.e., indices) such as C_{max}, C_{min}, and AUC to outcome, usually measured by changes in plasma human immunodeficiency virus (HIV) RNA or $CD4^+$ T lymphocytes. However, variability in drug exposure within and between patients can be an important factor in interpreting these data. Thus a comprehensive understanding of these PK-PD relationships and of exposure variability is important for making clinical decisions that maximize the likelihood of treatment success for every patient.

Defined Exposure (PK)-Response (PD) Relationships by Class

Protease inhibitors. PK-PD relationships have been well studied for commonly prescribed PIs. For atazanavir, AUC[87] but not C_{min}[88] significantly correlates with antiviral activity as well as hyperbilirubinemia, a common, atazanavir-specific adverse event.[87,89,90] The latter relationship is likely due to reduced activity in the metabolizing enzyme, UGT1A1, within a subset of patients carrying the genetic polymorphism, UGT1A1rs887829.[89] Between-study discrepancies have been noted for darunavir, where early reports described a significant PK-PD relationship[91] for twice-daily dosing, which was not substantiated in later studies for either once-daily or twice-daily dosing.[92,93] A potential explanation of this discordance is that high interpatient (between patients) and intrapatient (within the patient) variability in darunavir plasma concentrations obscures this relationship; also, high intrapatient variability has been previously associated with failure to achieve virologic suppression.[94] Although lopinavir/ritonavir C_{min} is an important predictor of response in antiretroviral treatment–experienced patients,[95,96] it is not correlated with response in antiretroviral-naïve patients.[97] This finding demonstrates the importance of viral resistance in influencing PK-PD relationships (see later discussion).

Nonnucleoside reverse transcriptase inhibitors. C_{min} is correlated with virologic response to nevirapine.[98] Historically, efavirenz plasma concentrations between 1000 ng/mL and 4000 ng/mL have been targeted to minimize the risk of virologic failure and central nervous system toxicity.[99] However, more recent reports exploring efavirenz combined with modern nucleoside reverse transcriptase inhibitors (NRTIs) suggest a C_{12h} cutoff between 0.47 ng/mL and 0.76 ng/mL as a more sensitive and specific efficacy threshold.[100,101] In a phase IIb study, no relationship was observed between rilpivirine exposure and virologic response rates.[102] All doses in this study produced plasma concentrations above the protein-adjusted EC_{50} and were likely at the plateau of the exposure-response curve. For etravirine, no relationship was observed between AUC or C_{min} and virologic response in treatment-naïve patients taking

400 mg once daily.[103] However, in treatment-experienced patients who are taking 200 mg twice daily, multiple studies describe C_{min} as a significant predictor of virologic response with a proposed target of >300 ng/mL.[92,104] These results suggest that C_{min} measurements may be useful for prediction of nonnucleoside reverse transcriptase inhibitor (NNRTI) responses.

Integrase strand transfer inhibitors. The role of C_{min} as a marker of efficacy of integrase strand transfer inhibitors may be less clear. In a comprehensive PK-PD analysis, dolutegravir C_{min} best predicted plasma viral load reduction on day 11 of monotherapy. The in vivo EC_{50} of 36 ng/mL was also identified as the potential C_{min} target.[105] Plasma PK-PD relationships correlating C_{min} with antiviral activity have also been described for once-daily dosing with elvitegravir and raltegravir.[106,107] However, this relationship is not observed for raltegravir with 400 mg twice-daily dosing[107,108] or with the new 1200-mg once-daily formulation,[109] both of which achieve C_{min} values that are greater than twofold to sixfold higher (543 nM and 113 nM, respectively) than the IC_{50} (31 nM) and the C_{min} of the 800-mg once-daily dose referred to previously (40 nM). Thus this absence is likely attributed to achieving exposures at the plateau of the exposure-response curve.

Entry inhibitors. The CCR5 inhibitor maraviroc also demonstrates a clear PK-PD relationship where AUC is predictive of response.[110]

Nucleoside reverse transcriptase inhibitors. Defining PK-PD relationships for NRTIs is more difficult because these drugs are intracellularly phosphorylated to their active diphosphate and triphosphate metabolites. Multiple rate-limiting phosphorylation steps, cellular membrane efflux transporter activity, and differential phosphorylation rates in activated versus quiescent $CD4^+$ cells[111-113] result in plasma parent drug concentrations that do not consistently correlate with metabolite concentrations.[114] However, a 50% increase in zidovudine triphosphate concentrations and a 33% increase in lamivudine triphosphate concentrations have been positively correlated with the rate of HIV-1 RNA decline after starting therapy.[114–116] For tenofovir, disoproxil fumarate, and emtricitabine, the plasma PK-PD relationship plateaus beyond the treatment doses of 300 mg and 200 mg once daily,[117–119] suggesting saturation of cellular phosphorylation processes. Therefore the active metabolite C_{min} associated with these doses (approximately 84 fmol/million cells and 4000 fmol/million cells, respectively) represent potential therapeutic targets. High plasma tenofovir concentrations have also been associated with increased risk of nephrotoxicity.[120,121] The novel prodrug formulation, tenofovir alafenamide, reduces this risk by maximizing cellular uptake, thereby minimizing plasma tenofovir exposure.[122] This case illustrates the use of a targeted-prodrug approach to alter biodistribution and PK-PD of a compound (tenofovir) to improve efficacy and safety of the compound.

Antiretroviral Therapeutic Drug Monitoring

With established PK-PD relationships, antiretroviral TDM can be considered. Some antiretroviral drugs (particularly PIs and raltegravir) can have significant intraindividual PK variability owing to food effects and other environmental influences (e.g., prescription and nonprescription medications, nutraceuticals) on DMEs and transporters.[123] This makes interpreting single drug concentrations challenging. Yet TDM for efficacy and toxicity can be warranted in certain clinical circumstances where virologic response may be unpredictable or antiretroviral options are limited. Current guidelines recommend TDM for pregnant patients who have risk factors associated with virologic failure (such as not achieving viral suppression during early stages of pregnancy); patients with pathophysiologic conditions that alter drug PK such as gastrointestinal, hepatic, or renal dysfunction; antiretroviral treatment–experienced patients; patients with clinically significant drug or food interactions; patients with concentration-dependent drug toxicities; patients with alternative dosing regimens or antiretroviral combinations; and treatment-adherent patients with lack of virologic response.[84] Based on convenience, trough concentration cutoffs for efficacy (and occasionally toxicity) have been offered for PIs and NNRTIs—mostly assigned by expert consensus[84]; the review by van Luin and colleagues[124] provides a consolidated list of these targets. An inherent limitation to using these concentration targets to guide clinical decision making is the inability to account for additive or synergistic drug interactions within the specific antiretroviral regimen being interrogated. Even so, TDM-guided therapy has been shown to be effective in certain clinical settings.[125,126]

