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Use of norfloxacin in poultry production in the eastern province of Saudi Arabia and its possible impact on public health

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Samples of market-ready chicken muscle and liver from 32 local broiler farms were first screened for antibiotic residues by microbiological assay. The antibiotic-residue-positive muscles and livers from 22 farms were further analysed for norfloxacin (NFX) residues by high performance liquid chromatography. NFX was detected in 35.0% and 56.7% of raw antibiotic-residue-positive muscles and livers, respectively. The NFX-positive muscles and livers were respectively obtained from 11 (50.0%) and 14 (63.6%) of the 22 antibiotic-residue-positive farms. Since the maximum residue limit (MRL) for NFX has not yet been fixed, the MRL for enrofloxacin was used in the study. All NFX-positive farms had mean raw tissue levels, which were 2.7- to 34.3-fold higher than the MRL. Although cooking markedly reduced NFX tissue concentrations, mean detectable levels remained above MRL in large proportions of NFX-positive samples and farms. Susceptibility patterns of Enterobacteriaceae isolates from chicken and human patients to NFX showed alarmingly high rates of resistance in chicken isolates especially among *Escherichia coli* (45.9%) and *Pseudomonas* spp. (70.6%) compared with patients' isolates (10.5% and 18.2%, respectively). The study reveals widespread misuse of NFX in the local poultry industry, which may pose a major risk to public health including possible stimulation of bacterial resistance and hypersensitivity reactions to fluoroquinolones. More prudent use of fluoroquinolones in food-producing animals is therefore recommended. Further, there is a need to establish MRL values for NFX.

Keywords: norfloxacin, poultry, enterobacteriaceae, Saudi Arabia, drug residues, fluoroquinolones

Introduction

Norfloxacin (NFX) is a fluoroquinolone antimicrobial agent with high activity against Gram-negative microorganisms, thus recommended for the treatment of human urinary tract infections (Childs 1993, Pfau and Sacks 1993, Pittman *et al.* 1993). The fluoroquinolones are relatively new antimicrobial agents and resistance to them remains largely low. However, there is a worrisome world-wide trend of increased resistance to these agents among bacteria responsible for both hospital- and community-acquired infections including methicillin-resistant *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia marcescens, Escherichia coli, Salmonella* spp., *Campylobacter* spp. and *Neisseria gonorrhoeae* (Acar and

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Goldstein 1997, Hayward *et al.* 1999, Sidorenko *et al.* 1999). Cross-resistance between fluoroquinolones has also been reported among a wide range of microorganisms including both Gram-positive cocci and Gram-negative bacilli (Barry and Fuchs 1997, Hoogkamp-Korstanje 1997, Peterson *et al.* 1998, Tankovic *et al.* 1999).

The use of antimicrobial agents in food-producing animals has recently become a very important public health issue. These agents are widely used for the prevention and therapy of infectious disease in farm animals, an important measure when raising animals under intensive husbandry methods of production (Swan 1969, Mercer 1975, Linton 1977, Johnston 1998). In addition, they are routinely used at sub-therapeutic levels as animal feed additives for their growth-promoting properties (Droumev 1983). These practices, however, carry many disadvantages including the stimulation of microbial resistance to antibiotics with the possible transfer of resistant pathogens from animals to humans (Holmberg *et al.* 1984, Trolldenier 1996, Anon. 1998, Tollefson *et al.* 1998). Further, the presence of drug residues in animal products may pose a potential health risk to the public (Mercer 1975, Linton 1977, Woodward 1991).

Fluoroquinolones were introduced for veterinary use in the eastern province of Saudi Arabia in the late 1980s. Norfloxacin, in particular, has been available since 1993 in pure powder form for addition to poultry feed or drinking water for prophylaxis or treatment of infections due to Gram-negative microorganisms.

This study was aimed at screening locally produced edible chicken tissues for the possible presence of norfloxacin residues at the time of marketing. The drug levels were also determined and compared with the permissible maximum residue limits (MRLs) of fluoroquinolones. In addition, the susceptibility patterns of Enterobacteriaceae isolated from chicken and human patients to norfloxacin were investigated.

