

# Antimicrobial Drugs

Dawn Merton Boothe

# 7

## Chapter Outline

### DRUGS THAT TARGET THE CELL WALL

- Beta-Lactam Antimicrobials
- Vancomycin
- Teicoplanin
- Fosfomycin

### DRUGS THAT TARGET RIBOSOMES (BACTERICIDAL)

- Aminoglycosides

### DRUGS THAT TARGET NUCLEIC ACIDS

- Fluorinated Quinolones
- Rifamycins
- Metronidazole

### DRUGS THAT TARGET FOLIC ACID

- Inhibitors of Folic Acid Synthesis:/Sulfonamide/  
Trimethoprim or Ormetoprim Combinations

### DRUGS THAT TARGET RIBOSOMES (BACTERIOSTATIC)

- Tetracyclines
- Phenicols
- Lincosamides: Lincomycin and Clindamycin
- Macrolides and Azalides
- Tylosin
- Ketolides

### MISCELLANEOUS ANTIMICROBIALS

- Oxazolidinones
- Topical Antimicrobials

The principles that guide proper antimicrobial selection are discussed in Chapter 6. This chapter focuses on the individual drugs or drug classes and their use to successfully treat bacterial infections. This includes not only resolution of clinical signs but avoidance of resistance. Characteristics discussed for each drug class include structure-activity relationship; the mechanism of antimicrobial action, including whether the drug is time- or concentration- dependent (Table 7-1); the spectrum of antimicrobial activity (Table 7-2), including pharmacodynamics (minimum inhibitory concentrations [MIC] (Tables 7-3 and 7-4) for selected organisms; mechanisms of antimicrobial resistance; clinically relevant aspects of the drug; the disposition of the drug in the patient (as it relates to both safety and efficacy); adverse drug effects; and drug interactions. The breakpoint MICs (the concentration at which an infecting isolate is considered susceptible or resistant to a drug of interest) are delineated in Chapter 6, Table 6-2). Pharmacokinetics were drawn from individual manuscripts, and the Antimicrobial's Monograph issue of the *Journal of Veterinary Pharmacology and Therapeutics*.<sup>2</sup> In addition, Albarellos<sup>1</sup> also has provided a review of disposition of selected antimicrobials; these have been included, when appropriate, in Table 7-1. Tissue distribution of antimicrobials is addressed when available; Table 7-5 provides information regarding the relative proportion of tissue versus serum concentrations of drugs, with a focus on body fluids and phagocytic cells. As a reminder (see Chapter 6), drug concentrations measured in tissue homogenates are minimally relevant to concentrations to which microbes are exposed. Data collected by ultrafiltration probes is preferred.

However, interstitial fluid is not free of factors that might preclude drug activity (i.e., proteins or ionization; see discussion of cefovecin in cats); as such, dosing errors should be on the side that increase concentrations in tissues. Therapeutic indications are offered when relevant. The dissociation constant of a drug (pKa) and selected information regarding the chemical characteristics of selected drugs or preparation stability are provided for selected drugs in Table 7-6. Doses are indicated in Table 7-7; however, doses ideally should be designed on the basis of intergration of pharmacokinetic (PK) and pharmacodynamic (PD) data (see Chapter 6). Treatment of specific infection is addressed by system in Chapter 8.

Chapter 6 addressed the importance of integrating PK and PD MIC data when designing a dosing regimen. The PK parameters on which integration is most commonly based are the maximum drug concentration (for both time-dependent and concentration-dependent drugs) and elimination half-life. The latter is particularly important for time-dependent drugs but will also increase area under the curve (AUC), which predicts the efficacy of selected concentration-dependent drugs (e.g., fluoroquinolones; see Table 7-1). Among the sources of PK data to be consulted beyond this chapter are the Antimicrobial Monographs published by the United States Pharmacopiea<sup>2</sup> in conjunction with the *Journal of Veterinary Pharmacology and Therapeutics*. The PD data on which integration is based ideally is the MIC of the isolate cultured from the site of infection in the patient. If not available, the high range of the MIC or the MIC<sub>90</sub> might be a reasonable population statistic surrogate indicator of "what is needed" (see Tables 7-3 and 7-4). When available, PD information for

Table 7-1 Pharmacokinetic Data for Selected Antimicrobials

Drug	Vd (L/kg)	Half-Life (hr)	Dose (mg/kg) <sup>†</sup> /C <sub>max</sub> (μg/mL)
Amikacin (CD, I)	0.23 (D)	1 [D] <sup>‡</sup>	20 (IV)/65
	0.18-0.38 <sup>§</sup>	1.3±0.3 (C, IV) <sup>102</sup>	10 (IM, D)/14
	17 (C) <sup>102</sup>	1.9±0.2 (C, IM, SC) <sup>102</sup>	10 (SC, D)/14 <sup>‡</sup>
			10 (SC, Greyhounds)/27
			F = 0.9 <sup>207</sup>
			10 (IV, Greyhounds)/49 <sup>103</sup>
			10 (IV, Beagles/35) <sup>103</sup>
			5 (IV,C)/22 (extrapolated) <sup>102</sup>
			5 (IM, C)/17 <sup>102</sup>
			5 (SC, C)/22 <sup>102</sup>
			10 (IM, C)/38.5 <sup>‡</sup>
		10 (SC, C)/39.6 <sup>‡</sup>	
		20 (IM, C)/65.6 <sup>‡</sup>	
		20 (SC, C)/67.9 <sup>‡</sup>	
Amoxicillin (TD, I) <sup>67</sup>	0.2 (D)	1	20 (IV)/13
		1.5 <sup>†</sup>	12.5 (PO)/5-6 (5.5)
			11 (SC, D)/7
			10 (SC, C)/7
Amoxicillin with clavulanic acid (TD, I) <sup>26</sup>	0.2	1-1.5	20 (IV)/13
		1.5 amoxicillin <sup>†</sup>	5 (PO)/4.5-6
		0.71 clavulanic acid	11 (SC, D)/7
			15 (SC, C)/10
			16.7 (PO, D)/11.4 amoxicillin <sup>†</sup>
		4.3 (PO, D)/2.06 clavulanic acid <sup>†</sup>	
Ampicillin (TD, I)	0.2-0.4 [D]	0.5-1.5	20 (IV)/50
	0.12 <sup>‡</sup> -0.22	0.8-1.1 (Nelis, 1992)	12 (SC)/14
	[C]	0.2 (D) <sup>‡</sup>	6.6 (SC)/7
		1.25 (C) <sup>‡</sup>	30 (PO)/10
			10 (PO)/3
			14-16(PO)/3.4-5.5 (Nelis, 1992)
Azithromycin (TD, S)	12 (Vd <sub>ss</sub> , D) <sup>269</sup>	29 (IV), 35 (PO) (D) <sup>269</sup>	24 (IV, D)/6.8 (F=0.97) <sup>269</sup>
	23 (Vd <sub>ss</sub> , C)	35 (C) <sup>270</sup>	24 (PO, D)/4.2 <sup>269</sup>
			5 (PO, C)/0.97±0.65 (F=0.58) <sup>270</sup>
Carbenicillin (TD, I)	0.19	0.25	
Cefaclor (TD, I)		2 [D] <sup>‡</sup>	25 (PO, D)/24.5
			44 (PO, D)/20
Cefamandole (TD, I)			10.7 (IV, D)/9.4
Cefazolin (TD, I) (first)	0.3-0.7	0.75-1.4	15 (IV)/45
			30 (IV)/90
			15 (SC)/25
			30 (SC)/50
Cefepime (TD, I)	0.14	1	14 (IV, D)/77 (extrapolated) <sup>28</sup>
Cefixime (TD, I) (third)	0.22 (Vd <sub>ss</sub> )	7-8 (D) <sup>‡</sup>	5 (PO, D)/2 <sup>‡</sup>
	8-18% f <sub>ub</sub>		5 (PO, 6 days [D])/4.8 <sup>‡</sup>
Cefodroxil (TD, I) <sup>62</sup> (first)		1.7 without food; 4 with food <sup>†</sup>	11 (PO, D)/10.5
			22 (PO, D)/16.3-18.6 <sup>‡</sup>
			44 (PO, D)/21
			30 (PO, D) 35 <sup>‡</sup>
			22 (PO, C)/17.4 <sup>‡</sup>
Cefotaxime (TD, I) (third)	0.48 [D]	0.75-0.8 (D) <sup>‡</sup>	50 (IV, IM, D)/41
	0.4 [D]	1 (C) <sup>‡</sup>	50 (IM, D)/47 <sup>‡</sup>
	(Vd <sub>ss</sub> )		50 (SC, D)/30 <sup>‡</sup>
	0.18 <sup>‡</sup> [C]		10 (IV, D)/35 <sup>*</sup>
			10 (IM, C)/36 <sup>‡</sup>
			50 (IM, C) 47 <sup>‡</sup>
			50 (SC, C)/30 <sup>‡</sup>

Table 7-1 Pharmacokinetic Data for Selected Antimicrobials—cont'd

Drug	Vd (L/kg)	Half-Life (hr)	Dose (mg/kg) <sup>1</sup> /C <sub>max</sub> (μg/mL)
Cefotetan (TD, I) (third)	0.25	0.9 1.1 (D) <sup>†</sup>	20 (IV, D)/43
Cefovecin (TD, I)	0.13 [D]	5.5 days (D) 6.9 days (C)	8 (SC, D)/121 (bound) <sup>20</sup> 8 (SC, C)/141 (bound) <sup>20</sup> 8 (SC, D)/4.2 (predicted unbound) (PI) <sup>20</sup> 8 (SC, C)/8.5 (predicted unbound) (PI) <sup>20</sup>
Cefoxitin (TD, I) (second)	0.32 [D] <sup>†</sup>	0.7-1.3 (D) <sup>†</sup>	60 (IV)/20 30 (IV)/10 30 (SC)/20 10 (SC)/15
Cefpodoxime (TD, I)	0.15 [D]	5.6 (PO, D) 4.7 (IV, D)	10 (PO, D)/16 (see text) 5 (PO, D) 8.2
Ceftazidime (TD, I) (third)	0.13-0.22	0.82	20 (IV)/49 30 (SC)/42.2 4.4 mg/kg, then 4.1 mg/kg/hr(CRI)/22.5
Ceftiofur (TD, I) (third) <sup>71</sup> (based on bioactivity) <sup>†</sup>		5-7 (D) <sup>†</sup>	(Bioactivity) 0.22 (SC, D)/1.7 <sup>†</sup> 2.2 (SC, D)/8.9 <sup>†</sup> 4.4 (SC,D)/27 <sup>†</sup>
Ceftizoxime (TD, I) (third)	0.26	1	20 (IV, D)/50
Ceftriaxone (TD, I) (third) <sup>68†</sup>	0.24 0.27 <sup>†</sup>	0.85 0.9 (IV) <sup>†</sup> 1.3 (IM) <sup>†</sup> 1.7 (SC) <sup>†</sup>	20 (IV)/45 50 (IM)/115 50 (SC)/69 F (IM, SC, D)=1.0
Cefuroxime (TD, I)			60 (IM, D)/79
Cephalexin (TD, I)(first) <sup>60,67</sup> (based on bioactivity) <sup>†</sup>	0.23	1.4-2.5* 1.3 (D) <sup>†</sup> 1.8 (D <sup>61</sup> increases to 2.6 at night) 4.7 <sup>59a</sup>	20 (IV, D)/41 20 (IV, D)/24 20 (PO)/20.3 F (PO, IM, D) = 0.6 22 (PO)/20 25 (PO, D)/18.8±2.8 <sup>6</sup> 40 (PO,D)/35 <sup>†</sup> 30 (PO)/28 15 (PO, C)/11-29 <sup>†</sup> 25 (PO, C)/15 <sup>†</sup> 20 (SC, C)/54 <sup>†</sup> 20 (IM, C)/61.8 <sup>†</sup> 10-15 (PO, D)/18.6 10 (SC, D)/24.9 <sup>†</sup> 10 (IM, D)/31.9 <sup>†</sup>
Cephalothin (first) (TD, I) <sup>62</sup>	0.43	0.7-0.85 1.7 without food, 2.8 with <sup>†</sup>	10 (IM)/9.3 20 (IV)/35 40 (IV)/45 20 (SC)/22 40 (SC)/30 30 (PO,D)/45 without food, 28 with food <sup>†</sup>
Cephapirin (TD, I) (first)	0.32	0.5	30 (IV)/26.9
Cephradine (TD, I)			50 (PO)/39
Chloramphenicol (TD, S)	0.85-1.77 [D] <sup>†</sup> 2.36 [C] <sup>†</sup>	1.2 [D] <sup>†</sup> 2.7± 0.7 <sup>254</sup> 3,3 (SC) (C) <sup>255</sup> 6.9 (IV) (C) <sup>255</sup>	33 (PO, D)/8/5 33 (SC,D)/15 50 (PO, D)/20±4 (large dogs) <sup>252</sup> 50 (PO, D)/27±7 (small dogs) <sup>252</sup> 20 (IV, C)/19.5±1.5 <sup>255</sup> 20 (IM, C)/18.6±2.6 <sup>255</sup> 20 (SC, C)/14.8±2.9 <sup>255</sup> 20 (PO,C)/9.8±2.6 <sup>255</sup> 50 total (PO, C) 8 to 25 (range) <sup>254</sup>

Continued

Table 7-1 Pharmacokinetic Data for Selected Antimicrobials—cont'd

Drug	Vd (L/kg)	Half-Life (hr)	Dose (mg/kg) <sup>1</sup> /C <sub>max</sub> (μg/mL)
Ciprofloxacin (CD, I) (see also enrofloxacin)	3 (D) <sup>†</sup>	2.2 (D at 2.5-10 mg/kg) <sup>‡</sup>	10 (IV, C)/2.53 (extrapolated from terminal component) <sup>195</sup>
	3.85 (C) <sup>†</sup>	4.9 (D at 10 mg/kg) <sup>‡</sup>	10 (PO, D)/1.4 <sup>‡</sup>
		5.3 (D at 20 mg/kg) <sup>‡</sup>	20 (PO, D)/2.8 <sup>‡</sup>
		8.9 (D at 40 mg/kg) <sup>‡</sup>	40 (PO, D)/6.6
		4.53 (C) <sup>†</sup>	23 (PO, D × 7 day)/5.68 10 (PO, C)/0.89 <sup>†</sup> (F = 0.3±0.1)
Clarithromycin (TD, S)	1.4 (Vd <sub>ss</sub> , D) <sup>†</sup>	3.9 (D) <sup>‡</sup>	10 (PO, D)/3.3 (F=0.7) <sup>‡</sup>
Clindamycin <sup>261,261</sup>	0.86 ± 0.35 (D) <sup>†</sup>	2 (IV) <sup>†</sup>	11 (PO, D)/5
		7.1 (IM) <sup>†</sup>	5.5 (PO, D)/2
		5 (SC) <sup>†</sup>	10 (IV, D)/18.8 extrapolated, 7.5 postdistribution <sup>†</sup>
		16.4±15.4 (C; capsule) <sup>261</sup>	10 (IM, D)/7.5 (F = 1.15) <sup>†</sup>
		7.5±1.7 (C; solution) <sup>261</sup>	10 (SC, D)/4.4 (F = 3.10) <sup>†</sup>
			15 (PO, C)/11 11 (PO, C)/9 11(PO, C, capsule)/7.4±1.7 <sup>261</sup> 11(PO, C, solution)/6.6±2.2
Cloxacillin (TD, I)	0.2	0.5	
Dicloxacillin (TD, I)	0.2	0.7	
Difloxacin (CD, I)		9.3 [D] <sup>‡</sup>	5 (PO, D)/1.1-1.8
		6.9±0.5 (Heinen, 2002)	10 (PO, D)/2.3
		8.5±0.54 (Frazier)	5 (PO × 5 d, D)/1.8 <sup>190</sup> 5 (PO × 3 d)/1.79 ± 0.11 <sup>115</sup>
Doxycycline (TD, S)	0.93 (Vd <sub>ss</sub> <sup>†</sup> )- 1.5 (D) (f <sub>ub</sub> = 9%) 0.65±0.09L/ kg (D) <sup>57</sup> 0.34 (Vd <sub>ss</sub> , C) <sup>‡</sup>	7-10 (D) <sup>‡</sup>	5 (PO, D)/5
		4.56±0.57 (D) <sup>57</sup>	1.1, 0.1 (IV & CRI; D)/1.4 unbound <sup>57</sup>
		4.6 (C) <sup>‡</sup>	2.5 (PO, D)/3
			5 (PO, C)/6
			2.5 (PO, C)/3 5 (PO, D)/3.5 <sup>158</sup> , (see also Chapter 8)
Enrofloxacin (CD, I)	2.6 3.7-7 (D, Vd <sub>ss</sub> ) <sup>‡</sup> 4 (C, Vd <sub>ss</sub> )	0.92 (2.5) <sup>7</sup>	2.5 (PO, D)/1
		2.02 (5) <sup>7</sup>	5 (PO, D)/1.6-2
		2.4/3.9 (at 5 mg/kg, D) <sup>†</sup>	5 (PO, D for 5 d)/1.4± 0.07 <sup>190</sup>
		4.1 (5 mg/kg, D) (Heinen, 2002)	5 (PO, D, for 3 d)/1.75±0.16 (Ciprofloxacin: 0.4) <sup>179</sup>
		2.6/6.3 (7.5mg/kg, D) <sup>83§</sup>	5.5 (PO, D for 7 d)/2.45 <sup>‡</sup>
		2.9/7.4 (10 mg/kg, D) <sup>83§</sup>	5.8 (PO, D for 7 d)/1.43(Cip: 0.36) <sup>‡§</sup>
		4.1/11.7 (20 mg/kg, D) <sup>83§</sup> 6.7/6.1 (at 5 mg/kg, C) <sup>†</sup>	7.5 (PO, D)/1.6 (Cip: 1) <sup>83</sup> 10 (PO, D)/1.7 (Cip: 1.2) <sup>83</sup> 11 (PO, D for 7 d)/4.56 <sup>‡</sup> 20 (PO, D)/4.2 (Cip: 1.9) <sup>83</sup> 2.5 (PO, C)/1.3 5 (PO, C)/2.5
Erythromycin (TD, S)	2.7 (Vd <sub>ss</sub> , D) <sup>†</sup> 4.8±0.9	1-1.5	10 (IV, D, lactobionate)/6.4±1.38 <sup>267</sup>
		1.7 (D) <sup>†</sup>	25 (PO, D, estolate tablet)/0.3±0.17 <sup>267</sup>
		1.35±0.4 (IV); <sup>267</sup>	10 (IV, D)/29 (C <sub>o</sub> ) <sup>269</sup>
		2.92±0.8 (estolate tablet) <sup>267</sup>	10 (PO, D)/4.9 <sup>269</sup>
		2.56±1.77 (ethylsuccinate suspension) <sup>267</sup>	20 (PO, D, ethylsuccinate suspension)/0.17± 0.09 <sup>269</sup> 20 (PO)/3.5
Florfenicol (TD, S)	0.6 (C) 1.45±0.8 (D) <sup>250</sup>	9.2 (IM, D)	20 (IV, D)/6.5 (at 1 hr; extrapolated from) <sup>250</sup>
		1.2 (IV, D) <sup>250</sup>	20 (PO, D)/6.4 <sup>250</sup>
		4 (IV, C)	20 (IM, D)/1.64
		5.6 (IM, C)	22 (IM, C)/20
		7.8 (PO, C)	22 (PO, C)/27

Table 7-1 Pharmacokinetic Data for Selected Antimicrobials—cont'd

Drug	Vd (L/kg)	Half-Life (hr)	Dose (mg/kg) <sup>1</sup> /C <sub>max</sub> (μg/mL)
Fosfomycin disodium phosphate (TD, I)	0.70 ± 0.15 (D) (Vd <sub>ss</sub> ) (Guiterrez, 2008)	See text	See text
Gentamicin (CD, I)	0.35±0.04 [D] <sup>97</sup> 0.18 (Vd <sub>ss</sub> [D]) <sup>†</sup> 0.14-0.2 [C]	0.87-1.36 (D) 1.1[D] <sup>†</sup> 1.25±0.3 (C, IV) <sup>101</sup> 1.27±0.26 (C, IM) <sup>101</sup> 1.14±0.11 (C, SC) <sup>101</sup>	4 (IV)/27 8 (IV)/44 3 (IV, D)/24 (extrapolated Co.) <sup>98</sup> 3 (IV, D)/14 (extrapolated from B) <sup>98</sup> 10 (IV, D)/28 3 (IM, SC, D)/10.5 <sup>98</sup> 4.4 (IM, D)/7.5 2.2 (IV, D)/6 10 (IV, C)/28 2 (IM, C)/4 3 (SC, C)/15-17 <sup>†</sup> 5 (IV, C)/35 (extrapolated from B) <sup>101</sup> 5 (IM, C)/21.6 <sup>101</sup> 5 (SC, C)/23.5 <sup>101</sup>
Imipenem/cilastin (TD, I)	0.32 (D)	0.83-0.92 (IM) 1.5 (SC)	30 (IV)/180 10 (IV)/65 <sup>56</sup> 5 (IM)/13.2 (D) F (IM, D) = 1.5 5 (SC)/8.8 (D)
Kanamycin (CD, I)	0.23-0.28	0.75-1 0.77-1 (D) <sup>†</sup>	7.5 (IM, D)/25.8 <sup>†</sup> 10 (IM, D)/27.6±7.5 <sup>†</sup> 15 (IM, D)/37.8 <sup>†</sup> 25 (IM, D)/55.6 <sup>†</sup> 39 (IM, D)/84.5 <sup>†</sup>
Levofloxacin (CD, I) (see also Ofloxacin)	1.75±0.42 (C)	8.4± 3.5 = 9.3 ± 1.6 (C)	10 (IV, C)/5.6± 1.4 (extrapolated from terminal curve) 10 (PO, C)/4.7± 0.9 <sup>200</sup> (F=0.86±0.44)
Linezolid (TD, I)	0.63 (D)	3.6 (D)	25 (IV, D)/63 10 (IV, D)/23 25 (PO, D)/26 (F=0.96)
Lincomycin (TD, S)			22 (PO)/1.2 15 (PO)/1
Marbofloxacin (CD, I)	1.2-1.37 (D) <sup>†</sup>	9.1-14.7 PO, (D) <sup>†</sup> † 9.0± 2 [D] (Heinen, 2002) 11.0± 0.94 (Frazier, 2000) 11.5 (at 1 mg/kg, SC, D) 13.4 (at 4 mg/kg, SC, D) 12.7 (C) <sup>†</sup>	2 (IV, D)/2.5 (extrapolated) <sup>188</sup> 1 (PO, D)/0.83 <sup>†</sup> 2 (PO, D)/1.38 <sup>†</sup> 2 (PO, D × 8 [D])/1.4 <sup>190</sup> 4 (PO, D)/2.9 <sup>†</sup> 5.5 (PO, D)/4.2±0.5 1 (SC, D)/0.78 2 (SC, D)/1.52 4 (SC, D)/3 5 (PO, D)/1.41±0.07 <sup>†</sup> 6.2 (PO, C)/4.8
Meropenem (TD, I)	0.37 0.34	0.67 0.73	20 (IV)/60 (extrapolated) and 24 in ICF 20 (SC)/26 (plasma) and 11 ICF <sup>57</sup>
Metronidazole (CD>TD, I>S)	0.95 (D) <sup>†</sup> 100	4.3 4.5(D) <sup>†</sup>	44 (IV)/60 44 (PO, D)/42
Minocycline (TD, S)	2 (D)	7-7.3 (D)	For urinary tract infection only
Nitrofurantoin (S)		4.6 (D)	20 (PO, D)/14.2± 3.4
Ofloxacin (CD, I) (racemic mixture of R and S levofloxacin)			

Continued

Table 7-1 Pharmacokinetic Data for Selected Antimicrobials—cont'd

Drug	Vd (L/kg)	Half-Life (hr)	Dose (mg/kg) <sup>1</sup> /C <sub>max</sub> (µg/mL)
Orbifloxacin (CD, I)		4.5 = 5.2 (C) <sup>‡</sup>	2.5 (PO, C)/2 <sup>‡</sup>
		5.4 = 5.6 (D) <sup>‡</sup>	2.5 (PO, D)/1.4-2.3 <sup>‡</sup>
		7.1 ± 0.42 (D)	2.5 (PO, D)/1.4 ± 0.07
		(Heinen, 2002)	
Oxacillin (TD, I)	0.3 (D)		40 (PO)/4.0 30 (PO)/3.0
Oxytetracycline (TD, S)	2 (D) <sup>‡</sup>	6 (D) <sup>‡</sup>	
Penicillin G (TD, I)	0.16	0.5 (D)	20,000 U/kg (IV)/30
			22,000 U/kg (SC)/14
Piperacillin (TD, I)			50 (IV)/250
			25 (IV)/125
Rifampin (CD, I)		8 (D) <sup>‡</sup>	10 (PO, D)/40 <sup>‡</sup>
Sulfadimethoxine (TD, S) <sup>‡</sup>		13.1 (D) <sup>‡</sup>	55 (PO, D)/67 <sup>‡</sup>
		10.2 (C) <sup>‡</sup>	
Sulfadiazine (TD, S) <sup>‡</sup>	1.02	9.8	
Sulfamethazine (TD, S) <sup>‡</sup>	0.5-0.6 (D)	16-17 (D)	
Tetracycline (TD, S)		1.6-2 (D) <sup>‡</sup>	20 (PO, D)/9
		2.5 (C) <sup>‡</sup>	13.75 (PO, D)/7
Ticarcillin (TD, I)	0.34 (D)	1-1.25	100 (IV)/200
			40 (IV)/80
Ticarcillin (TD, I) with clavulanic acid <sup>55</sup>		1-1.25 (ticarcillin)	100 (IV)/200
		0.40 (clavulanic acid)	40 (IV)/80
			F (IM, D) = 0.91 ticarcillin
			F (IM, D) = 0.65 clavulanic acid
Trimethoprim (TD)	1.49 (D)	2.5 (D)	
Tylosin (TD, S)	1.7(D) <sup>‡</sup>	0.9 (D)	10 (IM, D)/1.5 <sup>‡</sup>
Vancomycin (TD, I)		4-6	15 mg/kgq6 hr/40 peak 5 trough µg/mL

V<sub>d</sub>, Volume of distribution; C, cat; I, bactericidal; D, dog; IV, intravenous; IM, intramuscular; SC, subcutaneous; T, time dependent; PO, by mouth; T, bactericidal; V<sub>d<sub>ss</sub></sub>, volume of distribution at steady state; S, bacteriostatic; F, bioavailability; f<sub>ub</sub>, fraction unbound; PI, Package Insert, constant-rate infusion; IC<sub>F</sub>, intracellular fluid.

\*CD or TD = Concentration or time dependency (see Chapter 6). 1 C<sub>max</sub> refers to the maximum serum concentration obtained at the dose given by the route in parenthesis. Data refer to both cat and dog unless indicated otherwise (D=dog; C=cat). A new dose can be determined by proportionally changing the dose based on the desired change in C<sub>max</sub>. For example, a 20 mg/kg IV dose of amikacin resulted in C<sub>max</sub> of 40 µg/mL. If a patient is given 10 mg/kg IV amikacin, the resulting C<sub>max</sub> should approximate about 20 µg/mL. The data should be used in conjunction with a minimum inhibitory concentration (see Chapter 6, Table 6-2).

<sup>‡</sup>Source as indicated by drug name.

<sup>‡</sup>USP Veterinary Pharmaceutical Information Monographs—Antibiotics, *J Vet Pharmacol Ther* 26(Suppl 2), 2003.

<sup>‡</sup>Half-life or C<sub>max</sub> of ciprofloxacin (µg/mL) is that achieved from metabolism of enrofloxacin when enrofloxacin is administered at the indicated dose. The drugs should work in an additive or synergistic fashion.

<sup>‡</sup>90% protein binding in dog, 99% in cats; amount reported is peak concentration in transudate.

<sup>‡</sup>Static if sole agent, bactericidal if the sulfonamide is combined with a diaminopyrimidine (trimethoprim, ormetoprim)

canine and feline pathogens (e.g., see Table 7-3) is offered for selected drugs; in addition, relevant information from the human-medicine literature is provided (see Table 7-4). Care should be taken when extrapolating information regarding human pathogens to dogs and cats, although a growing amount of evidence suggests that relative susceptibility of isolates is similar for many drugs (indeed, isolates are likely to be shared), and the data are likely to include both patients that have previously received and not been exposed to antimicrobials. For time-dependent drugs, the relevant PD index (PDI) to be targeted is  $T > MIC$ , with a target of at least 50% to 75% of the dosing interval necessary to enhance efficacy, and longer to avoid resistance. An exception can be made for the carbapenems, for which  $T > MIC$  of 25% of the dosing interval is sufficient. For concentration-dependent drugs, the relevant PDI

is a  $C_{max}/MIC \geq 10$ .<sup>3</sup> This ratio should be reached at the site of infection. Alternatively, the AUC/MIC should target 125 to 250. Although as low as 30 has been supported for selected gram-positive drugs, this is particularly true for *Streptococcus pneumoniae*, which is an organism that is particularly problematic in humans. This low AUC/MIC may not be relevant to other gram-positive organisms, including other streptococci. Because availability of AUC data is limited, this chapter will focus on C<sub>max</sub>/MIC as the target for concentration-dependent drugs. For PDI for both time- and concentration-dependent drugs, doses should be modified as indicated by drug, host, and microbial factors.

The discussion of antimicrobial drugs is based on their classification by mechanism of action (Figure 7-1; see Table 7-1). The mechanism of action of each drug determines drug

Table 7-2 Spectrum of Antimicrobial Activity

Class	Drugs	MOA	G+	Stph	G-	Pse	An	My	Act	Noc	AM
<b>Beta-lactams</b>											
<b>Penicillins</b>											
Natural	Penicillin	Cell wall	3+	2+	1	N	3-4+	0	Y		
Semisynthetic	Dicloxacillin	Cell wall	4+	4+		N	N	0			
	Ampicillin	Cell wall	3+	2-3+	2-3+	N	3-4+	0	Y	Y	
	Amoxicillin	Cell wall	3+	2-3+	3+	N	3-4+	0	Y	Y	
	Amoxicillin-clavulanate	Cell wall	3+	3-4+	3+	N	3-4+	0	Y	Y	
	Ticarcillin	Cell wall	3-4+	4+	3-4+	Y	3-4+	0	Y	Y	
	Ticarcillin-clavulanate	Cell wall	3-4+	4+	4+	Y	3-4+	0	Y	Y	
Carbapenem	Meropenem	Cell wall	4+	4+	4+	Y	3-4+	0	Y	Y	
Monobactam	Aztreonam	Cell wall	N	N	4+	Y	N	0			
<b>Cephalosporins<sup>†</sup></b>											
First generation	Cephalexin	Cell wall	3+	3-4+	1-2+	N	1-2+	0	0		
	Cefazolin	Cell wall	3+	2-3+	2-3+	N	1-2+	0	0		
Second generation	Cefoxitin	Cell wall	2-3+	3-4+	3-4+	N	4+	0	0		
Third generation	Cefotaxime	Cell wall	3+	1-4+	1-4+	Y	3-4+	0			
	Ceftiofur	Cell wall	3+	2+	2-3+	N	2+	0			
	Cefpodoxime	Cell wall	3+	3-4+	3+	N	2-3+	0			
	Cefovecin	Cell wall	3+	3-4+	3+	N	2-3+	0			
Aminoglycosides	Gentamicin	Ribosomes 30&50	1+	4+ <sup>§</sup>	4+	3-4+	0	Y	N/Y*	Y	Y
	Amikacin	Ribosomes 30 & 50	1+	3-4+ <sup>§</sup>	4+	4+	0	Y		Y	
Fluorinated quinolones	Enrofloxacin <sup>†</sup>	Topoisomerases	1-2+	3-4+	3-4+	Y(C&S)	1+	Y	0	0	Y
	Pradofloxacin	Topoisomerases	3-4+	3-4+	3-4+	Y	3+	Y	0	0	Y
Sulfonamides	Sulfadiazine	Folic Acid synthetase	2-3+	2-3+	2+	N	2-3+	N	Y	Y	0
Pyrimethamine	Trimethoprim	Folic acid reductase				N					
Tetracyclines	Doxycycline	Ribosomes 30s	2-3+	2-3+	2-3+	N	2-3+	Y	C&S		Y
Phenicol	Choramphenicol <sup>‡</sup>	Ribosomes 50s	2-3+	2-3+	2-3+	N	2-3+	Y			
Macrolides	Erythromycin	Ribosomes 50s	3+	3-4+	1-2+	N	2-3+	Y	Y		Y
	Azithromycin	Ribosomes 50s	3+	2-4+	2+	N	2-3+	Y	Y		Y
Lincosamides	Clindamycin	Ribosomes 50s	4+	3-4+	1+	N	3-4+	Y	Y		
Nitroimidazoles <sup>*</sup>	Metronidazole	DNA-RNA	N	N	N	N	4+		C&S		Y
Oxazolidinones	Linezolid	Ribosomes 50s-70s	4+	4+	N	N	3+	Y	Y	Y	Y
Rifamycin <sup>§</sup>	Rifampin	RNA	3+	3+	N	N	Y	Y	N		Y
Glycopeptide	Vancomycin	Cell wall	4+	4+	N	N	Y	N	N		

MOA, mechanism of action; G+, gram-positive; G-, gram-negative; Stph, Staphylococcus; Pse, Pseudomonas; An, Anaerobes; My, Mycoplasma; Act, Actinomyces; Noc, Nocardia; AM, atypical mycobacterium; Y, yes; N, no; C&S, culture and susceptibility testing. 0, No efficacy; 1, poor; 2, fair; 3, good; 4, excellent.

\*Spectrum reflects inherent susceptibility and does not include acquired resistance.

<sup>†</sup>See text for specific differences, but in general enrofloxacin represents marbofloxacin, orbifloxacin, and difloxacin.

<sup>‡</sup>Generally ineffective toward enterococci.

<sup>§</sup>Generally not as sole therapy.

**Table 7-3** Susceptibility Data for Feline and Canine *Escherichia coli* Pathogens (n = 595)

Drug	Resistant <sub>BP</sub>	Mode	MIC <sub>50</sub>	MIC <sub>90</sub>	Range
Amoxi-clavulanate	≥32/16	4	4	32	0.5-2048
Ampicillin	≥32	2	4	512	0.25-512
Ticarcillin-clavulanate	≥128	2	2	64	2-2058
Meropenem	≥16	0.25	0.25	0.5	0.25
Cefotaxime	≥64	1	1	16	1-2048
Cefoxitin	≥32	4	4	32	0.5-2048
Cefpodoxime	≥8	0.5	0.5	256	0.12-512
Ceftazidime	≥32	0.5	0.5	16	0.25-512
Cephalothin	≥32	8	16	2048	1-2048
Gentamicin	≥16	1	1	8	1
Enrofloxacin	≥4	0.06	0.06	32	0.03-512
Ciprofloxacin	(≥4)	0.03	0.03	32	0.3-128
TMPS	≥4/c	0.06	0.06	2	0.06
Azithromycin	≥8	8	8	64	1-512
CHPC	≥32	8	8	32	2-2048
Doxycycline	≥16	1	2	32	0.25-1024

BP, Breakpoint; MIC, minimum inhibitory concentration; CHPC: chloramphenicol; TMPS, trimethoprim-sulfonamide combination. All MIC are in µg/mL. Data is likely to include isolates from dogs or cats exposed to antimicrobials  
 \*475 isolates are from the urinary tract. Data was generated by the author and includes isolates from animals exposed to antimicrobials.

**Table 7-4** Susceptibility Data for Selected Drugs and Selected Human Pathogens Associated with Skin and Soft Tissue Infections<sup>106</sup>

Drug	Resistant MIC <sub>BP</sub>	<i>Enterobacter</i> spp.		<i>E. coli</i>		<i>K. pneumoniae</i>		<i>P. aeruginosa</i>		<i>S. aureus</i>	
		MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>
Ciprofloxacin	≥4	≤0.25	0.5	≤0.25	>2	≤0.25	2	≤0.25	>2	≤0.25	>2
Levofloxacin	≥8	≤0.03	0.5	≤0.03	4	0.06	2	0.5	>4	0.25	>4
Doxycycline*/ Tetracycline	≥16	≤4	>8	≤4	>8					≤0.5	1
Amikacin	≥64	<1	2	2	4	1	2	2	4		
Gentamicin	≥16	≤1	≤1	≤1	2	≤1	4	2	>8	≤1	≤1
TMPS	≥4/76	≤0.5	1			0.05	1			≤0.5	≤0.5
Nitrofurantoin	≥128	≤32	64	≤32	≤32	≤32	64				
Clindamycin	≥16	>16	>16							0.12	>8
Erythromycin	≥16									0.5	>8
Linzeolid	≥8*			≤2	16					2	2
Rifampin	≥4									≤0.25	≤0.25
Vancomycin	≥32									1	1

MIC, Minimum inhibitory concentration; BP, breakpoint, TMPS, trimethoprim-sulfonamide. All MIC are in µg/mL. Data is likely to include isolates from dogs or cats exposed to antimicrobials.

\*When testing *Enterococcus* spp.

efficacy (i.e., bactericidal versus bacteriostatic) and mechanisms of resistance<sup>4</sup>; influences time- versus concentration-dependence and duration of postantibiotic effect; and, for some drugs, affects safety. Mechanisms of action also influence the selection of combination antimicrobial therapy. For drugs that are approved for use in humans but not animals and for which information regarding use in dogs and cats is not available, PK information in humans will be summarized.

### DRUGS THAT TARGET THE CELL WALL

#### Beta-Lactam Antimicrobials

The broad spectrum, low toxicity, and reasonable cost of beta-lactam antibiotics contribute to their frequent use for treatment of infections. In addition, their effects on cell wall synthesis result in their frequent selection for combination antimicrobial therapy. The beta-lactam antibiotics include the



**Table 7-5 Serum Concentration of Drugs Achieved in (Human) Tissues\***<sup>292</sup>

		ICF	Joint Fluid	Ascites	Pleural Fluid	Bronchial Secretions	Sinus Secretions	Middle Ear	CSF	Aqueous Humor	PMN or AM
Aminoglycosides	Amikacin	17	111 (4-7)	58 (5)	40, 21 (7.5)	21			35	0.5-4.5	
	Gentamicin	31	80	90 (0.5)	57	<8			2.5	22.5	21-73
Penicillins	Amoxicillin	40,76		83		4-23		24-50	10		
	Ampicillin	13	62	100		5	3	6-50	3.4		6
	Penicillin	17	93	39	67-88			35	8	2.5	37
	Ticarcillin	23,40,88		14		1					
	Piperacillin	35		55		4			29	4.5	<10
	Carbenicillin	22				1					
Carbapenems	Imipenem			85		20			8.5	8.5	33
	Meropenem	87 (4-12)				20-47			21	29	
Monobactam	Aztreonam	90		43	79	21			6.7	2.5	
First-generation cephalosporins	Cephalexin		66		30		10			2.5	55
	Cefodroxyl	70	98		114					42.5	
	Cefazolin	11	32 (0.5)		30	2			0	<2	<10
Cephamycins	Cefoxitin	45		117	31	11-16			52	7.5	
Oxyimino-cephalosporins	Ceftazidime	13,35		45	21	11			23.5	4.5-12.5	
	Cefotaxime	13,35	116	120 (6)	24	2		123	18-51	2.5	56
	Cefpodoxime				67 (6)						
Fluoroquinolones	Ciprofloxacin	80			26-126	26-89			25(1), 18-146 (8)	8.5-17.5	600-700
	Levofloxacin					188					1850
	Ofloxacin	49		69 (24), 93 (8)		60			42-72	14	815
Macrolides	Erythromycin base	46 (4-6)									1700-4600
	Erythromycin	6				25					
	Azithromycin	130				1692				9.3	900-70,000
Lincosamides	Clindamycin	9		44 (1-5)	92						1200
Tetracyclines	Doxycycline	47			36	17	57	42	14-22	10.5-13.5	
	Tetracycline					30	79-100			9.5-11.5	74
Sulfonamides	Sulfadiazine	50					20	57			
	Sulfamethoxazole	37						27	40		
	Trimethoprim	55					133	119			44-600
Rifampin		20 (6-9)		23 (4)	40				4		70-800
Fosfomycin		53			22					25.5	
Vancomycin			81	52	41-111				0	10.5	122
Linezolid			87			414					
Metronidazole								70	43	38.5	85-103
Chloramphenicol											964
Blisters, disks, threads											

ICF, Intracellular fluid; CSF, cerebrospinal fluid; PMN, polymorphic neutrophils. With noted exceptions (difference cited in parentheses), timing 1 to 2 hours. All concentrations are in µg/mL.

Table 7-6 Selected Chemical Characteristics of Antimicrobial Drugs

Drug	MW	Acid/Base	pKa	Predicted PC*
Amikacin	585	Base	8.1	0.0006
Amoxicillin	365	Acid	2.8, 7.2	0.0026
Clavulanate		Acid		0.069
Ampicillin	349	Acid	2.7, 7.3	7.58
Azithromycin	749	Base	8.74	1071
Cefaclor	385	Acid		
Cefadroxil	381	Acid		
Cefazolin	454	Acid		0.81
Cefpodoxime	557	Acid		1.12
Cefotaxime	455	Acid	3.35	
Cefovecin		Acid		
Cefoxitin	427	Acid	2.2	1.65
Ceftiofur	523	Acid		
Cephalexin	347	Acid	5.3, 7.3	
Cephapirin		Acid	2.15, 5.44	
Ciprofloxacin	331	Amphoteric	6.1, 8.6	0.27
Clindamycin	425	Base	7.7	57
Doxycycline	462	Amphoteric	3.4, 7.7, 9.7	0.91
Enrofloxacin	360	Amphoteric	6.0, 8.8	3.54 (actual)
Erythromycin	733	Base	8.8	234
Gentamicin	470	Base	8.2	0.02
Imipenem	317	Acid	3.2, 9.9	0.64
Kanamycin	484		7.2	
Levofloxacin	361			0.95
Linezolid	337			
Marbofloxacin	362	Amphoteric	6.2, 8.6	0.08
Meropenem	383			0.83
Metronidazole	171			0.69
Orbifloxacin		Amphoteric		
Penicillin	334	Acid	2.7	60
Piperacillin	517			4.67
Rifampin	822	Zwitterion	1.7, 7.9	229
Sulfadiazine	250	Acid	6.4	1.54
Sulfadimethoxine	310		6.2	
Tetracycline	444		8.3, 10.2	0.40
Ticarcillin	384			9.7
Trimethoprim	290	Base	7.6	18
Tylosin	916		7.1	
Vancomycin	1449	Amphoteric	7.8, 8.9 (Basic) 2.2, 9.6, 10.4, 12 (acid)	13

MW, Molecular weight; pKa, dissociation constant; PC, octanol-water partition coefficient. Note that the PC is dependent on ambient pH.

cephalosporins, penicillins (including combination penicillin/beta-lactamase inhibitors), carbapenems, and monobactams (see Table 7-1).

### Structure-Activity Relationship

Beta-lactam antibiotics contain a four-member beta-lactam ring as the active site. A second member ring establishes the drug as either a cephalosporin—one carbon larger—or

a penicillin (Figure 7-2).<sup>5-9</sup> Chemically, the beta-lactams are classified as weak acids (see Table 7-6). They include natural, and semisynthetic drugs (see Table 7-2). Penicillin is a natural drug derived from the molds of the genus *Penicillium*. Penicillin serves as a base for the semisynthetic aminopenicillins (ampicillin, amoxicillin), the extended-spectrum penicillins (carbenicillin, ticarcillin, piperacillin), the carbapenems (imipenem, meropenem), and the monobactams (aztreonam).