Alternative PK-PD Indices for Antiretroviral Therapy

Viral heterogeneity and drug resistance can impact the PK-PD relationship. Various resistance mutations can make a virus less sensitive to a drug, either completely (e.g., K103N mutation against nevirapine or efavirenz) or in an escalating fashion (e.g., accumulating mutations can confer increasing resistance to PIs). Therefore relating drug concentrations to the susceptibility of an individual patient's viral isolate has been explored. First described by Ellner and Neu,[127] the inhibitory quotient (IQ) integrates drug exposure (defined as total or protein-unbound AUC, C_{max}, or C_{min}) and viral susceptibility (expressed as the in vitro IC_{50}, IC_{90}, IC_{95}, or IC_{99}, with or without the presence of plasma proteins). The IQ is most commonly calculated as the ratio of the drug concentration at the end of the dosing interval (C_{min}) to the in vitro IC_{50}: C_{min}/IC_{50}. Several derivatives (nIQ, vIQ, and gIQ) have also been proposed to account for confounding factors in the PK-PD relationship, such as the presence of multiple mutations and the effect of protein binding.[128] The nIQ (normalized inhibitory quotient) was developed to eliminate protein binding confounding and is the ratio of C_{min} to the fold change in antiretroviral susceptibility (using virtual phenotype) related to a fixed ratio of the population mean antiretroviral C_{min} to the cutoff for resistance; for example, nIQ = (C_{min}/fold change in IC_{50}) ÷ (population C_{min}/fold change resistance cutoff).[129] The vIQ (virtual inhibitory quotient) is defined as the ratio of C_{min} to the IC_{50} of wild-type virus multiplied by the virtual phenotype (a calculated fold decrease in susceptibility mathematically derived from the individual patient viral genotype and matched to a genotype-phenotype database): C_{min}/IC_{50} · virtual phenotype. The gIQ (genotype inhibitory quotient) is calculated as the ratio of C_{min} to the number of clinically important PI mutations. Of all of these measures that incorporate PK and viral susceptibility into a PD target, the gIQ has found the most utility in clinical study.[128,130,131] The IQ and its derivatives, rather than individual PK parameters, may more strongly predict antiviral efficacy for darunavir, lopinavir/ritonavir, and etravirine in highly treatment-experienced patients.[91,95,96,132,104] There are, however, a number of limitations of using the IQ to predict virologic response. There is no current standardization for calculating or selecting the IQ or its derivatives for prediction of virologic response among clinical trials evaluating the IQ. Therefore collating information from the available literature is difficult. Also, there is no consensus on which IQ derivative achieves the best predictive power, and each derivative is accompanied by its own unique set of considerations. Regardless, since 2010 with more potent and easy-to-use antiretroviral therapy available, there has been less practical application of these techniques in clinical care.

Finally, Shen and associates[133] investigated mathematical approaches to characterizing antiretroviral PD and relative potency. The median effect model of dose response evaluates the slope of a dose-response curve using the proportion of affected virus, proportion of unaffected virus, drug concentration, and IC_{50}. In this model, slope is characteristic of the drug class, whereby antiretroviral drug classes with steeper slopes (e.g., PIs) require lower concentration/IC_{50} ratios for virologic suppression. However, this model does not take into account the multifactorial influences of antiretroviral PD durability such as tolerability, drug interaction potential, and genetic barriers to resistance. Additionally, some inconsistencies have been noted in the model. For example, although the model assigns raltegravir a comparatively low slope of 1.1 ± 0.05, clinically, raltegravir demonstrates rapid, potent, and sustained virologic suppression, suggesting that these models need more refinement to explain more of the variability in the model prediction.

Pharmacodynamics for Other Antiviral Drugs

PK-PD relationships have also been established in the treatment of other viral infections including hepatitis C virus (HCV), cytomegalovirus (CMV), and herpes simplex virus (HSV). Since the first direct-acting agents (DAA) were approved for HCV treatment in 2011, 10 more agents with improved potency have joined the market. Virologic suppression of these first DAAs, boceprevir and telaprevir, was highly dependent on C_{min}. This relationship was demonstrated in the phase II

telaprevir trial,[134] in which higher rates of sustained virologic response (SVR) were observed for a lower total daily dose divided three times daily compared with a higher total daily dose divided twice daily. With the improved potency and combination dosing of newer DAAs, PK-PD relationships have been more difficult to define. Specifically, multiple studies have failed to describe a predictable relationship for sofosbuvir-ledipasvir PK estimates and SVR.[135] Likewise, after correcting for covariates, a PK-PD analysis of paritaprevir, ombitasvir, ribavirin, and dasabuvir found that only ombitasvir AUC was predictive of SVR.[136] For paritaprevir, increased AUC was associated with increased probability of experiencing a grade 3 adverse event.[137]

Foscarnet, a polymerase and reverse-transcriptase inhibitor used to treat CMV, exhibits both a PK-PD and PK-toxicity relationship that strongly correlates AUC with the outcomes of increased days to progression of CMV retinitis and increased nephrotoxicity risk.[138] TDM is not well validated for foscarnet but has been successfully employed in patients receiving hemodialysis.[139] In this report, dose adjustments were made to achieve C_{max} concentrations of 500 to 800 μM between the proposed efficacy (CMV IC_{50} = 100–300 μM) and toxicity (>1000 μM) thresholds.