Methods

Specimen collection

A total of 242 muscle and 714 liver samples were obtained from randomly selected chicken that were ready for market during 49 inspection visits to 32 broiler farms in the eastern province of Saudi Arabia over a period of two years starting from January 1996. In addition, rectal swabs from randomly selected live healthy chickens were obtained. Urine specimens were also collected from patients attending at King Fahd Hospital of the University, A1-Khobar, Saudi Arabia, for treatment of urinary tract infections.

Microbiological assay of antibiotic residues

All chicken muscle and liver samples were first screened for antibiotic residues by microbiological assay. Pieces of muscle and liver (50–100 μg) were transferred into wells in Mueller Hinton agar plates (Oxoid, Unipath Ltd. UK) previously seeded with a reference strain of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtillis* (B.B.L 6633). The plates were then incubated at 37°C for 20 h. These tests were performed in duplicate for any bacterial strain and the appearance of an inhibition zone in any sample was considered to indicate the presence of antibiotic residues.

Norfloxacin concentration analysis

One hundred and twenty muscle and 120 liver samples found positive for antibiotic residues by microbiological assay were further subjected to analysis by high performance liquid

chromatography (HPLC) for norfloxacin residues. Portions of muscle and liver (20 g) were cooked in 100 ml of water at 100°C for 20 min. Homogenates of both raw and cooked tissues were prepared in 2.0 M sodium phosphate/sulphite buffer, pH 6.1 (1:10, w:v). The concentrations of NFX were then determined in both raw and cooked tissue homogenates as well as in the cooking fluid using a previously described HPLC method (Chan *et al.* 1989). The HPLC apparatus was LC Module-1 Plus (Waters Associates) equipped with a Novapak C_{18} column (Waters Associates), a UV/visible detector set at 340 nm and a personal computer software (Millennium v. 2.15). The mobile phase consisted of methanol:acetonitrile: 0.4 M citric acid (3:1:10) and was run at 1.2 ml min^{-1}.

Bacterial susceptibility tests

Specimens were directly inoculated on MacConkey agar, eosin, methylene blue and xylose lysine desoxycholate agar media. Growth was identified by standard laboratory methods including Gram stain and API 20E and API 10S systems (bioMérieux SA, Marcy I'Etoile, France). The susceptibility tests of Enterobacteriaceae isolated from chicken and human patients to norfloxacin (10 μg) were carried out using a Bauer–Kirby disc diffusion method (Bauer *et al.* 1966). The antibiotic discs were obtained from Oxoid (Unipath Ltd., Basingstoke, Hampshire, UK) and the results were interpreted according to the criteria recommended by the National Committee for Clinical Laboratory Standards (1993).

Statistical analysis

The results of NFX residue and susceptibility studies were statistically analysed using Student's *t*-test, with a significance level of $P < 0.05$.

Results

Norfloxacin residues

High performance liquid chromatographic analysis of the 120 antibiotic-residue-positive samples of each of muscle and liver revealed that norfloxacin residues were present in 42 (35.0%) raw muscle and 68 (56.7%) raw liver. The antibiotic-residue-positive samples were obtained from 22 (68.8%) of the investigated 32 broiler farms. Eleven (50.0%) and 14 (63.6%) of the 22 antibiotic-residue-positive farms tested positively for NFX in muscle and liver samples, respectively.

The mean NFX concentrations for farms ranged from 0.08 to 1.00 μg g^{-1} with an overall mean concentration (± SD) of 0.25 ± 0.28 in raw muscle and from 0.11 to 1.03 μg g^{-1} (mean ± SD = 0.48 ± 0.31) in raw liver (Table 1). These results indicate that the overall mean NFX concentration in raw liver was approximately two-fold higher than that in raw muscle. However, after cooking, mean levels fell markedly in muscle to 0.11 ± 0.05 μg g^{-1} and significantly ($P < 0.01$) in liver to 0.16 ± 0.12 μg g^{-1} (Table 1). Norfloxacin was also detectable in the cooking fluid of seven (31.8%) antimicrobial-residue-positive farms with an overall mean concentration (± SD) of 0.06 ± 0.04 μg g^{-1} (Table 1).