Table 7-7 Dosing Regimens of Selected Antimicrobials\*

Drug	Dose	Route of Administration	Frequency (hr)
Amikacin	15-22 mg/kg	IM, IV, SC	24 (consider monitoring)
Amoxicillin	20-30 mg/kg	IM, IV, PO, SC	6-12
Amoxicillin-clavulanic acid	10-30 mg/kg	PO	6-12
	62.5 mg/cat	PO	6-12
Ampicillin	20-60 mg/kg	PO	6-8
	10-50 mg/kg	IV	6-8
Ampicillin sulbactam	10-50 mg/kg	IM, IV	6-8
Ampicillin trihydrate	10-50 mg/kg	IM, SC	6-8
Amprolium	100 mg/dog	PO (on food or in water)	24 × 7-10 days
Azithromycin	5-10 mg/kg (D)	PO	12-24
	5-15 mg/kg (C)	PO	12-48
	15 mg/kg loading dose	PO	
Aztreonam	12-25 mg/kg	IM, IV	8-12
Baquiloprim-sulphadimethoxine or sulphadimidine	30 mg/kg	PO	24 × 2 days then q 48 × 10-21 days
Carbenicillin	15-110 mg/kg	IM, IV, SC	6-8
Carbenicillin indanyl sodium	10-55 mg/kg	PO	8
Cefaclor	4-20 mg/kg	PO (in a fasted animal)	8
Cefadroxil	20-35 mg/kg	PO	8-12
Cefamandole	6-40 mg/kg	IM, IV	6-8
Cefazolin sodium	10-25 mg/kg	IM, IV, SC	4-8
	10-22 mg/kg	IV	1-2 times during surgery
Cefepime	50 mg/kg	IM, IV	8
Cefixime hydrochloride	5-12.5 mg/kg	PO	12-24
Cefmetazole sodium	20 mg/kg	IV	6-12
Cefoperazone sodium	22 mg/kg	IV, IM	6-12
Cefotaxime sodium	20-80 mg/kg (D)	IM, IV, SC	4-12
Cefotetan disodium	30 mg/kg	IV, SC	8
Cefovecin	8 mg/kg	SC	2-14 days based on organism MIC
Cefoxitin sodium	15-30 mg/kg (D)	IM, IV, SC	6-8
	6-40 mg/kg (D)	IM, IV	6-8
Cefpodoxime proxetil	5-10 mg/kg	PO	12-24
Ceftazidime	15-30 mg/kg	IM, IV, SC	6-12
Ceftiofur	2.2-4.4 mg/kg	SC	12-24
Ceftizoxime	25-50 mg/kg	IM, IV	8-12
Ceftriaxone	15-50 mg/kg	IM, IV	12
	25 mg/kg	IM, IV	1-2 times during surgery
Cefuroxime axetil or sodium	10-30 mg/kg	IV, PO (with food)	8-12
Cephalexin	20-60 mg/kg	PO	6-12
Cephaloridine	10 mg/kg	IM, SC	8-12
Cephalothin	10-44 mg/kg	IM, IV, SC	4-8
Cephmandole	6-40 mg/kg	IM, IV	6-8
Cephapirin	10-30 mg/kg	IM, IV, SC	4-8
Cephradine	10-40 mg/kg	IM, IV, PO	6-8
Chloramphenicol palmitate	25-50 mg/kg (D)	PO	8
	50 mg/cat	PO	12
Chloramphenicol sodium succinate	25-50 mg/kg (D)	IV, SC, IM	6-8
	50 mg/cat	IV, SC, IM	12
Chlortetracycline	25 mg/kg	PO	6-8
Ciprofloxacin	10-50 mg/kg (D)	PO	12-24
	5-20 mg/kg (D,C)	IV	12-24

Continued

Table 7-7 Dosing Regimens of Selected Antimicrobials\* — cont'd

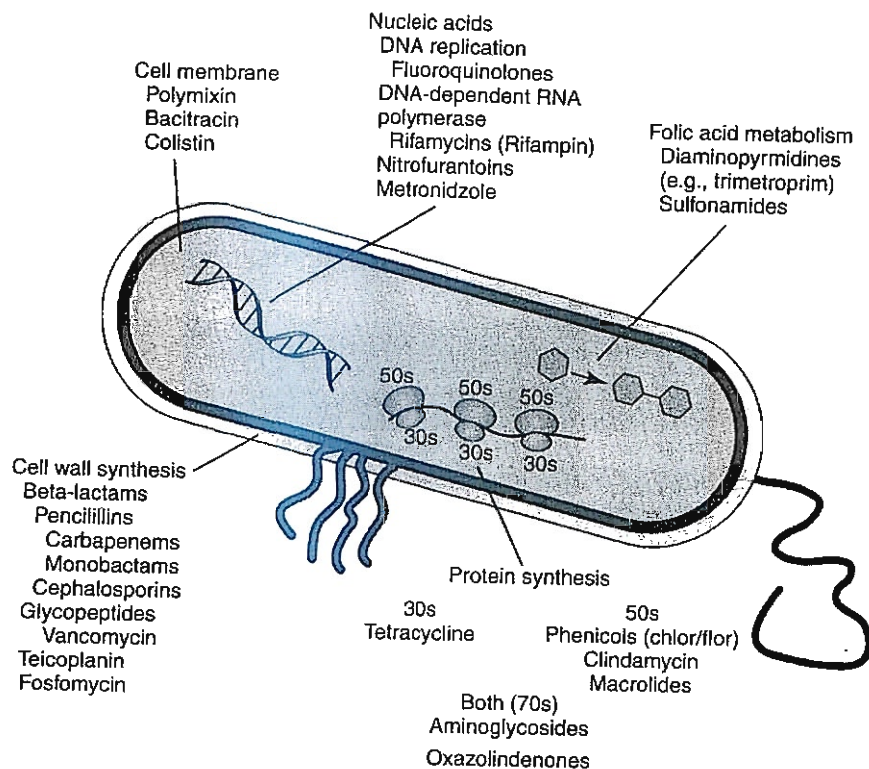
Drug	Dose	Route of Administration	Frequency (hr)
Clarithromycin	2.5-10 mg/kg (D)	PO	12-24
	62.5 mg/cat	PO	12 with clofazimine
	7.5 mg/kg (C)	PO	12 with metronidazole and amoxicillin
	5-10 mg/kg	PO	12 with rifampin and enrofloxacin
Clindamycin	5-20	PO	12-24
	25-50	PO	24 (for toxoplasmosis)
Clofazimine	4-8	PO	24
Cloxacillin	20-40 mg/kg	IM, IV, PO	4-8
Dapson	1.1	PO	8-12
Dicloxacillin	30-50 mg/kg	PO	6-8
Difloxacin	5-10 mg/kg	PO	24
Dihydrostreptomycin	20-30 mg/kg	IM, SC	24
Doxycycline	5-10 mg/kg	IV, PO	12-24
Enrofloxacin	5-20 mg/kg (D)	IM, IV, PO, SC	12-24
	5 mg/kg (C)	IM, IV, PO, SC	24
Erythromycin	10-22 mg/kg (D), maximum of 40 mg/kg	PO	8-12
	10-22 mg/kg (C)	IV, PO	8
	3-5 mg/kg (C)	IM	8
Ethambutol	15-25 mg/kg	PO	24-72
Fosfomycin	40-80 mg/kg	PO	12
Florfenicol	100-200 mg	IM, PO, SC	8 (D), 12 (C)
	25-50 mg/kg	PO, SC	8
	20 mg/kg (D)	IM, PO	6
	22 mg/kg (C)	IM, PO	12
Gentamicin	6-8 mg/kg	IV, IM, SC	24
	4-8 mg/kg (D), apply light coating	Topical	24
Hetacillin	20-44 mg/kg	PO on an empty stomach	8-12
Imipenem-cilastin	5-10 mg/kg	IM (using IM preparation), IV (slow), SC	6-8
Isoniazid	10 mg/kg (D)	PO	24
Kanamycin	10-20 mg/kg	IM, IV, SC	24
Levofloxacin	(Obtain MIC first) 10 mg/kg	IV, PO	24
Lincomycin	22-33 mg/kg	IM, IV, PO	12-24
Linezolid	10-20 mg/kg	IV, PO	12-24
Marbofloxacin	2.5-5.5 mg/kg	PO	24
Meropenem	12-40 mg/kg	IV, SC	8
Methanamine mandelate	16.5 mg/kg (D only?)	PO	24 (safety not established in cats)
Methicillin	20 mg/kg	IM, IV	6
Minocycline	12.5- 25 mg/kg (D)	PO	12
	5-12.5 mg/kg (D)	IV	12
Neomycin	7-10.5 mg/kg	IM, IV, SC	24, highly nephrotoxic
	10-20 mg/kg (dilute in water)	Per rectum	6
	10-20 mg/kg	PO	12
Novobiocin	10 mg/kg	PO	8
Ofloxacin	20mg/kg	PO	24
Orbifloxacin	2.5-7.5 mg/kg	PO	24
Oxacillin	22-40 mg/kg	IM, IV, PO	6-8

Table 7-7 Dosing Regimens of Selected Antimicrobials\*—cont'd

Drug	Dose	Route of Administration	Frequency (hr)
Oxytetracycline	55-82.5 mg/kg	PO	8
	7-12 mg/kg	IM, IV	12
Penicillin G, benzathine	50,000/kg	IM	2 days
Penicillin G, phenoxymethyl potassium	20-30 mg/kg	PO	6-8
Penicillin G, procaine	20,000-100,000 U/kg	IM, SC	12-24
Penicillin V potassium	10 mg/kg	PO	8
Piperacillin sodium	25-50 mg/kg	IM, IV	8-12
Piperacillin-tazobactam	3400-4500 g (D)	IV	6-8
Rifampin (in combination)	10-20 mg/kg	PO	8-12 (D), 24 (C), combined with a second antimicrobial?
Roxithromycin	15 mg/kg	PO	24
Spectinomycin	5-12 mg/kg	IM	12
Spiramycin	12.5-23.4 mg/kg	PO	24 × 5-10 days
Streptomycin	20-40 mg/kg	IM	24
Sulfadiazine	Initial dose: 220 mg/kg	PO	Once as loading dose (nocardiosis)
	Followed by: 50-110 mg/kg	PO	12 (nocardiosis)
	Loading: 50-100 mg/kg	PO	Once as loading dose (toxoplasmosis)
	Maintenance 7.5-25 mg/kg	PO	12 (toxoplasmosis)
Sulfadiazine/trimethoprim	50 mg/kg	IV, PO	12
	30 mg/kg (C) 30 mg/kg (D)	PO, SC IV, PO, SC	12 8-12
Sulfadimethoxine	25-100 mg/kg	IM, IV, PO	12-24
	Loading dose: 55 mg/kg	PO	Once as loading dose
Sulfadimethoxine/ormetoprim	27 mg/kg (D)	PO	24 × 14 days
	Loading dose: 55 mg/kg (D)	PO	Once as loading dose
	Followed by: 27.5 mg/kg (D)	PO	24 for a maximum of 21 days
Sulfaguanidine	100-200 mg/kg	PO	8 × 5 days
Sulfamethazine/sulfamerazine	Loading dose: 100 mg/kg	PO	Once as loading dose
	Followed by: 50 mg/kg	PO	12
Sulfamethoxazole	Loading dose: 100 mg/kg	PO	Once as loading dose
	Followed by: 50 mg/kg	PO	12
Sulfamethoxazole/trimethoprim	15 mg/kg	PO	12
Sulfasalazine	10-50 mg/kg (D), maximum of 3 g	PO	8-12, taper by 50% when response occurs
	10 mg/kg (D)	PO	8 until remission then taper to lowest effective dose
	250 mg (C)	PO	8 × 3 treatments then q24hr
	10-20 mg/kg (C)	PO	8-12 for 10 days then 24 hr
Sulfisoxazole	50 mg/kg	PO	8
Teicoplanin	3-12 mg/kg (D)	IM, IV	24
Tetracycline hydrochloride	10-33 mg/kg	PO	8-12
	7 mg/kg	IM, IV	8-12
	10-22 mg/kg (D)	PO	8-12
Ticarcillin or ticarcillin-clavulanic acid	40-110 mg/kg	IM, IV	4-8
	Initial dose: 15-25 mg/kg	IV (over 15 min)	Once
	Followed by: 7.5-15 mg/kg	IV CRI	—
Vancomycin	10-20 mg/kg (D)	PO	6 (For GI infections only)
	15 mg/kg	IV (over 30 min)	6

IM, Intramuscular; IV, intravenous; SC, subcutaneous; D, dog; C, cat.; PO, by mouth; CRI, constant-rate infusion.

\*Dosing regimens ideally are based on the minimum inhibitory concentration of the infecting microbe and the appropriate PDI (e.g.,  $C_{max}$ : MIC > 10 for concentration-dependent drugs and T > MIC of 25 to 100% depending on the drug)



**Figure 7-1** Drug mechanisms of action determine drug efficacy, bactericidal or bacteriostatic effects, mechanisms of bacterial resistance, and appropriateness of combination therapy. Occasionally, the mechanism of drug action predicts the mechanism of host toxicity.

Penicillin G is the basis for the definition of the international unit (IU) of penicillin, which is equivalent to 0.6 mg of the international pure crystalline sodium penicillin (1.6 IU/mg). The conversion of USP units varies with the salt, with 1 mg of penicillin G equivalent to the following units: sodium (1500-1750); potassium (1440-1680), and procaine (900-1050). As a group the natural penicillins are unstable and subject to hydrolysis at the beta-lactam ring. Degradation can occur when combined with other solutions. Degradation also occurs for most penicillins exposed to gastric acidity, precluding oral absorption.<sup>9</sup>

The cephalosporins are derived from a chemical produced by the fungus *Cephalosporium acremonium*. The six-member ring of the cephalosporins renders them more stable; this increased stability also causes them to be less susceptible to resistance. More than 22 cephalosporins are approved for use in the United States, including the cephamycins (e.g., cefoxitin, cefotetan) and oximino-cephalosporins (e.g., ceftazidime, cefotaxime, ceftiofur, cefpodoxime, cefovecin) (see Figure 7-2). The cephalosporins have been variably categorized, with the original "generation" designation being the most widely accepted (Table 7-8).<sup>8,10,11</sup> The designations began as an indicator of chronologic approval but have evolved such that each indicates relative resistance to beta-lactamase destruction; the first generation is most and the later generations least susceptible to destruction.<sup>10</sup> The advent of extended-spectrum beta-lactamases renders the classification less clear in that

these beta-lactams specifically target later-generation drugs. Spectrum and pharmacologic properties of drugs within the generations vary, particularly in the third or later generations. Reclassifying the cephalosporins into groups according to the route of administration, and spectrum has been proposed (see Table 7-8).

### Mechanism of Action

The mechanism of action of beta-lactams reflects interference with bacterial cell wall synthesis (Figure 7-3). The bacterial cell wall comprises several layers of a peptidoglycan matrix. The peptidoglycan strands are composed of five repeating disaccharide units of *N*-acetylglucosamine and *N*-acetylmuramate; these units are formed by the bacteria in stages. A pentapeptide, which ends with a D-Ala-D-Ala terminus, is attached to each of the repeating units of these disaccharides. The units are joined to form a chain or peptidoglycan strand. The resulting chains are then cross-linked to provide cell wall rigidity. Cross-linking between the D-Ala-D-Ala terminals is catalyzed by transpeptidase enzymes, one of several types of proteins that bind penicillin (referred to as *penicillin-binding proteins [PBPs]*) located in the cell wall (see Figure 7-3).<sup>12</sup> The bacterial substrate for the transpeptidase enzyme is the pentapeptide of the peptidoglycan and, specifically, the terminal amino acids D-Ala-D-Ala. The beta-lactam ring is the functional (active) group of all drugs in this class. It is structurally similar to the D-Ala-D-Ala terminus of the pentapeptide,

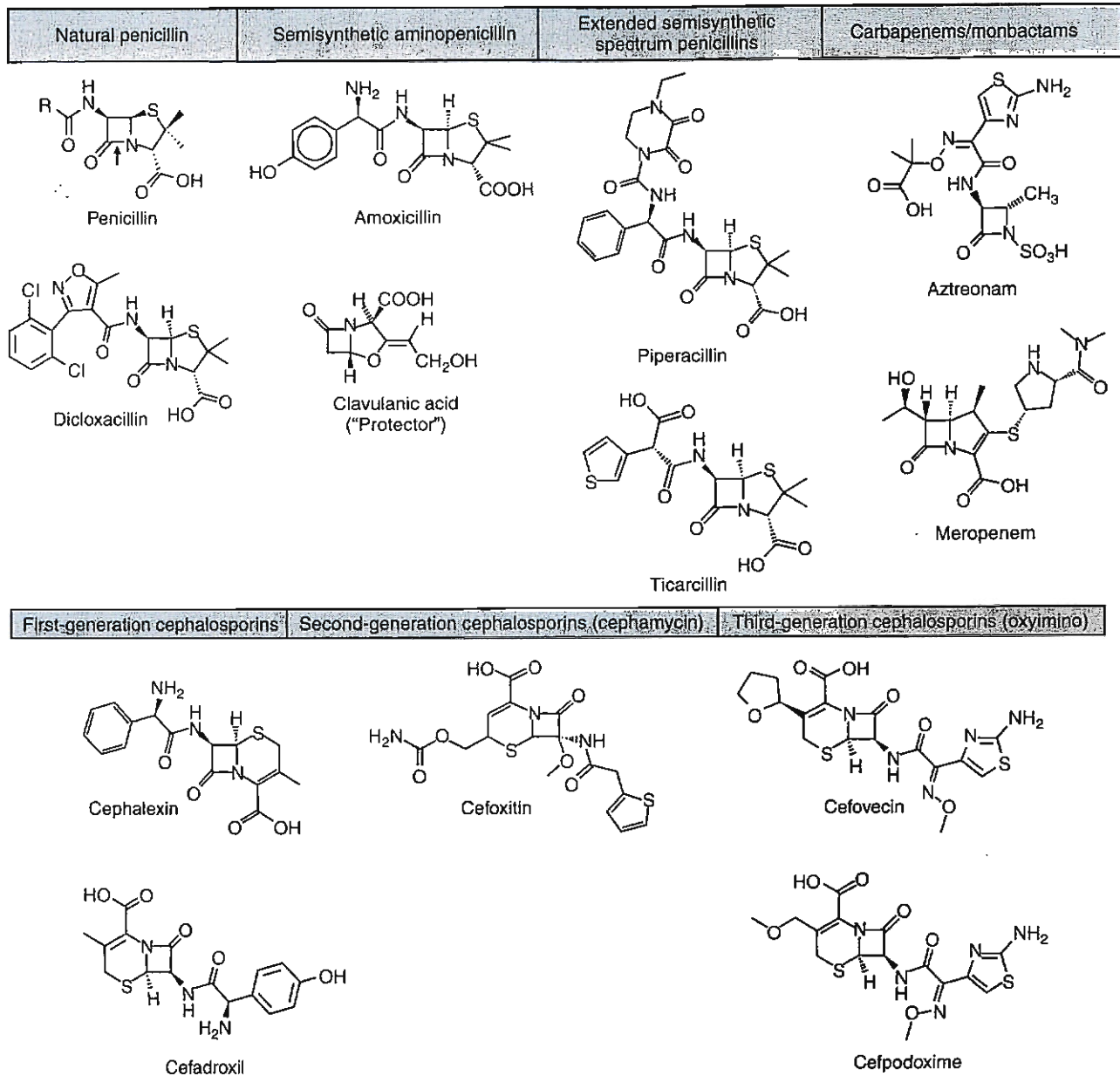


Figure 7-2 Beta-lactam antibiotics include the penicillins and cephalosporins. The four membered beta-lactam ring of each drug mimics the substrate of the transpeptidase enzyme (a penicillin-binding protein), and specifically the terminal portion of p-D-Ala-Asp-D-Ala (boxed inset). This ring structure also is the target of beta-lactamase enzyme destruction. Penicillins have an adjacent five-member ring, cephalosporins a seven-member ring. The addition of larger structures to the basic ring structure may help reduce emerging resistance by beta-lactamase rings but will not avoid methicillin resistance.

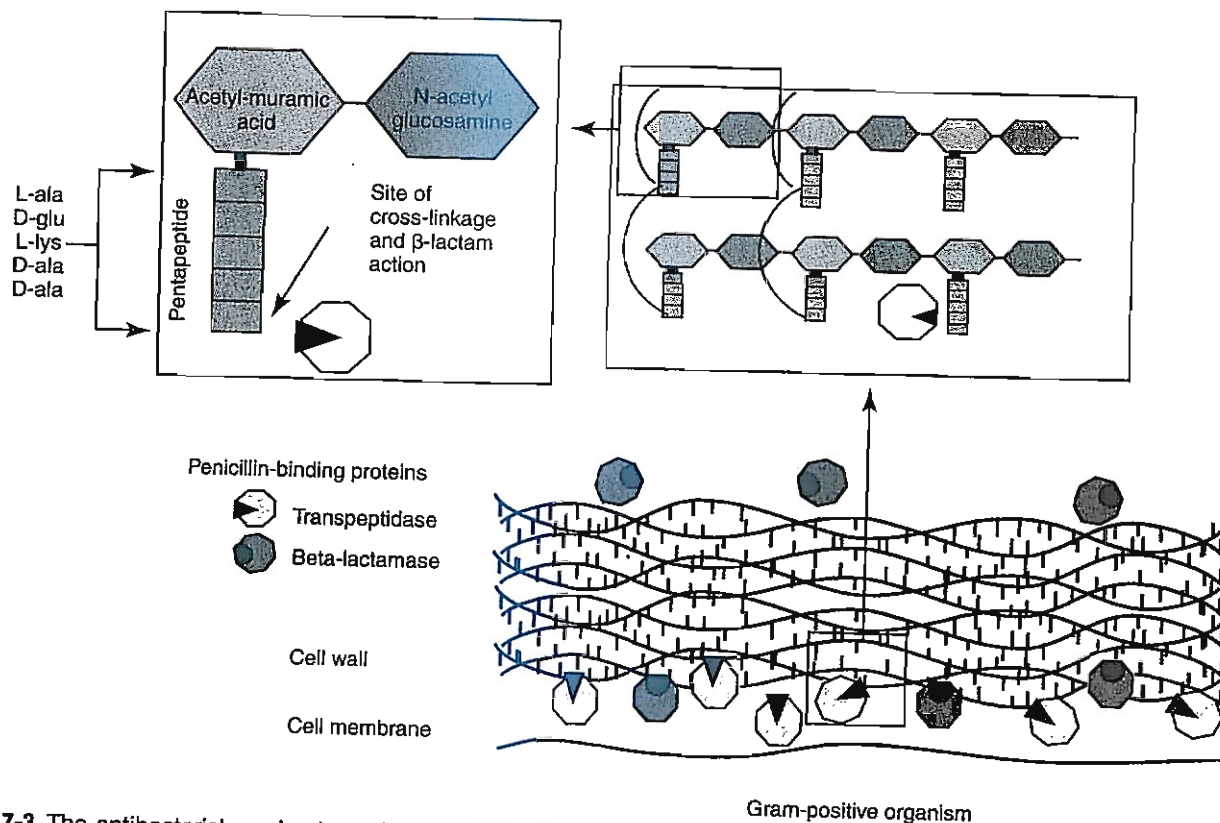
acting as a substrate and subsequently inhibiting the D-D transpeptidase enzyme (see Figure 7-3). In an actively growing cell, as peptidoglycan precursors increase in response to inhibition of synthesis, autolysins, particularly in gram-positive organisms, contribute to cell wall degradation. Degradation coupled with impaired cell wall synthesis causes the bacterial cell wall to lose rigidity. The cell becomes permeable to the surrounding environment, which, although isotonic to the host, is hypotonic to the organism. Influx of surrounding fluid into the hypertonic bacterial cell results in cytolysis, or

osmotic lysis, particularly in gram-negative organisms. Cell wall instability induces the secretion of autolysins, particularly in gram-positive organisms. Because organisms continually break down and rebuild cell walls, the efficacy of the beta-lactam antibiotic ideally is constantly present and, as such, this class of drugs is considered time-dependent (see Chapter 6). However, the duration that the plasma drug concentration (PDC) should be above the MIC varies with the drug, with the desired duration being 50% to 75% of the dosing interval for most drugs. However, T > MIC may be as little as 25% to 50%

**Table 7-8 Cephalosporin Grouping Based on Generation, Route, and Spectrum**

Group	Drug	Generation	Route	Resistance to Beta-Lactamases	Potency (Dose)	Spectrum
1	Cefazolin Cephalothin	First	Parenteral	Staphylococcal, not enterobacterial	Moderate	High activity against gram +
2	Cefadroxil Cephalexin Cephadrine	First	Oral	Staphylococcal, some enterobacterial	Moderate	High activity against gram + Some gram -
3	Cefoxitin Cefotetan Cefuroxime Cefamandole	Second	Parenteral	Many	Moderate	High activity against gram- and anaerobes
4	Ceftiofur, Cefotaxime Ceftriaxone	Third	Parenteral	Many	High	Gram -, Some gram +
5	Cefpodoxime Cefixime	Third	Oral	Many	High	High activity against gram -, some gram +
6	Ceftazidime Cefoperazone	Third	Parenteral	Many	High	High activity against gram-including <i>Pseudomonas</i> gram+
7	Cefepime Cefpirome	Fourth	Parenteral	Many	High	High activity against gram-

+, Positive; -, negative.



**Figure 7-3** The antibacterial mechanism of action of the beta-lactams. The pentapeptide containing the D-Ala-D-Ala terminus (the structure mimicked and thus inhibited by beta-lactam antibiotics) provides the cross-linking of the strands of the cell wall, which are critical to rigidity. Two types of penicillin-binding proteins (PB) are located in the cell wall of bacteria. Transpeptidase enzymes are responsible for catalyzing the cross-bridging between the pentapeptides thus providing rigidity; changes in the structure of these proteins confers resistance to methicillin (PB-2) or other drugs. Beta-lactamase penicillin-binding proteins destroy susceptible beta-lactam antibiotics.



for carbapenems because they are characterized by more rapid bacterial killing.<sup>13</sup> A longer  $T > MIC$  is indicated to decrease the risk of resistance.

Although all PBPs are able to covalently bind beta-lactam antibiotics, the numbers bound and subsequent activity vary among organisms. Up to nine PBPs are encoded by the genome of *Escherichia coli*; each PBP generally has subgroups. The diversity of PBPs is responsible, in part, for differences in the spectrum of activity of the beta-lactams. High-molecular-weight PBPs (1, 2, and 3) are essential for microbial growth and survival in *Staphylococcus* spp., whereas only PBPs 1 and 2 are critical for *Streptococcus* spp.; as such, these PBPs are the critical targets of antimicrobial therapy in these organisms.<sup>14</sup> In *E. coli* PBP-2 is essential for cell elongation and PBP-3 for cell division. Because PB-3 appears to complex with PBP-1, -4, and -7 as well as with other proteins,<sup>12</sup> effective antimicrobial binding to PBP-3 might have a greater impact than binding to other PBPs in *E. coli*. The PBP targeted is known for some drugs (e.g., cefpodoxime targets PBP-1a and 1b and PBP-3 (see package insert).

**KEY POINT 7-1** The mechanism of action of the beta-lactams necessitates the presence of the drug throughout most of the dosing interval.

Although beta-lactams are very effective antimicrobials, their unique mechanism of action increases the risk of therapeutic failure in certain conditions, independent of bacterial resistance. Efficacy, particularly toward gram-negatives, is reduced in a hypertonic environment (e.g., the renal interstitium of the normally functioning kidney, an abscess) because osmotic lysis may not occur. Slow growth impairs autolysin activity, which may result in the loss of the bactericidal effect of the beta-lactam antibiotic. Examples might include the combined use of a beta-lactam with a drug that slows growth of the organisms (i.e., a ribosomal inhibitor [see Figure 7-1]), or in a hypoxic environment (e.g., abscess).

### Spectrum of Activity

The spectrum of activity of beta-lactam antibiotics varies (see Table 7-2). PD data are available for both the dog and cat for limited drugs (Table 7-9), with selected information provided on human pathogens associated with skin or soft tissue infections (Table 7-10). Penicillin G, a natural antibiotic, is effective against selected gram-positive cocci and both gram-negative and gram-positive anaerobes, but it is beta-lactamase sensitive.<sup>5</sup> Selected enterococci are not susceptible to penicillin, and most staphylococci produce beta-lactamases. The gram-negative spectrum of penicillin G is limited but includes *Pasteurella multocida*. Penicillin V is an orally bioavailable natural penicillin, but its antimicrobial efficacy is reduced.<sup>9</sup> Beta-lactamase-resistant isoxazolyl-derivative penicillins include dicloxacillin, cloxacillin, methicillin, and oxacillin. These drugs are effective against gram-positive organisms, including *Staphylococcus* spp., and gram-negative and anaerobic organisms.

The spectrum of the beta-lactams was expanded with the production of the semisynthetic aminopenicillins. Amoxicillin and ampicillin (aminopenicillins) are considered broad-spectrum drugs; however, this classification has largely been muted by acquired resistance unless combined with clavulanic acid or sulbactam. They target PBP-1a. The anaerobic and gram-positive spectrum of penicillin G is maintained (although the aminopenicillins are slightly less efficacious against anaerobes). The aminopenicillins are generally effective against enterococci, although *Enterococcus faecium* often expresses resistance. In addition, many gram-negative organisms are added to the spectrum, including *E. coli*, *Pasteurella*, some *Proteus* species, *Klebsiella*, and selected others (e.g., *Salmonella*, *Shigella*). *Serratia*, *Enterobacter*, and *Pseudomonas* are not, however, included in the spectrum of the aminopenicillins. The spectrum of ampicillin is generally similar to that of amoxicillin, and it serves as the model drug for amoxicillin on culture and susceptibility (C&S) testing whereas amoxicillin-clavulanic acid indicates data for ampicillin-sulbactam. However, the potency of ampicillin generally is less than that of amoxicillin against enterococci and *Salmonella* but greater against *Shigella* and *Enterobacter*. The aminopenicillins are less effective compared with the penicillins against *Bacteroides fragilis*, although efficacy remains good to excellent.<sup>9</sup> Like penicillin, the aminopenicillins are beta-lactamase sensitive. Combination with a beta-lactamase protector (e.g., clavulanic acid or sulbactam) improves efficacy and thus broadens the spectrum against susceptible organisms that have acquired resistance through beta-lactamase production. This includes *Staphylococcus*, *E. coli*, *Klebsiella* spp., and some *Proteus* spp.<sup>15</sup> *Pseudomonas* spp. and other gram-negative organisms remain resistant.<sup>7,9</sup> Further modifications led to the extended-spectrum penicillins characterized by a markedly enhanced spectrum, particularly against gram-negative organisms, including *Pseudomonas aeruginosa*, *Serratia*, *Proteus* spp., some *Klebsiella* spp., *Shigella* spp., and *Enterobacter* spp. Examples include the carboxypenicillins carbenicillin and ticarcillin, with ticarcillin having two to four times higher activity toward *Pseudomonas* spp. than carbenicillin, and the ampicillin-derived ureidopenicillin piperacillin, which has the highest antipseudomonal activity.<sup>5,7,16,17</sup> The extended-spectrum penicillins are effective against anaerobic organisms, although they may be less effective than the natural penicillins. They maintain, however, good to excellent activity against *B. fragilis*.<sup>9</sup> The extended-spectrum penicillins are beta-lactamase sensitive; however, a ticarcillin/clavulanic acid combination product is available.

The carbapenems (imipenem and meropenem) and monobactams (aztreonam) represent the most recent members of the beta-lactam penicillins.<sup>18</sup> Imipenem targets PBP-1a, -1b, and -2, with its efficacy based on binding to PBP-2 and -1b. It is prepared in combination with cilastatin, which inhibits renal tubular degradation (metabolism by dehydropeptidase-1) of imipenem. As a result, drug half-life may be prolonged (although the clinical relevance of this effect in animals is questionable), and the formation of potentially nephrotoxic metabolites is reduced. Imipenem and meropenem have the broadest

Table 7-9 Susceptibility Data for Selected Beta-Lactams and Selected Feline and Canine Pathogens

		Amoxi-Clav	Cefadroxyl	Cefovecin	Cefpodoxime	Cephalexin
<i>Acinetobacter</i>	Mode		>32	16		>64
	MIC <sub>50</sub>	16/8	>32	16		>64
	MIC <sub>90</sub>	32/16	>32	32		>64
	Range	2/1-64/32	16->32	8-32		32->64
	n	16	16	16		16
	Species	D, C	D, C	D, C		D, C
	Source	1	1	1		1
	CLSI	Y	Y	Y		Y
<i>Bacteroides</i> spp.	Mode	≤0.5/0.25	0.5	0.25		≤0.5
	MIC <sub>50</sub>	≤0.5/0.25	0.5	0.25		1
	MIC <sub>90</sub>	≤0.5/0.25	16	2		16
	Range	≤0.5/0.25-8/4	≤0.25->32	≤0.06-8		≤0.5-64
	n	32	32	32		32
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
	<i>Bordetella bronchiseptica</i>	Mode				
MIC <sub>50</sub>		2				
MIC <sub>90</sub>		4				
<i>Clostridium</i> spp.	Mode	≤0.5/0.25	2	0.25		≤0.5
	MIC <sub>50</sub>	≤0.5/0.25	2	0.5		2
	MIC <sub>90</sub>	≤0.5/0.25	16	16		16
	Range	≤0.5/0.25-1/0.5	≤0.25->32	≤0.06->32		≤0.5->64
	n	15	15	15		15
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
	Coagulase-negative <i>Staphylococcus</i> spp.	Mode	≤0.5/0.25	1	0.12	
MIC <sub>50</sub>		≤0.5/0.25	1	0.12		1
MIC <sub>90</sub>		≤0.5/0.25	4	2		4
Range		≤0.5/0.25-1/0.5	≤0.25-8	≤0.06-8		≤0.5-16
n		89	89	89		89
Species		D, C	D, C	D, C		D, C
CLSI		Y	Y	Y		Y
Coagulase-positive <i>Staphylococcus</i> spp.		Mode	≤0.5/0.25	1	0.25	
	MIC <sub>50</sub>	≤0.5/0.25	1	0.25		1
	MIC <sub>90</sub>	≤0.5/0.25	2	0.5		2
	Range	≤0.5/0.25-16/8	0.5->32	0.12->32		≤0.5->64
	n	24	24	24		24
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
	<i>Corynebacterium</i> spp.	Mode	≤0.5/0.25		1	
MIC <sub>50</sub>		≤0.5/0.25	2	1		2
MIC <sub>90</sub>		2/1	32	4		64
Range		≤0.5/0.25-4/2	≤0.25->32	0.25->32		≤0.5->64
n		11	11	11		11
Species		D, C	D, C	D, C		D, C
CLSI		Y	Y	Y		Y

**Table 7-9 Susceptibility Data for Selected Beta-Lactams and Selected Feline and Canine Pathogens—cont'd**

		Amoxi-Clav	Cefadroxyl	Cefovecin	Cefpodoxime	Cephalexin
<i>Enterobacter</i> spp.	Mode	4/2	8	1		8
	MIC <sub>50</sub>	4/2	16	1		8
	MIC <sub>90</sub>	64/32	>32	32		>64
	Range	1/0.5->64/32	8->32	0.12->32		4->64
	n	39	39	39		39
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
<i>Enterobacter cloacae</i>	Mode	64/32	>32	2		>64
	MIC <sub>50</sub>	64/32	>32	1		>64
	MIC <sub>90</sub>	64/32	>32	2		>64
	Range	2/1->64/32	8->32	0.5-8		4->64
	n	20	20	20		20
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
<i>Enterococcus</i> spp.	Mode	1/0.5	>32	>32		>64
	MIC <sub>50</sub>	1/0.5	>32	>32		>64
	MIC <sub>90</sub>	1/0.5	>32	>32		>64
	Range	≤0.5/0.25-32/16	≤0.25->32	≤0.06->32		≤0.5->64
	n	45	45	45		45
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
<i>Enterococcus faecium</i>	Mode					
	MIC <sub>50</sub>	0.5				
	MIC <sub>90</sub>	1				
	CLSI					
<i>Escherichia coli</i>	Mode	4/2	8	0.5		8
	MIC <sub>50</sub>	4/2	8	0.5	0.25	8
	MIC <sub>90</sub>	8/4	16	1	0.5	16
	Range	1/0.5-64/32	4->32	0.12->32	0.12->32	2->64
	n	223	223	223	41	223
	Species	D, C	D, C	D, C	D	D, C
	CLSI	Y	Y	Y	Y	Y
<i>Fusobacterium</i> spp.	Mode	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>50</sub>	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>90</sub>	≤0.5/0.25	0.5	≤0.06		≤0.5
	Range	≤0.5/0.25-2/1	≤0.25-8	≤0.06-1		≤0.5-4
	n	66	66	66		66
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
<i>Klebsiella pneumoniae</i>	Mode	2/1	8	0.5		4
	MIC <sub>50</sub>	2/1	8	0.5		4
	MIC <sub>90</sub>	16/8	16	1		4
	Range	2/1-64/32	8->32	0.25-2		4-64
	n	16	16	16		16
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y

Continued

Table 7-9 Susceptibility Data for Selected Beta-Lactams and Selected Feline and Canine Pathogens—cont'd

		Amoxi-Clav	CefadroxyI	Cefovecin	Cefpodoxime	Cephalexin
<i>Klebsiella</i> spp.	Mode	2/1	8	0.5		4
	MIC <sub>50</sub>	2/1	8	0.5		4
	MIC <sub>90</sub>	2/1	8	1		4
	Range	1/0.5-4/2	8-16	0.25-1		2-4
	n	11	11	11		11
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
<i>Pasteurella multocida</i>	Mode	≤0.5/0.25	4	≤0.06		2
	MIC <sub>50</sub>	≤0.5/0.25	4	≤0.06	≤0.03	2
	MIC <sub>90</sub>	≤0.5/0.25	4	≤0.06	≤0.03	2
	Range	≤0.5/0.25-2/1	1-16	≤0.06-0.12	≤0.03-0.12	≤0.5-8
	n	188	188	188	32	188
	Species	D, C	D, C	D, C	D	D, C
	CLSI	Y	Y	Y	Y	Y
<i>Peptostreptococcus</i> spp.	Mode	≤0.5/0.25	16	0.5		16
	MIC <sub>50</sub>	≤0.5/0.25	8	0.5		8
	MIC <sub>90</sub>	≤0.5/0.25	32	1		16
	Range	≤0.5/0.25-2/1	≤0.25->32	0.12-2		≤0.5->64
	n	21	21	21		21
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
<i>Porphyromonas</i> spp.	Mode	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>50</sub>	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>90</sub>	≤0.5/0.25	1	≤0.06		≤0.5
	Range	≤0.5/0.25	≤0.25-1	≤0.06		≤0.5-2
	n	29	29	29		29
	Species	D,C	D,C	D,C		D, C
	CLSI	Y	Y	Y		Y
<i>Prevotella</i> spp.	Mode	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>50</sub>	≤0.5/0.25	4	0.25		≤0.5
	MIC <sub>90</sub>	≤0.5/0.25	32	4		64
	Range	≤0.5/0.25-1/0.5	≤0.25-32	≤0.06-8		≤0.5->64
	n	11	11	11		11
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
<i>Proteus mirabilis</i>	Mode	1/0.5	16	0.25		8
	MIC <sub>50</sub>	1/0.5	16	0.25	≤0.03	8
	MIC <sub>90</sub>	1/0.5	16	0.5	0.06	16
	Range	≤0.5/0.25-8/4	8->32	0.12-0.5	≤0.03-0.06	8-32
	n	110	110	110	14	110
	Species	D, C	D, C	D, C	D	D, C
	CLSI	Y	Y	Y	Y	Y
<i>Proteus</i> spp.	Mode	1/0.5	16	0.25		16
	MIC <sub>50</sub>	1/0.5	16	0.25		16
	MIC <sub>90</sub>	2/1	16	0.25		16
	Range	≤0.5/0.25-8/4	2->32	0.12-8		4/64
	n	71	71	71		71
	Species	D, C	D, C	D, C		D, C
	Source	1	1	1		1
CLSI	Y	Y	Y		Y	

**Table 7-9** Susceptibility Data for Selected Beta-Lactams and Selected Feline and Canine Pathogens—cont'd

		Amoxi-Clav	CefadroxyI	Cefovecin	Cefpodoxime	Cephalexin
<i>Staphylococcus aureus</i>	Mode	≤0.5/0.25	2	1		2
	MIC <sub>50</sub>	1/0.5	2	1	2	2
	MIC <sub>90</sub>	4/2	8	2	2	8
	Range	≤0.5/0.25-16/8	1->32	0.5->32	0.12-2	1->64
	n	36	36	36	19	36
	Species	D, C	D, C	D, C	D	D, C
	Source	I	I	I	P	I
<i>Staphylococcus intermedius</i>	CLSI	Y	Y	Y	Y	Y
	Mode	≤0.5/0.25	1	0.12		1
	MIC <sub>50</sub>	≤0.5/0.25	1	0.12	0.12	1
	MIC <sub>90</sub>	≤0.5/0.25	2	0.25	0.5	2
	Range	≤0.5/0.25-16/8	0.5->32	≤0.06->32	0.12->32	≤0.5-64
	n	231	231	231	118	231
	Species	D, C	D, C	D, C	D	D, C
<i>Streptococcus</i> spp.	Source	I	I	I	P	I
	CLSI	Y	Y	Y	Y	Y
	Mode	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>50</sub>	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>90</sub>	≤0.5/0.25	2	0.5		4
	Range	≤0.5/0.25-1/0.5	≤0.25-8	≤0.06-0.5		≤0.5-16
	n	27	27	27		27
<i>Streptococcus, beta-hemolytic</i>	Species	D, C	D, C	D, C		D, C
	Source	I	I	I		I
	CLSI	Y	Y	Y		Y
	Mode	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>50</sub>	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>90</sub>	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	Range	≤0.5/0.25	≤0.25-1	≤0.06-8		≤0.5-2
<i>Streptococcus canis</i>	n	22	22	22		22
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y
	Mode	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>50</sub>	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	MIC <sub>90</sub>	≤0.5/0.25	≤0.25	≤0.06		≤0.5
	Range	≤0.5/0.25	≤0.25-8	≤0.06-≤0.06		≤0.5-8
<i>Streptococcus</i>	n	66	66	66		66
	Species	D, C	D, C	D, C		D, C
	CLSI	Y	Y	Y		Y

Amoxi-Clav, Amoxicillin-clavulanic acid; MIC, minimum inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute; D, dog; C, cat; y, yes. All data (except cefpodoxime) is from Stegmann et al.<sup>20</sup>; data for cefpodoxime is from the package insert. All MIC are in µg/mL. Data is from dogs and cats considered to be antimicrobial free.<sup>20</sup>

antimicrobial spectrums available against bacterial organisms with cell walls, including *Pseudomonas* spp. Imipenem and meropenem are relatively resistant to beta-lactamase destruction. However, an extended beta-lactamase enzyme has recently been reported, particularly in *Klebsiella pneumoniae*, emerging as a nosocomial pathogen.<sup>19</sup> An advantage of the carbapenems has been their very low MICs (0.05 to 2 µg/mL) for most

susceptible organisms. Meropenem is generally similar to imipenem for empirical treatment of serious infections.

Aztreonam is a monobactam (see Figure 7-2), with a high affinity for PBP-3 and lesser affinity for PBP-1a. It is particularly effective against gram-negative aerobes, including *Pseudomonas* spp. but is ineffective against gram-positive organisms and anaerobes.

**Table 7-10** Susceptibility Data for Selected Human Pathogens Associated with Skin and Soft Tissue Infections<sup>160</sup>

Organism	Drug	Penicillin	Ampicillin	Imipenem	Meropenem	Piperacillin	Cefazolin	Cefoxitin	Ceftazidime
<i>Enterobacter</i> spp.	MIC <sub>50</sub>		>16	0.25	≤0.06	4(4)		>32	0.25
	MIC <sub>90</sub>		>16	0.5	0.12	128(64)		>32	>16
<i>Escherichia coli</i>	MIC <sub>50</sub>		4	0.12	≤0.06	2(1)	≤2	4	≤0.12
	MIC <sub>90</sub>		>16	0.25	≤0.06	>128(4)	16	16	0.5
<i>Klebsiella pneumoniae</i>	MIC <sub>50</sub>		>16	0.12	≤0.06	8(2)	≤2	2	≤0.12
	MIC <sub>90</sub>		>16	0.25	≤0.06	>128(16)	≥16	16	2
<i>Proteus</i>	MIC <sub>50</sub>								2
	MIC <sub>90</sub>								2
	MIC <sub>50</sub>			1	1	8(8)			16
<i>Pseudomonas aeruginosa</i>	MIC <sub>90</sub>			8	8	128 (>64)			
	MIC <sub>50</sub>	8	16	≤0.06			≤2		8
<i>Staphylococcus aureus</i>	MIC <sub>90</sub>	>32	>16	4			>16		>16

MIC, Minimum inhibitory concentration.

Parantheses refer to the combination of piperacillin with tazobactam.

The spectrum of the cephalosporins is more diverse than that of the penicillins and is not as easily categorized. Although generalizations regarding the spectrum of activity of each successive generation might be made, variability in efficacy among the drugs within and certainly among generations may result in therapeutic failure if attention is not paid to differences.<sup>9</sup> Thus either the package insert or C&S data should be consulted before selecting a cephalosporin, particularly beyond the first generation. In general, cephalosporins are ineffective against enterococci. With each successive generation, the cephalosporins become increasingly more resistant to beta-lactamase destruction, and all generations are generally more resistant as a class than are the penicillins. As such, they are often chosen as empirical first-choice treatment of *Staphylococcus* spp. Cephalothin (no longer commercially available) has been the drug designated by the Clinical Laboratory Standards Institute (CLSI; previously National Committee for Clinical Laboratory Standards [NCCLS]) as the model indicator for susceptibility for the first-generation cephalosporins. However, it does not represent the class equally. The aerobic spectrum of the first-generation cephalosporins is similar to that of the aminopenicillins,<sup>9</sup> although efficacy is more similar to amoxicillin-clavulanic acid combinations. First-generation cephalosporins such as cefazolin, cephalothin, and cephalixin are active (although not equally so) against gram-positive and gram-negative organisms such as *E. coli*, *K. pneumoniae*, and *Proteus mirabilis*. Among the first-generation drugs, cefazolin has better efficacy than cephalixin against gram-negative organisms (e.g., *E. coli*) but poorer efficacy against *Staphylococcus* spp.<sup>5,9</sup> Efficacy of cephalixin against *E. coli* is fair to poor. The anaerobic spectrum of the first-generation cephalosporins is fair but less than that of the aminopenicillins.

The second-generation cephalosporins, cefamandole, cefactor, cefoxitin, and others, are characterized by enhanced activity toward *Enterobacter* spp., some *Proteus* spp., *E. coli*, and *Klebsiella* spp.<sup>5</sup> Cefoxitin has an excellent anaerobic spectrum, particularly against *Bacteroides* spp.,<sup>5,8</sup> although it is less effective than first-generation drugs against gram-positive organisms. Third- (cefotaxime, ceftazidime, cefpodoxime, cefoperazone, cefovecin, and the oxa-beta lactam moxalactam) and fourth-generation (cefepime; not approved in the United States) cephalosporins are generally reserved for serious gram-positive or gram-negative infections (e.g., *P. aeruginosa*, *Enterobacter* spp., and *Serratia* spp.). However, although the efficacy of most of the second-plus generation cephalosporins against *E. coli* tends to be good to excellent, efficacy against *P. aeruginosa*<sup>9</sup> is variable, and cross-efficacy among members of these generations to any organism should not be assumed. For example, cefoperazone and ceftazidime are among the most effective drugs against *P. aeruginosa*, although efficacy is less than that of the newer extended-spectrum penicillins. Cefpodoxime and cefovecin are generally effective against *E. coli* but not effective against *Pseudomonas* spp. Selected third-generation cephalosporins (e.g., cefotaxime) are effective against anaerobic organisms, whereas others (e.g., ceftazidime, ceftriaxone, and cefpodoxime) are not. Ceftiofur is a third-generation cephalosporin approved for use for canine urinary tract infections. The antimicrobial spectrum of ceftiofur includes gram-positive (*Streptococcus* spp. and *Corynebacterium* spp.), gram-negative (*Pasteurella*, *E. coli*, and *Salmonella* spp. but not *Pseudomonas* spp.), and anaerobic organisms. Ceftiofur is effective against many staphylococcal organisms; however, selection against *Staphylococcus* spp. should be based on C&S data.<sup>9</sup> The first-generation drug cefazolin has been inappropriately promoted as a generic version of ceftiofur.<sup>19a</sup>

The spectrum of the third-generation drugs cefpodoxime and ceftiofur (the former approved in dogs and cats, the latter approved in dogs but used in cats) includes *Staphylococcus* spp.; ceftiofur is also approved for use in the treatment of *Streptococcus* spp. Both drugs are effective against a variety of gram-negative organisms, including *E. coli* and *Klebsiella*, but are not generally effective toward *Pseudomonas* spp. Stegemann<sup>20</sup> has provided PD statistics for a large number of organisms for ceftiofur, as well as selected other beta-lactams, some of which are provided in Table 7-9.