Finally, the interplay between PK and PD was illustrated in the observation that intravaginal administration of 1% tenofovir gel decreased risk for HSV type 2 (HSV-2) in the CAPRISA004 trial,[140] which aimed to evaluate the gel for HIV prevention. This finding was unexpected because oral dosing in women was known to achieve genital tract tenofovir concentrations of approximately 70 ng/mL (well below the estimated HSV-2 EC_{50} of 14,000–19,000 ng/mL).[141,142] However, topical administration resulted in genital tract concentrations that were approximately 3 logs higher than oral dosing. A subsequent PK-PD analysis and clinical trial (CAPRISA004) demonstrated that tenofovir 1% gel protected against HSV-2 acquisition with decreased risk among women achieving genital tract concentrations ≥10,000 ng/mL.[143] These findings illustrate the importance of a comprehensive understanding of the PK-PD relationship of antiretroviral and antiviral agents in the development of new drugs and new clinical applications for old drugs.

CONCLUSIONS

Optimal dose selection of an antiinfective agent for an individual patient is an indispensable goal of clinical practice. The study of the interrelationship between drug exposure and response through PK-PD analyses is now an established component of antiinfective drug development to meet this goal. This domain of pharmacology has developed to follow a pathway that integrates information from in vitro, in vivo, clinical, and in silico experiments to define a dosing regimen that increases the probability of effect and reduces the probability of toxicity in a population. However, various nonpharmacologic factors can influence efficacy and safety-related outcomes in individuals. These unmeasured or immeasurable factors can confound our assessment of the "true" exposure-response relationship. Clinical use of an agent in populations underrepresented in early studies leads to an identification of pharmacologic and nonpharmacologic factors that influence outcome. Thus our understanding of the specific antiinfective exposure-response relationship evolves with the clinical use of an agent. Continued innovations in genomic, assay, and computer software capabilities will foster individualized antiinfective dose selection. Importantly, discovery of the complex interactions between the metagenome and antiinfective agents may help to explain some of the interindividual variability in PK-PD and perhaps help direct antiinfective therapy more precisely.

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20 Penicillins and β-Lactamase Inhibitors

Yohei Doi

SHORT VIEW SUMMARY

PENICILLIN G

Usual Adult Dose: 12 to 24 Million Units/Day Intravenously (IV) in Equally Divided Doses Every 4 to 6 Hours
- Renal and hepatic failure: decrease dose in renal failure
- Cerebrospinal fluid (CSF) penetration: poor
- Adverse effects: hypersensitivity reaction, hyperkalemia (potassium salt), hypokalemia (sodium salt)

OXACILLIN

Usual Adult Dose: 1 to 2 g Every 4 Hours IV
- Renal and hepatic failure: no adjustment
- CSF penetration: poor
- Adverse effects: hypersensitivity reaction, interstitial nephritis

NAFCILLIN

Usual Adult Dose: 1 to 2 g Every 4 Hours IV
- Renal and hepatic failure: no adjustment
- CSF penetration: low
- Adverse effects: hypersensitivity reaction, interstitial nephritis, hypokalemia

AMPICILLIN

Usual Adult Dose: 2 g Every 4 to 6 Hours IV
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction

AMPICILLIN-SULBACTAM

Usual Adult Dose: 1.5 to 3 g Every 6 Hours IV
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction, diarrhea

AMOXICILLIN

Usual Adult Dose: 500 mg Every 8 Hours Orally or 1 g Every 12 Hours Orally
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction

AMOXICILLIN-CLAVULANATE

Usual Adult Dose: 500 mg Every 8 Hours Orally or 875 mg Every 12 Hours Orally
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction, diarrhea

PIPERACILLIN

Usual Adult Dose: 3 to 4 g Every 4 to 6 Hours IV
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction

PIPERACILLIN-TAZOBACTAM

Usual Adult Dose: 2.25 to 4.5 g Every 6 to 8 Hours IV
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: low
- Adverse effects: hypersensitivity reaction, diarrhea

CEFTOLOZANE-TAZOBACTAM

Usual Adult Dose: 1.5 to 3 g Every 8 Hours IV
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: not available
- Adverse effects: hypersensitivity reaction

CEFTAZIDIME-AVIBACTAM

Usual Adult Dose: 2.5 g Every 8 Hours IV Infused Over 2 Hours
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: not available
- Adverse effects: hypersensitivity reaction

MEROPENEM-VABORBACTAM

Usual Adult Dose: 4 g Every 8 Hours IV Infused Over 3 Hours
- Renal and hepatic failure: decrease dose in renal failure
- CSF penetration: not available
- Adverse effects: hypersensitivity reaction

PENICILLINS

Penicillin was discovered by Alexander Fleming from *Penicillium notatum* (now *Penicillium chrysogenum*) in 1928.[1] The work of Florey, Chain, and associates isolated penicillin and made possible the commercial production of penicillin G.[2] By the middle of the 1940s, penicillin G was available for general use in the United States, thus initiating the modern antibiotic era.

Chemistry

The basic structure of penicillins is a nucleus consisting of a thiazolidine ring, the β-lactam ring, and a side chain (Fig. 20.1). The core ring structures, particularly the β-lactam ring, are essential for antibacterial activity. The side chain determines in large part the antibacterial spectrum and pharmacologic properties of each particular penicillin.

Emergence of β-lactamase–producing organisms, particularly *Staphylococcus aureus,* prompted development of compounds resistant to hydrolysis by β-lactamases and the search for agents more active than penicillin G against gram-negative species. The isolation of the penicillin nucleus, 6-amino-penicillanic acid, from a precursor-depleted fermentation of *P. chrysogenum* made possible the production and testing of numerous semisynthetic penicillins, including methicillin, active against β-lactamase–producing *S. aureus;* ampicillin, active against selected gram-negative bacilli; and carbenicillin, active against *Pseudomonas aeruginosa.* Since then, numerous agents with different pharmacologic and antimicrobial properties have been developed.

Mechanism of Action

The antibacterial activity of penicillin, such as that for all β-lactam antibiotics, is triggered by its inhibition of bacterial cell wall synthesis. Although the precise mechanism by which penicillin kills bacterial cells is not known, stimulation of the production of deleterious hydroxyl radicals that irreversibly damage the cell appears to be a final common pathway of bactericidal, but not bacteriostatic, antibiotics, including the penicillins.[3]

The cell wall of both gram-positive and gram-negative bacteria is composed of peptidoglycan, which allows cells to contain and resist

high osmotic pressure.[4] The cell wall of gram-positive bacteria is a substantial layer 50 to 100 molecules in thickness, whereas in gram-negative bacteria it is only one or two molecules thick. An outer membrane lipopolysaccharide layer, not found in gram-positive bacteria, is present in gram-negative species. The basic subunit of the peptidoglycan component is a disaccharide monomer of *N*-acetylglucosamine (NAG, or GlcNAc) and *N*-acetylmuramic acid (NAM, or MurNAc) pentapeptide (Fig. 20.2). The cytoplasmic enzymatic reactions that generate cell wall precursors, the disaccharide monomer subunit translocation across the cytoplasmic membrane, and the transglycosylase reaction that links the subunit to the peptidoglycan polymer are not sensitive to penicillin.[5] Penicillin inhibits enzymes that catalyze the final step in bacterial cell wall assembly, which is the formation of the cross-links that bridge peptidoglycan, giving it its structural integrity.