Figures 1 and 2 display the mean detectable concentrations of norfloxacin in raw and cooked muscle and liver for each farm in comparison with the internationally recommended maximum residue limit (MRL) for enrofloxacin (0.03 μg g^{-1}). All farms with detectable levels of norfloxacin in both raw muscle and liver had mean concentrations that were 2.7- to 34.3-fold

Table 1. Summary of norfloxacin (NFX) residues in 120 samples of each of chicken muscle, liver and cooking fluid from 22 antibiotic-residue-positive broiler farms

	Muscle		*Liver*		
	Raw	*Cooked*	*Raw*	*Cooked*	*Cooking fluid*
Range of farm mean conc. ($\mu g\ g^{-1}$)	0.08–1.00	0.06–0.16	0.11–1.03	0.04–0.42	0.02–0.12 ($\mu g\ ml^{-1}$)
Overall farm mean conc. ± SD ($\mu g\ g^{-1}$)	0.25 ± 0.28	0.11 ± 0.05	0.48 ± 0.31	0.16 ± 0.12*	0.06 ± 0.04 ($\mu g\ ml^{-1}$)
% of farms positive for NFX	50.0	18.2	63.6	63.6	31.8
% of samples positive for NFX	35.0	14.2	56.7	44.2	26.7
% of NFX-positive samples with conc. above MRL	100.0	40.5	100.0	72.1	–

*$P < 0.01$ compared with raw liver.

higher than MRL value. Moreover, after cooking, mean NFX concentrations still remained above MRL in muscle in 4 (36.4%) of the NFX-positive farms while in liver in all 14 NFX-positive farms (Figs 1 and 2).

Patterns of susceptibility to norfloxacin

The susceptibility patterns of Enterobacteriaceae isolated from both chicken and human patients to NFX are summarized in Table 2. The overall resistance to NFX was significantly higher in

Table 2. Comparison of patterns of resistance to norfloxacin of Enterobacteriaceae isolates from chicken and patients

	Chicken isolates		*Patients isolates*		*t-test*	
Organism	*No. in test*	*% resistance*	*No. in test*	*% resistance*	*P value*	*Significance*[a]
Salmonella spp.	–	–	10	0.0	–	NA
Proteus spp.	230	2.2	11	15.4	0.1616	NS
E. coli	74	45.9	276	10.5	0.0000	S
Pseudomonas spp.	17	70.6	22	18.2	0.0002	S
Klebsiella spp.	12	25.0	89	7.8	0.1836	NS
Others	22	27.3	51	7.8	0.0628	NS
Overall resistance	355	16.9	461	10.0	0.0360	S

[a]S = significant, NA = not applicable, NS = not significant.