**KEY POINT 7-2** The spectrum of the penicillins becomes broader as the class "extends," whereas the spectrum of the later-generation cephalosporins varies with the drug.

### Resistance

Bacteria develop resistance to beta-lactams through four major mechanisms: altered or different PBPs such that antibiotic binding does not occur (e.g., staphylococcal organisms and penicillins; enterococcal organisms and cephalosporins); efflux through specific pumps; loss of or changes in porins (especially *P. aeruginosa*); and inactivation by beta-lactamases. Inactivation by beta-lactamases is most common. *Staphylococcus* resistance to penicillin appeared as early as 1942; by the late 1960s, more than 80% of medically relevant isolates were resistant to penicillin as a result of beta-lactamase production. Today more than 90% of isolates (human) produce penicillinase.<sup>21</sup> The approval of "protected" drugs (i.e., improved the efficacy of selected penicillins), but along with the cephalosporins, is likely to have contributed to the emergence of altered PBP. This most notorious mechanism of resistance has yielded methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

**Beta-lactamases.** Beta-lactamases are structurally and mechanistically similar to PBPs; indeed, certain PBPs are capable of beta-lactamase activity. Destruction of the beta-lactam (amide) ring reflects its hydrolysis (see Figure 7-2).<sup>22</sup> Currently, more than 400 distinct beta-lactamase enzymes are produced by gram-negative, gram-positive, and anaerobic organisms.<sup>23,24</sup> Selected examples are listed in Table 7-11. Although clearly a major mechanism of resistance in gram-positive organisms, beta-lactamase production is also the major mechanism by which gram-negative organisms develop resistance.<sup>22</sup> Beta-lactamase production occurs as a result of either chromosomal mutations, particularly in gram-positive organisms, or plasmid-mediated resistance in both gram-positive and gram-negative organisms. Beta-lactamases are either constitutive, already present in the cell wall (particularly in gram-negative organisms), or induced by the presence of the antimicrobial drug (in both gram-negative and gram-positive organisms).<sup>25</sup> Gram-negative bacteria have the added advantage of secreting beta-lactamases into the periplasmic space such that they are strategically placed before the antibiotic can penetrate the cell wall.<sup>9</sup> The beta-lactams are variably susceptible to destruction by beta-lactamases; microbes vary in which enzyme they produce and whether the enzyme is constitutive or inducible (see Table 7-11).

Two major types of beta-lactamases exist: serine-based enzymes and the metallo-beta lactamases. The latter contain a zinc atom that activates water as the destructive site (see Table 7-11).<sup>22</sup> Several schemes have been proposed to classify beta-lactamases. The most common scheme is based on the molecular structure (Amber Classification); however, classification according to the target substrate (Bush-Jacoby Classification) may be easier to follow (see Table 7-11). According to the Amber system, Class B enzymes contain the metallo-beta lactamases, but the other three classes are serine-based enzymes. These include classes A (TEM, SHV), C (ampC, targeting cephamycins [cefotetan, ceftiofur]), and D (OXA; targeting protected drugs, such as dicloxacillin, but also protectors such as clavulanic acid). The most prevalent beta-lactamases are class A penicillinases and cephalosporinases, including clinically relevant TEM-1 and 2 or SHV-1 enzymes found in *E. coli* and *K. pneumoniae*, and PC-1 enzymes produced by *S. aureus*.<sup>22</sup>

**KEY POINT 7-3** Microbes have been able to adapt to each pharmaceutical manipulation intended to decrease beta-lactamase activity.

TEM-1 and SHV-1 confer high-level resistance to penicillins and first generation cephalosporins but generally do not target the extended-spectrum (selected second- and third- or fourth-generation) cephalosporins or carbapenems. As such, the cephalosporins (cephalosporin C) are generally less impacted by beta-lactamases, particularly those produced by *Staphylococcus* species.<sup>10</sup> However, only a few cephalosporins are stable against anaerobic beta-lactamases. Selected semi-synthetic beta-lactams also are less impacted by beta-lactamases, including most third-generation cephalosporins and imipenem. The semisynthetic dicloxacillin (and oxacillin) is beta-lactamase resistant, with the exception of class D (group 2d). The combination of beta-lactam antibiotics with drugs that inhibit beta-lactamase activity (e.g., clavulanic acid, sulbactam, and tazobactam) increases the potency of the beta-lactam antibiotic, (but not the spectrum) toward susceptible organisms (see Tables 7-2, 7-9 and 7-10). Clavulanic acid irreversibly binds to some but not all beta-lactamases (see Table 7-11).<sup>26</sup> Combinations of beta-lactams with beta-lactamase inhibitors are particularly useful against mixed infections and have shown efficacy against selected multiresistant pathogens such as *Acinetobacter* spp. Aztreonam is generally resistant to beta-lactamase destruction but is susceptible to extended-spectrum beta-lactamases (ESBLs). The presence and diversity of beta-lactamases in canine and feline staphylococcal organisms has been described. As in other species, production is encoded by the *blaZ* gene, with all four classes of enzymes (A to D) represented genes for classes A, C, and D being plasmid mediated and class B chromosomally mediated.<sup>27</sup>

Microbes have adapted to each pharmaceutical manipulation intended to combat emergent resistance resulting from beta-lactamase destruction. Third-generation cephalosporins such as cefotaxime and ceftazidime initially were considered

Table 7-11 Classification of Beta-Lactamases, the Enzymes, and Drugs Targeted<sup>5,37</sup>

Group	Type	Subgroup	Class*	Enzyme Type	CA	Example Enzymes	Producing Organism	Target Drugs
1	Ser		C	Cephalosporinases	R	AmpC CMY-2	1-6	6-8 > 1,5
2	Ser		A,D			TEM, SHV		
		a	A	Penicillinases				
		b	A	Penicillinases		TEM-1 SHV-1 PC-1	1, 2 2, 1 7	1 1
				Cephalosporinases				
		be	A	Extended cephalosporinases		TEM, SHV variants SHV variants		2, 5
		br	A	Inhibitor resistant	R	TEM, SHV variants	1,2,3, others	5
		c	A	Carbenicillinase		PSE, CARB	4, vibrio	1 (carbenicillin)
		d	D	Oxacillinase	R	ARI (OXA)	2,4, 10	4,5
				Carbapenemases				
		e	A	Cephalosporinase	Sus	CTX-M PER	1, others	
		f	A	Carbapenemases		NCA IMI KPC GES SME	2, others	1,2,3,6
3	Met (ZN)		B	Metalloenzymes	R	IMP VIM SIM GIM	4, 5, 8, 9	1, 3, 5, 8
4	Ser		NA	Penicillinase	R			
<b>Keys</b>	<b>Example producers</b>					<b>Target drugs</b>		<b>Example drug</b>
1	<i>E. coli</i>			1		Penicillins (e.g., Amp, Amox, Pip)		
2	<i>Klebsiella</i>			2		Oxyimino monobactams		Aztreonam
3	<i>Proteus</i>			3		Carbapenems		
4	<i>Pseudomonas</i>			4		Oxazolympenicillins		Oxacillin
5	<i>Enterobacter</i>							Cloxacillin
6	<i>Serratia</i>							Dicloxacillin
7	<i>Staphylococcus</i>			5		Inhibitors		Clavulanic acid
8	<i>Bacillus</i>							Sulbactam
9	<i>Bacteroides</i>							Tazobactam
10	<i>Acinetobacter</i>			6		First-generation cephalosporins		
				7		Oxyimino-cephalosporins (1)		Ceftriaxime Cefotaxime Ceftriaxone Ceftazidime Cefpodoxime Cefovecin
				8		Cephameycins (7alphamethoxy)		Cefoxitin Cefotetan

\*Amber Classification System. See text for abbreviations for enzyme types.



indestructible by beta-lactamases.<sup>25</sup> However, high-level use has been accompanied by induction and selection for ESBLs in multiple-resistant coliforms,<sup>28</sup> particularly in those organisms that produce TEM and SHV enzymes. The genes encoding ESBLs are carried by large plasmids and are able to confer information between bacterial species and strains. The ESBLs are most commonly found in *Klebsiella* spp. (incidence in North America, 4.4%), *E. coli* (3.3% to 4.7%), or *P. mirabilis* (3.1-9.5%), but they also have been detected in other members of the family Enterobacteriaceae and in *P. aeruginosa* isolates.<sup>29-32</sup> The resistant gene codes for mutations in one or more amino acid (serine) substitutions in class A enzymes (TEM or SHV). The resultant change in configuration allows the enzyme to gain access to the drug despite the large oxyimino side chain of these newer-generation drugs.<sup>24</sup> Drugs amenable to destruction by ESBL include third-generation cefotaxime, ceftazidime and ceftriaxone, cefpodoxime, and (presumably) ceftovecin.<sup>22,33</sup> Selected fourth-generation drugs are also susceptible, including cefepime (no longer marketed in the United States).<sup>28</sup> Cephamycins (e.g., second-generation cephalosporins cefoxitin, cefotetan) do not appear to be destroyed (although they are destroyed by ampC). Monobactams (i.e., aztreonam) are destroyed. Carbapenems are generally not destroyed by ESBL, nor are beta-lactamase protectors such as clavulanic acid. The use of beta-lactamase protectors appears to reduce the clinical emergence of ESBLs and may reduce the emergence of other resistant pathogens such as *Clostridium difficile* and vancomycin-resistant enterococci.<sup>34</sup> However, the effect (e.g., of the beta-lactamase in the presence of ESBLs) is not always predictable. Decreased cephalosporin usage also reduces the advent of ESBLs.

Resistance to ESBLs often is incorporated in plasmids simultaneously conferring resistance to aminoglycosides and sulfonamides.<sup>22</sup> Further, ESBL resistance may be associated with non-plasmid-mediated resistance mechanisms such as occurs for quinolones.<sup>22</sup> An "inoculum effect" of ESBLs has been described for some drugs and may explain discrepancies among studies: the MIC of the organisms toward cephalosporins increases with a larger ( $10^7$ ) compared with smaller ( $10^5$ ) inoculum. Because susceptibility may depend on the size of the inoculum at the site of infection,<sup>22</sup> ESBLs may not be detected on routine C&S testing.<sup>31</sup> Lack of detection of ESBLs may also reflect different levels of activity against the different cephalosporins.

Detection of ESBLs has been based on double disk diffusion techniques. The susceptible cephalosporin (e.g., cefpodoxime, ceftazidime) is incubated with the isolate as the sole drug and in the presence of a beta-lactamase inhibitor; a substantial reduction in the MIC (e.g., fourfold to eightfold) with the combination drugs compared with the cephalosporin by itself indicates an ESBL.<sup>35,36</sup> Not all clinical microbiology laboratories have incorporated tests for ESBLs in routine testing procedures.<sup>22</sup> The presence of an ESBL should be suspected with organisms resistant to or with high MIC to cefotaxime but susceptible to beta-lactam/beta-lactamase combinations.<sup>22</sup> The detection of an isolate with ESBL in a patient with a serious gram-negative bacillary infection should lead to the use of

a carbapenem. However, a novel carbapenemase also has been described following isolation in *Serratia* spp., *K. pneumoniae* and *Enterobacter cloacae*.<sup>19,22,37</sup> Alternatively, combination of the cephalosporin with clavulanic acid should be considered.

**KEY POINT 7-4** Extended-spectrum beta-lactamases target later-generation cephalosporins but may be missed with susceptibility testing unless special tests are performed.

**Altered penicillin-binding proteins.** The advent of MRSA and multidrug-resistant *Enterococcus* spp. also has been associated with cephalosporins although it is likely that beta-lactamase inhibitors contributed to its emergence.<sup>25</sup> The approval of the cephalosporins in the 1980s was followed by the first MRSA epidemics in the mid-1980s in the United Kingdom; the use of second- and third-generation cephalosporins also was associated with an outbreak of MRSA in Japan.<sup>25</sup> In humans, mortality associated with *S. aureus* bacteremia is 20% to 40%; MRSA has become a leading cause of nosocomial infections in human medicine. The term MRSA was coined in the early 1960s, when these penicillinase-resistant drugs were relatively new, and refers to resistance expressed *in vitro* to methicillin.<sup>21</sup> Although this discussion will focus on MRSA, increasingly, methicillin resistance is being recognized in other species and much of this information is relevant to all methicillin resistant staphylococci (MRS). Over the 30 to 40 years since MRSA was identified, MRSA infections have led to increased mortality and morbidity. The sequelae of MRSA are worse than those associated with beta-lactamase resistance because no alternative therapy remains that is predictably effective.<sup>21</sup> In contrast to resistance resulting from penicillinase production which is generally considered low level, infection with MRSA is considered high-level resistance. Further, MRSA isolates are essentially multidrug resistant, that is, expressing resistance to classes other than beta-lactams.

MRSA and methicillin-resistant *Staphylococcus pseudintermedius* (MRSIG)<sup>38</sup> are indicated by the presence of the *mecA* gene. This gene encodes a mutation in penicillin-binding protein 2a, thus reducing its affinity for the beta-lactam ring, rendering the organism resistant to all beta-lactams. The *mecA* gene is carried on the staphylococcal chromosomal cassette (SCC); currently five SCC<sub>mec</sub> have been described.<sup>39</sup> Protectors such as clavulanic acid are also unable to bind and thus are ineffective.<sup>21</sup> Detection of MRSA or MRSIG (or methicillin-resistance in other staphylococci [MRS]) on C&S testing generally is based on resistance to oxacillin, which is more stable than methicillin in disks used for testing. However, variability in testing methods can profoundly alter results; therefore, cefoxitin might be a more appropriate indicator of multidrug resistance in these organisms.<sup>40</sup> Alternative procedures such as polymerase chain reaction or latex agglutination have been used to detect the gene responsible for the formation of penicillin-binding protein 2a (*mecA*) of MRSA, and other techniques such as pulsed-field gel electrophoresis or multilocus sequence typing identify the specific strain of MRSA (e.g., USA100 or USA300). It is likely that this area of diagnostics will be refined in the next decade and will be applied to other MRS.

**KEY POINT 7-5** Changes in the penicillin-binding protein (PBP2) by *Staphylococcus* spp. renders the microbe resistant to all beta-lactams.

Antimicrobials are associated with induction, selection, and propagation of MRSA. The wide use of cephalosporins, in particular, may have contributed significantly to the advent of MRSA. MRSA in human patients has evolved from a hospital-acquired (HA-MRSA; nosocomial) infection (usually USA100) that occurs most commonly in patients whose immune systems are compromised by a community-acquired infection (CA-MRSA), in which otherwise healthy persons are infected, usually in the skin or soft tissue. Crowded conditions, shared items, and poor hygiene increase the risk of CA-MRSA. It is CA-MRSA strain USA300 that appears to be most commonly associated with increased colonization in dogs and cats. In contrast, it is HA-MRSA (USA-100) that is most commonly associated with infections in dogs and cats.<sup>40a</sup> According to the Center for Disease Control, the incidence of MRSA doubled in human medicine between 1999 and 2006. The impact of MRSA (or other MRS) in veterinary medicine is increasingly problematic, not only because of its impact on the patient but also because of public health considerations. The *mec* gene has been detected in MRSA organisms infecting dogs,<sup>40-42</sup> and MRSA has been associated with infection in dogs.<sup>43</sup> However, MRSA also has been found in up to 4% of healthy dogs, with identification complicated by the need for multiple sampling sites (nasal and rectal or perineal). Risk factors for the presence of MRSA in pets or working dogs (e.g., detection and aid dogs) include contact with human hospitals (particularly if patients fed the dogs treats or were licked by the dogs) and children.<sup>42</sup> Infections have been isolated to family members and pets in the same household, but this is likely to reflect original transmission from humans to the pet.<sup>40-42,44</sup> It is likely that colonization is transient in animals. However, healthy pets have been demonstrated to be potential reservoirs for transmission of MRSA to healthy handlers and a potential health risk to immunocompromised patients (humans and presumably other animals in the household). According to the American Veterinary Medical Association, colonization by MRSA is suggested to be an occupational risk for veterinarians, although the frequency of infection associated with MRSA in veterinarians compared with other health professionals has not been documented.

MRSIG<sup>45</sup> has a prevalence of 0.58% to 2% in healthy dogs and up to 4% in healthy cats,<sup>42,46</sup> with the *mec* gene present in each canine MRSIG isolate in one study.<sup>47</sup> Human colonization with MRSIG is unusual.<sup>42</sup> However, MRSIG has been reported as a cause of infection in human patients,<sup>42</sup> and transmission from pets with pyoderma to humans has been confirmed.<sup>48,49</sup> Although the true public health significance of MRSA and MRSIG (or other multidrug-resistant organisms) in pets is not clear, the fear of infection may be as important as true risk, necessitating proper hygiene and other proactive measures such that human or animal health (including unnecessary euthanasia) is not risked.

The American Veterinary Medical Association offers a website that includes a discussion of MRSA zoonoses, including sources of guidelines that might decrease the risk presented to susceptible humans.<sup>49a</sup> Among the more important actions that can be taken is establishment of infection control policies and guidelines in each veterinary practice. In general, common sense approaches should prevail (e.g., minimizing intimate contact, maintaining good personal and environmental hygiene practices; see the three D's approach described in Chapter 6). This includes cleansing of hands of handlers and the paws (or body) of animals that might be exposed to MRSA, including those visiting human health care facilities. Immunocompromised patients are at most risk for MRSA infection acquired from an animal. In such cases the carrier or infected animal should be removed from the environment until successfully treated for MRSA. For dogs with skin infections, cultures are indicated to detect MRSA, particularly in animals for which infection does not resolve. Successful resolution of colonized or infected animals may require both topical (for skin infections) and systemic therapy. Evidence of successful treatment might be based on skin swabs of the ear, nose, and perianal region. Care must be taken to ensure that the laboratory providing culture procedures is well-versed in the diagnosis of MRSA, including speciation of coagulase-positive organisms.

**KEY POINT 7-6** Methicillin-resistant *Staphylococcus aureus* likely originated with a human, whereas Methicillin-resistant *Staphylococcus pseudintermedius* likely originated from a dog or cat.

The multidrug resistance associated with MRSA is now evolving toward other (non-beta-lactam) antimicrobials. This reflects, in part, other resistance genes in the gene cassette carrying the *mec* gene.<sup>42</sup> Drugs that are affected include fluorinated quinolones and aminoglycosides. Although newer fluorinated quinolones (e.g., levofloxacin) appear to be more effective than older drugs in vitro, particularly to *Staphylococcus*, whether this translates to better clinical efficacy is unclear.<sup>21</sup> Glycopeptides such as vancomycin are the initial drugs used to treat MRSA in humans, although increasingly vancomycin-resistant *Staphylococcus aureus* (VRSA) infections have emerged. Linezolid and rifampin are alternative drug choices.

Multidrug resistant *Enterococcus* spp. also is an emerging issue; its emergence also appears to be correlated to use of cephalosporins. *Enterococcus faecalis* more so than *Enterococcus faecalis* is likely to develop resistance, and speciating *Enterococcus* spp. susceptibility testing might be prudent. Resistance reflects a change in penicillin-binding protein (PB-V), and the risk is increased when drugs effective against *Enterococcus* spp. are used.

### Pharmacokinetics

The beta-lactams are weak acids, which favor oral absorption. Many of the beta-lactam antibiotics, however, are destroyed by the acidity of the gastrointestinal tract and thus cannot

be given orally. Penicillin exceptions include penicillin V, dicloxacillin, the aminopenicillins (ampicillin and amoxicillin, including combinations with clavulanic acid), and carbenicillin (indanyl form only; effective concentrations can be achieved only in urine). Lack of stability also may affect the shelf-life of reconstituted products; expiration dates should be adhered to as indicated for the reconstituted product. Orally bioavailable cephalosporins include cephalexin, cefadroxil, and cefpodoxime (third or fourth generation). The oral bioavailability of the cephalosporins also varies among drugs and species.<sup>5,9</sup>

Many beta-lactams are available as intravenous or parenteral preparations. Absorption from parenteral sites tends to be rapid and complete, with the exception of products that are specifically formulated to allow slow release (e.g., esterified penicillins). Although drug concentrations may persist in circulation longer than non-slow-release preparations (an appealing aspect for time-dependent antimicrobials), older dosing regimens were designed for efficacy against organisms considerably more susceptible to drugs at the time of approval compared with current microorganisms. Thus consideration should be taken to design the dose of these products to compensate for any increase in MIC that may have emerged since the approval of the labeled dose. Selected beta-lactams are highly bound to plasma proteins. Although binding limits distribution into tissues, it also contributes to a long disappearance half-life. Cefpodoxime and, to a greater degree, cefovecin are example of beta-lactams whose long half-life reflects slow release from intravascular protein.<sup>20</sup>

**KEY POINT 7-7** As water-soluble drugs, all beta-lactams distribute to extracellular fluid, do not penetrate sanctuary tissues well, and are renally excreted.

Distribution of beta-lactams is limited to extracellular fluid (volume of distribution [Vd or Vd<sub>ss</sub>] of unbound drug generally  $\leq 0.3$  L/kg), but, barring a marked host inflammatory response, adequate concentrations of unbound drug can usually be achieved in the interstitial fluid (the site of most infections) in many tissues (see Table 7-5).<sup>5,9</sup> Penicillins and cephalosporins are thus widely distributed throughout most extracellular body fluids, including kidneys, lungs, joints, bone, soft tissues, and bile.<sup>5,8,11</sup> Interstitial fluid concentrations in normal tissues generally can be predicted by, but are not necessarily equivalent to, the concentration of (unbound) drug in plasma. Comparisons of AUC frequently reveal interstitial fluids to be 30% or less than that in plasma. Among the first-generation cephalosporins, cefadroxil appears to have the better tissue-to-PDC ratio in humans (see Table 7-5). Neither penicillins nor cephalosporins traverse sanctuaries well, including mammary, prostatic, or blood-brain barriers. Imipenem, but generally not antipseudomonal penicillins such as ticarcillin and piperacillin, can reach effective concentrations in the brain. However, first- and second-generation cephalosporins should not be used for central nervous system (CNS) infections because many are destroyed by local enzymes or transported out of the CNS. Beta-lactams in general achieve

25% or less in bronchial secretions compared with PDCs (see Table 7-5).<sup>50-52</sup> Inflammation increases the penetration of many beta-lactams. For example, cefuroxime, cefotaxime, ceftriaxone, and ceftazidime can reach therapeutic concentrations when the cerebral spinal fluid (CSF) is inflamed.<sup>9</sup> Acute inflammation may also increase beta-lactam penetration of abscesses and pleural, peritoneal, and synovial fluids because of changes in vascular permeability. However, those drugs characterized by high binding to plasma protein will likewise be bound to inflammatory proteins. As response to therapy decreases, resolution of inflammation may decrease distribution. Further, if inflammation does not resolve but progresses, efficacy of beta-lactams is likely to decrease as a result of poor penetrability of lipid tissue. The beta-lactams do not significantly accumulate in phagocytic cells (see Table 7-5). Beta-lactams are concentrated in the urine, enhancing efficacy for cystitis; the clinician must not assume that the high concentration will be achieved in other tissues that also are infected (e.g., nephritis or other urinary tract sites, and even high urinary concentrations may be ineffective in the presence of biofilm (see Chapter 8).

The small Vd that characterizes the unbound beta-lactams contributes to their relatively short half-lives, which often are less than 1 to 4 hours (see Table 7-1). Slow release of highly-protein bound drugs will prolong presence in the plasma. Because beta-lactams in general do not exhibit a long postantibiotic effect, dosing intervals for such drugs may be inconvenient; for critical patients, administering the drug as a constant-rate infusion may be appropriate. The attributes of constant-rate infusion for critical human patients receiving beta-lactams with short half-lives are well recognized and have been demonstrated in animal models.<sup>3</sup> The advantages may reflect better steady-state concentrations of drugs in peripheral tissues. Exceptions occur for selected drugs that have a longer half-life, drugs characterized by metabolism to active metabolites, or slowly absorbed or released preparations. The former includes cefpodoxime (4- to 5-hour half-life and 80% to 90% protein bound) and cefovecin (approximate 4- to 5-day half-life and 90% to 99% bound to serum proteins in dogs or cats). Penicillins designed for slow release include slow-release esters (e.g., procaine or benzathine penicillins) or highly protein-bound drugs that may be slowly released from plasma to tissue (e.g., cefovecin). For the latter, generally either absorption or distribution, rather than elimination, half-life is prolonged, resulting in a "flip-flop" model (see Chapter 1). The beta-lactam antibiotics are eliminated, in general, by active tubular secretion in the renal tubules. Clavulanic acid, which is a beta-lactam antibiotic, albeit with poor efficacy by itself, is excreted primarily in the urine of dogs.<sup>53</sup> With the exception of hetacillin (no longer available), hepatic metabolism does not play a role in the elimination of the penicillins. Some cephalosporins are eliminated in the urine after deacetylation by the liver, often generate no active metabolites. Examples include cephalothin, cephalixin, cefotaxime, and ceftiofur. Imipenem is degraded to inactive metabolites in the kidney. Reabsorption from the urine is facilitated by an acid urinary pH. Deacetylation of ceftiofur results in an active metabolite; dosing regimens and C&S testing are

based on ceftiofur bioactivity.<sup>54</sup> Ceftriaxone and cefoperazone are eliminated in the bile in humans and appear to be at least partially eliminated in the bile in dogs.<sup>9</sup>

#### Disposition of selected beta-lactam antibiotics

**Penicillins.** Preparations of penicillin G intended for intramuscular use (e.g., procaine and benzathine) may be prepared as esters, which hydrolyze at variable rates and thus prolong absorption. Procaine penicillin is absorbed for at least 24 hours and benzathine penicillin for approximately 120 hours in some species.<sup>9</sup>

For the aminopenicillins the oral bioavailability of amoxicillin is greater than that of ampicillin and, unlike ampicillin, is not impaired by the presence of food.<sup>5</sup> Clavulanic acid appears to be about 30% to 65% orally bioavailable.<sup>15,53,55</sup> The absorption of both amoxicillin and clavulanic acid appears to occur through a saturable process. As with humans, a maximum rate may be reached in dogs at 10 mg/kg and 5 mg/kg, respectively. As the oral dose of amoxicillin reaches 25 mg/kg and clavulanic acid 6.25 mg/kg, amoxicillin may interfere with oral absorption of clavulanic acid. Thus ratios that favor clavulanic acid might be preferred to ensure sufficient absorption.<sup>26</sup> Other disposition parameters of the aminopenicillins are summarized in Table 7-1. The disposition of amoxicillin is such that care should be taken to ensure that underdosing does not occur. This is likely to require administration beyond the label dose (12.5 mg/kg, alone or as clavulanic acid). For treatment of *S. pseudintermedius*, Stegemann<sup>20</sup> has reported an MIC<sub>90</sub> of <0.5 µg/mL for amoxicillin-clavulanic acid (see Table 7-9). The MIC<sub>50</sub> and MIC<sub>90</sub> for amoxicillin-clavulanic acid and *E. coli* are 2 and 8 µg/mL, respectively. Integration of PK-PD for these organisms indicates that an alternative drug to amoxicillin with or without clavulanic acid might be considered; an exception might occur with UTI because higher drug concentrations will be achieved in the target tissue (urine). However, precaution is also suggested with this approach (see Chapter 8). Note that CLSI has recently re-set breakpoint MIC's such that many isolates considered susceptible before this change will now be considered resistant.

**KEY POINT 7-8** The low C<sub>max</sub> achieved for amoxicillin at the labeled dose coupled with its short half-life markedly increase the likelihood of therapeutic failure even for "susceptible" isolates.

**Carbapenems.** Both imipenem and meropenem have been studied in dogs.<sup>56,57</sup> Imipenem is minimally protein bound in dogs.<sup>56</sup> Peak concentrations (see Table 7-1) occur at 30 minutes for intramuscular and 50 minutes for subcutaneous administration. Extrapolated PDCs after intravenous administration appear to approximate 40 mg/L. The volume of distribution of 0.32 L/kg indicates distribution to extracellular fluid; clearance (CL) is 0.26 L/hr/kg. The elimination half-life varies almost twofold with the route (see Table 7-1). Bioavailability is high after intramuscular or subcutaneous administration.<sup>56</sup> In dogs given 5 mg/kg subcutaneously, targeting a 12-hour interval and a T ≥ MIC (25%) (acceptable for carbapenems),

the highest MIC that might be treated is 2 µg/mL. The dose should be increased (approximately 30%) to adjust for ≤70% drug movement from plasma into normal interstitial fluid, particularly if the drug is given subcutaneously.

Meropenem has been studied in dogs after single dose<sup>58</sup> and constant-rate infusion.<sup>57</sup> As with imipenem, it is minimally (12%) protein bound in dogs. Clearance is 5.6 to 6.5 mL/min/kg. After a dose of 20 mg/kg, mean meropenem (µg/mL) in interstitial fluid (using ultrafiltration techniques) was 24 ± 8 µg/mL. After subcutaneous administration, C<sub>max</sub> (µg/mL) in plasma and interstitial fluid, respectively, were 25 and 11 (ratio = 0.44), and AUCs were 63 and 43 µg · hr/mL, (ratio 0.68) respectively. The better ratio for AUC reflects a longer mean residence time in intracellular fluid (ICF) compared with plasma (2, 4, and 0.9 hours, respectively). Although interstitial fluid concentrations correlated very well with PDC, the doses based on plasma C<sub>max</sub> values might be increased at least 40% when basing dosing on PDC to compensate for differential distribution to extracellular sites of infection. The AUC in interstitial fluid after 20 mg/kg administered intravenously or subcutaneously was 73 µg · hr/mL, and 43 µg · hr/mL, respectively, indicating that intravenous administration might be preferred to subcutaneous administration from a cost standpoint. Note that the time to maximum concentration in interstitial fluid after subcutaneous administration was 3.7 hours (2 hours for intravenous administration), indicating a potential delay in response in the acute situation.<sup>58</sup> Based on plasma C<sub>max</sub> after 20 mg/kg administered subcutaneously in dogs, a 12-hour dosing interval, and T > MIC of 25%, the highest MIC that might be treated is 4 µg/mL. If concentrations are used to design the dosing regimen, the highest MIC that could be treated would be 1 µg/mL. Anuric renal failure in humans prolongs the half-life of meropenem fourfold.<sup>59</sup>

**First-generation cephalosporins.** Papich et al. described the tissue distribution of cephalexin.<sup>59a</sup> The ratio of cephalexin C<sub>max</sub> or AUC in plasma versus interstitial fluid were approximately 50% and 57%, respectively. The elimination half-life of cephalexin appears to be somewhat route dependent, being almost twice as long as after oral administration (150 minutes) compared with intramuscular or intravenous administration (80 minutes; see Table 7-1). However, Papich et al. reported a much longer half-life of 4.7 + 1 hours in dogs after oral administration of 25 mg/kg.<sup>59a</sup> Plasma clearance is 2.5 mL/min/kg.<sup>60</sup> Bioavailability approximates 60% after either oral or intramuscular administration.<sup>60</sup> Oral bioavailability in dogs is affected by the time of day of administration, with C<sub>max</sub> 22% lower in the evening; however, this is more than offset (as a time-dependent drug) by a prolongation of half-life by 50%.<sup>61</sup> The oral bioavailability of cephalexin also is affected by pretreatment with metaclopramide, which increases C<sub>max</sub> and AUC, respectively, by 17% and 25%.<sup>61</sup> Based on the original half-life reported for cephalexin, targeting T > MIC (50%), the maximum MIC that can be treated using an oral dose of 20 mg/kg is 1 µg/mL. This is equivalent to the MIC<sub>50</sub> but less than the MIC<sub>90</sub> (2 µg/mL) reported for *S. intermedius* and cephalexin in dogs.<sup>20</sup> A dose of 40 mg/kg is needed for twice-daily

dosing, or the interval should be reduced to every 8 hours. Doses would need to be further increased to compensate for differential distribution to tissues or other host or microbial factors. However, if the half-life of 5 hours is used, then twice-daily dosing of cephalexin will result in drug concentration in both plasma and interstitial fluid above the MIC<sub>90</sub> for *S. intermedius*<sup>20</sup> for 12 hours or more.<sup>59a</sup> Note that the MIC<sub>50</sub> and MIC<sub>90</sub>, respectively, for *E. coli* and cephalexin are 8 and 16,<sup>20</sup> indicating that this drug should not be used to treat infections associated with *E. coli*, including urinary tract infections.

Cephalothin (no longer available in the United States, although it remains the model drug for first-generation cephalosporins at the time of publication) has been studied in dogs after oral administration at 30 mg/kg. Food affects its absorption: C<sub>max</sub> of 45 µg/mL is reduced to 28 µg/mL with food at a T<sub>max</sub> of 1.7 and 2.8 hours, respectively. Elimination half-life is 1.8 and 2.6 hours without and with food, respectively.<sup>62</sup>

Cefadroxil achieves a C<sub>max</sub> of 35 µg/mL at a T<sub>max</sub> of 20 minutes after an oral dose of 30 mg/kg. Food minimally affects rate or extent of absorption according to one study, but it does increase half-life from 1.7 to 4 hours.

Cefazolin has been studied in two separate groups of canine patients undergoing elective orthopedic procedures. In one study<sup>63</sup> clinical canine patients (n = 15) undergoing total hip replacement were administered 22 mg/kg intravenously over 2 minutes at the time of surgical positioning; animals were dosed 2 more times.<sup>64</sup> The distribution of the central compartment (V<sub>c</sub>; before distribution) was 0.083 ± 0.008 L/kg. The distribution half-life approximated 5 minutes, and the elimination half-life approximated 45 minutes. Tissues from the coxofemoral joint capsule, acetabulum, and femoral cancellous bone were collected from each patient as the site was approached surgically; serum samples were collected at the same general time for each patient. Peak serum concentrations after the first dose were 178 ± µg/mL; tissue (homogenate) concentrations and mean time of collection were as follows: joint capsule, 58 + 5.7 µg/mL at 20 min, acetabulum 157 + 23 at 52 minutes and bone cancellous 227 + 29 at 68 minutes. Peak serum concentrations approximated 178 µg/mL (before distribution) and 119 µg/mL (after distribution). Based on simulations, ideal dosing was suggested to be either 22 mg/kg every 2 hours or 8 mg/kg every hour, to ensure drug concentrations remained above the MIC of *Staphylococcus* spp. (reported at 2 µg/mL).

**Second- and third-generation cephalosporins.** Cefuroxime is a second-generation cephalosporin approved for use in humans. Oral administration is in the form of the axetil ester; as a prodrug, desterification occurs before oral absorption. It has been studied both orally and parenterally in Beagles (n=6) as part of a toxicity study.<sup>64a</sup> Intravenous doses up to 500 mg/kg every 24 hours were well tolerated for 1 week. Jung<sup>65</sup> compared cefuroxime in serum to that in cortical tissues in dogs. At approximately 1.25 hours, after 10 and 20 mg/kg administered intravenously, serum concentrations were 12.5 and 28.7 µg/mL, respectively. The elimination half-life was 2.9 hours. Spurling<sup>66</sup> reported limited PDCs after oral administration in Beagles. Concentrations (µg/mL) after oral

administration of the axetil form at 100 or 400 mg/kg were approximately 28.7 ± 5, and 77 ± 17, respectively.<sup>66,67</sup>

**KEY POINT 7-9** The longer half-life of cefpodoxime renders it preferable to cephalothin for treatment of susceptible infections as long as a sufficiently high dose is used.

After a dose of 50 mg/kg ceftriaxone (third generation) was given to apparently healthy dogs, clearance was 3.61 ± 0.8 mL/kg/hr; T<sub>max</sub> occurred at 30 minutes compared with 90 minutes after subcutaneous administration. Pain occurred at the injection site after both intramuscular and subcutaneous administration, whereas intravenous administration was not associated with any adversity.<sup>68</sup>

Based on studies in dogs after an intravenous dose of 14 mg/kg, cefepime was distributed to a volume of 0.14 l/kg, suggesting that the drug might be protein bound. However, both the elimination half-life and MRT were short at 60 minutes. Clearance was 0.13 ± 0.04 l/kg/hr. The dose necessary to maintain the breakpoint MIC of 8 µg/mL for at least two-thirds of the dosing interval (above 2 µg/mL for the entire interval) (for humans) in dogs was recommended by the author to be 40 mg/kg every 6 hours.<sup>28</sup>

Ceftazidime is a third-generation drug characterized by an elimination half-life of 0.8 hours in dogs. After subcutaneous injection, T<sub>max</sub> occurs at 1 hour after administration of 30 mg/kg. When given an initial dose of 4.4 mg/kg followed by a constant-rate infusion of 4.1 mg/kg/hr for 36 hours, C<sub>max</sub> at steady state is 22.2 µg/mL. Total body clearance is 0.19 L/kg/hr.<sup>69</sup> The MIC<sub>90</sub> for clinical isolates (n = 101) of *P. aeruginosa* was ≤ 4 µg/mL.<sup>69</sup> Using 4 µg/mL as the basis for a subcutaneous dose of 30 mg/kg, only 3 half-lives can elapse for T = MIC, indicating a 6-hour dosing interval might be appropriate for *Pseudomonas* spp. Ceftazidime has been studied in cats (n = 5) after intravenous and intramuscular (30 mg/kg) administration.<sup>70</sup> After intravenous administration, the V<sub>d</sub> was 18 ± 0.04 L/kg; protein binding was not described. Plasma clearance was 0.19 ± 0.08 L/hr/kg, and elimination half-life was 0.77 ± 0.06 hour. After intramuscular administration, bioavailability was 82.47 ± 4.37%, resulting in a C<sub>max</sub> of 89.42 ± 12.15 µg/mL, at a T<sub>max</sub> of approximately 30 minutes. The authors indicated that for an 8- to 12-hour dosing interval, T > MIC would range from 35% to 52% of the dosing interval for intravenous and 48% to 72% for intramuscular administration for isolates with an MIC ≤ 4 µg/mL.

Ceftiofur is a third-generation drug approved for use in dogs for treatment of urinary tract infections. It has been studied at 0.22, 2.2, and 4.4 mg/kg administered subcutaneously in dogs (n = 9).<sup>71</sup> PDCs increase proportionately (see Table 7-1). It has a relatively long half-life compared with other cephalosporins, reflecting, in part, its active metabolite. Accordingly, a longer dosing interval is likely to be more reasonable for ceftiofur compared with the first-generation drugs. When administered subcutaneously, peak PDCs (C<sub>max</sub>) were 1.66 ± 0.2, 8.91 ± 6.42, and 27 ± 1 µg/mL at 0.22, 2.2, and 4.4 mg/kg, respectively.<sup>71</sup> At the C<sub>max</sub> of approximately 9 µg/mL at a dose of 2.2 mg/kg, targeting T > MIC of 50%, the highest MIC that can

be treated at 12-hour intervals is 4 µg/mL. At 4.4 mg/kg administered subcutaneously, the  $C_{max}$  disproportionately increases to 29 µg/mL, and the highest MIC that could be treated using the same targets is 16 µg/mL, which actually exceeds the  $MIC_{BP}$  ( $\geq 8$  µg/mL). Urine concentrations were also reported for ceftiofur bioactivity in the dog. At 24 hours, urine concentrations at 2.2 and 4.4 mg/kg were 8.1 and 29.6 µg/mL, respectively. These concentrations surpassed the the  $MIC_{90}$  for *E. coli* (4.0 µg/mL) and *P. mirabilis* (1.0 µg/mL).<sup>71</sup>

Cefpodoxime is a relatively new third-generation cephalosporin to be approved in dogs for treatment of canine pyoderma. Orally, it is administered as a prodrug, cefpodoxime proxetil, which is desterified in the gastrointestinal tract such that it is absorbed as cefpodoxime. According to the package insert and technical monographs, oral bioavailability in dogs is 63% and food does not impair absorption. At 10 mg/kg administered orally,  $C_{max}$  is variable at  $16.4 \pm 11$  µg/mL, suggesting that dosing should err on the high side for higher MIC;  $T_{max}$  occurs at 2 to 3 hours. Plasma clearance is 23 mL/hr/kg. Cefpodoxime is excreted largely in the urine with more than 75% excreted as the parent drug. The elimination half-life of 5.6 hours (MRT 9 hours) is longer than that of many beta-lactams; therefore a longer dosing interval is possible (i.e., 12 to 24 hours, depending on the dose and MIC of the infecting microbe). PDCs after 10 mg/kg appear to approximate 1 µg/mL at the end of a 24-hour dosing interval. Thus PDC will stay above the  $MIC_{90}$  for *E. coli* (0.5), and for *S. pseudintermedius* (0.5) well beyond the targeted  $T > MIC$  of 50% to 75%. (assuming MIC does not change dramatically overtime). However, at 5 mg/kg administered orally in dogs, the highest MIC that can be treated with a 12-hour dosing interval is 4 µg/mL, and with a 24-hour dosing interval, 2 µg/mL, both of which are still above the  $MIC_{90}$  of the approved pathogens. Cefpodoxime is well tolerated in dogs at doses as high as 400 mg/kg/day for 6 months.

Tissue kinetics of cefpodoxime compared with cephalexin have been described in dogs.<sup>59a</sup> The free and thus diffusible fraction of drug in plasma ranged from 9% to 34%. Maximum drug concentrations after administration of 8.5 mg/kg (single dose) in dogs ( $n = 6$ ) was (extrapolated from plot) approximately 10 µg/mL free drug ( $33 \pm 7$  µg/mL total) in plasma compared with  $4.3 \pm 1.9$  in interstitial fluid, suggesting less than 50% of the drug in plasma reaches interstitial tissues. Unbound AUC in plasma was not provided, but the disappearance half-life of cefpodoxime from interstitial fluid was twice as long as that from plasma ( $10 \pm 3$  hours versus  $5.6 \pm 0.9$  hours, respectively). The reason for this difference is not clear, although factors that influence diffusibility from tissue into serum might also influence antibacterial activity potentially precluding drug efficacy. Nonetheless, on the basis of these data, interstitial concentrations of cefpodoxime exceeded the  $MIC_{90}$  of *S. intermedius* and *E. coli* as reported on the package insert for 24 hours.<sup>59a</sup> This is in contrast to cephalexin, which is  $< 20\%$  bound to plasma proteins and for which interstitial concentrations exceeded the  $MIC_{90}$  for *S. pseudintermedius* (as reported by Stegemann<sup>20</sup>) for 12 hours but did not achieve the  $MIC_{90}$  for *E. coli*.

Cefovecin (third-generation) is the newest cephalosporin to be approved in dogs at the time of this publication. Its PD and PK have been very well described including either concentrations or bioactivity in interstitial fluid in dogs or cats in part because its disposition is complicated by extensive binding to plasma proteins.<sup>20,72,73</sup> Accordingly, care must be taken when designing dosing regimens to base decisions on unbound, rather than total, drug. Based on protein-binding studies (microdialysis) at cefovecin concentrations ranging from 10 to 300 µg/mL in dog plasma, 96% to 98% is bound at concentrations below 100 µg/mL, with the fraction increasing to 72% at 200 µg/mL and 56% at 300 µg/mL. Avid protein-binding results in a slow release and a long elimination half-life of 136 or 133 hours when given intravenously or subcutaneously, respectively. Protein-binding also affects  $T_{max}$ , which does not occur until 6 hours (based on total drug), and the apparent  $V_d$  (0.12 L/kg), which is higher than total blood volume but considerably lower than extracellular fluid volume.  $C_{max}$  of unbound, active drug approximates about 5 µg/mL. Predicted unbound concentrations suggest that  $T > MIC_{90}$  of *S. pseudintermedius* (0.25 µg/mL) occurs at approximately day 12 after dosing 8 mg/kg subcutaneously; however, this is reduced to day 8 on the basis of the lowest unbound concentration predicted by the 95% confidence interval of 1 µg/mL, which is the more prudent statistic to follow (see package insert). For organisms with  $MIC \geq 2$  µg/mL (see Table 7-9) (e.g., *S. aureus*, not an approved indication),  $T > MIC$  of mean (predicted) unbound drug at approximately 1 to 2 days; however, if based on the lowest (95% confidence interval) predicted unbound concentrations, 2 µg/mL would not be reached in plasma. In contrast, the  $MIC_{90}$  of *Streptococcus canis* (an approved indication) is much lower ( $< 0.06$  µg/mL); thus  $T > MIC$  exceeds 14 days even when based on the lowest predicted unbound concentration in plasma. The same is true for *Pasteurella*, the approved indication in cats; the targeted  $T > MIC_{90}$  is not reached until 12 days after treatment.

**KEY POINT 7-10** The high fraction of cefovecin binding to plasma proteins prolongs its half-life, but less than 10% of total drug is active.

Studies of unbound cefovecin in tissue have been published using tissue cage models in dogs.<sup>72</sup> The studies demonstrate that unbound cefovecin effectively moves from plasma into tissues, as indicated by antibacterial activity against *S. pseudintermedius* across time). After 8 mg/kg administered subcutaneously in dogs, cefovecin (total)  $C_{max}$  (total, µg/mL) was 116, 32, and 40 in plasma, transudate, and exudate, respectively, with elimination half-life from transudate similar to that in plasma (147 hours and 136 hours, respectively). Antibacterial activity was detectable in transudate at 4 hours; however,  $T_{max}$  of cefovecin antibacterial activity did not occur until approximately 2 days. Interestingly, antibacterial activity in transudate actually exceeded antibacterial activity in plasma at all time points after 8 hours and far

exceeded it from day 5 forward. Peak antibacterial effects for *S. pseudintermedius* persisted in transudate until day 10 after injection, with log 2 reduction in CFUs still present at day 18; activity was gone by day 21.