Peptidoglycan is composed of long β-linked polysaccharide chains of NAG and NAM pentapeptides whose synthesis is catalyzed by a series of Mur enzymes. Each pentapeptide consists of amino-acid residues alternating between L- and D-stereoisomers and terminating in D-alanyl-D-alanine (see Fig. 20.2). After translocation from the cytoplasm to the exterior of the membrane, a stem peptide of variable length and composition is attached to the third amino acid of this pentapeptide. Pentapeptides are then joined with stem peptides to form a cross-link between polysaccharide chains. This reaction is catalyzed by a transpeptidase that forms an amide bond between the terminal free amine group of a stem peptide and a penultimate D-alanine of a pentapeptide, displacing the terminal D-alanine in the process. This transpeptidation reaction is sensitive to inhibition by penicillin. There are distinct transpeptidases that provide for anchoring of new peptidoglycan to old, that cross link special structures, and that direct formation of the cell wall septum. Although there are penicillin-sensitive reactions, such as those catalyzed by carboxypeptidases, these reactions do not seem to be essential.

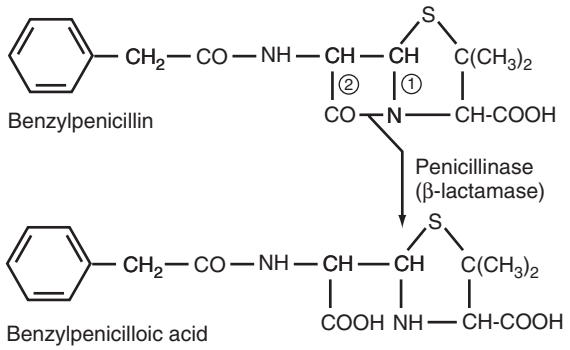

FIG. 20.1 Structure of penicillin and site of β-lactamase attack.

The penicillin-sensitive reactions are catalyzed by a family of closely related proteins, called penicillin-binding proteins (PBPs).[6] Bacteria produce four types of PBPs, which structurally resemble and likely are derived from serine proteases. High-molecular-weight PBPs (i.e., >50 kilodaltons [kDa]) and low-molecular-weight PBPs catalyze transpeptidation and carboxypeptidation reactions of cell wall assembly, respectively. Class A high-molecular-weight PBPs are bifunctional enzymes that have transpeptidase and transglycosylase domains that cooperatively interact during cell wall synthesis.[7] Signal-transducing membrane proteins bind β-lactams and generate a transmembrane signal leading to induction of certain β-lactamases and PBP2a, which mediates staphylococcal methicillin resistance.[8,9] β-Lactamases are PBPs that catalyze hydrolysis of the β-lactam ring. Except for β-lactamases, which may be either secreted or membrane associated, PBPs are membrane bound. PBPs are inhibited by β-lactam antibiotics through covalent binding of the active-site serine residue. Because the essential functions for survival of the cell generally reside with high-molecular-weight PBPs, it is binding to and inhibition of these PBPs that mediates the antibacterial activity of β-lactam antibiotics.

PBPs vary both in amounts present and in the physiologic functions they serve during cell wall assembly. They differ in their affinities for binding β-lactam antibiotics, which explains at least in part why β-lactam antibiotics differ in their antibacterial properties and spectrum of activity. Spratt's studies in *Escherichia coli* were the first to elucidate the different functions of PBPs.[10] Inhibition of PBP1b, which has transpeptidase activity, or a substitute enzyme 1a, results in cell lysis.[11] PBP1 is speculated to be important for cell elongation. Inhibition of PBP2 results in formation of round cells that eventually lyse, indicating that it has a role in cell elongation and in determining the rod size and shape in *E. coli*.[12] Inhibition of PBP3, also a transpeptidase, produces long, filamentous cells, indicating that it is important for the ordered process of cross-wall formation and cell division.[13] Low-molecular-weight PBPs are carboxypeptidases, which play a role in the maintenance of cell shape and septum formation.[7,14]

β-Lactam antibiotics produce their lethal effect on bacteria by inactivation of multiple PBPs simultaneously, but inhibition of cell wall synthesis by itself is not necessarily lethal. For example, cells that are not growing and cells that are osmotically protected survive the presence of penicillin. Unopposed action of autolysins occurring when PBPs are inhibited by β-lactam antibiotics may contribute to the antibacterial effect in some organisms. Cell lysis, although it certainly is lethal and often accompanies cell wall inhibition, is also not required for cell death. The lethal effect in both gram-positive and gram-negative organisms appears to be cell cycle dependent, with inhibition of PBPs leading to disruption of a crucial event probably at the time of cell division. This disturbed morphogenesis is hypothesized to initiate cell death.[3,15]

Resistance Mechanisms

Four mechanisms account for clinically significant bacterial resistance to penicillins and for other β-lactam antibiotics as well: (1) destruction of antibiotic by β-lactamase, (2) failure of antibiotic to penetrate the