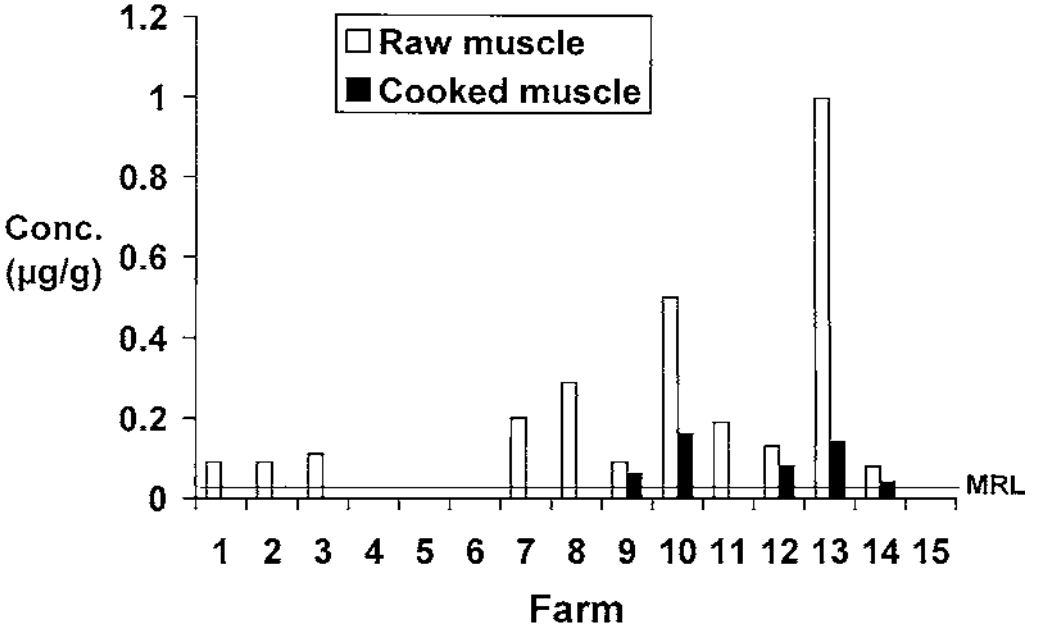

Fig. 1. Mean detectable concentrations of norfloxacin in raw and cooked chicken muscle in broiler farms in comparison with the maximum residue limit (MRL = 0.03 µg g^{-1}) of enrofloxacin.

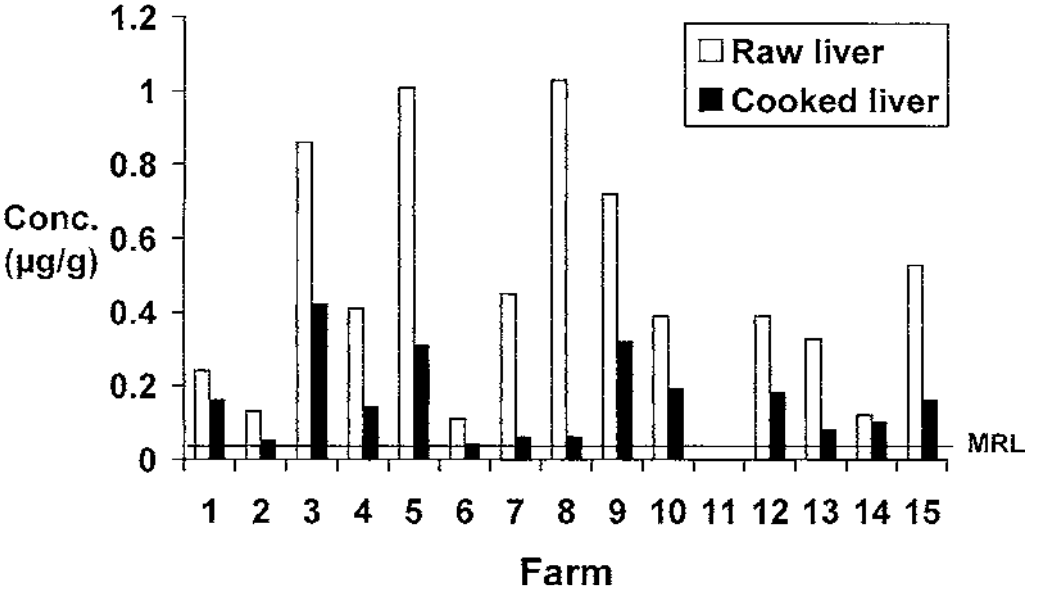

Fig. 2. Mean detectable concentrations of norfloxacin in raw and cooked chicken liver in broiler farms in comparison with the maximum residue limit (MRL = 0.03 µg g^{-1}) of enrofloxacin.

organisms isolated from chicken than in those from patients ($P = 0.036$). Significantly higher rates of resistance were especially noted among *E. coli* and *Pseudomonas* spp. chicken isolates compared with patients' isolates.

Discussion

The presence of antibiotic residues in food-producing animals has received enormous world-wide attention from local and international regulatory and public health agencies. This is owing to the importance of the issue and its possible significant impact on public health. Many reports indicated that microbial resistance to antibiotics may arise as a result of animal exposure to these agents and that the resistance may possibly be transferred to human pathogens (Sogaard 1973, Mercer 1975, Linton 1977, Holmberg *et al.* 1984, Bebora *et al.* 1994, Amara *et al.* 1995). In addition, human exposure to animal products containing significant levels of antibiotic residues may provoke immunological responses in susceptible individuals and cause disorders of intestinal flora (Linton 1977, Holmberg *et al.* 1984, Woodward 1991).