Urine concentrations of cefovecin have been reported in dogs after subcutaneous administration of 8 mg/kg. Peak urine (presumably unbound) concentrations of 66 µg/mL were achieved at 54 hours and approximated 2.9 µg/mL at 18 days.

These data support the use of cefovecin for treatment of susceptible isolates causing urinary tract infections. Cefovecin also is approved for use in cats. Compared with the dog, cefovecin at 8 mg/kg reaches a higher total plasma  $C_{max}$ ; however, it is 99% or more bound to plasma proteins in the cat. Although mean predicted unbound concentrations approximate 10 µg/mL, the predicted variability is great, yielding as little as 0.2 if based on the lower 95% confidence interval (see package insert). The elimination half-life in cats is slightly longer at 166 hours (compared with 136 hours in dogs). The  $T_{max}$  for plasma is only 2 hours in cats (compared with 6 hours in dog). Peak concentrations of cefovecin in transudate (occurring at 1 day) were approximately 65 µg/mL (compared with approximately 30 µg/mL in dogs). However, 99% of the drug in transudate also was bound, despite the assumption that transudate is protein free. Antibacterial studies were not performed in the transudate of cats and it is not clear what impact, binding has on transudate bioactivity. The concentration of free drug in transudate in cats approximated or exceeded the  $MIC_{90}$  ( $T > MIC_{90}$ ) *P. multocida* (0.012 µg/mL; the approved target organism in cats) for 10 days.

The percentage of a radiolabeled dose of cefovecin recovered in urine of dogs (approximately 28%) was only slightly higher than that in feces (24%), indicating that the impact of cefovecin on normal gastrointestinal microbiota may not necessarily be less than that of orally administered drugs. Although urine contamination of feces may have occurred during the collection process, a second peak in PDCs occurs in cats, indicating that enterohepatic circulation may occur.

**KEY POINT 7-11** Indiscriminate use of cefovecin must be avoided such that emergence of methicillin-resistant *Staphylococcus aureus* or methicillin-resistant *Staphylococcus intermedius* will not be facilitated.

Stegemann<sup>20</sup> has reported the PD activity of many anaerobic and aerobic gram-positive and gram-negative (potentially) pathogenic organisms collected from dogs and cats in the United States and Europe. Isolates were tested toward cefovecin, amoxicillin-clavulanic acid, cephalixin, and cefodroxil. The number of isolates in general for each organism exceeded 25, although exceptions exist (e.g., *Klebsiella*, coryneforms). *Acinetobacter* and *Enterococcus* spp. ( $n \geq 25$ ) were characterized by an  $MIC_{50}$  of 16 or higher, well above the  $C_{max}$  of unbound drug; cefovecin should not be used to treat infections caused by these organisms. For the remaining isolates, integration of PD data with PK data (see Table 7-6) reveals that  $T > MIC$  for cefovecin that is superior to the other three drugs studied.

Several considerations should be made when selecting cefovecin as empirical choice for treatment of (presumed) susceptible infections in the dog or cat. First, recognizing the historical relationship between cephalosporins and MRSA might lead to judicious, if not limited, use. Second, not all organisms are equally susceptible to cefovecin. Caution is recommended when using cefovecin for treatment of organisms whose  $MIC_{90} \geq 2$  µg/mL. Third, if the decision is made to redose cefovecin, doing so probably should be considered at 2 to 4 days rather than 7 to 14 days for those organisms whose MIC is equal to or greater than 2 µg/mL. The need for redosing might be limited to those patients at risk for persistent and thus resistant infections. A final consideration for cefovecin therapy is the time that must lapse to detectable (4 to 8 hours in plasma or transudate) and peak (2 to 3 days) antibacterial activity of cefovecin in interstitial fluid.<sup>72</sup> Cefovecin may not be a wise choice if rapid antibacterial efficacy is needed. This includes the surgical patient. Because of its long time to onset and persistence, cefovecin should not be used for surgical prophylaxis. Fourth, increasingly in human medicine, the duration of antimicrobial therapy is being shortened (e.g., to 5 days or less) for treatment of uncomplicated infections such that emergent resistance might be minimized (see Chapter 6); with cefovecin, "hit hard, get out quick" is not possible.

**Drug interactions.** The potential synergistic and antagonistic effects of beta-lactams with other antimicrobials was discussed in Chapter 6. Synergism resulting from enhanced antimicrobial uptake associated with altered cell wall permeability has been demonstrated for a number of antimicrobials. Antagonism should be anticipated with drugs whose impact slows organism growth (i.e., single subunit ribosomal inhibitors); efficacy of beta-lactams may be reduced to bacteriostatic rather than bactericidal effects. An exception may occur for chloramphenicol and selected Enterobacteriaceae (see the discussion of chloramphenicol). As weak acids, the beta-lactams may chemically interact with and inactivate weak bases (see the discussion of aminoglycosides). Inactivation occurs at high concentrations, as might occur with mixing of medications, or potentially, in urine. High protein binding of beta-lactams may result in drug interactions with other highly protein-bound drugs because of competition for protein-binding sites, as is exemplified for cefovecin. Drugs for which higher concentrations have been demonstrated when combined with cefovecin and include carprofen, furosemide, doxycycline, and ketoconazole (PI). It should be anticipated that concurrent use of cefovecin with other highly protein-bound drugs will result in increased free drug concentrations. Beta-lactams will compete for active tubular secretion proteins in the proximal tubule with other organic acids (e.g., penicillins, cephalosporins, nonsteroidal antiinflammatory drugs, sulfonamides, diuretics). The prototypic example drug is probenecid, the combination of which with penicillins was used therapeutically to prolong elimination before implementation of mass production technology. According to the package insert accompanying probenecid, combined use with penicillin results in a twofold to fourfold increase in penicillin drug concentrations.

### Adverse Effects

Mammalian cells lack a cell wall; therefore, the beta-lactam antibiotics are very safe. Diarrhea is a common side effect that may reflect altered intestinal microbial flora. Experimentally, co-oral administration with a recombinant beta-lactamase minimally altered fecal microflora but did not negatively influence PDCs.<sup>73a</sup> Increasing the ratio of amoxicillin to clavulanic acid reduces gastrointestinal upset in humans (but may decrease the absorption of clavulanic acid; see previous discussion), but ratios less than 4:1 can only be accomplished using human-approved drugs, whose equivalent bioavailability has not been established in dogs and cats. The role of probiotics in preventing diarrhea has yet to be established but warrants consideration. Hypersensitivity is an infrequent reaction and occurs less often with cephalosporins. Penicilloic acid (results from breakdown of the beta-lactam ring) is the more likely mediator of hypersensitivity reactions; it is generated from the activity of several beta-lactamase or other enzymes from various sources. Thrombocytopenia has been reported to occur with some members of this class. With the exception of the carbapenems and selected later-generation cephalosporins, the beta-lactams may cause endotoxin release (see Chapter 6), which may prove detrimental to the patient, although relevance to dogs and cats is not clear.<sup>74</sup> Penicillins, including imipenem, antagonize gamma-aminobutyric acid type A receptors and may thus lower the seizure threshold.<sup>75</sup> The risk may be greater in patients with renal disease.<sup>76</sup> Cephalexin can cause false glucosuria.<sup>77</sup>

### Therapeutic Use

The broad spectrum and wide safety margin of the beta-lactam antibiotics lead to their common use. Caution is recommended, however, when they are used to treat complicated infections without the benefit of C&S data. For many drugs, because of the short half-life,  $C_{max}$  achieved at recommended doses often is not sufficient to allow a convenient dosing interval. Exceptions occur for those cephalosporins with a long half-life or carbapenems for which  $T > MIC$  of 25% is acceptable. Resistance develops to beta-lactams relatively rapidly, and the drugs are not characterized by an excellent distribution pattern, with interstitial fluid concentrations of active drug often being 50% to 30% or less of plasma concentrations, depending on the tissue and the drug. Caution should be taken with third- and fourth-generation cephalosporins despite indications of susceptibility on culture data because of inducible ESBLs that require special testing, especially in the presence of a high infecting inoculum. The spectrum of natural penicillins is relatively narrow, particularly when considered in the context of resistance that has emerged through decades of use. Resistance to aminopenicillins also limits their use as empirical drugs of choice. Exceptions might include anaerobic infections. Because the extended penicillins are susceptible to beta-lactamase destruction, combination with a beta-lactamase protector (e.g., ticarcillin and clavulanic acid) or use of imipenem—which is inherently more resistant to beta-lactamase destruction—should

be considered. Imipenem or meropenem should be considered before other beta-lactams for treatment of infections associated with endotoxemia because either drug is associated with the least endotoxin release. Constant-rate infusion should be considered for those penicillins with a short half-life to maintain effective concentrations in the critical patient; alternatively, and preferably, carbapenems should be considered in lieu of penicillins. Use of beta-lactamase-protected products should be considered even in uncomplicated infections. Indiscriminate use of beta-lactams, and particularly cephalosporins, should be avoided to minimize the advent of MRSIG.

The first-generation cephalosporins have been excellent first-choice antimicrobials for many infections, including urinary, skin, and respiratory tract infections. Their relative resistance to beta-lactamases produced by *Staphylococcus* spp. leads to their frequent empirical selection for infections in which *Staphylococcus* spp. are assumed to be involved. However, their empirical use increasingly is being limited, particularly at dosing regimens currently recommended. Their efficacy against *Staphylococcus* spp. as well as against many gram-negative organisms leads to their selection for surgical prophylaxis. Cefovecin should not be included in this category because of its long time to antibacterial effect and time to maximum effect and the persistence of drug concentrations well beyond the immediate postoperative period. Of the second-generation cephalosporins, cefoxitin, which is not impacted by ESBL, might be more safely considered for empirical therapy requiring a broad-spectrum antimicrobial and for anaerobic infections. With the exception of *P. aeruginosa*, cefoxitin is effective against most other organisms. The use of other second-generation and the third-generation cephalosporins is best based on C&S data because the spectra of these drugs are so variable. Caution should accompany use of second- through fourth-generation cephalosporins when based on in vitro data that may not reflect the production of ESBLs. Note also that the (over) use of cephalosporins has been associated with the emergence of multidrug-resistant microorganisms, including MRSA, *Enterococcus* spp., and *P. aeruginosa*.<sup>25</sup>

Beta-lactams should be the first drugs considered for combination antimicrobial therapy (if used at appropriate dosing regimens). Their unique mechanism of action facilitates movement of other drugs into bacteria, which should facilitate efficacy of other antimicrobials. The risk of resistance should also be reduced as antimicrobial movement into the cell is improved. Beta-lactams are combined with drugs effective against gram-negative organisms when broad-spectrum therapy is needed, as in the case of life-threatening infections for which the causative organisms are not known, polymicrobial infections involving anaerobes and aerobes, or gram-positive and gram-negative organisms.

### Vancomycin

Vancomycin has had an important role in the treatment of human patients infected with methicillin-resistant staphylococci (see Chapter 6), but the advent of penicillinase-resistant



beta-lactams and the incidence of adverse reactions have curtailed its use. Vancomycin is a large glycopeptide with three components, each of which may be responsible for its antimicrobial action on bacterial cell walls (Figure 7-4).<sup>78</sup> The D-Ala-D-Alanine precursor of the pentapeptide fits into a pocket formed by the large molecule, sterically interfering with further cell wall elongation. The spectrum of activity of vancomycin is limited to *Staphylococcus* and *Streptococcus* spp. and anaerobes (see Table 7-4). Selected *Enterococcus*, *Clostridium*, and *Corynebacterium* spp. are also generally susceptible. With the exception of enterococcal organisms, the effects of vancomycin are generally bactericidal, although they act slowly. As with other cell wall-active antimicrobials, vancomycin exhibits time-dependent killing effects, with efficacy also related to AUC. Resistance has been impeded by the high specificity of the drug. Multiple mutations are required to change the enzymes currently targeted by vancomycin. Resistance that has developed by *E. faecalis* has resulted from synthesis of a new protein that interferes with vancomycin. More recently, vancomycin-resistant staphylococci have emerged. A strain of vancomycin-intermediate *S. aureus* (VISA) has been described, the mechanism of which includes thickening of the cell wall, coupled with "clogging" of the cell wall by vancomycin itself.<sup>79</sup>

Although vancomycin is available as an oral preparation, this preparation is intended for topical (gastrointestinal) administration, most commonly indicated for pseudomembranous colitis caused by *C. difficile*. Systemic effects require intravenous administration. Vancomycin is distributed to most body tissues. The exception is the CNS, unless the meninges are inflamed; even then only 30% or less will penetrate. It is renally eliminated; drug concentrations may become toxic if doses are not modified for the patient with renal disease.

The risk of nephrotoxicity is increased dramatically if the drug is given in combination with another nephrotoxic drug. Hypersensitivity in human patients warrants slow (60-minute) intravenous infusion of drug diluted in fluid. Ototoxicity has been reported in humans when concentrations reach 60 to 100  $\mu\text{g/mL}$ .<sup>80</sup> Its use for veterinary patients should be limited to treatment of organisms resistant to other drugs as based on C&S data.

### Teicoplanin

Teicoplanin is a mixture of several molecules (teicoplanins A<sub>2</sub> 1-5). The molecules compose a fused glycopeptide core ring structure (teicoplanin) to which are attached two carbohydrates (differing from those in vancomycin), mannose and n-acetylglycosamine, and an acyl (fatty acid). It is the latter structure that confers better lipid solubility compared with vancomycin. Its mechanism of action and impact on bacterial killing and spectrum is similar to those of vancomycin. Its use has largely been replaced by vancomycin or daptomycin.

### Fosfomycin

Fosfomycin is a phosphonic acid that contains a carbon-phosphorous bond (see Figure 7-12). It is a natural antibiotic produced by *Streptomyces fradiae*. Its in vitro spectrum is broad, and it expresses potential efficacy against isolates expressing multidrug resistance, including *E. coli* and gram-positive organisms. As a phosphoenolpyruvate analog, fosfomycin irreversibly inhibits phosphoenolpyruvate transferase, an enzyme that catalyzes the first step of cell wall peptidoglycan synthesis of microbial cell walls.<sup>81</sup> As a cell wall inhibitor, fosfomycin is bactericidal when present at the site of infection at therapeutic concentrations. Its

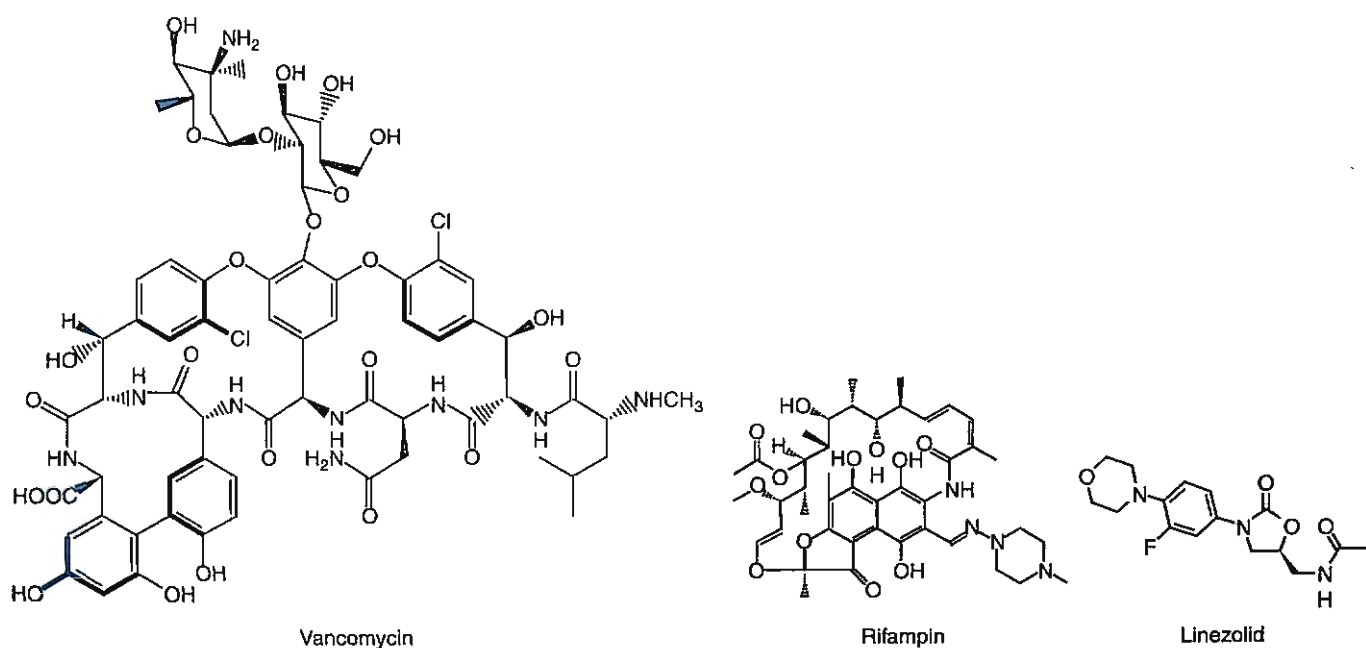


Figure 7-4 The chemical structure of selected drugs which target resistant gram-positive microbes.

irreversible nature contributes to a concentration-dependent effect. Fosfomycin exhibits in vitro activity against a broad range of gram-positive and gram-negative aerobic microorganisms associated with uncomplicated urinary tract infections. The MIC breakpoints reported for humans are 64 (S), 28 Intermediate (I), and 256 (R). Although its mechanism of action is similar to that of the beta-lactams, fosfomycin is not susceptible to destruction by any class of beta-lactamases. Rather, resistance to fosfomycin, which is unusual, reflects the FosX or FosA enzyme, which hydrolyzes the drug in a manner similar to that of glutathione S-transferases. The gene for this protein is chromosomally mediated. Thus when resistance does occur, it is usually only toward fosfomycin (single drug resistance) with cross-resistance not occurring between fosfomycin and other classes of antimicrobial agents. Therefore resistance is not associated with multidrug resistance.<sup>81</sup> Further, compared with susceptible strains, fosfomycin-resistant mutants are impaired, exhibiting poorer growth rates and reduced adherence to uroepithelial cells. Fosfomycin appears to reduce bacterial adherence to uroepithelial cells, and decreased adherence is facilitated by *N*-acetylcysteine<sup>82</sup> and urinary catheters.<sup>83</sup> Studies in humans have demonstrated that fosfomycin distributes well to soft tissues, reaching therapeutic breakpoints.<sup>84</sup> Other attributes of fosfomycin that support its use for treatment of *E. coli* urinary tract infections include renal excretion, synergistic interaction with several other classes of antimicrobials,<sup>86</sup> and preparation as a 3-g sachet (granules), which is mixed with water to orally deliver approximately 40 mg/kg (in humans).

The disposition of fosfomycin disodium (pure substrate) has been described in dogs ( $n = 8$ )<sup>88</sup> after intravenous, intramuscular, subcutaneous, and oral administration at both 40 and 80 mg/kg day for 3 days. Plasma protein binding was negligible; drug concentrations increased in a dose-dependent manner and did not change during the study period, including across each 3-day treatment period. At 40 mg/kg, peak PDCs ( $\mu\text{g/mL}$ ) were as follows:  $51.8 \pm 3.4$  (extrapolated peak PDC; Co, intravenous) and  $5.4 \pm 0.04$  (oral); and at 80 mg/kg,  $113 \pm 12$  (Co, intravenous) and  $10.8 \pm 0.5$  (oral). Oral bioavailability (F) was 30%. Clearance was  $14.9 \pm 1.26$  mL/kg/hr, elimination half-life was  $1.3 \pm 0.06$  hours, and mean residence time was  $1.62 \pm 0.4$  and  $5.2 \pm 0.7$  (oral).

The PD and PK of fosfomycin have also been studied by the author. The distribution MIC for fosfomycin for clinical *E. coli* isolates, regardless of the presence of multidrug resistance, appears to be well below the susceptible breakpoint ( $\leq 64$   $\mu\text{g/mL}$ ) for fosfomycin. In more than 100 clinical isolates collected from dogs and cats, the MIC range was 0.25 to 4  $\mu\text{g/mL}$ ; the MIC<sub>50</sub> and MIC<sub>90</sub> were, respectively, 1 and 1.5  $\mu\text{g/mL}$ . Fosfomycin tromethamine was administered as a single oral dose of 80 mg/kg. After oral administration,  $C_{\text{max}}$ , elimination half-life and mean residence time were  $66 \pm 21$  ( $\mu\text{g/mL}$ ),  $2.5 \pm 1.09$  hours and  $5.1 \pm 1.7$ , hours, respectively. Drug was detected at concentrations exceeding the MIC<sub>90</sub> of fosfomycin for multidrug-resistant *E. coli* (1.5  $\mu\text{g/mL}$ ) until 7 (2.5  $\mu\text{g/mL}$ ) and 12 hours (9  $\mu\text{g/mL}$ )

after intravenous and oral administration, respectively. Drug was present in urine at concentrations above 10  $\mu\text{g/mL}$  at 24 hr post dosing. Gastrointestinal upset manifesting as mild to moderate diarrhea was observed in 4 of the 12 dogs. Food decreased oral bioavailability: without food,  $109 \pm 31\%$  (95% confidence interval CI: 84%-135%) and with food,  $66 \pm 16\%$  (95% CI: 52%-79%). Gender had no impact on oral bioavailability. Kill studies in our laboratory indicate that for treatment of *E. coli*, the drug is not concentration dependent, as is suggested by other studies that indicate both time- and concentration-dependent effects.<sup>88,89</sup> Further studies are warranted to establish efficacy for treatment of multidrug-resistant-associated urinary tract infections.

Although fosfomycin is appealing for treatment of urinary tract infections and potentially other infections caused by multidrug-resistant isolates, differences in bioavailability (oral) among different fosfomycin salts necessitates that PK be the basis, particularly of oral dosing regimens in the dog. Its efficacy appears to be both time and concentration dependent; if the latter, this should facilitate efficacy despite the short-half-life of the drug.<sup>88</sup> The drug appears to interact in an additive to synergistic fashion with a number of other antimicrobials.

## DRUGS THAT TARGET RIBOSOMES (BACTERICIDAL)

### Aminoglycosides

Despite their potential nephrotoxicity, aminoglycosides remain the cornerstone of aerobic gram-negative therapy in many complicated or serious infections. Minor differences in the chemical structures of these drugs lead to differences in efficacy and toxicity. Clinically useful aminoglycosides include neomycin, gentamicin, amikacin, netilmicin, streptomycin (or dihydrostreptomycin), and tobramycin.

### Structure-Activity Relationship

Aminoglycoside compounds are composed of an amino sugar linked through glycosidic bonds to an aminocyclitol.<sup>5,90</sup> They vary in the amino sugar and the specific number and location of the amine groups (Figure 7-5). The different name endings indicate the microbe of origin for the natural antibiotic: The suffix "icin" (e.g., gentamicin) originates from *Micromonospora* sp., whereas the "mycin" suffix (e.g., tobramycin) derives from *Streptomyces*. Amikacin is a semi-synthetic derivative of kanamycin, and netilmicin, a semi-synthetic derivative of sisomicin. Tobramycin is most similar to gentamicin in both spectrum and toxicity. The aminoglycosides are polycationic, depending on the number of amine groups. Kanamycin and gentamicin have two amino sugars, whereas neomycin has three amino sugars. The amine group of gentamicins is variably methylated, yielding three different gentamicins. Streptomycin has a different aminocyclitol sugar compared with the other drugs, whereas spectinomycin is an aminocyclitol that does not contain any amino sugars.

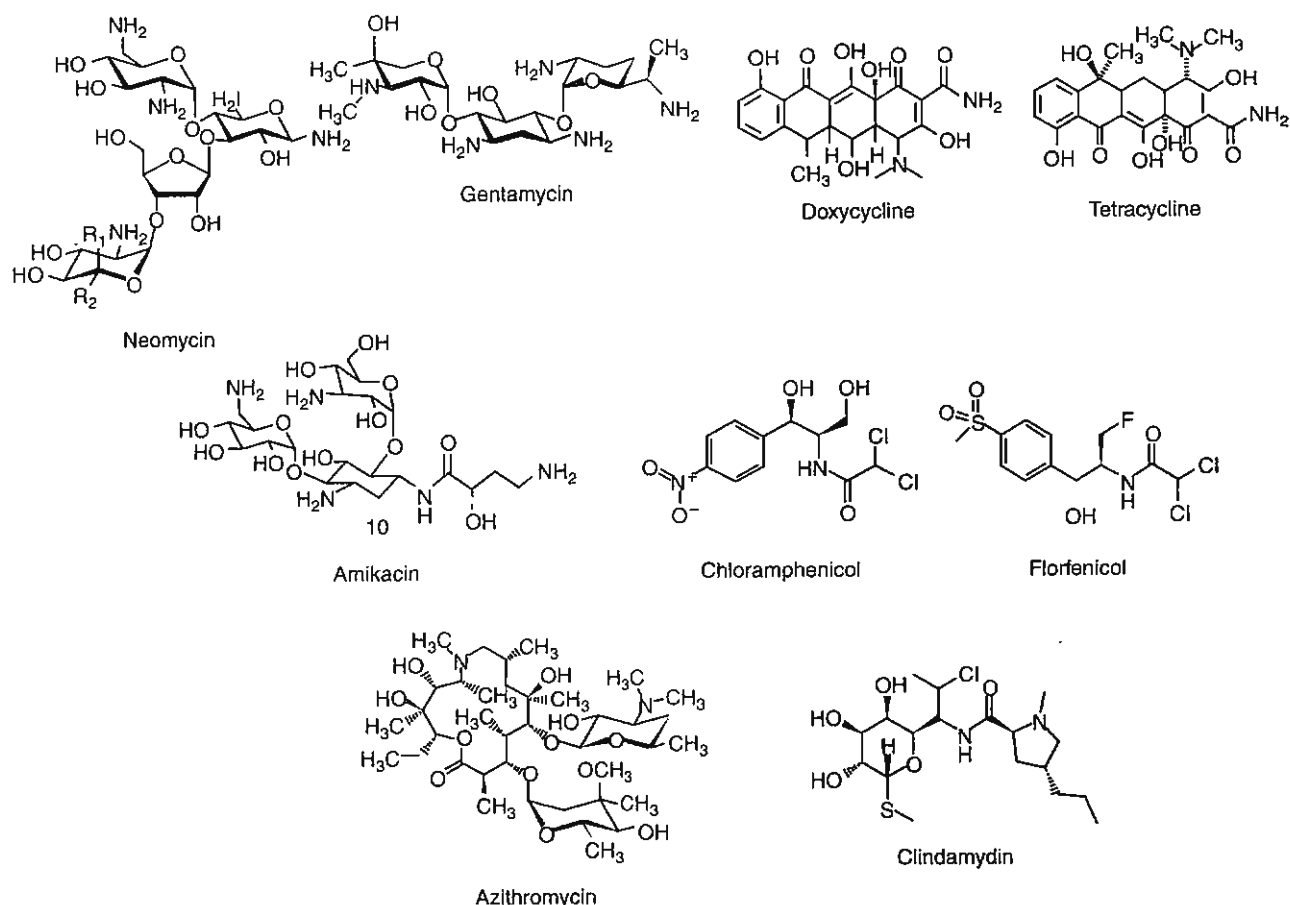


Figure 7-5 Chemical structures of ribosomal inhibitors.

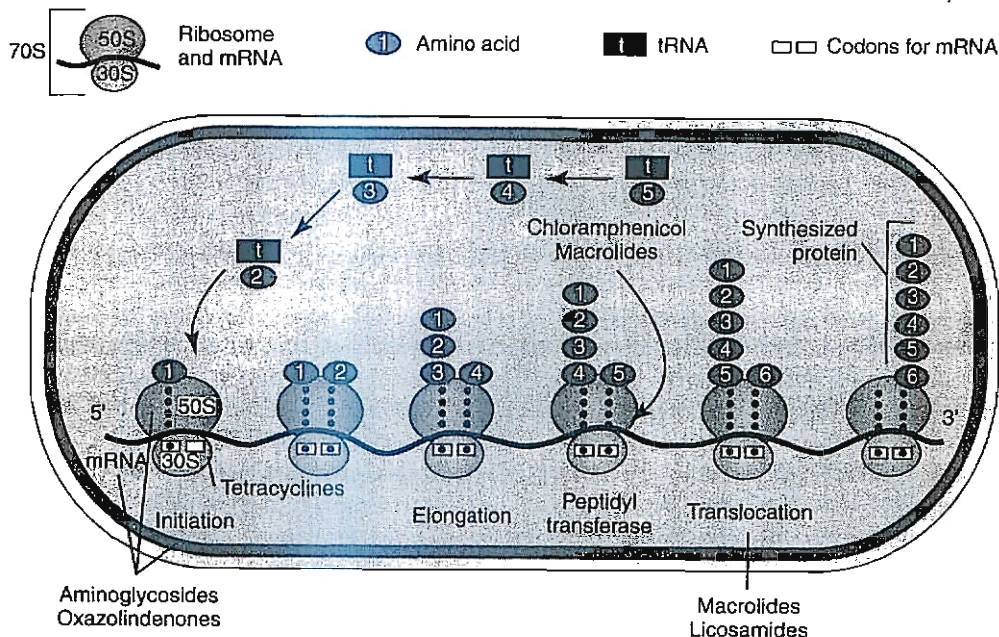
### Mechanism of Action

Aminoglycosides target bacterial ribosomes (Figure 7-6). The drugs enter gram-negative organisms initially through porins in the lipopolysaccharide layer. Subsequent penetration of aerobic bacteria at the level of the cell membrane appears to occur in three binding stages: the negatively charged moieties of phospholipids are first ionically attracted and bound by the positive moieties of the drug, followed by the lipopolysaccharides and finally membrane proteins. Energy-dependent uptake follows binding to lipopolysaccharides. An acidic environment external to the cell membrane has been associated with increased transport, perhaps because of an increase in the membrane potential differential. However, a lower pH more commonly has been associated with increased membrane resistance; the disparity may reflect the different molecules of each aminoglycoside. An alkaline environment consistently appears to facilitate transport as does movement of cations out of the cell membrane. Uptake depends on a membrane-bound respiratory protein that is lacking in anaerobic organisms, leading to inherent resistance. The system also is deficient in facultative anaerobes such as *Enterococcus* spp. Active transport depends on a high oxygen tension in the environment rendering obligate anaerobes inherently resistant, and facultative anaerobes resistant in an anaerobic environment.<sup>91</sup> Cations such as calcium and magnesium in the lipopolysaccharide covering and cell membrane repel the

aminoglycosides, impairing transport into bacterial cells (and renal tubular cells). Removal of calcium (e.g., through use of chelating agents such as ethylenediaminetetraacetic acid [EDTA]) or a decrease in serum calcium (i.e., hypocalcemia) facilitates aminoglycoside movement into the cell.<sup>5,90</sup> Hyperosmolarity and decreased pH also decrease drug movement into the cell.<sup>80</sup>

**KEY POINT 7-12** Efficacy of aminoglycosides is dependent on active transport. Accordingly, efficacy is markedly reduced to absent in an anaerobic environment, and anaerobes are not susceptible.

Once inside the cell, aminoglycosides bind to ribosomes (see Figure 7-6). Although their mechanism of action is not completely understood, aminoglycoside antimicrobials bind to the 30S ribosomal subunit, which, as the initiator of protein synthesis, plays a crucial role in providing high-fidelity translation of genetic material.<sup>92</sup> Binding is so effective that polyribosome formation is prevented, and protein synthesis is impaired because of altered synthesis and misreading. Thus, in contrast to most bacteriostatic drugs, which bind to 50S ribosomes, the aminoglycosides are more likely to achieve bactericidal concentrations safely in animals. Although only a small amount of aminoglycoside appears to penetrate the cell membrane, the initial impact on ribosomes is sufficient



**Figure 7-6** The mechanism of action of ribosomal inhibitors. The bacterial ribosome is a complex structure, composed of three RNA molecules (peptidyl and aminoacyl tRNAs and mRNA) and more than 50 proteins. The ribosome is formed as two subunits, 30S (including a 16S portion) and 50S (including a 5S portion; S referring to sedimentation rate), which join when protein synthesis is initiated and separate when completed. The process of initiation begins by the formation of a functional ribosome. The 30S subunit complexes with mRNA (which codes tRNA synthesis) and forms an initiation complex consisting of tRNA, the first amino acid (methionine), and three initiation factors (IF 1-3), one of which is an energy source, GTP. The initiation complex joins the 50S subunit, forming the (mature) 70S ribosome; it is the mature 70S ribosome that initiates protein synthesis. The mature ribosome is composed of an "A" or amino acid site (30S), the "P" or peptidyl site (50S), which contains a peptidyl transferase center; and an E or exit site adjacent to the P site. The aminoacyl tRNA carrying the amino acid binds to the A site, which then complexes to an elongation factor. Release of energy by GTP causes a conformational change or contracting motion, and the the peptide forming at the P site joins the amino acid at the A site. A nucleophilic attack initiated by the aminoacyl tRNA results in bonding of the amino acid to the growing peptide (transpeptidation); the growing peptide is then translocated to the P site. The elongation step is repeated until protein synthesis is completed.<sup>278</sup> The aminoglycosides inhibit ribosomal initiation (as the 30S subunit becomes activated to 70S); binding is irreversible, contributing to a bactericidal effect. Tetracyclines bind to the 16S portion of the 30S subunit of ribosomes, preventing the translocation of the amino acid from transfer RNA (tRNA) to the codon of messenger RNA (mRNA). Chloramphenicol and erythromycin prevent the transfer of peptides by binding to the 50S subunit. Erythromycin and clindamycin prevent translocation of the peptide. Drugs that act at the same site should not be used in combination.

to alter cell membrane proteins and permeability such that additional drug is able to penetrate the cell. Irreversible saturation of the ribosomes results in cell death and accounts for the concentration-dependent killing effects of the drugs; the irreversible nature of binding contributes to bactericidal effects.<sup>92</sup> Aminoglycosides are rapidly bactericidal, with efficacy and the postantibiotic effect of aminoglycosides correlating to peak concentrations, which ideally should be at least 10 times the MIC of the target organism.<sup>93-97</sup> Drugs that target the 50S ribosomal unit (e.g., chloramphenicol, linezolid) may interfere with intracellular movement and thus rapid killing effects of aminoglycosides.<sup>94</sup> Because toxicity of aminoglycosides is correlated with trough concentrations (later discussed as adverse effects of aminoglycosides), treatment is implemented with once-daily therapy at high doses. This approach is both clinically<sup>85,97,98</sup> and experimentally<sup>95,96,99</sup> equal to or more efficacious and safer than the traditional frequency of administration (i.e., two to three times daily). The appropriateness of this dosing method may vary with the organism and the immunocompetence of the patient.

**KEY POINT 7-13** The aminoglycosides exhibit a clinically important concentration-dependent postantibiotic effect.

### Spectrum of Activity

The spectrum of activity of aminoglycosides (see Tables 7-2 through 7-4, 7-9 and 7-10) includes most aerobic gram-negative bacteria, particularly *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Proteus* spp. and *Serratia* spp.<sup>5,16,80,91,101</sup> Newer aminoglycosides such as gentamicin, tobramycin, amikacin, and netilmicin have a wider spectrum compared with older compounds such as streptomycin and kanamycin. These drugs are also effective against selective aerobic gram-positive organisms, most notably *Staphylococcus* spp. However, they generally should not be used as sole agents against gram-positive organisms. Synergism against gram-positive isolates has been demonstrated when combined with penicillins or vancomycin.<sup>80</sup> Aminoglycoside activity against *Enterococci* spp. is adequate only when used synergistically with a cell wall-active antibiotic, such as beta-lactams or vancomycin.<sup>92</sup> Among the aminoglycosides, based on clinical isolates in humans, netilmicin has the lowest

MIC<sub>90</sub> toward *Enterococcus* spp. and, along with tobramycin, *Staphylococcus* spp. Of the aminoglycosides most commonly used in dogs and cats, gentamicin has a much lower MIC<sub>90</sub> than amikacin toward *Staphylococcus* spp., even accounting for differences in breakpoint MICs. Gentamicin is preferred to amikacin for treatment of *Staphylococcus* infections, based on a rabbit model of endocarditis.<sup>101</sup> Further, a recent comparison of activity of 1000 isolates also found gentamicin to be more effective than amikacin toward *Staphylococcus* spp.<sup>102</sup> In this same report, the authors noted that gentamicin also had lower MIC toward many enterobacteriaceae but that amikacin achieved higher serum concentrations (and has a higher breakpoint MIC), thus negating this benefit.<sup>102</sup> Gentamicin and tobramycin have a very similar spectrum toward gram-negative aerobes. They and amikacin are effective against *P. aeruginosa*, *Proteus* spp. and *Serratia* spp. Gentamicin is the least effective of the three against *P. aeruginosa* but most effective against *Serratia marcescens*.<sup>92</sup> Amikacin generally is most effective against *P. aeruginosa*. With the exception of *Pseudomonas* species (usually an obligate aerobe, although exceptions have been reported), these organisms are facultative anaerobes and, if cultured aerobically from an anaerobic environment, may fail to respond to aminoglycoside therapy in the patient. The aminoglycosides are also effective against *Nocardia* and selected atypical mycobacterial organisms.

**KEY POINT 7-14** In general, gentamicin may be more efficacious toward *Staphylococcus* spp. whereas amikacin may be more efficacious toward *Pseudomonas* spp.

### Resistance

Besides the inherent resistance of anaerobic organisms (owing to decreased active transport), resistance to aminoglycosides is acquired as a result of decreased cell entry; altered porin size in the gram-negative organism is less important.<sup>90</sup> Resistance also includes altered ribosomal structure (uncommon except for *Enterococcus* spp.) and, more commonly, destruction by microbial enzymes inside the cell. Resistance to gentamicin involving altered ribosomal structure by *Enterococcus* spp. generally affects all aminoglycosides, as well as penicillins and vancomycin. An exception is streptomycin, which is destroyed by a different enzyme and may remain effective toward *Enterococcus*.<sup>90</sup>

Enzymatic destruction is the most important mechanism of acquired resistance in clinical isolates, in part because it is acquired through conjugative plasmids. Resistance reflects enzyme modification of the amino or hydroxyl groups of the drugs. The modified drug can no longer bind to ribosomes. Impact on efficacy varies among the different aminoglycosides. For example, target sites of destruction by the enzymes are harder to reach with amikacin. Consequently, amikacin is less vulnerable to resistance than are other aminoglycosides and is frequently effective toward otherwise multidrug-resistant isolates.<sup>5,80,90</sup> At least three different enzyme classes exist, classified by phenotypes as to phosphotransferases, acetyltransferases, and nucleotidyltransferases. Among the aminoglycosides used clinically in veterinary medicine, gentamicin

and kanamycin more commonly act as substrates for phosphotransferases and acetyltransferases, whereas amikacin and tobramycin are more common substrates for the nucleotidyltransferases. Of the three enzymes, the phosphotransferases are more likely to be associated with high-level resistance.

Resistance to aminoglycosides by *Staphylococcus* spp. reflects chromosomal mutations in transmembrane potentials and thus drug uptake. Mutational resistance caused by changes in ribosome binding sites has been identified primarily against streptomycin, the use of which is limited. However, whereas the four gram-negative organisms most commonly causing (blood) infection in humans (*Pseudomonas*, *Klebsiella*, *E. coli*, and *Enterobacter*) remain susceptible (>95%) to the greatest number of aminoglycosides, up to 40% of *S. aureus* organisms are resistant to gentamicin. Current investigations are attempting to identify the mechanism by which enzymatic destruction of aminoglycosides might be inhibited, much the same as beta-lactamases have been used to prevent beta-lactam destruction.<sup>92</sup> Low-level resistance caused by multi-drug efflux mechanisms has been identified in *P. aeruginosa*, *Burkholderia* sp. (previously *Pseudomonas*), *Acinetobacter*, spp. and *E. coli*.<sup>92</sup>

**KEY POINT 7-15** Enzymatic destruction of aminoglycosides is increasingly limiting efficacy, particularly for gentamicin toward *Staphylococcus* spp.

Adaptive resistance has been described for the aminoglycosides (see Chapter 6). In humans up to 40 hours may need to elapse between doses for full bacterial susceptibility to commence.<sup>103</sup> This phenomenon supports the once-daily use of the aminoglycosides.

### Pharmacokinetics

The aminoglycosides are polar, water-soluble weak bases, and as such they are poorly absorbed from the gastrointestinal tract. An exception might occur in very young animals that are still absorbing colostrum or in the presence of inflammatory gastrointestinal disease.<sup>90,91</sup> Kanamycin, which is structurally very similar to amikacin, behaves similarly to amikacin.<sup>91</sup> Aminoglycosides are administered topically (including aerosolization and incorporation in beads) or parenterally but can be used orally for local bacterial cleansing of the gastrointestinal tract. However, absorption from body cavities may be sufficiently rapid to cause neuromuscular blockade.<sup>90</sup> Absorption will also occur when applied topically to large wounds with subcutaneous exposure; absorption may be sufficient to cause toxicity.<sup>104</sup>

Although aminoglycosides are distributed to extracellular fluids, their penetration into many tissues is considered poor (see Table 7-5). However, therapeutic concentrations can be attained in synovia and in pleural and peritoneal fluid, particularly if membranes are inflamed. Penetration of bronchial secretions is generally better than that of many beta-lactam antibiotics. However, therapeutic concentrations generally are not attained in CSF, ocular fluids, bile, milk, and prostatic secretions. Further, killing of intracellular (e.g., *Enterobacter* spp.) organisms may be limited.<sup>94a</sup> Intrathecal

administration has been indicated for CNS infections, but the advent of third- and fourth-generation cephalosporins and carbapenems has preempted this need.<sup>90</sup> Aminoglycosides are actively accumulated by renal tubular cells, but this may be of more relevance to toxicity rather than efficacy. In addition to anaerobic environments, the efficacy of aminoglycosides is reduced in an acidic environment such as might occur in the urine, ascitic fluid, and abscesses.

**KEY POINT 7-16** As water-soluble weak bases, aminoglycosides are not orally absorbed, do not penetrate tissues well, and are excreted in proportion to the glomerular filtration rate.

Drug elimination half-life of the aminoglycosides is generally less than 2 to 4 hours (see Table 7-1). The aminoglycosides are eliminated by glomerular filtration, which is a relatively inefficient process. Drug accumulates in acidic urine, and alkaline urine pH facilitates reabsorption. Urine concentrations have been described for selected aminoglycosides in dogs.<sup>105</sup> Dosing of gentamicin (6.6 mg/kg), tobramycin (3 mg/kg), and amikacin (15 mg/kg) subcutaneously in divided doses at 8-hour intervals (not recommended) for five consecutive doses and kanamycin at 11 mg/kg at 12-hour intervals (also not recommended) for 4 doses generated mean interval urine concentrations ( $\mu\text{g/mL}$ ) of  $107 \pm 33$  for gentamicin;  $66 \pm 39$  for tobramycin,  $342 \pm 153$  for amikacin, and  $473 \pm 306$  for kanamycin.<sup>105</sup>

The disposition of aminoglycosides varies somewhat among animals, primarily because of differences in glomerular filtration rates. Elimination is slower in larger animals because glomerular filtration rate decreases with body size; this may be offset by differences in  $V_d$ . Dosing based on metabolic rate normalizes the rate of elimination and might be considered in patients predisposed to aminoglycoside nephrotoxicity, although estimates of glomerular filtration rate based on extracellular fluid volume may be more accurate.<sup>106</sup>

A number of investigators have described the disposition of aminoglycosides in dogs or cats (Table 7-1). Gentamicin has been studied in dogs by multiple investigators. Riviere<sup>107</sup> described the disposition in 5-month-old Beagles ( $n = 11$ ). Clearance was  $4.1 \pm 0.6 \text{ mL/min}\cdot\text{kg}$  and  $V_d$  (area) was  $0.4 \pm 0.04 \text{ L/kg}$ . Elimination half-life was  $61 \pm 8$  minutes. Wilson<sup>108</sup> studied gentamicin (3 mg/kg) in dogs ( $n = 6$ ) after intravenous, intramuscular, and subcutaneous administration. After intravenous administration, clearance was  $2.29 \pm 0.48 \text{ mL/min}\cdot\text{kg}$  and  $V_{dss}$  was  $0.172 \pm 0.025$ . Bioavailability approximated 95% for both intramuscular and subcutaneous routes, yielding a  $C_{max}$  of approximately  $10 \mu\text{g/mL}$  for either route, with time to peak concentration for intramuscular administration being 27 minutes compared with 43 minutes for subcutaneous administration. The elimination half-life was  $54 \pm 15$  minutes. Albarellos<sup>204</sup> studied gentamicin after intramuscular administration of 6 mg/kg for 5 days. After day 1, assuming 100% bioavailability, clearance was  $1.24 \pm 0.6 \text{ mL/min}\cdot\text{kg}$  ( $1.10 \pm 0.4$  by day 5), and  $V_d$  (area) was  $0.084 \text{ L/kg}$  ( $0.1 \pm 0.05$  day 5). Mean residence time was  $1.48 \pm 0.54$  hour ( $1.77 \pm 0.48$

by day 5; significantly prolonged) and half-life ranged from 0.55 to 1.46 hours. For IV administration, the  $V_{dss}$  after intravenous administration was  $0.23 \pm 0.04 \text{ L/kg}$  and clearance was  $2.64 \pm 0.24 \text{ mL/min}\cdot\text{kg}$ .