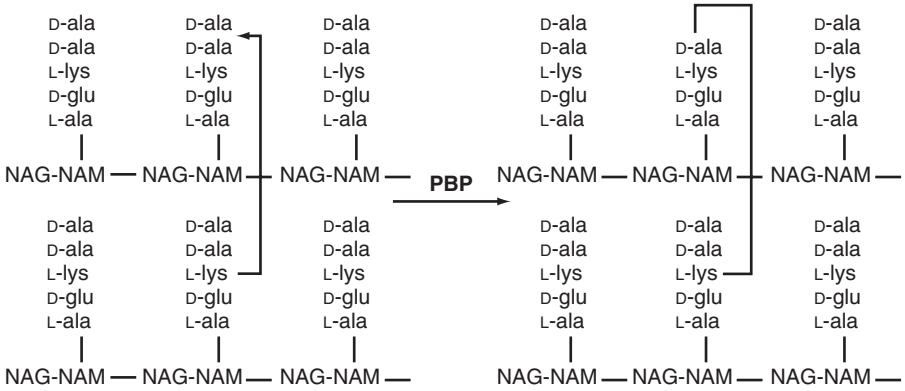

FIG. 20.2 Penicillin-binding protein *(PBP)* transpeptidation reaction that cross links bacterial cell wall. The structure for *Staphylococcus aureus* is shown. *NAG, N*-acetylglucosamine; *NAM, N*-acetylmuramic acid.

outer membrane of gram-negative bacteria to reach PBP targets, (3) efflux of drug across the outer membrane of gram-negative bacteria, and (4) low-affinity binding of antibiotic to target PBPs. β-Lactamase destruction of antibiotic is the most common mechanism of resistance, which in gram-negative bacteria, in general, and in *Pseudomonas aeruginosa,* in particular, is often accompanied by reduced permeability and augmented efflux.[16] β-Lactamases covalently react with the β-lactam ring, rapidly hydrolyze it, and destroy activity of the drug.

β-Lactamases can be categorized into one of four classes, Ambler classes A through D, based on amino-acid sequence similarity and molecular structure (Table 20.1).[17] Classes A, C, and D β-lactamases contain penicillin-binding motifs and are PBPs. They differ from other PBPs in that they typically are smaller, around 35 kDa versus greater than 50 kDa, and they are not cell wall–synthetic enzymes. They react with penicillin through the same series of reactions as other PBPs. There is initial, reversible binding and formation of the Michaelis-Menten complex, followed by acylation of the active-site serine and then followed by hydrolysis of the acyl intermediate in a deacylation reaction regenerating the active enzyme. Biochemically, the main distinction between cell wall–synthetic PBPs and β-lactamases is the rate of deacylation: The deacylation rate of penicillin-bound cell wall synthetic PBP is relatively slow, amounting to irreversible inhibition of its activity, whereas the deacylation rate of β-lactamase usually is orders of magnitude faster, rapidly hydrolyzing and turning over β-lactam molecules. Class B β-lactamases, although they also hydrolyze the β-lactam ring, structurally are unrelated to PBPs. They are zinc-dependent enzymes that use a different series of reactions to open the β-lactam ring and are inhibited by metal-chelating agents.

β-Lactamases that are most often clinically significant are class A or class C. Class A enzymes can be produced chromosomally or via a plasmid and typically hydrolyze penicillins preferentially, but some also have cephalosporinase or carbapenemase activity.[18] They are inhibited by β-lactamase inhibitors, such as clavulanic acid. Point mutations can render the enzyme resistant to inhibitors or extend the spectrum of activity to include third-generation cephalosporins and aztreonam, so-called extended-spectrum β-lactamases (ESBLs). Class C β-lactamases preferentially hydrolyze cephalosporins and are not inhibited by clavulanic acid. These β-lactamases usually are encoded on the chromosome and are inducible, although they may also be plasmid encoded and produced constitutively. Class B enzymes are intrinsically produced by some lactose-nonfermenting gram-negative species, such as *Stenotrophomonas maltophilia*, but it is the plasmid-mediated class B β-lactamases that have the broadest spectrum and are able to hydrolyze all β-lactams except aztreonam.

The outer membrane of gram-negative organisms is an important barrier to drug penetration and an important component of resistance.[19] β-Lactamases of gram-negative bacteria are located in the periplasmic space between the inner cytoplasmic membrane and outer lipopolysaccharide membrane and have been selected to be strategically concentrated to protect target PBPs from exposure to active β-lactam antibiotics. Small polar molecules (e.g., glucose, essential nutrients, β-lactam antibiotics) cross this barrier through protein channels called porins. Porins constrain entry of molecules into the cell according to size, structure, and charge. β-Lactam antibiotics that satisfy the entry requirements can traverse porin channels to the periplasmic space and bind to target PBPs. Absence or deletion of a critical porin, usually in the presence of a β-lactamase activity, can result in resistance.[20]

The third mechanism of resistance is efflux; the drug that enters the periplasmic space is pumped back across the outer membrane.[20,21] Efflux may operate independently of other mechanisms but more often exclusion of antibiotic by porins, destruction of antibiotic by β-lactamases, or both contribute to resistance by limiting periplasmic antibiotic concentration. Species differences in porins, pumps, β-lactamases, and target PBPs determine whether the organism is susceptible or resistant to a particular β-lactam.

The fourth general type of resistance mechanism involves production of a PBP that has low affinity for binding of β-lactam antibiotic.[22] The transpeptidase and transglycosylase activities of PBPs are essential in cell wall synthesis and assembly, and low affinity of PBPs toward β-lactam antibiotics allows the bacteria to circumvent their actions. This may be the result of mutations in PBP genes that lower binding affinity, as in penicillin-resistant pneumococci or *Neisseria* spp., or may be due to the presence of an extra, low-affinity PBP, such as PBP5 produced by *Enterococcus faecium* or PBP2a produced by methicillin-resistant staphylococci. Solution of crystal structures of low-affinity PBPs has identified critical molecular features and interactions that are responsible for resistance.[23,24] In the case of PBP2a, low-affinity binding of β-lactam antibiotic is mediated by structural changes that result in energetically unfavorable interactions between antibiotic and protein so that the active-site serine is inactivated not at all or too slowly to effectively block cell wall synthesis and bacterial growth.