In the present study, we examined locally produced chicken muscle and liver for the presence of norfloxacin (NFX). The results showed that a high proportion of the investigated broiler farms had detectable levels of NFX in raw muscle and liver at the time of marketing. Fluoroquinolones distribute widely into body tissues and are found in high concentrations in the excretory organs especially the liver and in the bile (Prescott and Baggot 1993). They are lipophilic compounds and are partially metabolized in the liver to active metabolites, which contribute to the pharmacological action of the parent drugs and the prolongation of their presence in the body (Prescott and Baggot 1993). Long withholding periods of 7 days for oral use of fluoroquinolones in farm animals have been recommended by the drugs' manufacturers.

Several organizations such as the Food and Agriculture Organization (FAO), World Health Organization (WHO), Veterinary Medicines Directorate (VMD) of the European Union and Food and Drug Administration (FDA) of the USA have set tolerance or maximum residue limits (MRLs), acceptable daily intakes (ADIs) and withholding times for pharmacologically active substances including antimicrobial agents. Different MRLs have been fixed for different quinolone compounds by the VMD of the European Union (Veterinary Medicines Directorate 1997). However, no MRL values have yet been fixed for norfloxacin or its parent compound, pefloxacin by any international regulatory agency. It is therefore prudent to use the lowest MRL value ($0.03\,\mu g^{-1}$) for quinolone compounds, which is that of enrofloxacin as the MRL for norfloxacin, an approach adopted in this study. The results showed that all raw NFX-positive muscle and liver samples had levels exceeding the MRL. Moreover, the overall farm mean concentrations of NFX in both raw muscle and liver were 8.3 and 16 times, respectively, higher than the MRL. These results confirm that NFX was heavily used in the investigated poultry farms. They also suggest that the recommended withholding time of 7 days for fluoroquinolones was either not strictly applied or may be insufficient for this drug. This, however, merits further investigation since NFX is a relatively new drug in veterinary practice.

The effect of cooking on NFX residues was also investigated in the same samples. Although cooking markedly reduced tissue concentrations of NFX, mean farm levels of the drug remained above MRL in 36.4% and 100% of NFX-positive farms in muscle and liver, respectively. These results demonstrate that NFX residues may persist in high concentrations in edible tissues even after cooking if the initial levels in raw tissues were excessively high. This may have serious implications for human health including the possible induction of hypersensitivity reactions to fluoroquinolones in susceptible individuals. It is therefore important to set MRL values for

norfloxacin and its parent compound, pefloxacin in animal-derived food products by the concerned international agencies.

NFX is currently recommended for the treatment of human urinary tract infections (Childs 1993, Pfau and Sacks 1993, Pittman *et al.* 1993) and bacterial enteritis (Bennish *et al.* 1992, Dupont and Ericsson 1993), which are commonly caused by Gram-negative organisms such as *E. coli, Pseudomonas* spp., *Klebsiella* spp. and *Shigella* spp. Although the rates of resistance of Enterobacteriaceae to NFX in this study remained relatively low among human isolates, they were excessively high in chicken isolates especially *E. coli, Pseudomonas* spp. and *Klebsiella* spp. Similarly, high levels of resistance to fluoroquinolones have also been observed among *E. coli* strains isolated from septicaemic and healthy chickens in Spain (Blanco *et al.* 1997). Increasing resistance to enrofloxacin among *E. coli* isolates, particularly from turkeys, was also noted in Germany (Trolldenier 1996). This may lead to the emergence of microbial resistance to quinolones in both avian and human pathogens. Indeed, exposure to norfloxacin has been shown to induce resistance to other fluoroquinolones and even to the structurally unrelated aminoglycosides in coagulase-negative staphylococci (Deshmukh *et al.* 1997). Moreover, the results of a previous study conducted in our institution on *E. coli* serotypes suggested that chicken might act as a possible source of pathogenic organisms in humans (AI-Ghamdi *et al.* 1999). The present study therefore lends support to the recent calls for a more prudent use of antibiotics in farm animals so as to stem the rise in microbial resistance to antibiotics (Anon. 1999, Fox 1999, Sainsbury 1999). This is particularly important with fluoroquinolones as new, highly effective antimicrobial agents.