Jernigan and coworkers<sup>100</sup> have described the disposition of several aminoglycosides in cats (see Table 7-1). After intravenous administration of gentamicin (3 mg/kg) in cats ( $n = 6$ ),  $V_{dss}$  was  $0.12 \pm 0.02 \text{ L/kg}$  and clearance was  $1.1 \pm 0.25 \text{ mL/kg}\cdot\text{min}$ . Bioavailability after subcutaneous administration was  $83 \pm 14.8\%$ . Gentamicin was also studied in cats ( $n = 6$ ) after intravenous, intramuscular, and subcutaneous administration of 5 mg/kg.<sup>110</sup> After intravenous administration,  $V_{dss}$  was  $0.14 \pm 0.02 \text{ L/kg}$  and clearance was  $1.38 \pm 0.35 \text{ mL/min}\cdot\text{kg}$ ; mean residence time was  $1.8 \pm 43$  hour. Bioavailability after intramuscular and subcutaneous administration was 67.8 and 76.2%, respectively. Tobramycin was studied in six cats after 5 mg/kg.<sup>111</sup> After intravenous administration,  $V_{dss}$  was  $0.19 \pm 0.03 \text{ L/kg}$  and clearance was  $2.21 \pm 0.6 \text{ mL/min}\cdot\text{kg}$ ; mean residence time was  $90 \pm 16$  minutes. Bioavailability after intramuscular and subcutaneous administration was 103% and 99% respectively; bioavailability was also measured at greater than 150% for both routes in one set of studies, perhaps indicating decreased clearance owing to nephrotoxicity. Finally, amikacin (5 mg/kg) was studied in cats ( $n = 6$ ) after intravenous, intramuscular, and subcutaneous administration.<sup>112</sup> After intravenous administration,  $V_{dss}$  was  $0.17 \pm 0.02 \text{ L/kg}$ , and clearance was  $1.46 \pm 0.26 \text{ mL/min}\cdot\text{kg}$ ; mean residence time was  $118 \pm 14$  minutes. Bioavailability after intramuscular and subcutaneous administration was  $95 \pm 20\%$  and  $12.3 \pm 33\%$ , respectively.

Disposition of the aminoglycosides appears to vary among breeds. Kukanich<sup>113</sup> has compared the PK of amikacin (10 mg/kg, administered intravenously) in Greyhounds and Beagles ( $n = 6$  each). The volume of distribution ( $\text{L/kg}$ ) was smaller (0.18 versus 0.23), but clearance was less (2.1 versus  $3.3 \text{ mL}\cdot\text{kg}/\text{min}$ ) in Greyhounds, thus elimination half-life did not differ (0.8 and 0.9 hour for Greyhounds and Beagles, respectively). The bioavailability of amikacin in Greyhounds after subcutaneous administration was approximately 90%. Although extrapolated time 0 PDC was reported for both species after intravenous administration (86 and 70  $\mu\text{g/mL}$ , respectively, for Greyhounds and Beagles), this is not an appropriate target on which to base  $C_{max}/\text{MIC}$  (i.e., the  $C_{max}$  should be measured after distribution has occurred). However, compartmental analysis yielded concentrations extrapolated from the terminal curve (presumably reflecting postdistributional concentration; see Table 7-1). On the basis of these data and a target  $C_{max}/\text{MIC}$  of 8 (rather than 10), the respective subcutaneous doses (mg/kg) of amikacin recommended by the authors to target an MIC of 2, 4, and 8  $\mu\text{g/mL}$ , respectively, were for the Greyhound 6, 12, and 24 and for the Beagle, 11.5, 22, and 40.

The influence of endotoxemia on gentamicin disposition has been described in cats.<sup>114,115</sup> Elimination half-life was shorter ( $77 \pm 13$  minutes before and  $65 \pm 14$  after), but this change is not likely to be significant, in part because neither  $V_{dss}$  nor clearance was significantly different.

The disposition of aminoglycosides also differs among ages. PDCs are less in the neonate and pediatric patient because greater total body water and extracellular fluid compartments increase the  $V_d$  of the drugs from 0.25 to 0.35 L/kg (see Table 7-1). Renal clearance of aminoglycosides is less. Thus for young animals the dose of aminoglycosides should be increased; although elimination half-life may be longer, the current use of a 24-hour interval should preclude the need to lengthen it further in the pediatric patient. Disposition is also altered by disease. Dehydration and obesity increase PDCs, which may be of benefit for these concentration-dependent drugs. Intensive fluid therapy or other syndromes associated with accumulation of fluid at a site to which aminoglycosides distribute and endotoxemia decrease plasma aminoglycoside concentrations.<sup>91</sup> Ascites also will increase the  $V_d$  and half-life of aminoglycosides.<sup>116</sup> Aminoglycosides may accumulate and cause nephrotoxicity in the fetus and should not be used during pregnancy.<sup>90</sup> Elimination is impaired in the patient with renal disease; dosing regimens are usually modified by lengthening the interval on the basis of serum creatinine concentration (see the section on therapeutic use).

### Adverse Effects

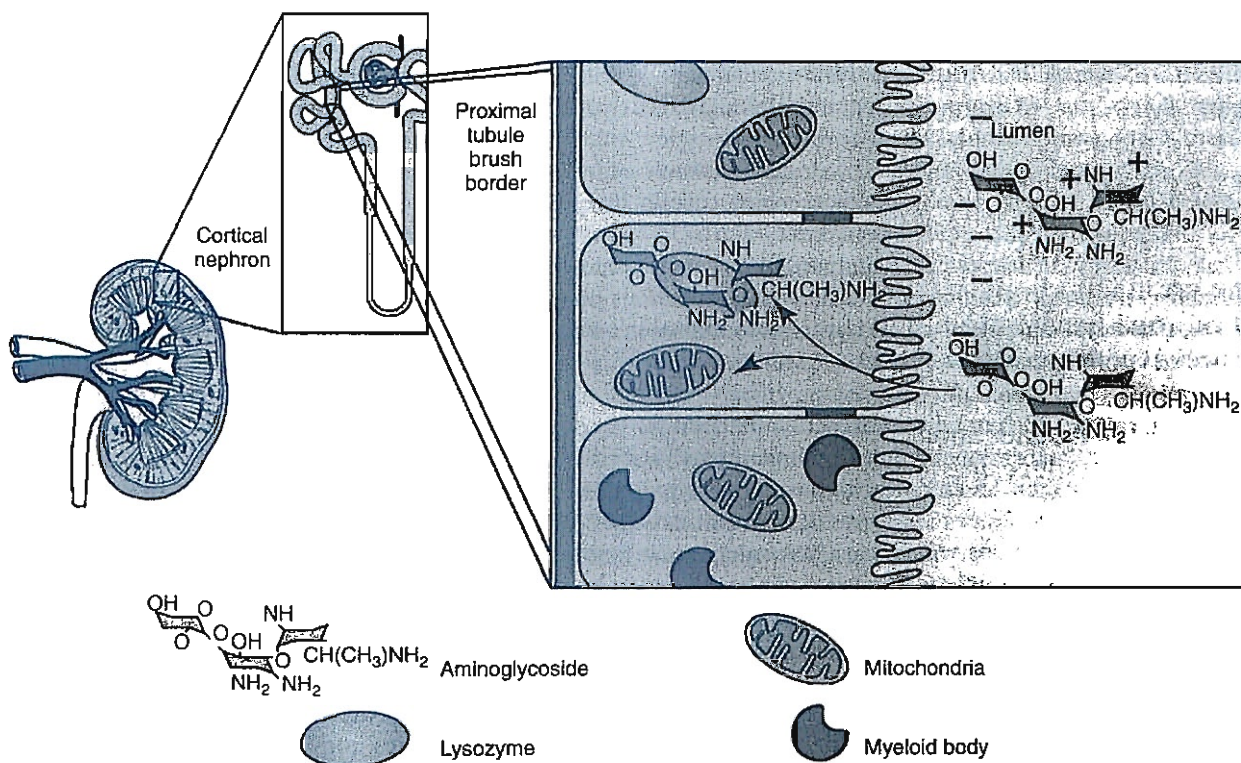
The aminoglycosides induce a glomerular and (principally) tubular nephrotoxicity; however, because of the regenerative capacity of the proximal tubule, toxicity is largely reversible

unless allowed to progress to an irreversible state (i.e., destruction of basement membrane). Toxicity results from active uptake into the renal tubular cell and disruption of cellular lysosomes (Figure 7-7). Impaired cellular respiration and synthesis of protective vasodilatory renal prostaglandins by the aminoglycoside may be important in the development of nephrotoxicity.

**KEY POINT 7-17** Aminoglycoside nephrotoxicity can be minimized if kidneys are allowed a drug-free period such that drug that has been actively accumulated in the kidney can be eliminated.

Reversible renal impairment occurs in up to 25% to 55% of human patients receiving aminoglycosides for more than 3 days, although the better-designed studies indicate a rate of 10% to 20%.<sup>90,117</sup> In humans aminoglycoside-induced nephrotoxicity is defined as an increase in serum creatinine concentration of 0.5 mg/dL in patients for which baseline concentration is < 3 mg/dL, or an increase in 1 mg/dL if the baseline is at or above 3 mg/dL.<sup>117</sup>

The exact mechanism of aminoglycoside-induced nephrotoxicity is not known. Toxicity begins as the anionic phospholipids of the renal tubular cell membranes attract and bind the cationically charged drugs. The relative nephrotoxicity of the different aminoglycosides reflects differences in their renal



**Figure 7-7** Nephrotoxicity of aminoglycosides occurs primarily in the proximal tubular cells. The cationic charge of the drugs is attracted to the anionic charge of the phospholipids in the cell membrane. The drug is actively accumulated in the cell by pinocytosis. Inside the cell the drugs accumulate in lysosomes, causing lysosomal disruption and release of myeloid bodies. Intracellular movement into lysosomes also limits intracellular efficacy. Mitochondrial function is also impaired. The effects of prostaglandin on renal blood flow may contribute to the toxicity of aminoglycosides. A number of factors increase or decrease the risk of toxicity (see text).

accumulation.<sup>90</sup> Nephrotoxicity may be related to the number of positively charged amino groups on the drugs; hence, neomycin is expected to be among the most nephrotoxic of the drugs.<sup>5</sup> An acidic local pH may enhance uptake by ionizing the aminoglycoside and thus increase the risk of toxicity. Of the clinically used aminoglycosides, neomycin is the most nephrotoxic and dihydrostreptomycin, the least. The nephrotoxicities of the other aminoglycosides are between these two extremes. Several studies have compared tobramycin and gentamicin (the latter is more concentrated), but controlled clinical trials in humans have failed to find a clinical difference in the nephrotoxicity potential between the two.<sup>90</sup> Studies comparing the nephrotoxic potential of amikacin with other aminoglycosides (but not gentamicin) also have been inconclusive.<sup>90</sup> A number of drugs increase the risk of nephrotoxicity (see the section on drug interactions).

The attraction of aminoglycoside cations to the renal tubular cell membrane can be competitively inhibited by divalent (e.g., magnesium or calcium) cations (e.g., ethylenediaminetetraacetic acid [EDTA]) or decreased in an alkaline urine (unionizing amine groups). Hypocalcemia or hypomagnesemia may increase the risk of aminoglycoside toxicity; in contrast, dietary calcium loading may protect against toxicity. Uptake of aminoglycosides also may be related to the amount of phosphatidylinositol in the cell membrane; the amount is disproportionately higher in renal cortex and cochlear tissues.<sup>91</sup>

Once the renal tubular cells are entered, aminoglycosides are then actively accumulated in the cell by pinocytosis; intracellular accumulation may result in concentrations greater than fiftyfold of that in plasma. Inside renal tubular cells, probably in part because of ion trapping, aminoglycosides are sequestered in lysosomes, which subsequently appear morphologically as myeloid bodies. The drugs are slowly eliminated in the urine as myeloid bodies, which contain drug, RNA, and DNA after the tubular cell dies.

The cause of tubular cell death induced by aminoglycosides remains unclear, although a number of cellular functions (in addition to lysosomal damage) are impaired; examples include phospholipases, sphingomyelinases, and ATPases. Mitochondrial respiration is decreased, impairing energy resources of the cell. Again, this may reflect interaction between the drug and mitochondrial cell membrane. Proximal tubular permeability may be impaired both directly as drugs interact with the cell membrane and indirectly as a result of impaired Na<sup>+</sup>, K<sup>+</sup>-ATPase activity. Aminoglycosides also alter glomerular function, perhaps by reducing the number and size of glomerular endothelial cells.<sup>91</sup> Finally, phospholipases important for renal prostaglandin synthesis are among the enzymes impaired by aminoglycosides. The initial decrease in glomerular filtration that accompanies aminoglycoside therapy may reflect the inability of the kidney to vasodilate in response to vasoconstrictor actions such as that signaled by angiotensin II.<sup>91</sup> This may reflect altered prostaglandin synthesis. As glomerular filtration declines, so may clearance of the aminoglycoside, thus increasing the risk of toxicity.<sup>90</sup>

The half-life of renal cortical aminoglycosides is approximately 100 hours. This and the fact that a critical

### Box 7-1

#### Minimization of Aminoglycoside Nephrotoxicity

1. Use once-daily therapy when appropriate.
2. Ensure adequate hydration status. If any doubt, or in a patient at risk, treat with sodium isotonic.
3. Maximize peak plasma drug concentration to ensure that  $C_{max}/MIC > 10$  and trough plasma drug concentration is  $< 2 \mu\text{g/mL}$ .
4. Monitor peak and trough concentration aminoglycoside concentration such that half-life and clearance can be followed.
5. Monitor urinary renal enzyme ( $\gamma\text{GT}$ ) to creatinine ratios, urine sediment, and serum creatinine.
6. Use the least nephrotoxic aminoglycoside (possible amikacin versus gentamicin).
7. Use the most effective aminoglycoside for the target organism (e.g., gentamicin for *Staphylococcus*, amikacin for *Pseudomonas*).
8. Use combination antimicrobial therapy, particularly with synergistic antibiotics and always for gram-positive organisms.
9. Avoid use of other nephrotoxic or nephroactive drugs, including antiprostaglandins, ACE inhibitors, and furosemide.
10. Administer during periods of activity (e.g., early morning in dogs, possibly evening in cats).
11. Treat with *N*-acetylcysteine.

aminoglycoside concentration must be reached before nephrotoxicity emerges generally preclude renal cortical nephrotoxicity before the first 3 days of therapy (Box 7-1).<sup>117</sup> No study has demonstrated a threshold of dosing or interval that ensures or predicts toxicity. Studies that have focused on aminoglycoside toxicity in dogs and cats have used dosing interval that ranges from 12 hours to constant intravenous infusion. Studies regarding aminoglycoside nephrotoxicity in cats have focused on doses of 35 mg/kg or more at intervals of 12 hours or less.<sup>91</sup> A bimodal course of aminoglycoside-induced nephrotoxicity has been described in the dog, with an initial subclinical phase characterized by a concentrating defect and an azotemic phase; different disease states might be predictable based on changes in pharmacokinetics.<sup>108</sup> Under experimental conditions, gentamicin at 4 mg/kg every 12 hours in dogs changes urine osmolarity within 7 days and an increase in serum creatinine by 17 days. Urinary prostaglandin E activity decreases before azotemia, which may be responsible for the state of nephrogenic diabetes insipidus. Whereas a single dose of 15 mg/kg gentamicin was associated with subclinical and morphologic changes in the kidney of young Beagles,<sup>119</sup> higher doses of 30 mg/kg administered at 8-hour intervals in dogs result in increases in urine gamma-glutamyltransferase within 2 days and serum creatinine within 9 to 12 days. Interestingly, a study that describes the disposition of gentamicins C1, C1a, and C2 in dogs found clearance of C1 to be twice as fast and  $V_d$  to be twice as high as for the other two gentamicins.<sup>120</sup> The investigators found that the renal binding of C1 is likely to be greater, suggesting that it is more likely to be nephrotoxic compared with C1a and C2.



Gentamicin (3 mg/kg) administered intravenously every 8 hours for 5 days to cats ( $n = 6$ ) was not associated with changes in serum or histologic indicators of renal or vestibular dysfunction.<sup>121</sup> Endotoxemia appears to cause more gentamicin renal medullary accumulation in cats but does not appear to be associated with increased renal pathology.<sup>115</sup> Tobramycin was associated with increased serum creatinine and/or BUN in 9 of 12 cats dosed twice with tobramycin despite washout periods,<sup>111</sup> suggesting that it may be more nephrotoxic than other aminoglycosides, at least in cats.

No indicator of renal damage induced by the aminoglycosides is sufficiently sensitive to prevent damage; indeed, damage will continue beyond detection with current methods. Changes in urine osmolality or sodium fractional clearance typical of the initial subclinical phase may detect a concentrating defect. However, this should be preceded by a release of renal tubular enzymes such as gamma-glutamyltransferase into urine. Measurement of the enzyme has been used experimentally to measure aminoglycoside toxicity. The enzymes increase within several days after damage has begun. However, 24-hour sample collection for these procedures is impractical. Measurement of the urine creatinine to gamma-glutamyltransferase ratio in spot samples of urine have proved useful in experimental models of aminoglycoside toxicity.<sup>123</sup> Ratios may not, however, change until several days after toxicity has begun.<sup>91</sup> A change in aminoglycoside clearance may be the most sensitive indicator of aminoglycoside toxicity (see Chapter 5.<sup>81,114,115</sup> In humans serum creatinine may increase up to 1 week after therapy is discontinued, indicating the potential for continued damage once the drug is discontinued,<sup>117</sup> presumably because accumulated drug remains in the tubules. Accordingly, nephrotoxicity is best avoided (see Box 7-1 and the section on therapeutic use).

The presence of renal disease is not a contraindication for aminoglycoside use, although it certainly raises the risk. Normograms have been designed in human medicine to reduce the risk of further damage (see the section on therapeutic use). The risk of nephrotoxicity is greater if any condition of the patient depends on renal prostaglandin formation, such as hypotension, shock, endotoxemia, renal or cardiac disease, or with concurrent drug therapy that impairs prostaglandin synthesis, such as nonsteroidal antiinflammatory drugs.<sup>5,126</sup> Metabolic acidosis (or an acidic urine pH) also predisposes the patient to aminoglycoside nephrotoxicity because drugs are ionized and attracted to the anionic changes of cell membranes.<sup>127</sup> Consequently, if the source of infection is in the urinary tract, maintaining an alkaline pH will enhance the efficacy of the aminoglycosides by facilitating their diffusion back into infected tissue (and bacteria), while decreasing renal tubular cell uptake of aminoglycosides, presumably because of decreased ionization of the drugs. Aminoglycoside toxicity was demonstrated to be temporal in rats,<sup>128</sup> being worse when rats were resting and least when active. Accordingly, dosing in the morning may be prudent for dogs; dosing at night might be considered for cats. Some patients (e.g., pediatric dogs <14 days of age, patients with diabetes mellitus or hypothyroidism) are protected against aminoglycoside- (gentamicin)-induced

nephrotoxicity because renal accumulation in the cortical tissues is limited.<sup>130,131</sup> Symptomatic hypomagnesemia, hypocalcemia, and hypokalemia associated with inappropriate urinary excretion of potassium despite low serum concentrations has been reported in humans after gentamicin therapy.<sup>132</sup> The magnitude correlated with the total cumulative dose of gentamicin. Risk factors included older age and long duration of therapy.<sup>123</sup> Note that hypomagnesemia and hypocalcemia may increase the risk of aminoglycoside toxicity by increasing the ease with which drugs enter the renal tubular cell.

**KEY POINT 7-18** The presence of renal disease is not a contraindication for aminoglycoside use, although it certainly raises the risk of adverse effects.

Studies have attempted to identify therapies that might treat or prevent aminoglycoside-induced nephrotoxicity. The role of prostaglandin analogs (e.g., misoprostol) in the prevention or treatment of aminoglycoside toxicity has not yet been established. Melatonin administered simultaneously to rats receiving gentamicin was associated with reduced nephrotoxicity.<sup>124</sup> Rate receiving L-Carnitine (40 to 200 mg/kg/day, injected) beginning 4 days before receiving doses of gentamicin ranging from 50 to 80 mg/kg had less nephrotoxicity (based on serum creatinine and histology) compared with untreated rats. Renal gentamicin concentrations were not different, suggesting that decreased aminoglycoside uptake by the renal tubular cell was not the mechanism of prevention. Proposed mechanisms were promotion of fatty-acid oxidation, increased mitochondrial ATP, and decreased formation of oxygen radicals.<sup>135</sup> Again, in rats, *N*-acetylcysteine (10 mg/kg intraperitoneally [IP]) protected against gentamicin (100 mg/kg subcutaneously/day  $\times$  5 days) induced nephrotoxicity.<sup>136</sup> This treatment apparently also has also been demonstrated to be otoprotective in human patients undergoing hemodialysis that are treated with gentamicin.<sup>137</sup> A federally funded human clinical trial is currently underway to validate the beneficial effects of *N*-acetylcysteine in patients with or at risk to develop aminoglycoside nephrotoxicity.

Aminoglycosides can cause an irreversible ototoxicity, although this is not likely to occur at therapeutic doses as long as trough concentrations are lower than 2 to 5  $\mu\text{g}/\text{mL}$  (lower should be targeted for gentamicin, higher for amikacin). However, a single dose of tobramycin was associated with ototoxicity in humans.<sup>80</sup> Like nephrotoxicity, ototoxicity reflects active uptake of the drug by hair cells of the cochlea. Both auditory and vestibular toxicity may occur. As with nephrotoxicity, the ototoxic potential of each drug varies. The drugs typically should not be given to a patient with a perforated eardrum. Aminoglycosides can cause neuromuscular blockade owing to impaired calcium release at myoneural junctions. The risk appears to be dose dependent and is greater with intravenous administration, in the presence of hypocalcemia, or when combined with other agents active at the myoneural junction (e.g., anesthetics, skeletal muscle relaxants). Neuromuscular blockade can be reversed by cholinesterase inhibitors and (cautiously) calcium.

### Drug Interactions

The risk of aminoglycoside ototoxicity and nephrotoxicity is increased when aminoglycosides are used in combination with one another or with nonsteroidal antiinflammatory drugs, diuretics (particularly loop-acting), angiotensin-converting enzyme inhibitors, amphotericin B, and other nephrotoxic (or nephroactive) or ototoxic drugs. The risk of neuromuscular blockade is increased with the combination of aminoglycosides and intravenous calcium, calcium channel blockers, and gas anesthetics and other neuromuscular blocking agents, including atacurium. Edrophonium will reverse the latter, whereas calcium supplementation can reverse any neuromuscular blockade.<sup>2</sup>

As weak bases, the aminoglycosides may chemically inactivate weak acids; inactivation has been documented *in vitro*<sup>138</sup> and *in vivo*<sup>139</sup> between tobramycin and extended-spectrum penicillins but not carbapenems.<sup>140</sup> Tobramycin appears more amenable to inactivation than does amikacin.<sup>139</sup> *In vivo* inactivation is more likely to occur in patients with renal disease for which PDC may be higher than in normal patients. Chemical inactivation might also occur in urine as higher concentrations are achieved. In general, the aminoglycoside is inactivated rather than the penicillin simply because the penicillin is present at much higher concentrations compared with the aminoglycoside.

Synergism between aminoglycosides and cell wall-active antimicrobials has been documented against *Enterococcus* spp. as well as some strains of Enterobacteriaceae, *P. aeruginosa*, staphylococci (including MRSA), and other microorganisms. However, these organisms are not always inhibited by the combination of aminoglycoside and cell wall-active compounds. Indeed, antagonism has been described between aminoglycosides and beta-lactams against a MRSA, presumably owing to induction of an aminoglycoside-modifying enzyme.<sup>92</sup>

### Therapeutic Use

Despite their ability to cause nephrotoxicity, the aminoglycosides remain the most effective drugs for the treatment of serious gram-negative infections. They are also effective (combination therapy recommended), against *Staphylococcus*, *Nocardia*, *Mycoplasma*, and selected *Mycobacteria* spp. Caution is recommended in their use for infections in tissues that are difficult to penetrate and infections that may be located in an anaerobic environment. Combination therapy and topical therapy (in concert with systemic therapy) should be considered whenever possible for serious or complicated infections or in the presence of intracellular infections. Aminoglycoside-impregnated calcium hydroxyapatite or methyl methacrylate beads and methyl methacrylate cement have been used with apparent success in orthopedic procedures (see Chapter 6).<sup>140a</sup> Aminoglycosides cannot be given orally with the intent of systemic effects, and their use might be limited to hospitalized patients. However, once-daily therapy increases the convenience and safety of outpatient aminoglycoside therapy.

The pharmacologic rationale for once-daily (also called extended-interval) dosing of aminoglycosides includes their concentration-dependent bacterial killing, minimization of the adaptive resistance, the presence of a postantibiotic effect,

and avoidance of renal cortical drug accumulation (i.e., providing a drug-free period to facilitate excretion) such that trough concentrations reach a low target.<sup>117</sup> As early as 1984,<sup>141</sup> a fixed-dose, prolonged interval was known to be safer than a reduced dose and fixed interval in regard to nephrotoxicity in dogs. Recent studies in dogs, humans, and experimental models have supported a 24-hour dosing interval (administering the total daily dose once a day) for aminoglycoside therapy. The once-daily dose of an aminoglycoside necessary to impair renal function has not been determined, in part because different drugs are studied at different doses and intervals. Because clinical patients are likely to be characterized by changes that predispose to toxicity, studies in normal animals may not be relevant. Once-daily administration of gentamicin was concluded to be safe for 5 days in dogs at a single daily dose of 6 mg/kg.<sup>108</sup> Maximum concentration ( $C_{max}$  ( $\mu\text{g/mL}$ ), was 9.2 at a  $T_{max}$  of 0.48 hours. Mean trough gentamicin serum concentrations were 0.1  $\mu\text{g/mL}$ . Although deemed safe, serum creatinine and urea nitrogen were increased and specific urine gravity decreased in one dog and granular casts were evident in two dogs.

Many clinical trials have been performed in humans to assess the safety and efficacy of once-versus multiple-daily dosing of aminoglycosides. Differences in objectives, patients, methodologies, and conclusions have led to confusion. Several meta-analyses have been performed in humans that focuses on clinical efficacy and either nephrotoxicity or ototoxicity in patients treated with aminoglycosides once versus multiple times daily. The number of trials included in each meta-analysis ranged from 21 to 26; the number of persons studied by each meta-analysis was 2100 to more than 3000. Barza's group<sup>142</sup> found that once-daily administration of aminoglycosides in patients without preexisting renal failure was as effective as multiple-daily dosing and was associated with a lower risk of nephrotoxicity and no greater risk of ototoxicity. Further, once-daily dosing was more convenient and less costly. A second (22 studies)<sup>143</sup> and third meta-analysis (26 studies)<sup>144</sup> found the rates of efficacy and toxicity were similar and convenience and reduced cost justified the once-daily approach. Another study found that gentamicin (once or multiple times daily) and ticarcillin-clavulanic acid, either alone or combined with gentamicin, was associated with the same efficacy and nephrotoxicity renal function was better preserved with either once-daily gentamicin combined with ticarcillin-clavulanic acid or ticarcillin-clavulanic acid alone.<sup>145</sup> However, in humans, experts continue to advise that extended-interval aminoglycoside dosing not be used in patients with endocarditis, mycobacterial infections, or burns. Further, a simple once-daily approach to aminoglycoside therapy should not be used if the patient's creatinine clearance is less than 20 mL/min or in patients in hemodialysis because of marked alteration of PK in these patients. Rather, monitoring should be the basis of dosing in these patients.<sup>146</sup> Further, for obese patients (actual body weight > 20% above ideal body weight [IBW]), the dose should be reduced using the following formula that adjusts weight: obese dosing weight =  $IBW + 0.4(\text{actual weight} - IBW)$ .<sup>147</sup> A number of normograms

have been developed for use in humans to support the design of aminoglycoside dosing regimens that will be effective yet safe in patients with renal disease. Generally, the normograms are based on creatinine clearance and other patient factors. However, in general, the normograms underestimate the dose necessary to achieve a therapeutic maximum drug concentration. Methods using probabilistic or deterministic methods are currently being investigated.<sup>148</sup> However, therapeutic drug monitoring continues to be the preferred method to allow calculation of individual patient PK.<sup>117,146</sup> Indeed, a meta-analysis that compared once-daily multiple-dosing therapy and dosing based on PK found that basing doses on individual PK was the safest approach to dosing with aminoglycosides.<sup>117</sup> AUC based on two time points has enhanced prediction of dosing regimens for aminoglycosides in children with cystic fibrosis.<sup>149</sup> However, the distribution phase of aminoglycosides is sufficiently slow that the first sample probably should be collected no earlier than 1 hour after dosing is complete. Monitoring peak (no earlier than 1 hour, to ensure complete distribution) and detectable trough concentrations (no later than 2 to 3 half-lives after the peak to ensure concentrations are still detectable) will allow estimation of half-life, and (if given intravenously) Vd and clearance (see Chapter 5). Pretreatment and posttreatment comparisons may be useful in the early detection of significant changes in renal function, which will also help guide safe therapy. The clinical pharmacologist offering recommendations will be able to determine these parameters regardless of the actual timing (i.e., 1 versus 1.5 hours for peak, 4 versus 8 hours for trough); however, accuracy in reporting the time that samples were collected is critical for proper recommendations when the samples are collected for determination of half-life.

Maintaining hydration is probably the single most important means by which the risk of aminoglycoside-induced nephrotoxicity can be minimized. Ototoxicity also can be minimized by hydration and avoidance of topical administration, particularly in the presence of a perforated tympanum. Although gentamicin is the most economical aminoglycoside, amikacin should be considered for serious infections because of its improved resistance to antimicrobial destruction and better efficacy against some organisms, including *P. aeruginosa*. The aminoglycosides are often used in combination with other antimicrobials that have a less comprehensive gram-negative spectrum. As with imipenem, the aminoglycosides cause minimal endotoxin release in patients suffering from gram-negative infections associated with a large inoculum.<sup>74</sup>

## DRUGS THAT TARGET NUCLEIC ACIDS

### Fluorinated Quinolones

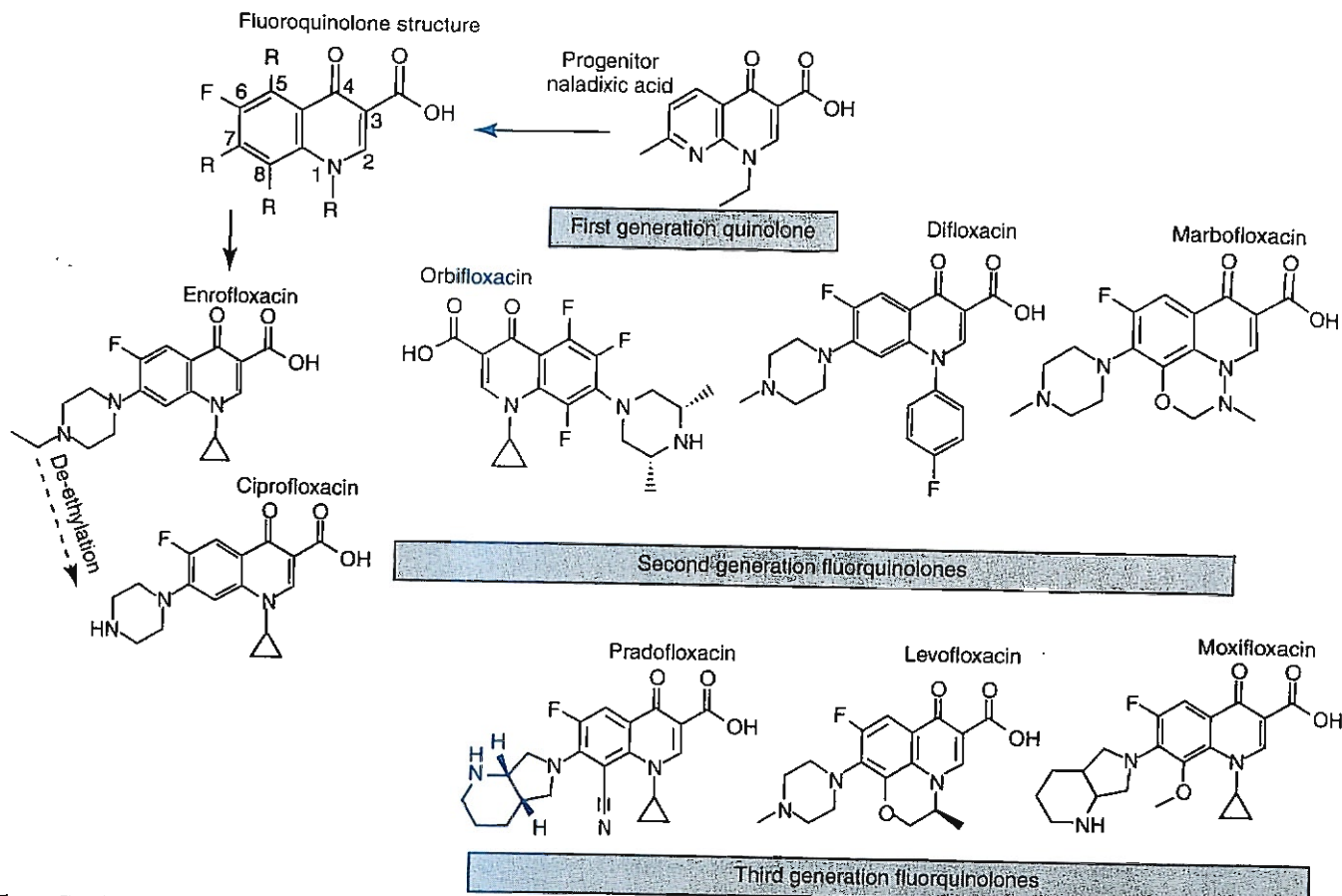
The fluorinated quinolones (FQs) are among the most recent classes of antimicrobials to be developed for treatment of bacterial infections. These synthetic drugs are minimally toxic yet have been effective in the treatment of many aerobic gram-negative organisms and selected gram-positive organisms. The desire to expand their spectrum of activity and the advent of resistance has led to innovated structural changes.

### Structure-Activity Relationship

A review of the development of FQs is worthwhile, not only to facilitate understanding of their actions but also to provide insight regarding the advantages of so-called designer drugs. Two decades elapsed between the development of nalidixic acid, the progenitor of the FQs, and norfloxacin, the first of the FQs to be approved for use. Among the FQs currently used for treatment of susceptible infections in dogs and cats, ciprofloxacin was first approved for use in humans in 1986, with its veterinary counterpart, enrofloxacin, rapidly following in 1991. Extensive use of these drugs has exposed the need for improvements and newer clinical indications; pharmaceutical companies have been attentive to addressing these needs.

Nalidixic acid is the progenitor of the FQs (Figure 7-8). Synthetic manipulations, including but not limited to the addition of a fluorine atom, have broadened the antibacterial spectrum; enhanced tissue penetrability; reduced (some) side effects (perhaps while contributing to others); and, most recently, decreased the risk of resistance. Currently marketed FQs generally consist of a quinolone ring nucleus, the target of most initial structural manipulations (Figure 7-9), or a naphthyridone ring structure, which replaces the nitrogen at carbon 8 on the quinolone structure (enoxacin, tosufloxacin, trovafloxacin, and gemifloxacin). The quinolone nucleus contains a carboxylic acid group at position 3 and an exocyclic oxygen at position 4 (hence the term "4-quinolones"); these are the active DNA gyrase binding sites, and thus these sites generally are not chemically manipulated. The structures yield two pKas for most FQs, rendering them amphoteric; they can act as weak bases, weak acids, or neutral compounds. For example, the carboxylic acid of enrofloxacin has a pKa of 6 and the amine group a pKa of 8.8. The side chain attached to the nitrogen at position 1 affects potency. The ethyl group at this position on nalidixic acid and the first of the clinically used FQs, norfloxacin, was replaced with a bulkier group (e.g., the cyclopropyl group of ciprofloxacin), which enhanced both gram-negative and -positive spectra. Substitution at position 5 also improved the gram-positive spectrum; however, it was the addition of a fluorine atom at position 6 that profoundly enhanced the gram-positive spectrum. The addition of a piperazyl ring, containing a heterocyclic nitrogen, at position 7 also was a critical improvement. This addition improved bacterial penetration (potency) and added *P. aeruginosa* to the gram-negative spectrum. The combination of the fluorine atom with a piperanyl ring produced the "breakthrough" class of FQs used today; norfloxacin was the first of these FQs to be approved in the United States.

Chemical manipulations continue to improve the FQs in terms of spectrum, potency, and avoidance of resistance. Substitutions on the piperazyl (e.g., ofloxacin, its L isomer, levofloxacin, and sparfloxacin) enhance the gram-positive penetration, whereas substitutions at position 8 enhance anaerobic activity (e.g., sparfloxacin, pradofloxacin, moxifloxacin). Substitutions at these sites with halogens such as chlorine or fluorine (e.g., 8-chloroquinolones or 8-fluoroquinolones [sparfloxacin]) result in ultraviolet unstable compounds (particularly the chloro substitution), which can cause phototoxicity. In contrast, substitution of a methoxy-group at the 8 position



**Figure 7-8** Various substitutions of the core chemical structure of the fluorinated quinolones have improved their spectrum, efficacy, and tissue penetration. The efficacy of the fluorinated quinolones depends on the ketone group at position 4 and on the carboxylic acid at position 3 (necessary for inhibition of DNA gyrase). The combination of the fluorine at position 6 (which markedly expanded the gram-positive spectrum) and the substitution of a piperyl ring at position (which enhanced efficacy towards *Pseudomonas aeruginosa* as well as increased tissue penetrability) represented a “breakthrough” for the fluorinated quinolones (e.g., enrofloxacin and its active metabolite, ciprofloxacin). Substitutions at position 8 increase the anaerobic spectrum (e.g., pradofloxacin). The addition of larger side chains may impair microbial resistance mechanisms.

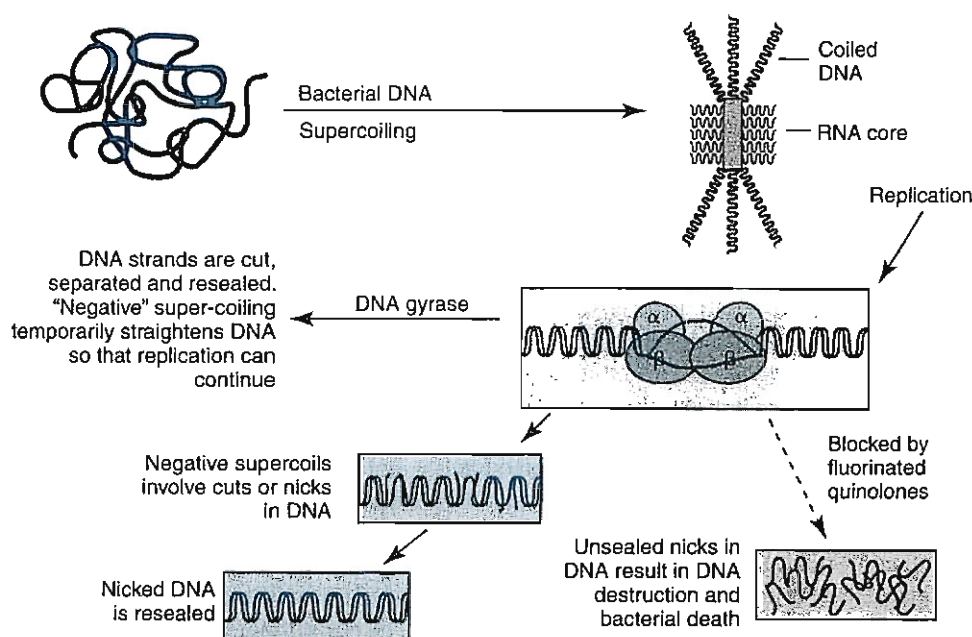
(e.g., moxifloxacin, gatifloxacin) confers good anaerobic activity but without risk of phototoxicity. Recent improvements (in human medicine) focus on increasing the efficacy of FQs toward pneumococci and MRSA, as well as other gram-positive cocci, Enterobacteriaceae, *Pseudomonas*, and anaerobes, and methods by which resistance might be minimized.

**KEY POINT 7-19** Chemical manipulations of fluorinated quinolones improve potency, broaden the spectrum, and decrease resistance.

Four drugs are currently approved for oral use in small animals in the United States: enrofloxacin (the first approved, for both dogs and cats, also approved for injectable [SC] use in dogs), followed rapidly by orbifloxacin (dogs and cats), difloxacin (dogs), and marbofloxacin (dogs and cats) (see Figure 7-8). Pradofloxacin may be undergoing consideration for approval for use in dogs in the United States. Variations in the chemical structures of these drugs may result in subtle differences in potency, efficacy, and tissue distribution. Human-marketed FQs, particularly ciprofloxacin and increasingly

levofloxacin, continue to be prescribed by veterinarians. Care should be taken to ensure that differences in disposition between humans and dogs or cats are considered when using these drugs. In their guidance to industry, the FQs have been indicated by the Food and Drug Administration (FDA) as “drugs of interest”; as such, veterinary use of these or newer FQs approved for use in humans is likely to draw scrutiny by allied health professions, including regulatory agencies. Note that use of drugs intended for human use (including cheaper generic drugs) instead of veterinary drugs solely because the former are less expensive is likely to be a disincentive for veterinary manufacturers with regard to future approvals of drugs for animals. Further, Animal Medical Drug Use Clarification Act stipulates that the conditions under which extra-label drug use is allowed include the lack of availability of a veterinary approved drug that meets the patient’s needs. Extra precautions should be taken when prescribing human-medicine drugs to ensure judicious use.

Because enrofloxacin was the first of the veterinary FQs to be approved for use in dogs and cats, it often is the gold standard on which subsequent drug approvals are based and



**Figure 7-9** The mechanism of action of fluorinated quinolones. During DNA synthesis, the double strands of circular bacterial DNA are in a tightly (negatively) coiled state (negative referring to the direction of the coils). The DNA strands are “unzipped” to allow either messenger RNA or a new DNA strand to be synthesized. The unzipping induces stress and the subsequent formation of positive supercoils, that ultimately must be removed. DNA gyrase, a topoisomerase, directs double-stranded breaks in the DNA. After DNA synthesis, the daughter chromosomes are unlinked by topoisomerase IV. Both DNA gyrase and topoisomerase IV are essential to bacteria and either or both are targeted by the fluorinated quinolones. Drugs that target both enzymes may require multiple mutations for resistance to emerge.

upon which clinical trials evaluating FQ efficacy are based. Because it is structurally similar to ciprofloxacin and because it is metabolized (up to 50% of the AUC of bioactivity) to ciprofloxacin in many species, much of the PD information in the human literature regarding efficacy for ciprofloxacin is applicable to enrofloxacin. However, exceptions occur, particularly with regard to PK considerations. Further, some differences exist in regard to pharmacodynamics between ciprofloxacin and enrofloxacin. Although marbofloxacin has been approved for a shorter period in the United States compared with enrofloxacin, it has been used since 1994 in Europe, and a considerable amount of information is available regarding this drug. In contrast, less information is available for orbifloxacin and particularly difloxacin.

### Mechanism of Action

The FQs currently are the only veterinary-approved antimicrobials that directly inhibit DNA synthesis. Bacterial DNA, is circular and can be up to 1.3 mm long, necessitating a negatively supercoiled state surrounding the RNA core (see Figure 7-9).<sup>150,151</sup> During DNA synthesis, the double strands of DNA must be uncoiled or “unzipped” to allow either messenger RNA to interpret or a new DNA strand to be synthesized. The unzipping of the double strands induces positive supercoiling, which leads to undue stress in the individual strands. Accordingly, DNA gyrase (topoisomerase II), directs double-stranded breaks in the DNA, thus inducing a negative supercoil configuration, balancing the positive supercoils. Once DNA polymerase passes through a break in the strand, the break is

repaired. Topoisomerase IV separates the daughter DNA molecules produced by DNA replication.<sup>152</sup> Both DNA gyrase and topoisomerase IV are essential to bacteria replication; both are targeted by FQs either individually or sequentially, depending on the drug and organism.<sup>151</sup>

Bacterial topoisomerases are ATPase-dependent enzymes. Each exists as a tetramer consisting of two A and two B subunits. For DNA gyrase, the subunits are encoded by the genes *gyrA* (2517 bp) and *gyrB* (2060 bp), respectively, and for topoisomerase *parC* and *parA*, respectively. The primary enzyme responsible for activity varies with the organism and influences the target of the FQ. DNA gyrase is the primary target in *E. coli*, other gram-negative organisms, and *Mycobacterium tuberculosis*, whereas topoisomerase IV is the primary target of *S. aureus* and (probably) other gram-positive organisms.<sup>151,153</sup> The efficacy of the FQs against various microbes can be explained, in part, by the presence or absence of the target enzymes, as well as drug preference for different enzymes (which in turn can be related to chemical structure [see above]). For example, unlike most other bacteria, *M. tuberculosis* lacks topoisomerase IV and might be less susceptible than other microbes that have both targets. Ciprofloxacin prefers topoisomerase IV, whereas moxifloxacin prefers DNA gyrase. Accordingly, bactericidal activity of moxifloxacin might be (and clinically appears to be) better compared with ciprofloxacin against *M. tuberculosis*. Efficacy of FQs is related to the number of molecules that interfere with the target topoisomerase; interference is irreversible, resulting in concentration-dependent effects.

**KEY POINT 7-20** Differences in efficacy of the fluorinated quinolones reflects, in part, the preferred topoisomerase targeted by the drug.

The MICs of the FQ for susceptible organisms tend to be low compared with most other antimicrobial drugs. DNA gyrase actions are inhibited at concentrations of 0.1 to 10  $\mu\text{g/mL}$ . The precise mechanisms by which FQs kill are not fully understood, but strand breakage, autolysis associated with SOS DNA repair systems, and blockade of replication by the gyrase FQ complex may cause bacterial inhibition without bacterial killing.<sup>154</sup> However, the concentration of FQs necessary to inhibit the growth of organisms (MIC) is very close to that necessary to kill the organism (MBC). Although mammal DNA replication also depends on a topoisomerase, its function is somewhat different. More important, affinity of host topoisomerases is less than 0.001 of that of bacterial DNA gyrase. Thus the unique mechanism of action of the FQs renders rapid bactericidal activity with minimal effects on the host. The time to effect for FQs is very short (30 minutes); their rapidity of action often is the reason for preference of these drugs compared with other equally but more slowly effective antimicrobials (e.g., amoxicillin-clavulanic acid combinations for the treatment of selected pyodermas). Interestingly, cellular factors such as intracellular magnesium concentration, salt, and ATP may influence the affinity of FQs for their target enzymes; the clinical implications of this observation are not clear.<sup>151</sup>

**KEY POINT 7-21** The irreversible interaction between drug and topoisomerase results in a concentration-dependent effect for the fluorinated quinolone.