TABLE 20.1 Classification of β-Lactamases

AMBLER MOLECULAR CLASS	MAJOR SUBTYPES[a]	PREFERRED SUBSTRATES	INHIBITOR[b]	MAIN GENETIC LOCALIZATION	REPRESENTATIVE ENZYME(S)
A	Gram-positive β-lactamase 2a	Penicillins	Clavulanic acid	Chromosome or plasmid	PC1
	Gram-negative β-lactamase 2b	Penicillins, early cephalosporins	Clavulanic acid	Plasmid or chromosomal	TEM-1, SHV-1
	Extended-spectrum β-lactamase 2be	Penicillins, extended-spectrum cephalosporins, aztreonam	Clavulanic acid	Plasmid	TEM-24, SHV-12, CTX-M-15
	Inhibitor-resistant TEM β-lactamase 2br	Penicillins	Clavulanic acid[c]	Plasmid	TEM-30, SHV-10
	Carbenicillin-hydrolyzing β-lactamase 2c	Carbenicillin	Clavulanic acid[c]	Plasmid	PSE-1, CARB-3
	Cephalosporin-hydrolyzing β-lactamase 2e	Extended-spectrum cephalosporins	Clavulanic acid	Chromosome	CepA
	Carbapenem-hydrolyzing β-lactamase 2f	Carbapenems	Avibactam[d]	Chromosome or plasmid	KPC-2, SME-1
B	Metallo-β-lactamase 3a	All β-lactams except monobactam	EDTA, divalent cation chelators	Chromosome or plasmid	IMP-1, VIM-2, NDM-1
C	AmpC-type β-lactamase 1	Cephalosporins	Cloxacillin, avibactam	Chromosome or plasmid	AmpC, CMY-2
D	Oxacillin-hydrolyzing β-lactamase 2d	Oxacillin	Clavulanic acid[c]	Chromosome or plasmid	OXA-1, OXA-10
	Extended-spectrum β-lactamase 2de	Extended-spectrum cephalosporins	Clavulanic acid[c]	Plasmid	OXA-11, OXA-15
	Carbapenem-hydrolyzing β-lactamase 2df	Carbapenems	None	Plasmid	OXA-23, OXA-40, OXA-48

[a]The updated Bush-Jacoby group[3] is indicated.
[b]Tazobactam and sulbactam have activities similar to those of clavulanic acid.
[c]Indicates relatively weaker inhibition.
[d]Avibactam generally inhibits β-lactamases inhibited by clavulanic acid.
EDTA, Ethylenediaminetetraacetic acid.

Classification

Penicillins can be conveniently divided into five classes on the basis of antibacterial activity with considerable overlap among the classes: (1) natural penicillins—penicillin G and penicillin V; (2) penicillinase-resistant penicillins—methicillin, nafcillin, and isoxazolyl penicillins; (3) aminopenicillins—ampicillin and amoxicillin; (4) carboxypenicillins—carbenicillin and ticarcillin; and (5) acyl ureidopenicillins—azlocillin, mezlocillin, and piperacillin. The carboxypenicillins and ureidopenicillins are also referred to as antipseudomonal penicillins. Differences within a class are principally pharmacologic, although one compound in a class may be more active than another.

The susceptibility profiles of various species of microorganisms are shown in Tables 20.2 to 20.4. Natural penicillins are most active against non–β-lactamase–producing gram-positive bacteria, anaerobes, and selected gram-negative cocci, such as *Neisseria* spp. Gram-positive bacteria inhibited by penicillin G tend to be more susceptible to penicillin G than to semisynthetic penicillins.[25] Penicillin V (used orally) can be substituted for penicillin G, except against gram-negative species, because it is less active than penicillin G against *Neisseria* and *Haemophilus* spp. Semisynthetic penicillinase-resistant penicillins are the drugs of choice only for penicillin-resistant *S. aureus* and *Staphylococcus epidermidis*. They are also active against streptococci but not against enterococci. Aminopenicillins possess the same spectrum as penicillin G, plus they are more active against gram-negative cocci and Enterobacteriaceae that do not produce β-lactamase.[26] Carboxypenicillins and ureidopenicillins have activity against some ampicillin-resistant aerobic gram-negative bacteria, such as *P. aeruginosa.* Carboxypenicillins are less active than the ureidopenicillins against streptococci and *Haemophilus* spp. Anaerobic gram-positive species are susceptible to the penicillins. Gram-negative anaerobic bacteria are susceptible to most penicillins, with the exception of isolates of *Bacteroides fragilis*, other *Bacteroides* spp., and some *Prevotella* spp., which produce chromosomal class A β-lactamase, and are inhibited by high levels of penicillin G or the semisynthetic antipseudomonal agent piperacillin.[27]

Pharmacologic Properties

Penicillins differ markedly in their oral absorption (Table 20.5). Acid-labile compounds, penicillin G, methicillin, and antipseudomonal penicillins are poorly absorbed. Acid-stable compounds can have major differences in oral absorption. Semisynthetic penicillins, except nafcillin, are well absorbed. Ampicillin is only partially absorbed (30%–55%), and food decreases absorption, whereas amoxicillin has higher oral bioavailability (74%–92%) that is not affected by food.[28,29]

Orally absorbed penicillins yield peak concentrations 1 to 2 hours after ingestion. Peak serum levels are delayed after ingestion with food, and peak levels also are lower, except for amoxicillin. Penicillin G procaine and benzathine penicillin G, repository forms of penicillin G, are absorbed more slowly from intramuscular (IM) sites than are the crystalline salts. Lidocaine can also be used as a diluent for IM injection of antipseudomonal penicillins.

Penicillins are bound to serum proteins in varying degrees, ranging from 17% for the aminopenicillins to 97% for dicloxacillin (see Table

TABLE 20.2 Usual Minimal Inhibitory Concentrations for Penicillins Against Cocci (μg/mL)

ORGANISM	PENICILLIN G	PENICILLIN V	AMPICILLIN, AMOXICILLIN	OXACILLIN[a]	PIPERACILLIN
Streptococcus pneumoniae[b]	0.03	0.03	0.03	0.13	0.05
Streptococcus pyogenes	0.015	0.015	0.03	0.13	0.2
Streptococcus agalactiae	0.06	0.03	0.12	0.13	0.2
Viridans group streptococci	0.06	0.12	0.12	0.5	0.25
Enterococcus faecalis	2	4	1	16	4
Enterococcus faecium	>16	>16	8	>16	>16
Peptostreptococcus spp.	0.13	0.13	0.13	2	0.5
Staphylococcus aureus[c]	0.03	0.03	0.12	0.13	0.8
Staphylococcus epidermidis[c]	0.015	0.03	0.03	0.13	0.8
Neisseria gonorrhoeae[d]	0.015	0.03	0.2	0.4	0.03
Neisseria meningitidis	0.03	0.12	0.12	0.1	0.01

[a]Oxacillin is representative of all antistaphylococcal penicillins; not active against methicillin-resistant strains.
[b]Penicillin-susceptible strains only; 10% or more of strains are penicillin-resistant in the United States (see www.cdc.gov/abcs).
[c]Penicillin and methicillin-susceptible strains only; most strains are resistant.
[d]Penicillin-susceptible strains only; 10% or more of strains are penicillin-resistant in the United States (see www.cdc.gov/std/).
Data from references 132–151.