Conclusion

The results of this study indicate that there is widespread misuse of fluoroquinolone agents by poultry producers in the eastern province of Saudi Arabia. The study also stresses the need to establish MRL and ADI values for norfloxacin and its parent compound, pefloxacin. In addition, resistance to norfloxacin in chicken Enterobacteriaceae is alarmingly high with potentially significant consequences for the emergence of resistance in human pathogens. It is therefore important to restrict the use of fluoroquinolones in food-producing animals in order to maintain their viability as effective therapeutic agents.

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References

Acar, J.F. and Goldstein, F.W. (1997) Trends in bacterial resistance to fluoroquinolones. *Clin. Infect. Dis.* **24 (Suppl. 1)**, S67–73.

AI-Ghamdi, M.S., EI-Morsy, F., A1-Mustafa, Z.H., AI-Ramadhan, M. and Hanif, M. (1999) Antibiotic resistance of *Escherichia coli* isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia. *Trop. Med. Int. Health* **4**, 278–283.

Amara, A., Ziani, Z. and Bouzouba, K. (1995) Antibioresistance of *Escherichia coli* strains isolated in Morocco from chickens with colibacillosis. *Vet. Microbiol.* **43**, 325–330.

Anon. (1998) Overuse of antibiotics in livestock results in resistant strains. *Public Health Rep.* **113**, 104.

Anon. (1999) Government advisory committee calls for a reduction in antibiotic use. *Vet. Rec.* **145**, 266–267.

Barry, A.L. and Fuchs, P.C. (1997) Antibacterial activities of grepafloxacin, ciprofloxacin, ofloxacin and fleroxacin. *J. Chemother.* **9**, 9–16.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turk, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **45**, 493–496.

Bebora, L.C., Oundo, J.O. and Yamamoto, H. (1994) Resistance of *E. coli* strains, recovered from chickens, to antibiotics with particular reference to trimethoprim sulfamethoxazole (Septrin). *East Afr. Med. J.* **71**, 624–627.

Bennish, M.L., Salam, M.A., Khan, W.A. and Khan, A.M. (1992) Treatment of shigellosis. III. Comparison of one-or two-dose ciprofloxacin with standard 5 day therapy. A randomized blinded trial. *Ann. Intern. Med.* **117**, 727–734.

Blanco, J.E., Blanco, M., Mora, A. and Blanco, J. (1997) Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. *J. Clin. Microbiol.* **35**, 2184–2185.

Chan, C.Y., Lam, A.W. and French, G.L. (1989) Rapid HPLC assay of fluoroquinolones in clinical specimens. *J. Antimicrob. Chemother.* **23**, 597–604.

Childs, S.J. (1993) Fleroxacin versus norfloxacin for oral treatment of serious urinary tract infections. *Am. J. Med.* **94**, 105S–107S.

Deshmukh, S.R., Nagoba, B.S., Wadher, B.J. and Tumane, P. (1997) Norfloxacin induced resistance to fluoroquinolones and structurally unrelated antimicrobial agents in coagulase negative staphylococci. *Ind. J. Med. Res.* **106**, 461–464.

Droumev, D. (1983) Review of antimicrobial growth promoting agents available. *Vet. Res. Commun.* **7**, 85–99.

Dupont, H.L. and Ericsson, C.D. (1993) Prevention and treatment of traveler's diarrhoea. *New Engl. J. Med.* **328**, 1821–1827.

Fox, M.W. (1999) Applauds increased regulation of antimicrobials. *J. Am. Vet. Med. Assoc.* **214**, 1615.

Hayward, C.L., Erwin, M.E., Barrett, M.S. and Jones, R.N. (1999) Comparative antimicrobial activity of gatifloxacin tested against *Campylobacter jejuni* including fluoroquinolone-resistant clinical isolates. *Diagn. Microbiol. Infect. Dis.* **34**, 99–102.