The efficacy of the FQs occurs, in part, because of a long postantibiotic effect, which also is concentration dependent. Depending on the organisms, drug, and concentration, the postantibiotic effects can approximate 5 to 8 hours.<sup>155-159</sup> The efficacy of the FQs appears to correlate more closely with peak concentrations (i.e., concentration dependent) than with duration of PDC above the MIC.<sup>160,161</sup> Consequently, efficacy is more likely when  $C_{\text{max}}/\text{MIC}$  exceeds 10 or more. However, duration of time that PDCs are above the MIC (AUC/MIC) also is an effective predictor of efficacy, and may be better than  $C_{\text{max}}/\text{MIC}$  for selected organisms.<sup>162</sup> Analysis of multiple studies focusing on the best predictor of successful bacterial killing indicated that the area under the inhibitory curve (AUC), an index that is similar to AUC/MIC (see Chapter 6) was the best predictor of efficacy. If AUC is greater than 100 but less than 250, bacterial killing is slow (evident by day 7 of therapy), whereas an AUC greater than 250 produced rapid killing, with eradication occurring within 24 hours. The effect occurred for both gram-negative and gram-positive organisms.<sup>153</sup> These data suggest that the most effective use of the FQs is to administer at a dose that will achieve rapid killing. A comparison of  $C_{\text{max}}/\text{MIC}$  or AUC/MIC may be helpful in comparing relative efficacy among the FQs used to treat feline or canine pathogens<sup>164</sup> (see Table 7-12).

### Spectrum of Activity

The (human-medicine) FQs have been categorized into 3 to 4 generations based on their spectrum of activity (see Figure 7-8).<sup>165</sup> Although not often used, the classification is helpful for perspective on the development of the FQs. The spectrum of nalidixic acid, the first-generation drug, is narrow. However, it was improved through pharmaceutical manipulation, yielding the second-generation drugs. This generation is exemplified by the human-marketed drug ciprofloxacin and the current veterinary FQs approved for use in dogs and cats. Their spectrum includes a broad gram-negative and less broad gram-positive spectrum. Third-generation drugs include levofloxacin, the L-isomer of ofloxacin, sparfloxacin, gatifloxacin, and moxifloxacin. This generation is characterized by enhanced potency, improved spectrum (which includes anaerobes), and reduced resistance. The fourth-generation drugs are characterized by the broadest spectrum and are exemplified by trovafloxacin. Each generation has been designed such that drug molecules target specific molecules of the target enzymes, thus increasing efficacy, and for some reducing the emergence of resistance.

The second-generation veterinary FQs have been referred to as broad in spectrum, but this term is appropriate only when referring to the gram-negative spectrum; the term *broad* is more appropriate for third-generation drugs, for which their currently is no veterinary approved example in the United States. The gram-positive spectrum is more selective, and anaerobes, in general, are not susceptible. However, other microbes are targeted, including cell wall-deficient microbes and mycobacterium. Organisms particularly susceptible to FQs include *Pasteurella* (among the lowest MICs), *E. coli*, *Klebsiella* spp. *E. cloacae*, *P. mirabilis*, *Citrobacter freundii*, and *S. marcescens*. *Pseudomonas* spp. also is included in the spectrum but generally is characterized by the highest MICs, with efficacy toward *Pseudomonas* spp. varying with the individual drugs (Table 7-12; see also Tables 7-3 and 7-4).<sup>154</sup> Among the drugs used in dogs or cats, ciprofloxacin, enrofloxacin, and marbofloxacin tend to have the lowest MICs. Ciprofloxacin is most potent toward gram-negative isolates, particularly for *E. coli* and *P. aeruginosa*.<sup>164,166</sup> The gram-positive spectrum includes *Staphylococcus* spp. and some *Corynebacterium*. The FQs have exhibited variable efficacy against *Streptococcus* species and *E. faecalis*.<sup>156,167</sup> Other susceptible organisms generally include *Campylobacter*, *Salmonella*, *Shigella*, and *Yersinia*. Efficacy of the FQs toward leptospirosis is supported by limited studies. Some rickettsial organisms may be susceptible; in vitro data and limited in vivo data indicate potential efficacy against organisms causing ehrlichiosis and Rocky Mountain spotted fever.<sup>168</sup>

**KEY POINT 7-22** The second-generation fluorinated quinolones have a broad gram-negative spectrum and a more limited gram-positive spectrum and are generally not effective toward anaerobes.

Integration of PK and PD of the FQs reveals some differences in predicted efficacy among the FQs used in cats and dogs toward organisms within the spectrum (see Table 7-12).

Table 7-12 Pharmacodynamic Data for Selected Fluoroquinolones and Selected Feline and Canine Pathogens<sup>(164, 180)</sup>

Organism	Enrofloxacin			Orbifloxacin			Difloxacin			Marbofloxacin			Pradofloxacin		Ciprofloxacin			
	n	MIC <sub>50</sub>	MIC <sub>90</sub>	n	MIC <sub>50</sub>	MIC <sub>90</sub>	n	MIC <sub>50</sub>	MIC <sub>90</sub>	n	MIC <sub>50</sub>	MIC <sub>90</sub>	n	MIC <sub>50</sub>	MIC <sub>90</sub>	n	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Bordetella</i>	25/54	0.25	0.5	54	0.5	2	54	2	4	54	0.25	0.5	54	0.12	0.25			
<i>Enterococcus</i>	40/41	1	1	41	4	4	41	2	4	41	2	2	94	0.12	0.25			
<i>Escherichia coli</i>	61	0.0625	≥64	28/155	0.12	0.39/0.25	61	0.0625	≥64	61/45	0.06/≥64		155	≤0.015	0.03			
<i>Klebsiella</i>	32/58	0.06	0.12/0.06	58	0.12	0.25	8/58	0.25	0.11/5	11/58	0.03	0.06	58	0.06	0.06	51	0.0625	
<i>Mycoplasma</i>	32/70	0.12	0.5/0.25	70	0.25	0.5	70	0.25	0.5	70	0.12	0.5	70	0.03	0.06			
<i>Pasteurella</i>	32	≤0.03	0.03	32	≤0.06	≤0.06	32	≤0.06	<0.05	32	≤0.03	0.06	≤0.03	≤0.015	≤0.015			
<i>Proteus mirabilis</i>	88/28	0.125	0.25/1	15	1	8	28	0.0625	0.5	35/18	0.125	0.125/1	93	0.125	0.25	28	0.0625	0.5
<i>Pseudomonas aeruginosa</i>	94/58	1/0.5	>2/8	94/34	4	>8/16	94/58	2/0.125	>4/2	94/38	1/0.5	1/4	94	1	>2	58	0.125	2
<i>Staphylococcus intermedius</i>	119/200	0.12	0.25/0.12	51/15	0.5	0.39/0.5	19	0.25	0.25/2	135/200	0.25	0.25	200	0.06	0.06	19	0.125	0.125
<i>Salmonella</i>	15	≤0.03	0.25	14	0.12	0.12	14	0.25	0.25	14	0.25	0.25	14	0.06	0.12			
<i>Staphylococcus</i>	120/16	0.5	≥64	8	1	ND	193/16	ND/0.25	0.46/32	14	0.5	0.25/64		0.06	0.12	16	0.25	32
<i>Streptococcus</i>	33/20	05/0.25	1	33/10	1/0.25	2/≥64	33/20	0.5/0.125	1	33/13	0.5	1/4	33	0.12	0.12	20	0.125	1
		MIC	MPC		MIC	MPC		MIC	MPC		MIC	MPC		MIC	MPC		MIC	MPC
<i>E. coli</i> ATCC8739*		0.03-0.06	0.3-0.35		0.25	1-1.25		0.125-0.5	1.5-1.6		0.03	0.25-0.3		0.014-0.03	0.2-0.25		0.015-0.03	0.1-0.15
<i>S. aureus</i> ATCC 6538		0.06-0.125	0.5-0.61		0.5	8-9		0.125	16-18		0.15-0.5	3-3.5		0.03-0.06	0.5-0.6		0.25-0.5	5
<i>Staphylococcus intermedius</i> ATCC 29663		0.06-0.125	1		ND	ND		ND	ND		0.05	ND		0.03	0.15		0.125	ND

MIC, Minimum Inhibitory concentration; MPC, mutant prevention concentration.

Based on PK reported either in the literature or on the package insert, two PDIs were determined: the  $C_{\max}/\text{MIC}$  (target 10) or  $\text{AUC}/\text{MIC}$  (target 125). The PDIs were compared among drugs for the susceptible isolates of each organism at the lowest and highest labeled dose for each drug. In general, at the low dose the only organism for which the target PDIs were reached for all drugs was *E. coli*. For all other organisms, even at the high dose, targets were reached consistently only for ciprofloxacin, enrofloxacin, and marbofloxacin.<sup>154</sup> The authors concluded that the highest dose of the FQ is generally recommended when possible and that enrofloxacin, marbofloxacin, and ciprofloxacin performed in vitro better than difloxacin and marbofloxacin.

Levofloxacin is a human-marketed third-generation FQ that increasingly is being used in dogs and cats. It is twice as potent against gram-positive isolates (topoisomerase IV) and equally potent against gram-negative isolates (DNA gyrase) compared with ciprofloxacin, although more recent data suggest that this is not consistent (see Table 7-4).<sup>169</sup> For example, the  $\text{MIC}_{50/90}$  ( $\mu\text{g}/\text{mL}$ ) for human organisms isolated from skin or soft tissue infections are as follows: *S. aureus* (0.25, > 4), *E. coli* ( $\leq 0.03$ , 4), or *P. aeruginosa* (0.5, > 4).<sup>170</sup> The potential efficacy of levofloxacin cannot be assessed for dogs because PK have not been established and neither  $C_{\max}$  nor AUC is available. Kinetics have been reported for levofloxacin in the cat, but at 10 mg/kg, the  $C_{\max}$  does not reach the  $\text{MIC}_{90}$  for *Staphylococcus* or *Pseudomonas* spp. The  $C_{\max}/\text{MIC}_{90}$  is only 1 (rather than the target  $\geq 10$ ) for *E. coli*. The target  $\geq 10$  would be reached based on the  $\text{MIC}_{50}$  for *Staphylococcus* spp. and *E. coli* but not for *Pseudomonas* spp. The safety of levofloxacin in cats at doses that will be necessary to reach the target PDI has not been established. These data suggest that PK and PD studies are needed in the dog before levofloxacin is used and that the organisms against which levofloxacin is used in cats at the dose of 10 mg/kg should be characterized by an MIC of 0.5  $\mu\text{g}/\text{mL}$  or less. Once-daily administration was demonstrated to be more effective against *Staphylococcus* spp., including an MRSA isolate, compared with twice-daily dosing.<sup>169</sup>

Anaerobic organisms have been considered generally resistant to the FQs. However, the spectrum of the newer drugs, particularly those substituted at position 8, has been expanded to include anaerobes. Levofloxacin, sparfloxacin, grepafloxacin, and pradofloxacin each has greater activity against anaerobes compared with older drugs. This includes the *B. fragilis* group, as well as *Clostridium*, *Peptostreptococcus*, *Prevotella*, and *Fusobacterium* spp.<sup>171</sup>

The FQs are effective against mycobacterial organisms. However, using *M. tuberculosis* as an example, the MIC ( $\mu\text{g}/\text{mL}$ ) for the newer FQs are lower compared with the second-generation drugs: 1  $\mu\text{g}/\text{mL}$  for levofloxacin, 0.1 to 0.5 for sparfloxacin, 0.2 to 0.25 for gatifloxacin, and 0.12 to 0.5 for moxifloxacin, compared with 0.5 to 4 for ciprofloxacin.<sup>154</sup> Of the FQs, gatifloxacin and moxifloxacin have been demonstrated to exceed the mutant potential concentration (MPC; see Chapter 8) for *M. tuberculosis*. Like other organisms, and despite their slow growth, the activity of FQs against *Mycobacterium* spp. is

concentration dependent. However, tubercular organisms are able to enter a dormant, persistent, and antimicrobial-resistant phase, necessitating long-term therapy.

Each of the veterinary FQs has been approved with a "flexible" dosing regimen, indicating low to high doses, with the choice depending on the MIC of the infecting organism. However, as previously discussed, increasing evidence suggests that the highest concentration should be targeted whenever possible. The concept of the MPC emerged in the context of emerging FQ resistance in mycobacteria. Targeting simply the MIC is likely to select for stepwise mutants (see Chapter 6).<sup>162</sup> Flexibility also occurs for the interval: for enrofloxacin and orbifloxacin, the label allows once- or twice-daily dosing, whereas for marbofloxacin and difloxacin, the dose is limited to once a day. Because FQs are concentration dependent, administration of the total daily dose as a once-daily dose is generally preferred, as has been demonstrated for ciprofloxacin<sup>173</sup> and levofloxacin.<sup>169</sup> *P. aeruginosa* is an example of an organism whose tendency toward resistance suggests the higher, once-daily dose.<sup>174</sup> Because efficacy of an FQ is based on  $\text{AUC}/\text{MIC}$  as well as  $C_{\max}/\text{MIC}$ , a second dose (not half the dose twice) might be considered, particularly for selected organisms (e.g., *S. aureus*).

**KEY POINT 7-23** Failure to achieve the mutant prevention concentration (MPC) may allow emergence of multistep mutants.

The amphoteric nature of the FQs complicates the impact of pH on efficacy. For example, difloxacin was shown to be most potent (based on MIC differences) at a pH of 7.1 compared with 5.9 or 7.9, with a fourfold increase in the MIC at the alkaline pH occurring for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *S. intermedium*.<sup>159</sup>

### Resistance

A major advantage of the FQs promoted during marketing, was the lack of clinically relevant plasmid-mediated quinolone resistance. Rather, the major mechanism of resistance reflects genetic mutations in the target topoisomerase enzymes (e.g., DNA gyrase [topoisomerase II] and topoisomerase IV). However, several observations dampen the importance of the predominance of mutational, rather than plasmid-mediated, resistance. First, history has demonstrated that resistance of any antimicrobial (plasmid or otherwise) may take several decades of intense antimicrobial use, suggesting that, as with other antimicrobials, the use of FQs ultimately was to be limited by resistance. Secondly, resistance to norfloxacin emerged as little as 3 years after its approval, regardless of the mechanism. This rapid development of resistance foretold a similar problem with other second-generation FQs. Thus, as the medical community enters the third decade of ciprofloxacin use in human medicine and the second decade of FQ use in veterinary medicine, increasing resistance, albeit not necessarily plasmid mediated, has emerged and is limiting the widespread effective use of these drugs in both human and veterinary medicine. Finally, plasmid-mediated resistance has appeared



and plays a role in horizontal transmission of FQ resistance.<sup>165</sup> The development of FQ resistance by human bacterial organisms has influenced the decision to ban extralabel use of FQs in food animals or use as food additives (i.e., growth promoters). Clinically, the increasing pattern of resistance for veterinary FQs and ciprofloxacin has emerged toward several organisms, including *S. aureus*, *P. aeruginosa*, *E. coli*, and other gram-negative organisms (see Chapter 6). In chronic otitis of dogs, 14% of *S. pseudintermedius* cultured from the middle ear and more than 65% of *Pseudomonas* spp. cultured from the external and middle ear were resistant to enrofloxacin.<sup>175</sup> A prospective study of more than 300 organisms submitted to commercial laboratories found nearly 30% of *E. coli* resistant to all veterinary FQs, as well as ciprofloxacin. The MIC<sub>90</sub> for *Pseudomonas* surpassed the CLSI MIC breakpoint for all drugs except ciprofloxacin, and for *E. coli* and *Staphylococcus* spp. (not including *S. intermedius*) exceeding it for all drugs by fourfold to eightfold.<sup>154</sup> A subsequent prospective study of more than 350 *E. coli* isolates (collected from all body tissues, with the vast majority associated with urinary tract infections) found that 30% demonstrated an MIC<sub>90</sub> greater than 32 µg/mL (MIC breakpoint ≥ 4 µg/mL), with regional geographical differences demonstrated.<sup>166</sup> Resistance to FQs is associated with FQ use; in humans a single dose of ciprofloxacin lead to FQ-resistant microorganisms.<sup>167</sup> That FQ resistance can be associated with FQ use in dogs was demonstrated by Debalvalya et al.<sup>178</sup> Close to 100% of fecal *E. coli* developed high level resistance to FQs (associated with multi-drug resistance) within 3 to 9 days of therapy of enrofloxacin in dogs (5 mg/kg every 24 hours).

Susceptibility data from laboratories that test both ciprofloxacin and enrofloxacin may report susceptibility to ciprofloxacin but resistance to enrofloxacin. Interpretive standards on culture reports for ciprofloxacin are based on human data and may not take into account differences in oral bioavailability, just as standards for enrofloxacin do not include bioactivity contributed by ciprofloxacin. Although ciprofloxacin is more potent toward *E. coli* and *Pseudomonas aeruginosa* compared to enrofloxacin, the difference is usually within 1 tube dilution. A prospective study compared the proportion of resistance and the relative susceptibility (efficacy) among ciprofloxacin, difloxacin, enrofloxacin (alone or with ciprofloxacin), marbofloxacin, and orbifloxacin FQs toward six organisms collected from canine and feline patients.<sup>154</sup> The proportion of resistant isolates, which was based on CLSI interpretive criteria, did not differ among drugs, suggesting that expression of resistance by an isolate to one (second-generation) FQ might be prudently interpreted as resistance to all, despite the not uncommon finding of susceptibility to ciprofloxacin and resistance to another FQ (e.g., enrofloxacin).

Three major mechanisms of FQ resistance have been identified,<sup>55,167</sup> with the most studied being changes in the structure of the target topoisomerase enzymes. However, mutations, which impart resistance within the FQ class of drugs, are often accompanied by decreased expression of porin membranes and increased activity of efflux pumps, which imparts multidrug resistance.<sup>155,169</sup> Thus far, resistance

to FQs acquired through changes in DNA gyrase has been documented clinically only after chromosomal point mutations; at least 10 different mutations have been identified so far. Resistance is stepwise, with the first step occurring primarily through mutations that reduce FQ affinity for the preferred topoisomerase target, which varies with the organism. Gram-negative bacteria tend to more commonly target DNA gyrase; changes occur more often in the *GyrA* subunit compared with *GyrB*.<sup>141</sup> The primary target of gram-positive organisms tends to be changes in topoisomerase IV, targeting *parC* and *parE* followed by changes in DNA gyrase. Recent evidence suggests that the drug (and its primary target) select for the mechanism of resistance.<sup>141</sup> High-level resistance generally reflects a second step mutation that leads to additional changes in the amino acid sequence of either (the alternate) topoisomerase target, thus further decreasing affinity, or the generation of efflux pump mechanisms. The MIC of the organisms progressively increases with each step. The role of reduced porin membranes and efflux pumps in FQ resistance was more recently discovered. Gram-negative isolates are associated with both mechanisms of reduced drug accumulation (i.e., porins and pumps), as well as decreased lipids in the lipopolysaccharide covering, impeding drug transport; gram-positive isolates (*S. aureus*) have been associated with increased drug efflux.<sup>145,147,167</sup> The efflux pumps affect multiple drugs, contributing to multidrug resistance, including resistance to drugs structurally unrelated to FQs.<sup>167,169, 169a</sup> These include tetracyclines, phenicols, and macrolides. Beta-lactams may also be involved; resistance to antiseptics and disinfectants may occur. Expression of the pump is chromosomally mediated. For example, mutations in the *mar* operon may induce the *acrAB* proteins of a stress-induced efflux pump, resulting in high-level resistance, even for isolates with no or single mutations in topoisomerase.<sup>155</sup> Plasmid-mediated quinolone resistance (PMQR), associated with the *qnr* gene, has recently been identified in clinical bacterial isolates, generally associated with class I integrons. However, while initially rare, in 2003, several strains of *E. coli* and *Klebsiella* spp. were found to transmit *qnr* resistance, and isolates have since been identified in the United States. The author has reported a high incidence of PMQR in clinical canine and feline *E. coli* isolates.<sup>165a</sup> Resistance mediated by PMQR and *qnr* tends to be low level and thus may be difficult to detect on C&S testing. Mechanisms include production of a protein that prevents quinolone binding to the target, and enzymatic destruction of the drug. Its impact appears to be related to its ability to increase the incidence of spontaneous mutations and facilitation of altered porin or efflux protein activity. Despite its low level, PMQR resistance associated with *qnr* appears to affect other drug classes, including cephalosporins (including second- and third-generation), aminoglycosides, and potentiated sulfonamides.

The emergence of stepwise resistance is generally indicated by an increase in the MIC of the organism toward the drug. In human medicine, isolates characterized by an MIC greater than 0.125 µg/mL for ciprofloxacin are treated as "reduced susceptibility," indicating that a first step toward mutation (or

resistance) has occurred, whereas isolates greater than 2 µg/mL are considered to have "high-level" resistance.<sup>165</sup> These reports are likely, in part, to be the basis of "susceptible" MIC breakpoint promulgated by CLSI. However, it is important to note that despite a susceptible designation for some isolates, reduced susceptibility is an indication that resistance has begun and use of a FQ should be done cautiously and judiciously. Actions such as using a second dose or using the drug in combination with a second, synergistic drug should be strongly considered. Current clinical microbiology laboratories often do not perform susceptibility testing at concentrations below 0.125 to 0.25 µg/mL for FQs, thus precluding the identification of isolates that are characterized by reduced susceptibility. Thus it is important to note that reduced susceptibility to an FQ of interest may characterize a "susceptible" isolate, and use of FQs should be done judiciously.

The term MPC was coined after substantial evidence emerged that resistance to FQs reflects multistep or stepwise selection of mutants when the FQ is used therapeutically at a dose that targets the MIC of a cultured infecting microbe (see Chapter 6).<sup>172</sup> At drug concentrations below the MPC, first step mutants will continue to grow in the absence of effective host response, and may replace the wild-type (nonmutant) population.<sup>180</sup> Consequently, the MPC, rather than the MIC, ideally is targeted with drug therapy. Predicting the MPC on the basis of MIC is not possible; the relationship between the two appears to be larger for gram-positive than gram-negative isolates, and varies among the FQs (see Table 7-12).<sup>180</sup> Among the veterinary FQs, using quality assurance isolates, the ratio of MPC to MIC seems to be similar for gram-negative isolates, being less than 10, and the MPC might be reasonably targeted with doses that are within recommendations based on a  $C_{max}/MIC$  ratio of 10. However, Pasquali<sup>181</sup> demonstrated that the MPC/MIC for *E. coli* was fourfold to sixteenfold higher for enrofloxacin compared with ciprofloxacin. In this study the authors found that targeting the MPC for *P. aeruginosa* was not effective, postulating that the reason reflects efflux pump activity rather than point mutation (the basis of the MPC theory) as the major mechanism of resistance. Enrofloxacin and pradofloxacin have the lowest MPC/MIC ratio for gram-positive isolates; concentrations necessary to target the MPC for gram-positive isolates may be achievable with these drugs but may not be achievable at recommended doses, particularly for difloxacin and orbifloxacin. Use of the highest dose of any FQ is recommended because of the risk of resistance. If reduced susceptibility is suspected (e.g., MIC > 0.25 µg/mL), then the addition of a second dose or use as part of combination therapy might be prudent. Combination therapy has been described as a mechanism to reduce emergent resistance to FQs. For example, in an in vitro model, rifampin prevented emergence of resistance to ciprofloxacin.<sup>169</sup> The addition of a FQ decreased the advent of resistance to cephalosporins in another study.<sup>182</sup>

Newer drugs, including gemifloxacin, trovafloxacin, gatifloxacin, and pradofloxacin, may target both DNA gyrase and topoisomerase IV. Thus for these drugs, multistep resistance may be necessary to neutralize their antibacterial effects.

Newer FQs appear to avoid resistance because their stereochemistry interferes with altered porin sizes and efflux mechanism. For example, for pradofloxacin the cyclopropyl ring at N1 provides bacterial killing, but the diazabicyclononyl moiety at C7 appears to physically block porins.<sup>154</sup> Wetzstein<sup>180</sup> compared the MPCs for older and newer FQs. That resistance may be more likely with older compared with newer drugs was suggested by an in vitro study,<sup>169</sup> in which resistance could be induced for ciprofloxacin but not levofloxacin. However, surveillance studies in humans infected with *Streptococcus* spp. as well as other isolates, report variable findings, including lower, similar, or higher rates of resistance for levofloxacin, compared with ciprofloxacin.<sup>169,183,184</sup> Because resistance is likely to emerge even to the newer FQs, use based on C&S testing and design of a dosing regimen that targets the MPC as much as possible is prudent.

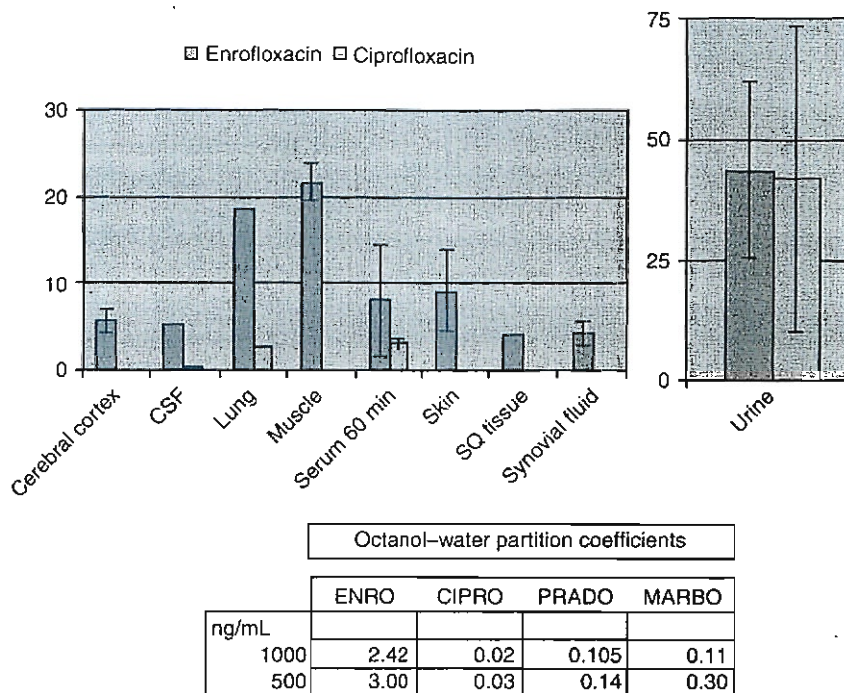
FQ resistance by *Mycobacterium* spp. occurs primarily as part of multidrug-resistant tuberculosis, which develops when an FQ is used as the only active agent in a failing multidrug regimen.<sup>154</sup> Thus combination with traditional antitubercular drugs (isoniazid, rifampin) enhances antimicrobial efficacy.<sup>154</sup>

### Pharmacokinetics

The PK of the veterinary FQs are largely comparable among the drugs, particularly if structurally similar, although individual differences may become important for some infections. Maximum drug concentrations of the FQs do not always increase linearly with dose (see Table 7-1) This may reflect, for some drugs, variability in peak concentrations measured among different investigators, including different analytical methods. In particular, attention must be paid to the method of drug detection, with those based on bioactivity (i.e., bioassay) frequently yielding higher concentrations if an active metabolite is present (e.g., enrofloxacin and ciprofloxacin).

**KEY POINT 7-24** The fluorinated quinolones are characterized by good to excellent tissue distribution because of their lipid solubility and accumulation in phagocytic white blood cells.

The only injectable preparation approved for dogs is for enrofloxacin, although an injectable preparation is available for human FQs, including ciprofloxacin. All remaining veterinary FQs approved in dogs or cats are available for oral administration. Enrofloxacin is available as a topical combination preparation. Marbofloxacin, enrofloxacin, difloxacin, and orbifloxacin are characterized by close to 100% oral bioavailability in young adult animals. A number of factors, however, influence absorption of FQs in general, and several drugs specifically. Magnesium and aluminum decrease oral absorption, and food may also, which may be undesirable for concentration-dependent drugs. The oral bioavailability of FQs may not be predictable, with extrapolation among species not recommended. For example, norfloxacin is characterized by 60% or less oral bioavailability in dog, and ciprofloxacin, generally less than 60%. Extrapolation of levofloxacin between humans and cats appears to be more appropriate than that of



**Figure 7-10** Selected tissue homogenate concentrations of enrofloxacin 2 hours after intravenous administration of 20 mg/kg. Concentrations in fluids are most relevant to bacterial exposure. Ciprofloxacin concentrations reflect metabolism of enrofloxacin to ciprofloxacin. Octanol-water partition coefficients suggest that enrofloxacin would distribute best into fluids at physiologic pH.

ciprofloxacin. Oral absorption also may be impaired in neonates, as has been demonstrated for enrofloxacin.<sup>185</sup>

As a class, the FQs are well distributed to most body tissues (see Table 7-5). Protein binding of enrofloxacin, ciprofloxacin, and marbofloxacin in dogs is  $34 \pm 2\%$ ,  $18.5 \pm 2\%$ , and  $21 \pm 6\%$ , respectively.<sup>176</sup> Although the Vd of the drugs ranges from a low of 1.12 (marbofloxacin) to a high of 3.2 (difloxacin), the clinical relevance of these differences is not likely to be a sufficient cause to select one over another. The respective PCs for selected FQs have been variably reported, with enrofloxacin characterized by the highest lipophilicity of the three: 2.4, 0.02, and 0.11;<sup>187</sup> and 3.54, 0.07, and 0.08, for enrofloxacin, ciprofloxacin, and marbofloxacin, respectively (Figure 7-10).<sup>176</sup> However, as with Vd, predicting tissue distribution based on PC is difficult. This reflects, in part, the common use of homogenate data for solid tissues. Homogenate data include both interstitial fluid and ICF. As such, drugs that penetrate cell membranes and accumulate in cells, not necessarily in active form, may be characterized by higher concentrations compared with drugs that distribute to interstitial fluid only. Intracellular trapping of drugs may limit access to microbes in interstitial fluid, although movement from the cell back into interstitial fluid may prolong the presence of drug in interstitial fluid by slow release from the cell. The relevance of the data is then influenced by the location of the infection (i.e., intracellular versus extracellular) and host (e.g., inflammation) or microbial (e.g., biofilm) factors that might affect efficacy. Fluid tissue concentrations (based on homogenate data) are generally greater in organs of elimination compared with plasma for all FQs. Solid tissue concentrations are often higher (e.g., if drug is trapped in the cells), particularly for

the liver and kidney (organs of elimination) but also spleen and lung (perhaps reflecting phagocytic cell accumulation), prostate (perhaps reflecting ion trapping), and muscle.<sup>161,188</sup> Homogenate tissue data are available on the package inserts of several of the veterinary approved FQs. Interestingly, the concentration of difloxacin in cortical bone (but not bone marrow), exceeds that in plasma by threefold, but did not change across a 24-hour period. This might suggest that FQs (or difloxacin) bind to bone, which may preclude activity. Frazier and coworkers<sup>189</sup> compared the disposition and homogenate tissue concentrations of difloxacin (5 mg/kg), enrofloxacin (5 mg/kg; ciprofloxacin also measured), and marbofloxacin (2.75 mg/kg) after multiple dosing (5 days) in the same dogs using a randomized crossover design (21 day washout period); drugs were detected using HPLC. Their studies demonstrate that the FQs accumulate in tissues with multiple dosing. Concentrations increased in the skin to reach a 4-day peak that exceeded the 1-day concentration by at least threefold. The concentrations in skin ( $\mu\text{g}/\text{mL}$ ) at 1 and 4 days were, respectively, as follows: marbofloxacin (1.87 and 4.9), enrofloxacin (1.38 and 5.99), ciprofloxacin (0.2 and 0.5 for a total bioactivity of 1.59 and 6.9), and difloxacin (1 and 3.8). Urine concentrations also were higher at day 4 compared to day 1, with the magnitude varying for each drug. The concentrations in urine ( $\mu\text{g}/\text{mL}$ ) were at 24 and 98 hours, respectively: marbofloxacin (14 and 50), enrofloxacin (0.14 and 1.83) plus ciprofloxacin (5.61 and 33.3 for a total bioactivity of 5.9 and 39), and difloxacin (0.56 and 1.8).

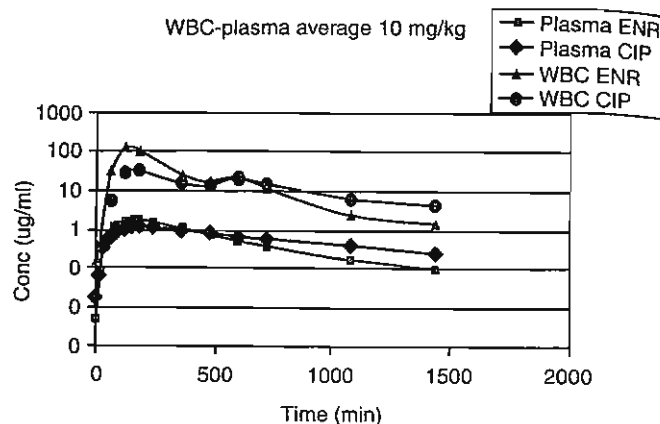
Homogenate data has been reported for enrofloxacin in anesthetized dogs ( $n = 4$ ) receiving 20 mg/kg of enrofloxacin IV dogs.<sup>188</sup> The 1- and 2-hour serum concentrations

**Table 7-13** Concentrations and Tissue to Serum Ratio for Enrofloxacin and Ciprofloxacin\*

Tissue	Enrofloxacin (µg/mL)	Ratio	Ciprofloxacin (µg/mL)	Ratio
Cerebrospinal fluid	785.8	0.5	59	0.3
Joint fluid	650	0.5	170	0.7
Urine	2827	2.0	21806	94
Aqueous humor	226	0.2	64	0.3
Bile	136182	95.0	50008	216
Serum	1433	1.0	230.85	1.0

\*3 hours after 4 days of oral and 1 day of intravenous 5 mg/kg enrofloxacin.

were 8.2 and 6.4 µg/mL (ciprofloxacin 3.1 and 2.8 µg/mL), respectively. Homogenate tissue to plasma ratios at 2 hours from lowest to highest were, in order, tracheal cartilage (0.2), aqueous humor (0.3), synovial fluid and subcutaneous tissue (0.4), peritoneal fluid and CSF (0.5), and brain (0.6).<sup>178</sup> For fluids located in sanctuaries, at 1 hour aqueous humor (n = 2) achieved 2.5 µg/mL enrofloxacin and 0.5 µg/mL ciprofloxacin; peak CSF concentration of 5.3 µg/mL occurred at 2 hours (one dog). For aqueous humor a second study documented, 0.23 µg/mL of enrofloxacin and 0.064 µg/mL of ciprofloxacin 3 hours after 4 days of oral and 1 day of intravenous dosing at 5 mg/kg.<sup>180</sup> The ratio of tissue to plasma concentrations were similar for ciprofloxacin and enrofloxacin (see Table 7-13). Another study documented that marbofloxacin (2 mg/kg, administered intravenously) achieves 0.41 µg/mL in aqueous humor at 3.5 hours in dogs.<sup>191</sup> Other ratios of plasma to tissue enrofloxacin after 20 mg/kg administered intravenously<sup>192</sup> included ligament (0.6), ear cartilage (0.7), and bone marrow (0.8). Concentrations in the prostate were 2.5-fold higher and urine 4.5-fold higher than in plasma (urine concentration of 45 µg/mL). Interstitial fluid concentrations of enrofloxacin (and formed ciprofloxacin) and marbofloxacin have also been measured using ultrafiltration. After 10 mg/kg enrofloxacin administered intravenously, the ratio of C<sub>max</sub> in interstitial fluid (2.41 µg/mL) compared with plasma (5.54 µg/mL) was 0.47; the ratio for AUC, however, was 1.3, indicating that the drug appears to stay longer in interstitial fluid compared with plasma.<sup>177</sup> A second study<sup>176</sup> determined plasma to interstitial fluid ratios after 5 mg/kg, administered orally, for marbofloxacin (approximating the highest labeled dose) and enrofloxacin (the lowest once-daily dose). Plasma to interstitial fluid C<sub>max</sub> ratio was 0.75 for marbofloxacin and 0.7 for enrofloxacin plus ciprofloxacin and for AUC was 1.11, for marbofloxacin and 1.3 for enrofloxacin and ciprofloxacin. The higher AUC for marbofloxacin reflected in part the higher C<sub>max</sub> but also a longer elimination half-life (8.5 hours) compared with enrofloxacin (3 hours). All FQs that have been studied thus far (enrofloxacin, marbofloxacin, pradofloxacin, and ciprofloxacin) accumulate in phagocytic WBCs; concentrations may be up to 140-fold higher compared with plasma



**Figure 7-11** Enrofloxacin is metabolized by de-ethylation to ciprofloxacin. The two compounds will act in an additive fashion. The dotted line in the top graph indicates the predicted amount of bioactivity resulting from both enrofloxacin and its active metabolite, ciprofloxacin, after administration of 10 mg/kg. The longer half-life of ciprofloxacin can contribute to a longer duration. The graph demonstrates the accumulation of both enrofloxacin and ciprofloxacin in white blood cells (top two plots).

(see Table 7-5).<sup>164,193-196</sup> Drug in phagocytes will be distributed to sites of inflammation, thus increasing concentrations at the site of infection.<sup>196</sup> Impact on intracellular killing is controversial. Whereas some studies have demonstrated that FQs retain intracellular killing effects compared with macrolides,<sup>197</sup> another in vitro study demonstrated reduced intracellular killing ability for a variety of FQs.<sup>184</sup>

**KEY POINT 7-25** The bioactivity of enrofloxacin can be doubled by formation of its more potent metabolite, ciprofloxacin.

The organ of elimination varies among the FQs. Difloxacin is eliminated almost exclusively by hepatic metabolism to inactive metabolites. Orbifloxacin is 40% eliminated unchanged in the urine. Marbofloxacin (clearance of 1.6 L/min) is largely excreted into the urine. However, up to 15% is metabolized in the liver to inactive metabolites,<sup>198</sup> with the proportion changing in the presence of renal disease.<sup>198</sup> Enrofloxacin also is eliminated in the urine as the unchanged drug, although approximately 25% of the drug is metabolized to ciprofloxacin, which subsequently achieves concentrations severalfold higher than enrofloxacin (Figure 7-11; see also Figure 7-8 and Table 7-13). Therapeutic concentrations of ciprofloxacin can be achieved in other tissues after administration of enrofloxacin, depending on the target organism.<sup>161,193,199</sup> The parent and metabolite should act in an additive fashion.<sup>160</sup> Because ciprofloxacin is characterized by a longer half-life than enrofloxacin in dogs (see Table 7-1), as a metabolite, ciprofloxacin can double the AUC of enrofloxacin bioactivity (see Table 7-1 and Figure 7-11).<sup>193</sup> Longer elimination half-lives also characterize difloxacin and marbofloxacin compared with orbifloxacin and enrofloxacin, contributing to higher AUC for these drugs (see Table 7-1). Elimination half-lives are somewhat dose

dependent,<sup>193</sup> at least for enrofloxacin and ciprofloxacin (see Table 7-1). Alkaline urine increases the passive reabsorption of FQs from the renal tubules and may also prolong the elimination half-life. The longer half-lives should increase efficacy by increasing the likelihood that the drug will achieve the target AUC/MIC. Heinen<sup>200</sup> compared PDIs among the FQs after oral administration, using a bioassay that detects both parent compound and active metabolites (see Table 7-1). Based on MIC<sub>90</sub> determined for *E. coli* and *Staphylococcus* spp. (from isolates before 1999), for no drug was the targeted AUC/MIC achieved for *Staphylococcus* spp. and only enrofloxacin achieved the C<sub>max</sub>/MIC for *Staphylococcus* spp. Enrofloxacin (5 mg/kg) had the highest C<sub>max</sub>/MIC toward *E. coli*, followed by marbofloxacin (2 mg/kg) and orbifloxacin (2.5 mg/kg); difloxacin (5 mg/kg) did not reach the targeted C<sub>max</sub>/MIC or AUC/MIC for either organism. A similar pattern of efficacy was found among the veterinary FQs by Boothe using isolate MIC and reported C<sub>max</sub>,<sup>164</sup> with enrofloxacin plus ciprofloxacin > ciprofloxacin > marbofloxacin > orbifloxacin > difloxacin being the general pattern of magnitude in PDI. However, the higher dose was generally needed to reach desired targets for PK/PD indices; isolates had been collected from 1998 to 2000 suggesting the likelihood of achieving targeted PDI with current isolates is less likely.

The disposition of enrofloxacin in neonatal kittens differs from that in adults, appearing to be age dependent even in the pediatric patient.<sup>185</sup> Administration of 5 mg/kg to 2- to 8-week-old kittens<sup>185</sup> revealed a shorter half-life at all ages but a Vd<sub>ss</sub> that was less at 2 to 4 weeks and greater at 6 to 8 weeks compared with that of adults. Accordingly, C<sub>max</sub> was lower in the 6- to 8-week-old kittens. Enrofloxacin was generally poorly bioavailable at all ages.

**Pradofloxacin.** Pradofloxacin is a newer-generation FQ that may be undergoing approval in animals in the United States and the European Union. Structurally, it is characterized by a cyclopropyl ring at N1 (see Figure 7-8) that increases bacterial killing. A diazabicyclononyl moiety at C7 appears to physically block drug efflux through porins and targets both topoisomerases such that mutation must be multistep.<sup>154</sup> Its spectrum includes *P. aeruginosa*. However, many anaerobes also will be effectively targeted. At 3 mg/kg orally for 5 days in dogs, C<sub>max</sub> was 1.7 ± 0.9 µg/mL and 6.2 ± 2.3 µg/mL in dogs (n = 6); half-life was 10 ± 7 hr at 3 mg/kg and 5.9 ± 1.5 hr at 12 mg/kg.<sup>190</sup> The long half-life results in an AUC/MIC that is favorable compared with the other FQs. Pradofloxacin also has been studied in anesthetized dogs. It appears to be well distributed among the tissues.<sup>190</sup> Aqueous humor concentrations achieved 0.32 µg/mL after 5 days of administration (4 oral followed by 1 IV) at 5 mg/kg of pradofloxacin.

**Ciprofloxacin.** Although ciprofloxacin has been studied in dogs following intravenous and oral<sup>202</sup> administration (Table 7.1), the studies used different animals, and limited information is available on its oral bioavailability in dogs. However, reports provided by the manufacturer indicate that ciprofloxacin is only 33% to 40% bioavailable in dogs<sup>203</sup> compared with nearly 80% to 100% in humans. Oral absorption

of ciprofloxacin in dogs involves a dose-dependent nonlinear component that may affect its oral absorption.<sup>202</sup> Oral and bioavailability of ciprofloxacin in cats (using pure powder in gelatin capsules) appears to be less than that in dogs, being 20% ± 11% following single dosing and 33% ± 12% after multiple dosing.<sup>205</sup> Oral absorption was characterized by marked interanimal variability, suggesting that oral absorption may be minimal in some cats.<sup>205</sup> This suggests that oral ciprofloxacin should be avoided in cats, and oral dosing in both cats and dogs should err on the side of higher doses to compensate for unpredictable oral bioavailability. More than several human-marketed generic preparations of oral ciprofloxacin are now available at a greatly reduced cost compared with oral enrofloxacin. However, whereas bioequivalence of a generic product must be proved to the pioneer product, this proof is generated only in the species in which the drug is approved. That the PK behavior of an orally administered generic drug will behave the same way in a nonapproved species should not be assumed.

The disposition of ciprofloxacin has been described in cats after intravenous administration of 10 mg/kg (see Table 7-1). In cats Vd<sub>ss</sub> of ciprofloxacin is 3.85 ± 1.34 L/kg, and plasma clearance is 0.64 ± 0.28 L/hr/kg, which exceeds the normal feline glomerular filtration rate (0.15-0.25 L/h/kg), suggesting that active tubular secretion occurs.<sup>206</sup> AUCs after intravenous and oral administration are 17 ± 5 and 3 ± 1.2 µg·hr/mL, respectively, in cats. Drug accumulation was not significant after seven oral administrations.<sup>205</sup> Ciprofloxacin is metabolized into active (in humans) and inactive metabolites (N-oxide [the primary metabolite in dogs] and N-desmethyl). However, high concentrations of unchanged drug are achieved in urine, as is demonstrated after administration of enrofloxacin (see Figure 7-10).

**Levofloxacin.** Levofloxacin is the optical S-isomer of the racemic drug substance ofloxacin (see Figure 7-8). Compared with older FQs, its spectrum includes mycoplasma and gram-negative organisms, but the spectrum is broader toward gram-positive organisms and includes anaerobes.<sup>207</sup> Ofloxacin is marketed as the levo isomer (i.e., levofloxacin) rather than the racemic mixture because the L-isomer is much more active against bacterial pathogens than the R-isomer. In humans levofloxacin is well absorbed orally, is distributed to a volume of 1.1 L/kg, and is renally excreted. Concentrations in the CSF approximate 16% of that in plasma, suggesting that the drug may not be well distributed into sanctuaries. Excretion is correlated with creatinine clearance, and half-life is prolonged with renal disease, requiring dose adjustments in patients with significant renal dysfunction.<sup>208</sup>

Because of its spectrum and improved antibacterial activity compared with veterinary FQs, levofloxacin has been used anecdotally in dogs but does not appear to have been studied in dogs. However, ofloxacin (but not its isomers) has been studied after oral administration in young and mature Beagles.<sup>209</sup> Peak concentrations (measured by HPLC) at 20 mg/kg were 14.2 ± 0.4 µg/mL. The dispositions of the L- and D-isomer are likely to differ, precluding prediction of the proportion of the C<sub>max</sub> represented by levofloxacin. However, even if 100% of

the drug is the L-isomer, concentrations are still well below the MIC<sub>90</sub> of levofloxacin. The disposition of levofloxacin has been well described in cats on the basis of a bioassay after intravenous and oral administration,<sup>210</sup> and it does not appear to be substantially different from that in humans. In cats the drug is well, albeit slowly, absorbed orally (T<sub>max</sub> 1.6 hours), with bioavailability at 87%. The drug is rapidly distributed, reaching a V<sub>dss</sub> of 1.75 L/kg; clearance is 0.14 L/hr/kg, and mean residence time is 13 hours (see Table 7-1). The C<sub>max</sub> following oral administration was 4.7 µg/mL, indicating that the drug should be used in cats only for organisms with an MIC of 0.5 µg/mL or less.