TABLE 20.3 Activity of Penicillins Against Selected Bacilli and Anaerobic Organisms

	MEAN MINIMAL INHIBITORY CONCENTRATION (μg/mL)			
ORGANISM	Penicillin G	Ampicillin, Amoxicillin	Oxacillin[a]	Piperacillin
Clostridium perfringens	0.5	0.1	0.25	0.25
Corynebacterium diphtheriae	0.1	0.2	3.1	
Listeria monocytogenes	0.25	0.5	4	2
Haemophilus influenzae[b]	1	0.25	32	0.1
Prevotella melaninogenica	0.1	0.1	0.5	0.25
Fusobacterium nucleatum	0.1	0.1	0.13	0.06
Bacteroides fragilis	32	16	64	20

[a]Oxacillin is representative of all antistaphylococcal penicillins.
[b]β-Lactamase–producing strains occur and are resistant to the penicillins.
Data from references 133, 134, 141, 143, and 152–160.

TABLE 20.4 Activity of Penicillins Against Enterobacteriaceae, *Acinetobacter* spp., and *Pseudomonas aeruginosa*

	MEAN MINIMAL INHIBITORY CONCENTRATION (μg/mL)			
ORGANISM	**Penicillin G**	**Ampicillin, Amoxicillin**	**Oxacillin[b]**	**Piperacillin**
Escherichia coli	200	>200	200	32
Klebsiella spp.	50	50	>200	16
Enterobacter spp.	>200	>200	—	4
Citrobacter freundii	—	100	—	6
Serratia marcescens	>200	>200	—	4
Morganella morganii	>200	>200	>200	2
Proteus mirabilis	3.1	6.3	>200	1
Proteus vulgaris	400	>200	>200	4
Providencia spp.	50	>200	>200	4
Salmonella enterica	12.5	6.3	>200	4
Shigella spp.	25	6.3	>200	2
Acinetobacter spp.	100	50	>200	25
Pseudomonas aeruginosa	>200	>200	>200	32

[a]Some of the data are historic and may not reflect current activity.
[b]Oxacillin is representative of all antistaphylococcal penicillins.
Data from references 133, 138, and 161–172.

TABLE 20.5 Pharmacokinetic Properties of Penicillins

				PEAK SERUM LEVELS				SERUM $T_{1/2}$ (h)	
	ORAL ABSORPTION (%)	**FOOD DECREASES ABSORPTION**	**PROTEIN BINDING (%)**	**Total Drug (μg/mL)**	**Free Drug (μg/mL)**	**PERCENTAGE OF DOSE METABOLIZED (%)**	**PERCENTAGE EXCRETED IN URINE UNCHANGED (%)**	**Normal (CrCl > 90 mL/min)**	**With Renal Failure (CrCl < 10 mL/min)**
Penicillin G	15–30	Yes	60	1.5–2.7[a]	0.6–1.0	19	15–30	0.7	6–10
Penicillin V	60	No	80	3–5[a]	0.8	56	26	0.52–0.78	
Oxacillin	33	Yes	90	5.6[a]	0.39	49	17	0.4–0.7	0.5–1
Cloxacillin	49	Yes	93	7–14[a]	0.6	22	38	0.5	0.8
Dicloxacillin	37	Yes	96	15–18[a]	0.6	10	33	0.8	1–1.5
Flucloxacillin	44	Yes	96	11–20[a]	0.6	10	41	0.8	2.9
Nafcillin	Variable, low	Yes	90	4.3[b]			9 (PO) –31 (IM)	0.55–1	1.2
Ampicillin	33–54	Yes	20	2–6[a]	3.2	21	26–43	1–1.3	8–20
Amoxicillin	74–80	No	20	7–8[a]	6	28	50–64	1–1.3	16
Piperacillin	Nil		48	58.3[c]			67.5	0.867	3.33

[a]Peak level after 500-mg dose taken fasting.
[b]Peak level after 500-mg intramuscular injection.
[c]Peak level after 2-g intravenous infusion.
CrCl, Creatinine clearance; *IM,* intramuscular; *PO,* orally.
Data from references 173–181.

20.5). The major protein to which they bind is albumin.[25] Only unbound drug exerts antibacterial activity. However, protein binding is a reversible process, and it is possible for bound penicillin to be released and then to kill bacteria in tissue or in the bloodstream. The major mechanism by which most of these drugs are removed from the body is by excretion as intact molecules via the kidney. Penicillins are metabolized to a minor degree.[30] Even minor differences in metabolism can result in clinically significant differences in half-life in the presence of renal failure. Biliary excretion of penicillins does occur, but it probably is important only for nafcillin and the antipseudomonal penicillins.

Penicillins are cleared by glomerular filtration, and they also are rapidly secreted into urine by renal tubular cells, and hence they have a short half-life, ranging from less than 30 minutes for penicillin to 70 minutes for carbenicillin. The ability of the renal tubular cells to secrete penicillin varies with the agents, but up to 4 g of penicillin G per hour can be excreted. Secretion can be blocked by probenecid, an inhibitor of organic acid secretion by tubular cells, which prolongs the serum half-life of all the penicillins.[31] Probenecid may also increase free penicillin concentration by competing for binding sites on albumin. Renal excretion of all penicillins in neonates is markedly less than in older children because tubular function is not fully developed at birth. Therefore dosing of penicillins must be modified when these drugs are given to neonates or low-birth-weight infants.