Holmberg, S.D., Osterholm, M.T., Senger, K.A. and Cohen, M.L. (1984) Drug-resistant Salmonella from animals fed antimicrobials. *New Engl. J. Med.* **311**, 617–622.

Hoogkamp-Korstanje, J.A. (1997) In-vitro activities of ciprofloxacin, levofloxacin, lomefloxacin, ofloxacin, sparfloxacin and trovafloxacin against Gram-positive and Gram-negative pathogens from respiratory tract infections. *J. Antimicrob. Chemother.* **40**, 427–431.

Johnston, A.M. (1998) Use of antimicrobial drugs in veterinary practice. *Br. Med. J.* **317**, 665–667

Linton, A.H. (1977) Antibiotics, animals and man. An appraisal of a continuous subject. In *Antibiotics and Antibiosis in Agriculture* (M. Woodbine, ed.), pp. 315–343. London: Butterworths.

Mercer, H.D. (1975) Antimicrobial drugs in food producing animals. Control mechanisms of governmental agencies. *Vet. Clin. North Am.* **5**, 3–5.

National Committee for Clinical Laboratory Standards (1993) *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 5th edn. *Approved Standard M2-A5.* Villanova, PA: NCCLS.

Peterson, L.R., Postelnick, M., Pozdol, T.L., Reisberg, B. and Noskin, G.A. (1998) Management of fluoroquinolone resistance in *Pseudomonas aeruginosa*–outcome of monitored use in a referral hospital. *Int. J. Antimicrob. Agents* **10**, 207–214.

Pfau, A. and Sacks, T.G. (1993) Single dose quinolone treatment in acute uncomplicated urinary tract infection in women. *J. Urol.* **149**, 532–534.

Pittman, W., Moon, J.O., Hamrick, L.C. Jr., Cox, C.E., Clark, J., Childs, S., Pizzuti, D., Fridricks, J. and St Clair, J. (1993) Randomized double-blind trial of high- and low-dose fleroxacin for complicated urinary tract infection. *Am. J. Med.* **94**, 101S–104S.

Prescott, J.F. and Baggot, J.D. (1993) Fluoroquinolones. In *Antimicrobial Therapy in Veterinary Medicine*, 2nd edn (J.F. Prescott and J.D. Baggot, eds), pp. 252–262. Iowa: Iowa State University Press.

Sainsbury, D.W. (1999) EU ban on four antibiotic feed additives. *Vet. Rec.* **144**, 80.

Sidorenko, S.V., Gel'fand, E.B. and Mamontova, O.A. (1999) Hospital infections caused by *Pseudomonas aeruginosa*. Significance in intensive therapy. *Anesteziol. Reanimatol.* **3**, 46–54.

Sogaard, H. (1973) Incidence of drug resistance and transmissible R factors in strains of *E. coli* isolated from faeces of healthy pigs. *Acta Vet. Scand.* **14**, 381–391.

Swan (1969) *Report of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine*. **Cmnd 4190**. London, HMSO.

Tankovic, J., Bachoual, R., Ouabdesselam, S., Boudjadja, A. and Soussy, C.J. (1999) In-vitro activity of moxifloxacin against fluoroquinolone-resistant strains of aerobic gram-negative bacilli and *Enterococcus faecalis*. *J. Antimicrob. Chemother.* **43**, (Suppl. B), 19–23.

Tollefson, L., Angulo, F.J. and Fedorka-Cray, P.J. (1998) National surveillance for antibiotic resistance in zoonotic enteric pathogens. *Vet. Clin. North Am. Food Anim. Pract.* **14**, 141–150.

Trolldenier, H. (1996) Development of resistance in infectious agents of agricultural animals in Germany (1990–1994)–a review. *DTW. Dtsch. Tierarztl. Wochenschr.* **103**, 256–260.

Veterinary Medicines Directorate (VMD) (1997) *List of Pharmacologically Active Substances for which Maximum Residue Limits Have Been Fixed.* **Regulation 2377/90, Annex I.** Brussels: VMD, European Commission.

Woodward, K.N. (1991) Hypersensitivity in humans and exposure to veterinary drugs. *Vet. Hum. Toxicol.* **33**, 168–172.