### Drug Interactions

The FQs inhibit selected hepatic drug-metabolizing enzymes and are known to prolong the elimination of selected drugs. Theophylline toxicity has been documented in humans and dogs (see Chapter 2) simultaneously receiving theophylline and ciprofloxacin or enrofloxacin.<sup>211</sup> Marbofloxacin also impairs the elimination of theophylline in dogs, but the effect is dose dependent, being absent at 2 mg/kg. However, at 5 mg/kg, theophylline clearance is decreased by 26% (compared with 50% reduction by enrofloxacin at 5 mg/kg IV once a day for 5 days), resulting in a change in theophylline half-life from 3.6 to 5.4 hours and a change in C<sub>max</sub> from 32 (no marbofloxacin) to 44 µg/mL (5 mg/kg marbofloxacin).<sup>212</sup> Ciprofloxacin has been associated with increased cyclosporine concentrations, prolonged anticoagulant effects of warfarin, and enhanced hypoglycemic effects of oral hypoglycemics and insulin. Presumably, enrofloxacin and other FQs might have similar effects. Because of chelation by magnesium, calcium, and other cations, drugs such as antacids, sucralfate, and multiple vitamins should not be administered orally at the same time as a FQ. Because FQs competitively inhibit gamma-aminobutyric acid receptor binding, drugs that act similarly (e.g., selected nonsteroidal antiinflammatory drugs) when used in combination may increase the risk of seizure or other CNS activity. Enrofloxacin has been associated with false glucosuria.<sup>77</sup>

**KEY POINT 7-26** Fluorinated quinolones can impair the metabolism of selected drugs.

The use of FQs in combination with other antimicrobials may result in synergistic activity (e.g., aminoglycosides for gram-negative organisms; beta-lactams for gram-positive or gram-negative organisms) (see Chapter 6) or antagonistic (e.g., ribosomal inhibitors).

### Adverse Effects

Adverse reactions to the FQs do not reflect interaction with mammalian topoisomerases. Most adverse reactions are predictable and can be prevented with proper administration. Gastrointestinal upset manifested by vomiting, nausea, and possibly diarrhea may occur after any route of administration but particularly oral administration. The intramuscular administration of enrofloxacin frequently causes pain on

injection. Nausea and vomiting have been reported when the intramuscular solution is given intravenously and may reflect mast cell degranulation and histamine release. The intramuscular solution also is very alkaline (pH 10). Diluting the drug in saline and administering it over a 30-minute period may reduce nausea and clinical signs consistent with an anaphylactoid response. FQs have been associated with allergic reactions; however, the lack of previous exposure in some (human) patients (and in the author's experience with ciprofloxacin) suggests an anaphylactoid rather than anaphylactic reaction.<sup>213</sup> Acute cardiovascular toxicity (hypotension, decreased left ventricular function) has been described for levofloxacin (Freedom of Information [FOI]) after an intravenous bolus (≥6 mg/kg) or intravenous infusion (≥20 mg/kg, but not ≤10 mg/kg). Increased circulating histamine concentrations accompanied the high-dose intravenous infusion, indicating a potential anaphylactoid reaction at 10, 15, 30, and 60 mg/kg intravenous bolus. Death occurred in dogs in association with neurologic and cardiac signs at 200 mg/kg, administered intravenously. Enrofloxacin also is available as a more concentrated solution (100 mg/mL) approved for use in cattle. However, it is prepared in an arginine-based vehicle, which is painful on injection and will cause perivascular inflammation if given parenterally by any route other than intravenous. Ulcers may occur if the large animal preparation is given orally.

**Cartilage deformities and ligament and tendon repair.** The FQs are associated with cartilage damage in dogs (and other species) (see package inserts). Enrofloxacin's original package insert cited clinical signs indicative of cartilage damage in Beagle puppies within 3 days of treatment at 12.5 mg/kg. Lesions have been documented in dogs treated with other FQs. For levofloxacin, arthropathies occurred in juvenile dogs at ≥10 mg/kg/day for 7 days (FOI). Lesions in adult dogs require much higher concentrations, as was demonstrated for levofloxacin: the no-observed-effect level was 3 mg/kg/day in normal 7- to 8-month-old dogs compared with 30 mg/kg/day in normal 18-month-old dogs. The arthropathic potential of ofloxacin (the racemic mixture of levo and the R-isomer of ofloxacin) also has been studied in dogs.<sup>209</sup> At 20 mg/kg for 8 days, eight out of eight 3-month-old animals developed histologic lesions, whereas only two developed clinical signs; the associated serum ofloxacin concentration was 14 µg/mL. The mechanism of cartilage damage is not known, although the most likely mechanism appears to be chelation of magnesium ions leading to dysfunction of integrins. These cell membrane proteins regulate a variety of cellular functions, including chondrocyte adherence to extracellular matrix and proteoglycan synthesis.<sup>215</sup> Magnesium-deficient diets in juvenile rats led to cartilage damage similar to that caused by FQs.<sup>216</sup> Indeed, magnesium supplementation may reverse the effects of FQs on canine chondrocytes.<sup>217</sup> Dogs may be among the most sensitive and the most likely to exhibit clinical lameness caused by FQ-induced cartilage damage.<sup>204</sup> Note that cartilage lesions as a result of FQs might be considered when FQs are used in any situation that involves growing or repairing cartilage, such as septic or immune-mediated arthritis and potentially osteoarthritis. Lesions have also been reported in other

species, including humans.<sup>218</sup> Use of chondroprotectants (i.e., polysulfated glycosaminoglycans) might be considered if FQ therapy must be instituted in growing dogs or other situations involving cartilage growth or repair.

The FQs appear to negatively affect healing in damaged ligaments.<sup>219</sup> Connective tissue proteins decreased by up to 73% in dogs treated with as little as 30 to 200 mg/kg ciprofloxacin orally. Lesions were similar to those produced in magnesium-deficient dogs, suggesting that FQs induce tendon or ligament damage by antagonizing magnesium effects in the affected tissues.

The impact of FQs on bone repair also may be of concern. Based on experimental fracture healing in rats receiving placebo, cefazolin, or ciprofloxacin (50 mg/kg every 12 hours subcutaneously for any of the aforementioned drugs), fracture callus healing appeared to be impaired by FQs.<sup>218</sup> In vivo studies in dogs of the effects of ciprofloxacin at 30 to 200 mg/kg/day orally (equivalent to approximately 15 to 65 mg/kg bioavailable drug) in dogs on either a normal or magnesium-deficient diet found a number of proteins were decreased in both groups at all doses, including collagen, elastin, and fibronectin.<sup>219</sup> Of these effects, the authors concluded that magnesium deficiency increases the risk of impaired healing in the presence of FQs.

**Seizures and other central nervous system disorders.** Seizures and other CNS disorders have been precipitated in human and veterinary patients<sup>220</sup> and animal models receiving FQs;<sup>221</sup> predisposing factors include a preepileptic state, high doses, and concurrent use of nonsteroidal antiinflammatory drugs.<sup>220</sup> Newer drugs may be more likely to cause CNS side effects.<sup>222</sup> FQs (and imipenem) inhibit GABA release, leading to hyperexcitability;<sup>76</sup> inhibition of N-methyl-D-aspartate or adenosine may also be involved.<sup>143</sup> FQs also lower seizure threshold and impede neuromuscular transmission. Peripheral neuropathies are a recognized side effect of FQs in humans.<sup>13,223</sup> Clinical signs in humans have been described as severe, involving multiple organs. Onset is described as rapid (within 24 hours of onset of therapy; 84% afflicted within 1 week) and long term in duration, with symptoms lasting more than 3 months in 71% of afflicted patients and more than 1 year in 58%. The majority of cases involved levofloxacin (64%), despite ciprofloxacin (21%) being the most commonly prescribed drug. The most frequent complaints included both sensory (tingling, burning, or numbness) and motor (musculoskeletal, cardiovascular, skin, gastrointestinal [cramping]) abnormalities; symptoms were described as severe in 80% of the patients.

**Dose-dependent retinal degeneration.** Dose-dependent retinal degeneration has been associated with use of FQs in cats. The incidence of ocular toxicity is very rare, occurring in 1 of 125,000 cats receiving enrofloxacin. The incidence at high doses is sufficiently low that toxicity was not detected in preapproval toxicity studies. During preapproval in cats, 25 mg/kg/day for 30 days and 125 mg/kg for 5 days were not associated with detectable toxicity. It is not clear whether ocular toxic-specific outcomes were addressed. Doses in clinical reports<sup>224</sup> in which ocular toxicity occurred (retrospective study) ranged

from 4.6 to 54 mg/kg/day, with duration of dosing ranging from 4 to 120 days. Clinical signs began with mydriasis, rapidly followed by acute blindness. Age may be a factor, with cats younger than 9 years seemingly requiring a higher (>20 mg/kg) dose. Diseases associated with changes in disposition that might result in high plasma enrofloxacin concentrations (e.g., renal disease, heart disease) may also increase the risk. Intravenous administration may increase the risk, further supporting the concentration dependence of toxicity.

Experimental studies by Bayer Animal Health in young, apparently healthy cats at 5, 20, and 50 mg/kg/day for 21 days found electroretinography changes in one of six cats at 20 mg/kg and severe changes in six of six cats within 1 week at 50 mg/kg. Manufacturers of other veterinary FQs have likewise performed follow-up ocular toxicity studies. Marbofloxacin was not associated with lesions in young cats treated with up to 27 mg/kg/day for 6 weeks or 55 mg/kg/day for 14 days. Orbifloxacin was not associated with lesions at 15 mg/kg/day orally for 30 days, but changes occurred at 45 and 75 mg/kg.<sup>225</sup>

**KEY POINT 7-27** Among the fluorinated quinolones currently approved for use in cats in the United States, marbofloxacin appears to be the least likely to cause retinal degeneration in cats.

The mechanism of ocular toxicity appears to reflect a mutation in four amino acids of an efflux protein in the blood-retina barrier, rendering it ineffective. Effective protein activity is absent in all cats. (personal communication, Dr. Katrina Mealey, Washington State University). The FQs are structurally similar to compounds known to cause accumulation in lysosomes of retinal pigment cells and subsequent ocular toxicity. Additionally, FQs have a predilection for pigmented cells of the eye. The FQs also have been associated with phototoxicity. The combination of FQs with ultraviolet radiation produces both a time- and concentration-dependent ocular toxicity, with a methyl group at position 8 of the quinolone ring reducing the risk.<sup>226</sup> Reducing exposure to sunlight (dosing at night, or keeping cats indoors) might be prudent for cats receiving FQs.

**Induction of bacteriophage supergenes.** Induction of bacteriophage supergenes has been associated with the use of FQs, and in dog bacterial isolates, specifically enrofloxacin. Shortly after approval of enrofloxacin in Canada, seven canine cases of streptococcal toxic shock syndrome (STSS) and/or necrotizing fasciitis (NF) were reported; four of the dogs had been treated with enrofloxacin in the early stages of infection. Treatment was not only ineffective, but the syndrome appeared to be worsened by the antimicrobial therapy.<sup>227</sup> Further investigation has provided some insight into the possible relationships between STSS and NF and bacteriophage supergenes in *S. canis*. Using polymerase chain reaction analysis, 22 of 23 *S. canis* isolates in one study exhibited a bacteriophage-encoded streptococcal superantigen gene. Under culture conditions, induction of the bacteriophage by enrofloxacin at therapeutic concentrations resulted in a 58-fold enhancement

of expression of the gene.<sup>228</sup> Apparently, the FQ stimulates autoingestion of a repressor protein that otherwise would prevent the bacteriophage from becoming lytic. FQs apparently also can induce bacteriophage lysis and enhanced Shiga toxin production in *E. coli*. For example, ciprofloxacin-treated mice experimentally colonized by Shiga-toxigenic *E. coli* died while their untreated colonized cohorts did not; increased Shiga toxin was demonstrated in their feces. However, induction requires ideal conditions, being dependent in part on stage and rate of growth and ideal drug concentration; conditions favoring bacteriophage induction in clinical patients have not yet been described.

### Therapeutic Use

The FQs originated from nalidixic acid, itself a by product of chloroquine.<sup>150</sup> Nalidixic acid is characterized by a narrow spectrum, and its use was limited to treatment of urinary tract infections. Modifications of chemical structures increasingly have improved the drugs, yielding drugs that have among the broadest of antibacterial spectrums. However, caution should be exercised with selected drugs because efficacy toward specific organisms (e.g., *Pseudomonas*, spp. anaerobes) varies. The FQs also are characterized as a class among those with the greatest tissue and antimicrobial distribution patterns. However, differences in tissue distribution (e.g., enrofloxacin versus ciprofloxacin, bone distribution of difloxacin) does indicate prudence when comparing FQ use. The rapid bactericidal effect of FQs is of clinical benefit in life-threatening situations or immune-suppressed patients; concentration dependence allows once-daily dosing that improves owner compliance. Intracellular accumulation of these drugs supports use for recurrent infections caused by intracellular organisms or at sites characterized by marked inflammation. Plasmid-mediated resistance has been slow to develop, although increasingly resistance, particularly that associated with multidrug resistance, is limiting FQ use. Oral bioavailability allows prolonged administration on an outpatient basis. However, bioavailability of the different drugs varies among the species, and good oral bioavailability should not be assumed. Rather, extrapolation of oral doses should be based on scientific studies. The unique mechanism of action of these drugs renders them appealing for combination antimicrobial therapy.

However appealing these numerous attributes of the FQs, common use of these drugs is discouraged. Widespread use—and abuse—of these drugs in the past 2 decades has proved that antimicrobial resistance can and will occur. Resistance, when it does occur, is often associated with multidrug resistance affecting chemically unrelated drugs. The emergence of MDR with newer FQs needs to be assessed. Confirmation of the need for the drug and attention to MPCs (see Chapter 6) in the design of the dosing regimen should be two hurdles that are consciously addressed each time these drugs are considered. The metabolism of enrofloxacin to ciprofloxacin and the reduced oral bioavailability of ciprofloxacin in dogs and cats coupled with the importance of ciprofloxacin as a human-medicine drug call for extra caution to be taken. Once the

decision is made to use an FQ, strict adherence to the principles of antimicrobial therapy, with a special focus on proper dosing regimens, is paramount to protecting this class of antimicrobial drugs, which is so critical to the medical community.

### Rifamycins

Rifamycins are macrocyclic antibiotics produced by *Amycolatopsis mediterranei*. Several semisynthetic derivatives of natural rifamycins (rifamycin SV, Rifampin, rifampicin, rifamiderifamide) have been used as extended-spectrum antibiotics.<sup>150</sup> Rifampin is among them. A large molecule (MW 823; see Figure 7-4) as with all rifamycins, it inhibits the B subunit of DNA-dependent RNA polymerase, suppressing RNA synthesis. Because mammalian RNA polymerase does not bind to rifamycins, its inhibition requires much higher concentrations. Rifampin can achieve bactericidal concentrations in some tissues. Effects are concentration-dependent for mycobacterium but unclear for other organisms. However, resistance develops very rapidly, markedly curtailing its use, and in general, rifampin should be used only in combination with other effective antimicrobials. Resistance may develop in as little as 2 days when it is used as the sole antimicrobial; rifampin is used experimentally to study mutation frequencies in some organisms. The use of rifampin as sole agent for treating pyoderma is addressed in Chapter 8. Resistance generally reflects a single mutation that changes the affinity of the target enzyme for the drug. Resistance (and efficacy) can be decreased with combination therapy with a number of drugs, including erythromycin, most beta-lactam antibiotics, chloramphenicol, doxycycline, and selected aminoglycosides. Rifampin has shown some efficacy against fungal microorganisms.

### Spectrum

The spectrum of activity of rifampin includes primarily gram-positive (especially *Staphylococcus* spp.) organisms (see Table 7-4). However, it also is effective against *Mycobacterium*, *Neisseria*, and *Chlamydia* spp. and has been used to treat *Clostridium* and *Bacteroides* species. Rifampin has limited activity against gram-negative organisms (including *Brucella*). Resistant gram-negative organisms include *E. coli*, *Enterobacter* spp. *K. pneumoniae*, *Proteus* spp. *Salmonella* spp., and *P. aeruginosa*. However, an Internet search reveals a number of papers that indicate efficacy toward *P. aeruginosa* when combined with a number of other drugs. Highly susceptible gram-positive organisms are considered to have an MIC of 0.25 µg/mL or less; MICs are often less than 0.1 µg/mL. In contrast, the MIC of gram-negative organisms is generally 8 to 32 µg/mL; the higher MICs reflect limited penetration of gram-negative organisms. A dose of 10 mg/kg in the dog achieves a  $C_{max}$  of 40 µg/mL (see Table 7-1); accordingly, its use for gram-negative isolates (and ideally, all isolates) should be based on C&S testing.

**KEY POINT 7-28** Rapid resistance to rifampin limits its use to combination therapy only.



### Pharmacokinetics

Rifampin may be administered intramuscularly, intravenously, or orally with systemic effects. Oral absorption of rifampin is incomplete in humans (~40%) with peak plasma concentrations occurring in 2 to 4 hours. Concurrent feeding may reduce or delay absorption. Because it is a substrate for P-glycoprotein,<sup>229</sup> oral absorption may be much higher in dogs exhibiting P-glycoprotein deficiency. Approximately 75% to 80% of rifampin is bound to plasma proteins. Rifampin is very lipid soluble, distributing well to most body tissues. It concentrates in white blood cells and is characterized by immunomodulation.<sup>230</sup> Because rifamycins penetrate tissues and cells to a substantial degree, they are particularly effective against intracellular organisms. Rifampin is rapidly eliminated after acetylation to a metabolite (desacetyl rifampin) that is equal in efficacy to the parent compound. Whether the dog is a deficient acetylator of rifampin is unclear. Both the parent and metabolite are excreted in the bile (supporting its use for cholangitis in humans); the parent compound and metabolite undergo enterohepatic circulation. The elimination half-life of rifampin is dose dependent, being about 8 hours in dogs.

### Adverse Effects

Rifampin is usually well tolerated and produces few side effects. However, gastrointestinal disturbances and abnormalities in liver function (icterus) have been reported in humans and may lead to discontinuation of therapy. Hypersensitivity reactions can also result from rifampin administration, and renal failure is a possible consequence when intermittent dosage schedules are followed. Partial, reversible immunosuppression of lymphocytes occurs. Urine, feces, saliva, sputum, sweat, and tears are often colored red-orange by rifampin and its metabolites; urine may stain. Plasma will also be orange and may be misinterpreted as hemoglobinemia. CNS depression after intravenous administration and temporary inappetence may occur. Interestingly, intermittent administration (less than twice weekly) increases the risk of side effects in humans, resulting in a flulike syndrome that is associated with clinical signs indicative of a drug reaction (eosinophilia, thrombocytopenia, hemolytic anemia [note potential for orange discoloration of plasma] and renal disease).<sup>150</sup> In a limited number of dogs, marked increases in serum alkaline phosphatase have been observed by the author. No other liver enzyme or function tests were affected, and dogs did not become clinically ill. The increase may reflect induction of the enzymes (much the same as glucocorticoids or phenobarbital), but monitoring of hepatic function may be prudent in at-risk dogs receiving rifampin.

### Drug Interactions

Rifampin is a broad, potent inducer of microsomal enzymes, including CYP1A2, 2C9, 2C19, and 3A4;<sup>150</sup> as such, it will shorten the elimination half-life of a number of drugs and may increase the risk of toxicity associated with drug metabolism.<sup>229</sup> Therapeutic failure may occur for other drugs metabolized by the liver if modifications in dosing regimens are not made. Rifampin PDCs will decrease after multiple dosing

because of induction, with plasma elimination half-life of rifampin progressively shortening by approximately 40% during the first 2 weeks of treatment in humans. Other affected drugs include the imidazoles, cyclosporine, digoxin, and several sodium channel- and beta receptor-blocking cardiac antiarrhythmics. Endogenous substrates of hepatic metabolism also may be affected; several steroids will be more rapidly catabolized.<sup>150</sup> Withdrawal syndromes have been reported in humans receiving opioid analgesics.<sup>150</sup> Because rifampin is a substrate for P-glycoprotein, dogs with the MDR-1 (ABC) deletion will have an increased risk of adverse reactions; the risk is increased if rifampin is used in combination with other drugs that interact with this protein. Finally, rifampin also has decreased biliary secretion of some compounds, notably contrast imaging media.<sup>150</sup> Rifampin has been used in combination with a number of drugs to enhance efficacy (and reduce resistance; see the section on resistance) for treatment of MRSA, VRE, and *Mycobacterium* spp. and others. Use in combination with doxycycline has been recommended for canine brucellosis, although clinical efficacy has not been demonstrated.<sup>2</sup>

**KEY POINT 7-29** Rifampin is a potent inducer of drug-metabolizing enzymes.

Two other rifamycins are approved for use in humans. Rifabutin is a derivative of rifampin that is characterized by less induction of drug-metabolizing enzymes. Used for the treatment of *Mycobacterium* spp., it is characterized by unique side effects, including polymyalgia, anterior uveitis, and others. Rifapentine is used to treat tuberculosis associated with human immunodeficiency virus infections in humans. Its longer half-life allows once-weekly dosing, and its impact on drug-metabolizing enzymes has been described as intermediate.<sup>150</sup> Rifaximin is a semisynthetic derivative of rifamycin that is not orally absorbed. It is indicated for treatment of enteric pathogens, including *Campylobacter*, *C. difficile*, *E. coli*, *Helicobacter pylori*, and *Salmonella* and *Shigella*.<sup>231</sup> A potential advantage of rifaximin is an apparent minimal long-term effect on the gastrointestinal flora: both *E. coli* and *Enterococcus* spp. were minimally affected after 3 to 14 days of therapy. Resistance to rifaximin seems to emerge only slowly, compared with systemic use of rifampin.<sup>231</sup> Indications in humans have been a variety of (nonbloody) diarrheas, including small bowel overgrowth, intestinal gas, and inflammatory bowel disease.

### Metronidazole

Metronidazole is derivative of the antibiotic azomycin (2 nitro-imidazole) secreted by a streptomycete (Figure 7-12).<sup>232</sup> A number of other nitroimidazoles were developed from azomycin.<sup>232</sup> Among the other closely related imadazoles used outside the United States are tinidazole, and benznidazole, the latter being used to treat acute Chagas disease. Metronidazole impairs microbial RNA and DNA synthesis but must first undergo nitrous reduction in the organism. As such, metronidazole is a prodrug, with efficacy depending on the

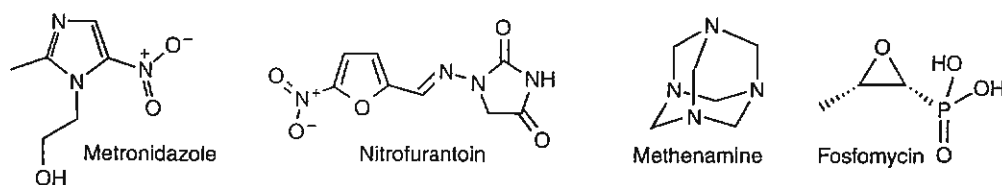


Figure 7-12 Chemical structure of miscellaneous antimicrobials.

nitrous group and a low redox potential that can be achieved only in an anaerobic environment.<sup>233</sup> Only organisms that live in a low-oxygen environment have developed anaerobic energy or electron-generating pathways (e.g., ferredoxins) capable of generating single electrons. Transfer of the electron to the nitrous group of metronidazole results in a highly reactive nitro radical ion. Although DNA is the primary target, other macromolecular structures may be targeted. Metronidazole will be regenerated on death of the microbe, thus facilitating its efficacy. Efficacy appears to be predominantly bactericidal, although actions may be bacteriostatic toward some organisms (e.g., *Eubacterium* spp.). Metronidazole acts as a concentration-dependent drug against trichomoniasis, and, although this is not always clear, it also appears to be concentration dependent when treating other microbes. However, time dependence has also been ascribed<sup>234</sup> (e.g., *Clostridium* and its efficacy appear to be similar if administered once or twice daily).<sup>235</sup>

**KEY POINT 7-30** Metronidazole is effective only against anaerobic bacteria.

### Spectrum

Metronidazole is rapidly bactericidal against all gram-negative (e.g., *B. fragilis*) and most gram-positive (e.g., *Clostridium* spp.) anaerobic bacilli, generally at MIC equal to or less than 8 µg/mL. Microaerophilic microbes such as *Helicobacter* and *Campylobacter* spp. are susceptible. Metronidazole is effective toward a number of protozoa, with efficacy dependent on the nitro group at position 5 and enhanced with substitutions at the 2 position.<sup>236</sup> Susceptible infections include trichomoniasis (MIC of 0.05 µg/mL if anaerobic conditions), amebiasis, and giardiasis (1 to 50 µg/mL).

### Resistance

Aerobes and facultative anaerobic bacteria lack electron transport systems necessary to generate single electrons and thus are resistant to metronidazole. Further, in higher oxygen environments, oxygen will compete for the electrons generated by anaerobic organisms, thus decreasing efficacy of metronidazole. Higher doses are necessary if the infection occurs in an environment of 1% or more oxygen.<sup>232</sup> Interestingly, protozoa may develop resistance to metronidazole in patients with impaired oxygen-radical scavenging abilities.<sup>232</sup> Microbes also acquire resistance by decreasing proteins that generate the electrons (e.g., ferredoxin). The mechanism of bacterial resistance is not totally clear, but increased production of

interfering enzymes is likely. Resistance by *Helicobacter* spp. can be rapid.

### Pharmacokinetics

Metronidazole is well distributed to all body tissues and can penetrate the blood–brain barrier. It is minimally protein bound (in humans). Elimination is dose dependent and occurs primarily by hepatic metabolism. At least one metabolite has 50% of the activity of the parent compound toward trichomonads. Intestinal microbes can produce a small amount of the reduced (active) metabolites. Peak concentrations in dogs after 44 mg/kg reached 42 µg/mL. Vd is 0.95 ± 0.1 L/kg, and clearance is 2.5 ± 0.54 mL/kg/min.<sup>237</sup> Oral bioavailability is variable, ranging from 59% to 100%. Elimination half-life in one study was 4.5 ± 9 hours (see Table 7-1). Metronidazole disposition has been described in the cat after single intravenous (5 mg/kg) administration as the salt-free product and then at 20 mg/kg orally of the benzoate salt (12.4 mg/kg active drug).<sup>238</sup> Extrapolated plasma concentration after intravenous administration at time 0 averaged 7.8 ± 2 µg/mL; Vd was 0.7 ± 0.3 L/kg, and plasma clearance was 91 mL/kg/hr. Elimination half-life and mean residence time were 5.3 ± 0.7 and 7.6 ± 1 hours, respectively. The benzoate salt was fairly well absorbed but was characterized by clinically significant variability, with a bioavailability of 65% ± 27% (range 28% to 80%). The C<sub>max</sub> also varied, with a mean of 8.8 ± 5.4, reflecting a range of 4.9 to 17.8 µg/mL; T<sub>max</sub> also varied from 1 to 8 hours (mean 3.6 ± 2.9 hours). Elimination half-life and mean residence time after oral administration were 5.2 ± 0.5 and 8.7 ± 1.3 hours, respectively.

### Adverse Effects

Metronidazole may discolor urine (red-brown).<sup>232</sup> More problematic adverse reactions include gastrointestinal upset (including hepatotoxicity when given at high doses) and CNS adversities, including seizures.<sup>237,239</sup> The risk of neurotoxicity is increased with intravenous administration; as such, oral administration is the preferred route whenever possible. The caustic nature of the intravenous solution also necessitates slow intravenous administration. The mechanism of neurotoxicity is not known, but in mice degenerative lesions have been demonstrated in the Purkinje cells, vestibular tracts, and several nuclei associated with equilibrium and fine motor control. These areas are also the site of the majority of gamma-aminobutyric acid–minergic receptors. In humans, characteristic lesions seen on magnetic resonance imaging indicate that the cerebellum may be most sensitive to damage; because interstitial edema was evident, with axonal swelling was suggested as a cause.<sup>240</sup>

In dogs, seizures are indicative of toxicity. One study in dogs ( $n = 21$ ) induced seizures at doses of 60 to 110 mg/kg for a total of 10 to 110 days.<sup>241</sup> The most common clinical signs were vertical nystagmus, ataxia, inability to walk ( $\geq 50\%$  each), and paraparesis (30%); less frequent neurologic signs included tetraparesis, hypermetria, tremors, head tilt, torticollis, and opisthotonus. Treatment with diazepam proved effective based on a shorter response time (resolution of debilitating clinical signs; 13 hours versus 4.5 days) as well as recovery time (return to normalcy; 11 versus 36 hours). The dose of diazepam was approximately 0.5 mg/kg, administered intravenously followed by oral administration every 8 hours for 3 days. Neurologic reaction to metronidazole has also been reported in cats ( $n = 2$ ). The dose and duration associated with clinical signs were 111 mg/kg body for 9 weeks followed by 222 mg/kg/day for 2 days in one cat and 58 mg/kg for 6 months in the second.<sup>242</sup> Clinical signs in cats included ataxia, altered mentation, and progression to seizures. Neurologic signs resolved within days of discontinuation of the drug and supportive therapy. Histologic lesions have also been described in another 14-year-old cat that developed fatal presumed metronidazole toxicity after treatment for inflammatory bowel disease at 73 to 147 mg/kg/day. Among the neurologic clinical signs was acute tetraparesis; lesions included diffuse, multifocal areas of necrosis throughout the brainstem.<sup>243</sup>

Metronidazole as either the free form or when administered as the benzoate salt was genotoxic (disruptive of lymphocytic DNA) but not cytotoxic to feline polymorphonuclear cells. Genotoxicity resolved within 7 days after the drug was discontinued.

### Preparations

Metronidazole is available as either a hydrochloride (used in the approved product) salt (oral or intravenous) or, in pure drug substrate form (i.e., for compounding), the benzoate salt. It can be administered as a loading dose infused over 30 to 60 minutes in fluids, followed by an intravenous drip. It also can be given intermittently as an 8- to 12-hour maintenance dose as long as the infusion takes place slowly. For intravenous administration the dose should be neutralized with sodium bicarbonate and mixed with lactated Ringer's solution, saline, bacteriostatic water, or 5% dextrose in water (see package insert). Because intravenous administration of metronidazole is complicated, oral administration is preferred whenever possible.

The benzoate salt of metronidazole, which is not commercially available, is less bitter tasting and more tolerable than the commercially available hydrochloride salt. The oral disposition of the benzoate salt was previously described.<sup>238</sup> However, the benzoate moiety is larger than the hydrochloride moiety, representing 38% of the drug product. As such, when dosed on total drug weight, the dose of metronidazole benzoate should be 1.6 times the dose of metronidazole hydrochloride.<sup>244</sup> Further, the benzoate must be removed by desterification before its absorption; it is not clear if oral administration of the benzoate form will be as effective against gastrointestinal microbes compared with a nonbenzoate form.

Metronidazole (not studied as a salt) has been demonstrated to be stable in solutions when stored at 40° C for 90 days.<sup>245</sup> However, it reacts with the aluminum of needles or other canulas. Metronidazole is subject to drug interactions associated with inhibition (e.g., cimetidine) or induction (e.g., phenobarbital, prednisone, rifampin) of drug-metabolizing enzymes.

Metronidazole is available as a topical gel, which provides wound odor control. Although it can be prepared as a transdermal PLO gel, studies by the author demonstrated minimal absorption when applied to the pinna of the ear for 3 weeks at 15 mg/kg.

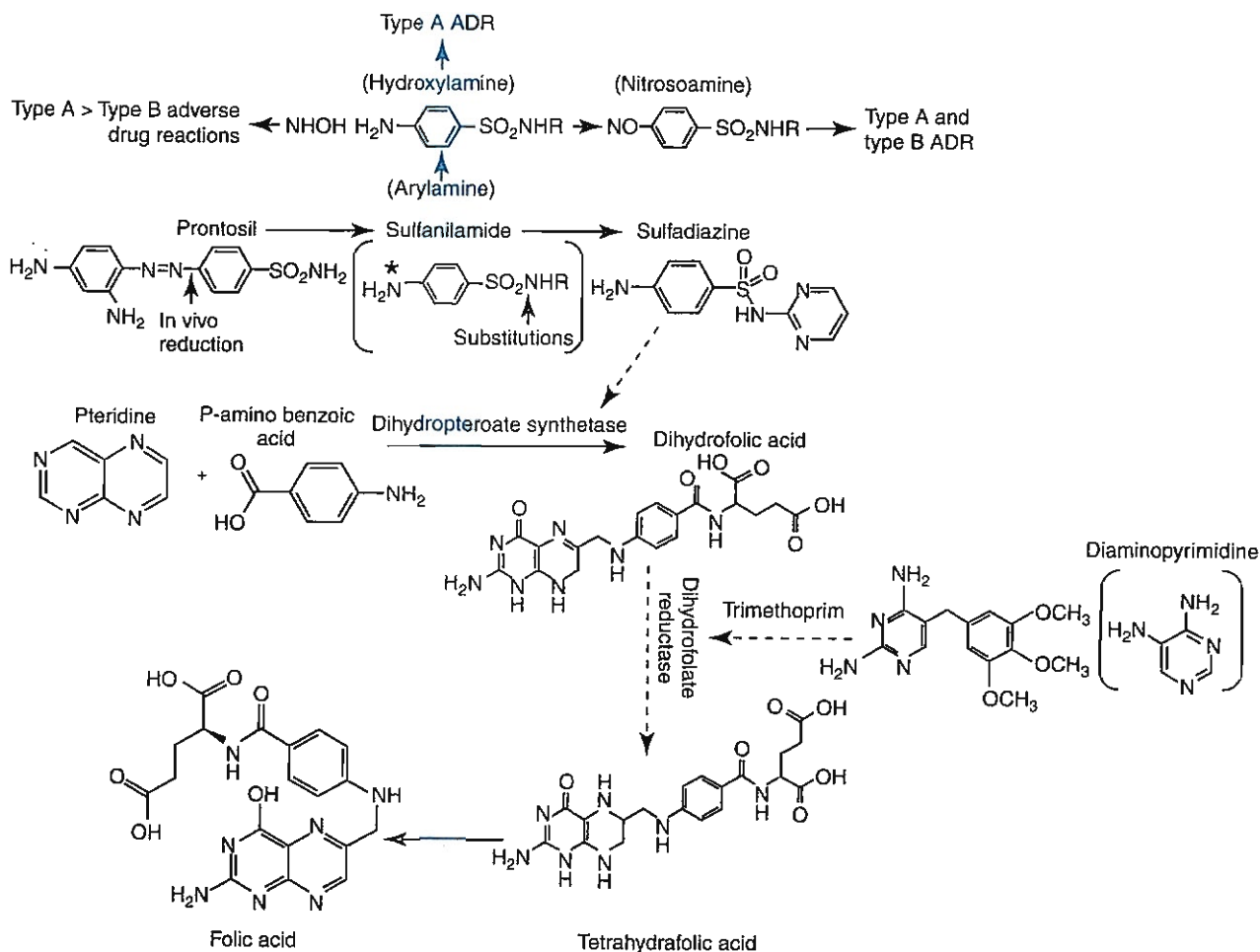
Metronidazole is a drug of choice for treating infections caused by obligate anaerobes, particularly those associated with gastrointestinal flora. Increasingly, it is used in lieu of oral vancomycin to treat *C. difficile*. Frequently, it is cited as a treatment for inflammatory bowel diseases in animals or humans (particularly Crohn's disease). Its efficacy may reflect, in part, immunomodulatory properties (see Chapter 19) or its ability to target those microbes most likely to produce inflammatory mediators.

## DRUGS THAT TARGET FOLIC ACID

### Inhibitors of Folic Acid Synthesis:/Sulfonamide/Trimethoprim or Ormetoprim Combinations

The sulfonamides are the oldest group of antibiotics used therapeutically. All sulfonamides that are currently used were derived from the first clinically relevant sulfonamide, sulfanilamide, itself a derivative of the azo dye prontosil. The discovery of its efficacy in vivo but not in vitro indicated that metabolism by the host was necessary for efficacy and contributed to the understanding of the role of drug metabolism in bioactivation. Once the metabolite was identified as the active drug, a number of manufacturers produced hundreds of different sulfonamide antimicrobial preparations. The FDA had not yet been empowered by Congress to evaluate drug safety, resulting in the lack of safety limitations. Among the vehicles in which drugs were prepared was a product containing ethylene glycol. The subsequent death of more than 100 persons, including children, ingesting the product contributed to congressional approval of the Food Drug and Cosmetic Act of 1938. It was this act that empowered the FDA to evaluate drugs for safety before marketing.

The sulfonamides were the first group of commercially available antimicrobials used systemically.<sup>246</sup> Their use was somewhat curtailed by the advent of the penicillins, only to increase again in the 1970s with their combination with the diaminopyridine trimethoprim. Not surprisingly, long-term use of these drugs has contributed to the development of resistance that has limited their clinical use.<sup>246</sup> However, a decline in their use, in part because of concerns regarding drug allergies, probably has contributed to a decline in resistance. Sulfonamides generally are used in combination with diaminopyrimidines for treatment of bacterial infections, with use of sulfonamides as sole agents generally limited to treatment of coccidiosis.



**Figure 7-13** The mechanism of action of the sulfonamides and the diaminopyrimidines. By itself, either type of drug is bacteriostatic, but the two-point sequential inhibition of folic acid synthesis results in bactericidal effects. The progenitor of the sulfonamides is sulfanilamide (*inset*). As such, all are arylamines. Metabolism of the arylamine to a hydroxylamine and nitroso compound contributes to the toxicities, including drug allergies, associated with sulfonamides.

### Structure Chemistry Relationship

As derivatives of sulfanilamide, all sulfonamides have the same nucleus. Functional groups have been added to produce compounds with varying physical, chemical, pharmacologic, and antibacterial properties, but the active amine is in position 4 and any substitutions at this position must be freed *in vivo* (Figure 7-13). Although amphoteric, sulfonamides generally behave as weak organic acids and are much more soluble in an alkaline than in an acidic environment. Those of therapeutic interest have  $pK_a$  values between 4.8 and 8.6. Water-soluble sodium or disodium salts are used for parenteral administration. Such solutions are highly alkaline, somewhat unstable, and readily precipitate out with the addition of polyionic electrolytes. In a mixture of sulfonamides (e.g., the sulfapyrimidine group), each component drug exhibits its own solubility; therefore, a combination of sulfonamides is more water soluble than a single drug at the same total concentration. This is the basis of triple sulfonamide mixtures used clinically (primarily in large animals). The N-4 acetylated sulfonamides, except for the sulfapyrimidine group (sulfadiazine), are less water soluble than their nonacetylated forms. Highly insoluble

sulfonamides are retained in the lumen of the gastrointestinal tract for prolonged periods and are known as "gut-active" sulfonamides. Most sulfonamides used clinically for treatment of bacterial infections are "potentiated." The "potentiator" of sulfonamides is a diaminopyrimidine; examples include trimethoprim, ormetoprim, and pyrimethamine (the latter being the preferred drug for toxoplasmosis) (see Figure 7-13).

### Mechanism of Action

Folic acid is an essential bacterial substrate necessary for protein and nucleic acid metabolism. Bacterial synthesis of folic acid is accomplished in several sequential steps (see Figure 7-13). The sulfonamides are structurally similar to PABA and act as competitive substrates (antimetabolites) for the synthetase enzyme. Among the many sulfonamides used clinically are sulfadiazine, sulfamethoxazole, sulfachlorpyridazine, sulfadimethoxine, and sulfasalazine; sulfisoxazole is the model drug upon which C&S testing is based.

Because folate metabolism is required for many cellular functions, bacterial growth is inhibited; consequently, the antibacterial effects of sulfonamides as sole agents are

bacteriostatic. The diaminopyrimidines trimethoprim and ormetoprim also impair folic acid synthesis but at a different point in the metabolic pathway. They prevent the conversion of dihydrofolate to tetrahydrofolate by inhibiting the reductase enzyme. By themselves, these drugs also are bacteriostatic. It is the combination of a sulfonamide antimicrobial with a diaminopyrimidine antimicrobial ("potentiated") that results in subsequent two-point inhibition of bacterial folic acid synthesis and thus bactericidal rather than bacteriostatic activity (see Figure 7-13).<sup>5,246</sup> Mammalian cells are not affected by these drugs because they are dependent on dietary sources of folic acid; in contrast, microbes cannot use external sources of the substrates. Further, the affinity of bacterial enzymes for the drugs is much higher than the mammalian enzymes. The competitive nature of the mechanism of killing activity of potentiated sulfonamides leads to a time-dependent effect. High inoculums may require higher doses for efficacy.

**KEY POINT 7-31** The combination of the sulfonamide and the diaminopyrimidine results in bactericidal effects.

### Spectrum of Activity

The spectrum of activity of sulfonamides is considered broad, but efficacy is variable because of acquired resistance. However, a decline in their use during the last decades (due to concerns regarding allergies) appears to be associated with an increased in susceptibility for a number of organisms. The spectrum of combined products includes gram-positive, gram-negative, and anaerobic organisms. The sulfonamides exhibit good to moderate activity against *E. coli*; *Enterobacter* spp.; *Klebsiella* spp.; *Proteus* spp.; *Pasteurella* spp.; and anaerobic organisms such as *Actinomyces*, *Bacteroides*, *Fusobacterium* spp., and selected clostridia.<sup>247-249</sup> The spectrum of these drugs does not include *Serratia* spp., *P. aeruginosa*, *Rickettsia*, or *Mycoplasma* spp. The sulfonamides exhibit good efficacy against *Brucella* spp., *Actinomyces* spp., and selected protozoal organisms such as *Pneumocystis carinii* and *Cryptosporidium* spp. Some *Chlamydia* spp. are susceptible to sulfonamides, whereas others are not. The difference appears to be based on whether the organism can obtain folic acid from the host.<sup>249a</sup> *Mycoplasma* organisms are not susceptible to sulfonamides. By itself, trimethoprim has a potency that is twentyfold to 200-fold less than that of sulfonamides.<sup>246</sup> Potentiated sulfonamides are generally useful for uncomplicated infections of many body systems.

### Resistance

Inherent resistance to sulfonamides reflects, in part, the ability of the microbe to make use of host folic acid. Resistance to the sulfonamides and to trimethoprim or ormetoprim occurs relatively rapidly. Chromosomal resistance results in impaired drug penetration, reduced affinity of the enzyme for the substrate, or increased bacterial production of PABA. Plasmid-mediated resistance occurs rapidly because of altered drug penetration and decreased affinity of the enzyme for the substrate. Resistance to one sulfonamide generally results in

resistance to all sulfonamides.<sup>246</sup> The increasing emergence of resistance has sharply curtailed the use of these drugs. The role of trimethoprim/sulfonamide combinations for the critically ill patient or for chronic infections should be based on C&S information because of the incidence of resistance.

### Pharmacokinetics

The sulfonamides are generally rapidly and completely absorbed after oral administration, although there are exceptions (see the discussion of structure and chemistry). Trimethoprim and ormetoprim are well absorbed after oral administration. Sulfasalazine is poorly absorbed as an intact molecule and is used primarily for gastrointestinal diseases. After oral administration sulfasalazine is partially absorbed in the small intestine. It undergoes enterohepatic circulation and ultimately is eliminated in the urine. Most of the drug (70%) is metabolized by colonic bacteria to its component parts: sulfapyridine and 5-aminosalicylic acid. Sulfapyridine is rapidly absorbed and subsequently eliminated in urine. The 5-aminosalicylic acid may provide the major therapeutic benefit for chronic inflammatory bowel disease.<sup>246</sup>

Solutions intended for parenteral administration must be buffered to prevent pain and irritation caused by the alkalinity of the compounds. Topical administration is not recommended because of the effects of these drugs on wound healing. An exception is made for silver sulfadiazine and mafenide, which are used primarily for burn patients in human.<sup>246</sup> Sulfadiazine is combined with silver in a topical otic preparation approved for use in dogs. Protein binding of the sulfonamides varies from 15% to 99%. Examples include sulfadiazine at 30% to 50% bound, sulfadiazine, at greater than 75%, and sulfasalazine up to 99% bound. Protein binding contributes to a relatively long half-life, allowing for convenient dosing intervals.

**KEY POINT 7-32** The disposition of the sulfonamides, which are time dependent, is markedly variable among members of this drug class.

The tissue penetrability of the sulfonamides varies. All are distributed at least to extracellular fluid. Sulfamethoxazole (the model drug for susceptibility testing) is limited to interstitial fluid, whereas sulfadiazine is distributed to total body water.<sup>246</sup> Sulfadiazine penetrates most body tissues extremely well, including the prostate.<sup>250</sup> The penetration of these drugs varies with the sulfonamide component. Prostatic penetration is facilitated by a high  $pK_a$ . Sulfadiazine ( $pK_a$  6.4) is among the best distributed sulfonamides but only achieved 11% of serum concentration in the prostate of dogs in one study (the original reference for this study could not be found). Drugs with a more basic  $pK_a$  may appear to better penetrate the prostate, although this may reflect ion trapping in prostatic fluids. Sulfadiazine can attain therapeutic concentrations in CSF, particularly if given intravenously, and is the preferred sulfonamide for CNS infections.<sup>246</sup> Trimethoprim achieves tissue concentrations four times higher than that in plasma. The combination of a sulfonamide with a diaminopyrimidine at a ratio of

1:5 trimethoprim/sulfonamide results in a bactericidal effect and a tissue distribution ratio of 1:20 in most tissues.<sup>5</sup> This ratio, however, is described in humans, and information in dogs or for sulfadimethoxine and ormetoprim does not appear to be available.