Reduction in renal function must be taken into account in the administration of certain penicillins. For creatinine clearance (CrCl) greater than 10 to 20 mL/min, only minor adjustments in the dosage

TABLE 20.6 Antibiotic Dosage Change in Renal Disease and After Dialysis

	DOSE CHANGE IN RENAL FAILURE		
AGENT	**Creatinine Clearance 30–50 mL/min**	**Creatinine Clearance 10–29 mL/min**	**DOSAGE FOR HEMODIALYSIS**
Penicillin G	NC	1–2 × 10^6 units/6 h	0.5–2 × 10^6 units/6 h; dose after HD
Penicillin V	NC	250 mg/6 h	250 mg/6 h; dose after HD
Ampicillin	NC	0.5–2 g/12 h	1 g/12–24 h; dose after HD
Amoxicillin	NC	500 mg/12 h	500 mg/12-24 h; dose after HD
Cloxacillin	NC	NC	NC
Dicloxacillin	NC	NC	NC
Flucloxacillin	NC	NC	NC
Nafcillin	NC	NC	NC
Oxacillin	NC	NC	NC
Piperacillin	NC	3 g/8–12 h	3 g/12 h; 1 g after HD

HD, Hemodialysis; *NC,* no change in dosing.

of other penicillins are required. In the presence of anuria, reduction is necessary in the total daily dose of the natural penicillins, of many of the penicillinase-resistant penicillins, and of the aminopenicillins (Table 20.6).

Peritoneal dialysis removes variable amounts of the penicillins. After hemodialysis, which yields a CrCl of 10 to 15 mL/min, and during continuous venovenous hemofiltration, with a 25 mL/min CrCl, the dose of penicillin G, ampicillin, amoxicillin, and piperacillin must be adjusted, but the dose of nafcillin or the isoxazolyl penicillins need not be adjusted.

Penicillins are well distributed to most tissues, including lung, liver, kidney, muscle, and placenta.[32] The levels of penicillins in bones are variable.[33] The levels of penicillins in abscesses and middle ear, pleural, peritoneal, and synovial fluids are sufficient to inhibit most susceptible bacteria.[32,34] Most penicillins are relatively insoluble in lipid and penetrate cells poorly. Distribution of all the penicillins to eye, brain, cerebrospinal fluid (CSF), or prostate is insufficient in the absence of inflammation.[32] Inflammation alters normal barriers, permitting entry of penicillins, which achieve concentrations in CSF that range between 5% to 10% for penicillin G and 13% to 14% with ampicillin.[35] Penicillins with low rates of protein binding reach levels in fetal serum equivalent to levels in maternal serum 30 to 60 minutes after injection. In contrast, the highly protein-bound semisynthetic penicillins achieve low concentrations in both amniotic fluid and fetal serum.[36]

Urinary concentrations of penicillins are high except for nafcillin, even in the presence of moderately reduced renal function, but in people with rates of CrCl less than 10 mL/min the urinary levels may not exceed those in the blood. Cortical and medullary concentrations of penicillins exceed serum levels.[37]

Most penicillins are actively secreted unchanged into the bile, yielding biliary concentrations well in excess of those in serum. The biliary levels of penicillin G and ampicillin are at least 10 times those in the serum, and the levels of nafcillin in bile are as high as 100 times the simultaneous serum level.[38] In the presence of common duct obstruction the levels of penicillins in bile are markedly reduced.[39] Because the biliary transport system is a saturable one, with drug doses producing very high serum levels, the biliary levels are not significantly increased over those measured at lower serum levels. Reabsorption and reexcretion is minimal.[40]

Untoward Reactions

The most important adverse effects of the penicillins are hypersensitivity reactions, which range in severity from rash to anaphylaxis[41] (Table 20.7) (see Chapter 23). Penicillins can act as haptens to combine with proteins contaminating the solution or with human proteins after the penicillin has been administered to humans. Penicilloyl and penicillanic acid derivatives are the major determinants of penicillin allergy. The penicilloyl determinant, which is produced through opening of the β-lactam ring, thereby allowing amide linkage to body proteins (Fig. 20.3), is the most important antigenic component. Penicillanic acid and its derivatives are produced when reconstituted penicillins break down in solution from acidity or temperature elevation. Minor determinants of allergy are benzylpenicillin itself and sodium benzyl penicilloate, which can act either as sensitizing agents or on their own elicit an allergic reaction. Both major and minor determinants may be involved in anaphylactic reactions, as well as in urticarial reactions. These reactions are mediated by immunoglobulin E (IgE) antibody. A person who has been sensitized by the hapten-carrier complex can have a reaction to penicillin alone or to penicillin that has formed dimers or polymers in solution. Although a history of penicillin allergy is quite common, less than 2% will have an allergic reaction if challenged, but the risk is higher for those with history of an immediate hypersensitivity reaction or a positive skin test.[42]

Serum sickness may occur with penicillins, but it is exceedingly uncommon (<0.01%).[43] The illness is characterized by fever, urticaria, joint pains, and angioneurotic edema. Exfoliative dermatitis and Stevens-Johnson syndrome are rare forms of allergic reactions to penicillins. The morbilliform eruptions that develop after penicillin therapy probably are due to IgM antibodies to the benzyl penicilloyl hapten and to the minor determinants. In many patients these rashes disappear, even if the penicillin is continued, owing to the production of IgG-blocking antibody. There is a risk, however, that the rash could progress to generalized desquamation. If a hypersensitivity reaction does occur, epinephrine given IM or intravenously (IV) usually aborts the reaction. Antihistamines and corticosteroids have not been shown to be of benefit.

Another allergic reaction to penicillins is that of allergic vasculitis with the development of cutaneous and visceral lesions similar to that found with polyarteritis nodosa.[44] This reaction is also extremely uncommon (<0.01%).[45]

Hematologic toxicity is rare, although neutropenia has been encountered with the use of all types of penicillins, particularly when large doses are used.[46] The mechanisms of the neutropenia are unknown, and white blood cell counts return to normal rapidly if the offending agent is discontinued. Sometimes a lower dose of drug can be used without production of neutropenia. Coombs-positive hemolytic anemia occurs rarely.[47] All penicillins at high concentrations bind to the adenosine diphosphate receptor site in platelets, preventing normal platelet aggregation.[48] Clinically significant bleeding may occur more commonly with penicillins than cephalosporins.[49]

Renal toxicity from penicillins is variable, ranging from allergic angiitis to interstitial nephritis.[50] Interstitial nephritis is seen with all penicillins and was particularly common with methicillin, which is no longer available for use. The clinical syndrome is one of fever, macular rash, eosinophilia, proteinuria, eosinophiluria, and hematuria. Initially the reaction is one of nonoliguric renal failure with a decrease in CrCl and a rise in serum urea nitrogen and serum creatinine concentrations. This reaction can progress to anuria and renal failure. Biopsy specimens