Sulfonamides that undergo hepatic metabolism are generally acetylated. All sulfonamide antimicrobials are arylamines. The dog lacks some genes that encode for N-acetyltransferases responsible for metabolism of arylamines.<sup>251</sup> Thus metabolism in the dog may involve other pathways, facilitating the formation of potentially nitroso metabolites that are responsible for allergic or other idiosyncratic reactions (see the section on adverse reactions) (see Figure 7-13).<sup>252</sup> Drugs are renally excreted as either the parent compound or the conjugated metabolite by either glomerular filtration or active tubular secretion. Both passive reabsorption and enterohepatic circulation can prolong the elimination half-life of selected sulfonamides.<sup>246</sup> Acetylated metabolites of sulfonamides are often less soluble than the parent compounds, which increases the risk of renal damage should drug precipitate and form crystals. However, this is unlikely in dogs because of deficient acetylation. The risk is reduced in other species because of the use of combination products, which reduces the total amount of dose needed for efficacy. The elimination half-lives of the drugs vary with the sulfonamide component and among the species. The duration at which sulfonamides remain in the body leads to classification as short-acting (12 hours or less: sulfacetamide, sulfathiazole, and sulfisoxazole), intermediate-acting (12 to 24 hours: sulfadimethoxine, sulfisoxazole, sulfamethoxazole, sulfapyridine, sulfamethazine, and sulfadiazine), and long-acting (longer than 24 hours).<sup>246</sup> In the dog, according to the package insert, sulfadimethoxine concentrations are 39 µg/mL 24 hours after dosing. Peak ormetoprim at 2 hours was 1.09 µg/mL in dogs but was 0.09 µg/mL at 24 hours, indicating a half-life of about 6 hours. It is not clear whether the differences in half-life between sulfadimethoxine and ormetoprim "match" in terms of ideal proportion throughout the labeled 24-hour dosing interval.

### Adverse Effects

Reactions to sulfonamide antimicrobials reflect the greatest proportion of antimicrobial adversities in the dog.<sup>252</sup> The adversities to sulfonamide antimicrobials but not other sulfonamides (e.g., nonsteroidal antiinflammatories, zonisamide, furosemide) probably reflect the basic structure of the sulfanilamide molecule, which is an arylamine, in which the amine group is directly attached to the benzene ring (see Figure 7-13). The susceptibility of dogs to sulfonamide toxicity may reflect the species' deficiency in acetylation and specifically N-acetylation. The proposed mechanism of toxicity reflects shunting of the sulfanilamide arylamine to an oxidative phase I pathway (see Figure 7-13). Oxidation of the arylamines yields hydroxylamine, a metabolite that can be cytotoxic at high concentrations; the metabolite also is somewhat allergenic. Hydroxylamine can be further metabolized (often spontaneously) to a nitroso compound, which is somewhat cytotoxic but is more immunogenic

than the hydroxyarylamine. The potential role of the arylamine as a cause of sulfonamide toxicity is supported by the lack of apparent toxicity by other sulfonamide drugs used in dogs, which, lacking a primary arylamine, are not converted to hydroxylamine. The likelihood of adversity may be related to the type of metabolites formed and the rate of acetylation. As such, the likelihood of toxicity occurring may vary among the sulfonamide antimicrobials. The mechanism of hypersensitivity may reflect haptenization of the metabolite and a subsequent T-cell response, although other mechanisms (e.g., humoral response or cytotoxicity) may contribute.<sup>252</sup> Deficiencies in glutathione, ascorbic acid, or other radical scavengers may increase the risk of either type A or B reactions; the role of supplementation in preventing or treating adversities apparently has not been addressed scientifically but may be prudent. Controversy exists as to whether the parent sulfonamide might be immunogenic.<sup>252</sup> The "potentiator" may also be responsible for some reactions; for example, trimethoprim has been associated with skin eruptions or hepatopathy in humans; further, use of sulfadiazine as the sole coccidiostat in dogs has not been associated with drug allergies.

**KEY POINT 7-33** Adversity to the sulfonamides probably reflects their metabolism to toxic or allergenic metabolites.

**Type A (I) Adverse drug reactions.** With the exception of thyroid-gland suppression, sulfonamides, and sulfadiazine in particular, appear to be free of type A or I adverse drug reactions at doses higher than those used therapeutically. For example, suppression of the thyroid gland was the only adverse effect evident in dogs treated with sulfadiazine at 300 mg/kg a day for 20 days. Any sulfonamide, including antimicrobial drugs, may profoundly alter thyroid physiology at high doses (25 mg/kg twice daily). The sulfonamide is a reversible substrate inhibitor of thyroid peroxidase, preventing the iodination and coupling of tyrosine residues necessary for formation of thyroxine and thyronine.<sup>253</sup> Whereas labeled doses of sulfadiazine and trimethoprim do not appear to cause thyroid suppression at least for 4 weeks, clinical hypothyroidism has occurred in one dog treated with trimethoprim sulfadiazine at 48 mg/kg/day for 10 weeks. Experimentally induced suppression of thyroid hormone (T<sub>4</sub>) synthesis occurred in 57% of dogs treated for pyoderma at 60 mg/kg/day for 6 weeks. Decreased thyroid hormone synthesis generally will be clinically relevant by 3 weeks of therapy but may take 6 to 8 weeks or longer and will return to normal within 3 weeks after therapy is discontinued.

Aplastic anemia has been reported in dogs receiving 30 to 60 mg/kg of sulfadiazine a day,<sup>252</sup> although the role of allergy versus folic acid deficiency was not documented. Because mammalian cells can use dietary folic acid, supplementation might be considered, particularly for patients that develop anemia (normocytic rather than megaloblastic)<sup>252</sup> consistent with folic acid deficiency while receiving a sulfonamide. Cats appear to be more sensitive to the effects of trimethoprim/sulfonamide combinations. Doses of 300 mg/kg per day for 10 to 30 days orally resulted in lethargy, anorexia, anemia, leukopenia, and increased blood urea nitrogen. Before the advent of

triple and potentiated sulfonamide preparations, crystalluria was a common type I side effect, with subsequent renal damage. Nonetheless, with high doses of any sulfonamide product, prudence dictates that the hydration status of the animal be normal, particularly if urinary pH is acidic.

**Type B (II) Adverse drug reactions.** Although the sulfonamides are generally safe drugs, the advent of hypersensitivity drug reactions (immunologic) has limited their use. Immune-mediated diseases of the skin, kidney, liver, and eye are not dose dependent.

Sulfonamide antimicrobial toxicity in animals has been reviewed.<sup>252,254</sup> The incidence of systemic sulfonamide toxicity in dogs has been reported as 0.25%. In a study of dogs (n = 40), inclusion criteria included clinical signs consistent with a drug allergy and treatment with a sulfonamide antimicrobial for at least 5 days.<sup>254</sup> The breeds most often represented were Golden Retrievers, Miniature Schnauzers, German Shepherd Dogs, Labrador Retrievers, and Samoyeds, with Miniature Schnauzers and Samoyeds being overrepresented. The lack of representation by Doberman Pinschers was suggested to reflect decreased treatment of this breed with sulfonamides. Ages ranged from 6 months to 14 years (mean  $5.7 \pm 3.2$ ), and neutered female dogs were overrepresented (60%). Three sulfonamides were represented, with 64% of afflicted dogs receiving sulfamethoxazole, 23% sulfadimethoxine, and 13% sulfadiazine; either trimethoprim or ormetoprim also were administered. No information was available regarding the proportion of sulfonamides prescribed to dogs. The frequency of each drug being administered was not determined. Doses ranged from 23 to 81 mg/kg/day, and time of onset ranged from 5 to 36 (mean 12) days. The most common clinical signs and the proportion of animals afflicted were fever (55%), thrombocytopenia (54%), hepatopathy (28%), neutropenia (27%), keratitis sicca (25%), and hemolysis (22%). Facial palsy was an unusual clinical sign. Other clinical signs included arthropathy, uveitis, skin and mucosal lesions, proteinuria, facial palsy, hypothyroidism, pancreatitis, facial edema, and pneumonitis. Dogs with hepatopathy or thrombocytopenia had a significantly lower recovery rates.<sup>254</sup> Dogs with hepatopathy also tended to have received the highest doses, suggesting that a toxic metabolite might be responsible and the adversity might be, in part, type A rather than type B (i.e., dose dependent and thus predictable). The fact that some animals developed adversities in as little as 5 days might also support a type A or idiosyncratic type B reaction, rather than allergy. Large breeds, with Doberman Pinschers overrepresented, appear to be at greater risk for developing arthropathy (as reviewed by Trepanier).<sup>252</sup>

Keratoconjunctivitis sicca is a more common side effect of sulfonamides in dogs, occurring in as many as 15% of animals receiving sulfonamides.<sup>254</sup> It has been reported in dogs after treatment with sulfasalazine, sulfadiazine, and sulfamethoxazole. The reaction may reflect direct cytotoxicity to the lacrimal gland rather than an allergic reaction, but nonetheless, time of onset may be months to years after therapy is initiated. Female dogs may be at greater risk. Resolution of clinical signs is more likely if the inciting drug is discontinued early; otherwise,

normal function may not recur once the drug is discontinued. Prognosis is more favorable for younger dogs receiving the drug for a short period of time.

### Drug Interactions

The sulfonamides have been associated with a number of drug interactions in humans.<sup>2</sup> Inhibition of elimination with subsequent prolonged or increased effects have been reported for oral hypoglycemic agents, dapson when combined with trimethoprim, folate antagonists (increased risk of megaloblastic anemia), methanamine (increased risk of crystalluria), procainamide (decreased metabolism when combined with trimethoprim), and warfarin (increased anticoagulant activity with trimethoprim). In contrast, increased elimination has been reported for cyclosporine when combined with either sulfonamides or trimethoprim.<sup>2</sup>

### Therapeutic Use

Because of the advent of resistance, the use of sulfonamides is limited to uncomplicated infections of most body systems. The concentration in urine supports the use of potentiated sulfonamides for urinary tract infections. Trimethoprim/sulfonamide combinations are indicated for treatment of infections caused by susceptible bacteria in difficult-to-penetrate tissues such as the prostate and CNS.<sup>246</sup> These drugs are among the drugs of choice for treating *Nocardia* and *Actinomyces* spp. Synergistic effects have been cited toward these organisms when used in combination with beta-lactam antibiotics.

## DRUGS THAT TARGET RIBOSOMES (BACTERIOSTATIC)

### Tetracyclines

Tetracyclines historically have been widely used, but development of resistance has largely curtailed empirical use in the last decade. However, the decline in use appears to have led to a decrease in resistance, and susceptibility increasingly is demonstrated through C&S data, potentially leading once again to more common use of these drugs.

### Structure-Activity Relationship

Three naturally occurring tetracyclines are obtained from *Streptomyces*: chlortetracycline (the prototypic drug but no longer available in human-medicine preparations), oxytetracycline, and demethylchlortetracycline (see Figure 7-5). Several tetracyclines have been derived semisynthetically (tetracycline from chlortetracycline, rolitetracycline, methacycline, minocycline, doxycycline, lymecycline, and others). Elimination half-lives permit a further classification into short-acting (tetracycline, oxytetracycline, chlortetracycline), intermediate-acting (demethylchlortetracycline and methacycline), and long-acting (doxycycline and minocycline) formulations. All of the tetracycline derivatives are crystalline, yellowish, amphoteric substances that, in aqueous solution, form salts with both acids and bases. They characteristically fluoresce when exposed to ultraviolet light. The most common salt form is the hydrochloride, except for doxycycline, which also is available as

doxycycline hyclate. The tetracyclines are stable as dry powders but not in aqueous solution, particularly at higher pH ranges (7-8.5). Preparations for parenteral administration must be carefully formulated, often in propylene glycol or polyvinyl pyrrolidone with additional dispersing agents, to provide stable solutions. Tetracyclines form poorly soluble chelates with bivalent and trivalent cations, particularly calcium, magnesium, aluminum, and iron. Doxycycline and minocycline exhibit the greatest liposolubility and better penetration of bacteria.

### Mechanism of Action

Tetracyclines bind bacterial ribosomes and impair protein synthesis (see Figure 7-6). Bacterial ribosomal activity was described in the section on aminoglycosides. The tetracyclines bind to the 16S portion of the 30S ribosomal subunits, preventing access of the amino-acyl tRNA to the acceptor site on the mRNA ribosome complex<sup>80</sup> (see Figure 7-6). Because tRNA binding is prevented, amino acids cannot be added to the peptide chain, and protein synthesis is impaired. Tetracyclines are bacteriostatic in action and should not be used in the immunocompromised patient, whether disease or drug induced (i.e., glucocorticoids or anticancer drugs). Their effects are described with other bacteriostatic ribosomal inhibitors as time dependent but are probably related to AUC. The tetracyclines also inhibit matrix metalloproteinases, an action separate from their antibacterial properties.

### Spectrum of Activity

Tetracyclines enter cells either through porins or active transport pumps.<sup>80</sup> They are considered broad spectrum (see Table 7-2), being effective against gram-positive, gram-negative, anaerobic organisms, as well as cell wall-deficient and rickettsial organisms and others. Their spectrum includes gram-negative organisms, particularly *Pasteurella* spp., and often *E. coli*, *Klebsiella*, and *Salmonella* spp. *P. aeruginosa* is generally not included; although susceptibility may be indicated on C&S data, caution should be exercised when selecting tetracyclines. They generally are intrinsically more effective against gram-positive organisms (see Tables 7-3 and 7-4). As such, *Staphylococcus* and *Streptococcus* spp. generally are included in the spectrum. However, the broad general use of these drugs has led to resistance by many organisms and use against gram-positive organisms should be based on C&S testing. The spectrum of action also includes *Chlamydia*, *Mycoplasma*, *Rickettsia*, and *Hemobartonella* organisms. Spirochetes (*Borrelia*, *Leptospirosis* spp. also are generally susceptible, and several mycobacterial organisms are susceptible. Tetracyclines target *Brucella* spp.) although in human medicine generally they are combined with rifampin or gentamicin. Tetracyclines generally are effective toward actinomycosis and are generally considered more effective than chloramphenicol.<sup>80</sup>

Tigecycline is a glycylcycline, a class of drugs that are synthetic analogs of the tetracyclines. Specifically, it is a glycolamide derivative of minocycline. The spectrum of this class is similar to that of the tetracyclines; however, they often remain effective against strains that have developed resistance to tetracyclines through increased efflux transport mechanisms.<sup>80</sup>

### Resistance

Resistance to tetracyclines is plasmid mediated and inducible.<sup>80</sup> Most resistance to tetracyclines results from either decreased influx or increased transport of the drug out of the microbial cell. Other mechanisms include altered binding site (which may reflect a mutation) and enzymatic destruction. Cross-resistance does not necessarily occur and depends on the mechanism. Drugs that minimize the impact of efflux pumps have been developed, including the glycylcyclines.<sup>255</sup> These drugs also have a higher binding affinity than tetracyclines.

### Pharmacokinetics

The oral absorption of tetracyclines is variable, with chlortetracycline being the least bioavailable, oxytetracycline more so, and doxycycline the most lipid soluble of the tetracyclines, being 100% bioavailable. Absorption is decreased in the presence of divalent and trivalent cations such as those present in milk products or antacids; exceptions occur for doxycycline and minocycline. Tetracyclines, particularly doxycycline, are widely distributed to most body tissues, and theoretically, inflammation need not be present for distribution into the brain<sup>80</sup> (see Table 7-5). Drugs will distribute through the placenta into the fetus and into milk. Doxycycline is able to penetrate cell membranes and thus gain access to intracellular organisms. Doxycycline is 99% protein bound, which prolongs its elimination half-life; note that concentrations in body fluids (see Table 7-5) are likely to reflect unbound drug, whereas that in plasma may reflect bound drug, decreasing ratios. Tetracyclines, with the exception of lipophilic tetracyclines such as minocycline and doxycycline, do not penetrate the CSF. The latter drugs are thus preferred because of better tissue penetrability for treatment of infections caused by susceptible bacteria in difficult-to-penetrate tissues, reaching 30% to 40% of plasma concentrations. Minocycline is characterized by a larger Vd in people than is doxycycline, suggesting the potential of better tissue penetrability, but may also be more bound to bone or other tissues containing cations. Tetracyclines accumulate in reticuloendothelial cells.<sup>80</sup> Tetracyclines are incorporated into forming bone and the enamel and dentin of teeth and cause discoloration of teeth upon eruption. The age at which this occurs in dogs and cats is not clear.

**KEY POINT 7-34** Among the tetracyclines, doxycycline and minocycline stand out for their lipid solubility and biliary excretion.

Doxycycline (PC 0.68 and pKa 3.09)<sup>57</sup> was studied in the dog in both plasma and interstitial fluid (using ultrafiltration) after intravenous and constant-rate infusion (to allow establishment of steady-state concentrations). The drug is 91% bound to plasma proteins in dogs, resulting in a total AUC difference sixfold higher in plasma compared with interstitial fluid. Further, the interstitial fluid C<sub>max</sub> (of unbound drug) was only 0.14 µg/mL at steady-state conditions; in contrast, PDCs extrapolated from the terminal component of the elimination curve was 1.6 µg/mL. The concentration of interstitial fluid



drug was equivalent to the concentration of unbound drug in plasma.<sup>57</sup> Vd of unbound drug was  $0.65 \pm 0.08$  L/kg; clearance was  $1.66 \pm 2.21$  mL\*kg/min.

With the exception of doxycycline and minocycline, the tetracyclines are eliminated by both renal (approximately 60%) and biliary (40%) excretion. Presumably, minocycline is eliminated essentially in the bile, whereas the route of elimination of doxycycline is less obvious. In humans it is eliminated by both renal (41%) and biliary (59%) mechanisms. In dogs intestinal elimination of the unchanged drug appears to be the predominant route, with only about 16% of a given dose being excreted unchanged in the urine. Tetracyclines undergo enterohepatic circulation. Toxic concentrations may accumulate in patients with renal disease. Differences that justify use of minocycline instead of doxycycline are difficult to ascertain. Adverse reactions to minocycline may, however, be more likely.

The tetracyclines are available as intravenous, parenteral, and ocular preparations. Tetracyclines should not be given intramuscularly because of local tissue damage and irritation. For the same reason, tetracyclines are not indicated for topical treatment other than the eye.

### Adverse Effects

Tetracyclines cause several adverse effects in small animals. Toxicity may be worsened in patients with renal disease because of decreased elimination. Gastrointestinal upset follows direct irritation of the gastrointestinal mucosa after oral administration; administration of doxycycline with food will reduce gastrointestinal side effects. Rarely, hepatotoxicity may occur. Rapid intravenous administration may result in collapse. Although the likelihood of this occurring in small animals is not clear, prudence dictates slow administration of a diluted solution (i.e., 1:10) when tetracyclines are administered intravenously. Although the mechanism is not certain, calcium binding may be important. Intravenous administration of tetracycline has caused anaphylactic shock in dogs. Diluting fluids should not contain calcium or other cations to which tetracyclines might chelate. Hypersensitivity has also been reported in a dog after intramuscular administration of tetracycline. Minocycline may be more likely to cause allergic drug reactions in dogs. Lesions characterized by erythema of the skin and mucous membranes occurred in dogs after administration of most doses of minocycline. Anemia may also occur (10 mg/kg, administered intravenously). Brown to gray discoloration of teeth may occur because of chelation of tetracyclines in calcium deposits of dentin and, to a lesser degree, enamel. Tetracycline and oxytetracycline cause a yellow discoloration, whereas chlortetracycline produces a gray-brown discoloration; of all the tetracyclines, oxytetracycline causes the least tooth discoloration. Because chelation might occur in forming dentin as well as enamel, tetracyclines should be avoided from 3 weeks' gestation to at least 1 month after birth. Among the lipid-soluble tetracyclines, doxycycline may be less likely to cause discoloration. In humans minocycline may stain teeth regardless of tooth development because of chelation with iron; the drug probably has not been used

sufficiently in animals to determine whether a similar effect will occur. Other side effects caused by tetracyclines include drug fever (in cats), an antianabolic effect, and a Fanconi-like syndrome in the kidneys, with the latter more likely with expired or degraded tetracyclines.<sup>255</sup>

Doxycycline has been associated with esophageal erosions in cats (and humans).<sup>80</sup> In a study of 30 cats, no orally administered tablets had passed in 30 seconds; only 40% had entered in 5 minutes. In contrast, 90% of tablets passed within 30 seconds when followed with 6 mL of water, with 100% passage at 90 seconds. For capsules only 17% had passed by 30 seconds, but 93% had passed by 60 seconds.<sup>256</sup> The impact of esophageal damage is not unique to doxycycline; other drugs are ulcerogenic because of local effects. Indeed, the cat has been used as a model to assess the ulcerogenic potential of orally administered drugs.<sup>257</sup> For doxycycline, the risk may be decreased with use of the monohydrate salt. In the event that erosions do occur, among the treatments to consider would be pentoxifylline.<sup>258</sup>

**KEY POINT 7-35** Doxycycline is only one of many drugs that might cause esophagitis in the cat.

### Drug Interactions

Because of chelation with cations (magnesium, calcium, aluminum, and so on), tetracyclines should not be simultaneously administered with cation-containing drugs (e.g., antacids, sucralfate, buffered aspirin, calcium-containing supplements, fluids). Cholestyramine may also bind to tetracyclines. Tetracyclines, with the exception of doxycycline, should not be administered with food.

Because tetracyclines bind to the 30s ribosomal subunit, combination with antimicrobials that target the 50s subunit might be considered (e.g., the phenicols, macrolides, and lincosamides) with scientific support. One study indicates an in vitro synergistic effect of the combined use of doxycycline and azithromycin against *P. aeruginosa*.<sup>259</sup>

### Therapeutic Use

The therapeutic indications for tetracyclines are many but have decreased in recent years because of the advent of resistance. Treatment of microbial infections is best based on C&S data. Doxycycline is the preferred tetracycline because of its ability to move intracellularly compared to other tetracyclines. Doxycycline generally is indicated among first-choice therapies for obligate intracellular organisms, including ehrlichiosis, Rocky Mountain spotted fever, chlamydiosis, mycoplasmosis, and hemobartenellosis. Doxycycline also has been used to treat canine brucellosis. Other potential indications include leptospirosis and Lyme disease.

### Phenicols

Chloramphenicol has been widely used in the past, but the development of resistance and human toxicity to chloramphenicol have severely curtailed its use and commercial availability. Florfenicol is a commercially available thiamphenicol derivative approved for treatment of bovine respiratory

diseases complex. A sulfonyl group replaces the aromatic ring nitro group that is otherwise associated with chloramphenicol's irreversible bone marrow suppression in humans (see Figure 7-5). As chloramphenicol increasingly is difficult to obtain commercially, florfenicol may find a niche for use in small animals, particularly cats, in which the disposition is more predictable than with dogs.

### Mechanism of Action

Like tetracyclines, chloramphenicol and florfenicol bind bacterial ribosomes and impair protein synthesis (see Figure 7-6). However, binding occurs at the 50s subunit with inhibition of peptidyl transferase. Actions are bacteriostatic in action, and these drugs should not be used in immunocompromised patients. As with other bacteriostatic ribosomal inhibitors, the effects of chloramphenicol and florfenicol should be considered time dependent. As with tetracyclines, although host ribosomes do not bind as effectively as do bacterial ribosomes, some host ribosomal activity will be impaired. Binding sites for chloramphenicol are close to those for clindamycin, which it competitively inhibits.<sup>80</sup> Chloramphenicol also inhibits mitochondrial protein synthesis in mammalian cells, with erythropoietic cells particularly sensitive.

### Spectrum of Activity

Chloramphenicol is considered broad spectrum (see Table 7-1), being effective against gram-positive, gram-negative, and anaerobic organisms. *P. aeruginosa* is generally not included. The spectrum of action also includes *Chlamydia*, *Mycoplasma*, *Rickettsia*, and *Hemobartonella* organisms. As previously noted, tetracyclines are considered more effective than chloramphenicol for the latter organisms, but chloramphenicol tends to be more clinically effective for other organisms. The spectrum of activity of florfenicol is similar to chloramphenicol; although the anaerobic spectrum has not been described, it is assumed to be similar. The MIC for florfenicol of small animals generally reflects 1.0 to 8.0 µg/mL.

### Resistance

Resistance to chloramphenicol is caused by destruction (acetylation) of the drug by microbial enzymes. The fluorine ring of florfenicol may impair bacterial acetylation, and thus florfenicol is more resistant to bacterial deactivation;<sup>260</sup> selected organisms resistant to chloramphenicol may be susceptible to florfenicol.<sup>2</sup>

### Pharmacokinetics

Chloramphenicol is very well absorbed after oral administration in its crystalline form. Many of the originally-approved preparations are no longer available in the United States. The liquid form is less well absorbed, so much so that the palmitate form should not be used for cats because of variability in oral absorption. The chloramphenicol succinate ester is the water-soluble form intended for injection (see Table 7-1). The succinate must be hydrolyzed by plasma, hepatic, pulmonary, or renal esterases before activity. Chloramphenicol palmitate is a suspension for oral administration. Its ester is hydrolyzed

by small intestinal lipases; the freed chloramphenicol is then orally absorbed. The freed chloramphenicol is among the most lipid soluble of the clinically used drugs and achieves moderate to high concentrations in most body tissues, including the CSF. It is, however, unlikely to achieve bactericidal concentrations in most tissues, including the CNS. Most of the drug is eliminated by hepatic metabolism. Glucuronidation is a major route of elimination of chloramphenicol. Cats eliminate chloramphenicol more slowly because of deficiencies in both phase I and phase II metabolism. Greater concentrations may occur in cat urine than in dog urine as a result.<sup>261</sup> Pediatric patients also may not eliminate chloramphenicol as efficiently as young adult dogs.

**KEY POINT 7-36** Although chloramphenicol is bacteriostatic, its excellent tissue distribution and relatively long half-life render it potential appealing choice in immunocompromised patients.

Chloramphenicol was studied after single oral dose as the commercially available Chloromycetin (50 mg/kg) in dogs.<sup>262</sup> Although pharmacokinetics were not reported, the  $C_{max}$  (µg/mL) at  $T_{max}$  were, respectively, for Greyhounds with feeding,  $21.6 \pm 4.8$  at 1.5 hours, or without feeding,  $18.6 \pm 6.7$  at 3 hours; large dogs (22-26 kg),  $20.0 \pm 4.8$  at 1.5 hours; and small dogs (11.4 to 15.5 kg),  $27.5 \pm 7.0$  at 3 hours. Peak concentrations were notably higher in small dogs than large dogs. Half-life in Greyhounds was 3.2 hours in fasted dogs versus 1.9 hours in fed dogs; the elimination half-life (based on noncompartmental analysis of published data) in large dogs was 2.3 hours compared with 3.4 hours in small dogs. Average half-life among all groups was  $2.7 \pm 0.7$  hours; mean residence time was  $4.6 \pm 0.67$  hours. Neither oral bioavailability nor clearance was determined.

During approval for use in humans, chloramphenicol was studied in dogs.<sup>263</sup> Chloramphenicol was measured on the basis of an analytic procedure that detected chloramphenicol and its metabolites; therefore the relevance of the data must be considered. Homogenate tissue concentrations were described after subcutaneous administration of 35 mg/kg for 2 dogs: at 1.5 and 3 hours, plasma concentrations were 21 and 13, respectively, yielding an elimination half-life of 2.9 hours. Concentrations in the brain and CSF at the same time were 15 and 8 µg/mL (brain) and 7 and 9 µg/mL (CSF), yielding a 3-hour plasma:tissue ratio of 0.7. A second study measured chloramphenicol using a bioassay. However, only a single dog was studied after oral administration of 150 mg/kg of the crystalline powder form. The  $C_{max}$  was 45 µg/mL at 4 hours and approximately 15 µg/mL at 8 hours, yielding a disappearance half-life of 2.5 hours. This should extrapolate to a  $C_{max}$  of approximately 14 µg/mL when 50 mg/kg is administered. Although 54% of the drug was eliminated in the urine, only 6.3% of the drug in the urine was pharmacologically active. Intravenous administration of 50 mg/kg yielded an initial plasma concentration of approximately 39 µg/mL and a concentration of approximately 5 µg/mL at 8 hours, yielding a half-life of about 1.5 hours.<sup>263</sup>

Chloramphenicol has been studied in cats ( $n = 5$ ). Oral administration of the crystalline powder in capsules yielded  $C_{max}$  ( $\mu\text{g/mL}$ ) of 43 to 62 at 40 mg/kg, 25 to 42 at 20 mg/kg tid, and 8 to 25 at 50 mg bid.<sup>264</sup> Cats were also dosed with succinate intravenously, intramuscularly, subcutaneously, or orally (crystalline powder in capsules).<sup>265</sup> Concentrations at 30 minutes ( $T_{max}$  for each route except oral) were, respectively ( $\mu\text{g/mL}$ )  $19.5 \pm 1.5$  (intravenous),  $18.6 \pm 2.6$  (intramuscular),  $14.8 \pm 2.9$  (subcutaneous) and  $9.8 \pm 2.61$  (oral) after administration of 20 mg/kg ( $n = 5$ ). The mean half-life of all three routes was  $4.4 \pm 1.38$ ; range was 3.3 hours for subcutaneous and 6.9 hours for intravenous. AUC for each route was similar (lowest at  $55 \pm 7 \mu\text{g}\cdot\text{hr/mL}$  for intravenous, highest at  $67 \pm 9$  for subcutaneous). Finally, the bioavailability of the palmitate salt suspension is poor in cats, particularly in the fasted state.<sup>266</sup> Peak concentrations of the crystalline form following 100 mg/cat was  $25 \pm 5$  (fasted) or  $31 \pm 3$  (fed) versus  $6.5 \pm 1.3$  (fasted) and  $16 \pm 3$  (fed) for the succinate form.

Florfenicol has been studied in dogs and cats.<sup>260,267</sup> In dogs, although predictable PDCs ( $1.64 \mu\text{g/mL}$ ) are achieved at 20 mg/kg after intramuscular administration, concentrations are unpredictable after subcutaneous administration. The drug appears to follow a "flip-flop" model, with the elimination half-life in dogs following intramuscular administration much longer (9 hours) compared with intravenous administration (<1 hour). A second study determined the oral bioavailability and described in more detail the PK of florfenicol (based on HPLC) in dogs ( $n = 6$ ) after intravenous and oral administration (20 mg/kg).<sup>267</sup> Florenicol clearance was  $1.03 \pm 0.49 \text{ L}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$ , and the  $V_{d_{ss}}$   $1.45 \pm 0.82 \text{ L/kg}$ . The elimination half life is  $1.11 + 0.94$  hour after intravenous and  $1.24 \pm 0.64$  after oral administration. Oral bioavailability was  $95 \pm 11\%$ , with  $C_{max}$  reaching  $6.18 \mu\text{g/mL}$  at a  $T_{max}$  of 0.94 hour. Florfenicol amine is a major metabolite of florfenicol, with a longer half-life in dogs (2.26 hours), but it has only 1/90 the activity of the parent compound, and its contribution to microbiological activity is considered negligible. Dogs showed no evidence of side effects after either intravenous or oral administration. The disposition of florfenicol by the intramuscular route appears to be more predictable in cats than dogs, with  $C_{max}$  (22 mg/kg) reaching  $20 \mu\text{g/mL}$  after IM administration and  $27 \mu\text{g/mL}$  after oral administration (see Table 7-1).<sup>267</sup> Oral administration was based on a solution of 100 mg/mL. The elimination half-life was 8 hours in cats after oral administration, supporting a 12-hour dosing interval. The distribution volume in cats is supportive of a lipid-soluble drug. Adverse reactions were not noted in the six cats studied.

### Adverse Effects

A major toxic concern with chloramphenicol for humans is both reversible dose-dependent and irreversible dose-independent (rare) bone marrow suppression. Reversible bone marrow suppression can also occur in animals. Dose-dependent bone marrow effects may reflect suppression of bone marrow precursor cells after mitochondrial damage. Irreversible bone marrow suppression may reflect reduction of the  $\text{NO}_2$  group to a toxic metabolite that causes stem cell

damage. Irreversible suppression should be avoided with florfenicol, which lacks the  $\text{NO}_2$  group. Although cats appear more sensitive to chloramphenicol-induced reversible bone marrow suppression than do dogs, toxicosis appears rapidly reversible once the drug is discontinued. Toxicity to chloramphenicol occurs in cats after 7 days of therapy at 50 mg/kg, administered intramuscularly. The drug can, however, be used for 7 to 10 days safely in cats after oral administration of the crystalline form (capsules) at the rate of 50 mg/cat.<sup>265,266</sup> The antianabolic effects of chloramphenicol may result in impaired protein synthesis in the patient; however, despite earlier concerns, impaired immune response to vaccines does not appear to occur.

**KEY POINT 7-37** Irreversible bone marrow suppression has resulted in limited availability of chloramphenicol products.

### Drug Interactions

Because they compete for the same ribosomal binding site, chloramphenicol should not be used in combination with macrolides. Because they target two different ribosomal sites, the combination of chloramphenicol with tetracyclines is appealing. Interestingly, the combined use of chloramphenicol with penicillins has been demonstrated to enhance penicillin (bacteriostatic) activity in Enterobacteraceae that are otherwise resistant to penicillins because of beta-lactamase production. Chloramphenicol inhibits product of beta-lactamases in these organisms.<sup>267a</sup> Chloramphenicol is a potent inhibitor of drug-metabolizing enzymes and inhibits the hepatic metabolism of other drugs, potentially causing toxicity should drug concentrations increase. Prolonged sleeping times have been documented after administration of pentobarbital to dogs and cats also receiving chloramphenicol;<sup>268</sup> chloramphenicol has markedly prolonged phenytoin half-life<sup>269</sup> and phenobarbital half-life (see Chapter 2) in dogs. Phenobarbital-induced sedation and ataxia have occurred in as few as 3 days of chloramphenicol therapy. Chloramphenicol decreases the rate of elimination of digoxin.<sup>270</sup>

**KEY POINT 7-38** Chloramphenicol is a potent inhibitor of drug-metabolizing enzymes.

### Therapeutic Use

Chloramphenicol has been commercially available as a palmitate (oral) salt and a sodium succinate injectable preparation. Florfenicol is commercially available only as solution intended for (intramuscular) injection, which has been studied in dogs and cats.

### Lincosamides: Lincomycin and Clindamycin

The lincosamides, including lincomycin and its congener, clindamycin, are large glycosidic antimicrobials that contain an amino side chain (see Figure 7-5). They are often used in humans as penicillin substitutes to minimize the risk of penicillin hypersensitivity. The lincosamides inhibit the 50s subunit of the bacterial ribosomes but at a site distinct from that bound by the macrolides or chloramphenicol (see Figure 7-6). Peptidyl transferase is subsequently inhibited. Efficacy

is reduced when the lincosamides are used concurrently with macrolides. The ribosomal action of the lincosamides results in a bacteriostatic action against susceptible organisms at recommended doses. Clindamycin is generally bacteriostatic but can be bactericidal at concentrations that can be achieved in some tissues. As with other bacteriostatic drugs, the lincosamides are classified as time dependent, implying that plasma or tissue drug concentrations should exceed the MIC of the infecting organism for the majority of the dosing interval; efficacy also may be related to the AUC/MIC.

The spectrum of the lincosamides varies with the drug. Clindamycin is more effective against susceptible bacteria compared with lincomycin and also has greater activity toward anaerobes. The spectrum of clindamycin includes aerobic gram-positive cocci, including *Staphylococcus* and *Streptococcus* spp. as well as *Nocardia* spp. and anaerobic organisms including *B. fragilis*, *Fusobacterium* spp., *Clostridium perfringens*, *Peptostreptococcus*, and *Actinomyces* spp. Clindamycin also is effective against cell wall-deficient organisms such as *Mycoplasma* spp. Plasmid-mediated resistance reflects changes in the ribosomes and appears to be increasing against *Staphylococcus* spp. and *Bacteroides* spp. Resistance to one lincosamide generally results in resistance to others. Occasionally, resistance to macrolides may confer resistance to clindamycin if the mechanism reflects methylation of the ribosome.<sup>80</sup> Clindamycin is not a substrate for the macrolide efflux pump.

Because of its anaerobic and gram-positive spectrum, clindamycin often is chosen as one component of combination antimicrobial therapy. This combination also has been used to target *P. aeruginosa*; although generally ineffective as a sole agent, clindamycin may alter adherence of the microbe to epithelial cells, facilitating killing by the alternative drug.

### Pharmacokinetics

Only oral preparations of clindamycin are approved in the dog and cat; an injectable preparation is approved for use in humans. Both clindamycin and lincomycin are bioavailable after oral administration, although clindamycin is more so. Food does not impair the absorption of clindamycin but does appear to impair absorption of lincomycin. Clindamycin is available as the hydrochloride, palmitate, or phosphate salts. The palmitate form is an oral prodrug, with the ester being rapidly hydrolyzed to yield free drug. The phosphate form is intended for parenteral administration, including subcutaneous, intramuscular (although it is painful), and intravenous routes. In the cat administration of 5.5 and 11 mg/kg orally generates serum concentrations above the MIC of most *S. pseudintermedius* organisms and previously *S. aureus*, but it is likely that resistance has resulted in less favorable PDI. Higher doses (11 to 20 mg/kg) will generate concentrations above the MIC of most susceptible anaerobes (see Table 7-1). In dogs oral administration of 11 mg/kg every 12 hours has been sufficient for treatment of most *Staphylococcus* spp. infections, but current MIC<sub>90</sub> for clindamycin and susceptible *Staphylococcus* spp. have not been reported. As a time-dependent drug, decreasing the interval to 8 to 6 hours may increase efficacy. Clindamycin is highly (>90%) protein bound. Distribution of the lincosamides

includes most body tissues, with excellent concentrations being achieved in the skin and bones. However, it does not substantially penetrate the brain or CSF, although it can achieve concentrations effective for toxoplasmosis.<sup>80</sup> Its Vd in both dogs and cats approximates 1.5 L/kg. Clindamycin has been cited for its efficacy in the treatment of chronic gingivitis or periodontal disease. Unlike many other drugs with a favorable spectrum, it is able to penetrate the biofilm that protects the causative organisms. Accumulation of clindamycin in white blood cells up to fortyfold or more may increase the probability of reaching bactericidal concentrations at some sites of infection. The lincosamides are eliminated primarily by biliary excretion.

After administration of 10 mg/kg intravenously, intramuscularly, and subcutaneously in dogs, in addition to C<sub>max</sub> and elimination half-life (see Table 7-1), the following were achieved: T<sub>max</sub> occurred at 73 ± 16 min (intramuscular) or 47 ± 20 min (subcutaneous) and CL (mL/min/kg) 6.1 ± 1.1. The elimination half-life may vary with the route (see Table 7-2), as does mean residence time at 143 ± 34, (intravenous), 700 ± 246 (intramuscular), or 364 ± 147 (subcutaneous) minutes. Bioavailability was 115% after intramuscular and 310% after subcutaneous administration. The long half-life coupled with the highest C<sub>max</sub> suggests that the subcutaneous route of administration is the preferred parenteral route for clindamycin.<sup>272</sup> The reason for the very high bioavailability after subcutaneous administration is not clear, although enterohepatic circulation is anticipated to increase bioavailability regardless of route of administration.

Clindamycin disposition has been reported in cats after oral administration of either a capsule or aqueous solution (see Table 7-1).<sup>271</sup> Peak PDCs are equivalent for both preparations, but a longer half-life for the capsule may contribute to a (not statistically significant) greater AUC for the capsule (42.6 ± 12.2) compared with the solution (35 ± 9.2). The lack of statistical difference may reflect the marked variability in half-life mean residence time for both preparations, which was approximately 6.5 hours.

### Adverse Reactions, Drug Interactions, and Indications

Pseudomembranous colitis is a reported side effect in humans caused by overgrowth of *C. difficile*. The negative impact on the intestinal microbiota may persist for more than 2 weeks.<sup>80</sup> Because of similar mechanisms of action, this drug should not be combined with chloramphenicol or erythromycin. It has been combined with aminoglycoside treatment of polymicrobial infections involving gram-negative and anaerobic organisms. The use of clindamycin as combination antimicrobial therapy was addressed in the preceding section. Because of its ability to impair pili formation and thus adherence to tracheal epithelium, clindamycin has been associated with treatment of cystic fibrosis associated with *P. aeruginosa* in humans, generally in association with antipseudomonadal antimicrobials.<sup>273</sup> However, the macrolides are more generally accepted for this use. The use of clindamycin as part of combination chemotherapy targeting protozoal disease (toxoplasmosis) is addressed in Chapter 12).

## Macrolides and Azalides

### Structure–Activity Relationship

The macrolides are named for their chemical structure, composed of a very large lactone (MW >750 to >1000) ring attached to a number of sugars.<sup>274</sup> They include the azalides, which contain a nitrogen in the ring structure (see Figure 7-13). No macrolide derivative is approved for use in dogs or cats at the time of this publication. Human-medicine drugs include the 14-member rings erythromycin and clarithromycin and the 15-member ring azithromycin (an azalide semisynthetic derivative of erythromycin), spiramycin, and dirithromycin (a pro-drug converted to the active erythromycylamine). The methyl group that distinguishes clarithromycin from erythromycin and the additional methyl group on azithromycin increases acid stability and enhances tissue distribution. Telithromycin is a ketolide macrolide (discussed later). Tylosin, a drug approved for use in food animals, is used to treat intestinal disorders, largely in dogs. Of the human drugs, erythromycin (first-generation), azithromycin, and to a lesser extent, clarithromycin (second-generation) are used in dogs and cats.<sup>274</sup> Tilmicosin is approved for use in selected food animals, but toxicity precludes its use in the injectable form in dogs and cats; information is not available regarding safety of other preparations. The second-generation macrolides differ from erythromycin only by the addition of a methyl group substitution. However, this simple change improves acid stability and tissue penetration. Further, because the methyl group enhances interaction with bacterial ribosomes, the spectrum also is improved.<sup>274</sup>

**KEY POINT 7-39** Efficacy of the very lipid-soluble macrolides and clindamycin is facilitated further by accumulation in phagocytic white blood cells.

### Mechanism of Action

Macrolides inhibit bacterial ribosomal action by binding to the 50s subunit of susceptible organisms (see Figure 7-6), and impairing the translocation step of protein synthesis. The azalides macrolides bind the ribosome at two sites.<sup>275</sup> Although macrolides are classified as bacteriostatic in vitro, they are bactericidal against very susceptible organisms. Further, selected drugs (e.g., azithromycin) accumulate in selected tissues at bactericidal concentrations. All macrolides generally accumulate in phagocytic white blood cells, which may facilitate distribution to the site of infection. Efficacy is enhanced in an alkaline pH, probably because of increased diffusion of the nonionized drug into organisms; as such, intracellular activity may be decreased in phagocytic cells. The antibacterial effects of the macrolides vary with the drug and are time dependent for erythromycin; antibacterial effects for azithromycin and clarithromycin are time dependent for some organisms and concentration dependent for others.

### Spectrum of Activity

Like the lincosamides, the macrolides are often used in humans as penicillin substitutes to minimize the risk of penicillin hypersensitivity. Organisms are considered susceptible to the

macrolides at an MIC below 2 µg/mL. For the first-generation drugs, gram-positive organisms accumulate erythromycin at concentrations that exceed that of gram-negative organisms by a hundredfold. As such, erythromycin is most effective against gram-positive organisms. *Streptococcus* spp. are susceptible at a range of 0.015 to 1 µg/mL, although resistance is increasing. Many *Staphylococcus* organisms have remained susceptible to erythromycin, but MIC ranges of 0.12 to > 128 µg/mL for *S. aureus* indicate an increasing trend of resistance. Among the staphylococci, *S. pseudintermedius* remains the most susceptible. *P. multocida*, *Bordetella pertussis*, and *Mycoplasma* spp. are among the organisms susceptible to erythromycin. However, use should be based on C&S testing. Erythromycin generally is effective against anaerobic organisms, with the exception of *Bacterioides* spp. Macrolides are generally effective against *Campylobacter* spp.

The azolides were designed to overcome barriers presented to penetration of gram-negative organisms. Thus the spectrum of azithromycin and clarithromycin increases, particularly in terms of gram-negative bacteria, although efficacy toward selected gram-positive microbes may decrease, requiring higher MIC.<sup>275</sup> The actions of the azolides are bactericidal for *Streptococcus pyogenes* and *S. pneumoniae* but bacteriostatic toward staphylococci and most aerobic gram-negative organisms. Clarithromycin is effective at lower concentrations than erythromycin against *Streptococcus* and *Staphylococcus* spp. but is similar to erythromycin in efficacy against other organisms. Azithromycin has less activity against gram-positive organisms compared with erythromycin and greater activity against selected gram-negative organisms and *Mycoplasma* spp.<sup>80</sup> Although the spectrum of the macrolides generally includes *Actinomyces* spp., efficacy is generally less for *Nocardia* spp. Clarithromycin and azithromycin are effective against the *Mycobacterium avium* complex, *Mycobacterium leprae*, and *Toxoplasma gondii*. Compared to erythromycin, azithromycin and clarithromycin have enhanced activity against selected protozoa (e.g., *T. gondii*, *Cryptosporidium* spp.).

Controversy surrounds the classification of macrolides as either concentration or time dependent. The macrolides do exhibit a postantibiotic effect, with that of clarithromycin and azithromycin being longer than that of erythromycin. Azithromycin appears to be bacteriostatic against *Staphylococcus* or *Streptococcus* spp.; in vitro killing did not increase in a dose-dependent manner, suggesting that the drug is a time-dependent antimicrobial.<sup>276</sup>

### Resistance

Acquired mechanisms of resistance to macrolides include pump-driven drug efflux from the cell (particularly in staphylococci, group A streptococci, and *S. pneumoniae*) and altered ribosomal targets (methylase enzyme; MLSB phenotype) that also confer resistance to lincosamides, which bind at the same ribosomal site. Efflux pumps contribute to resistance in *E. coli* as well.<sup>179</sup> Chromosomal mutations in *Bacillus subtilis*, *Campylobacter* spp., and gram-positive cocci alter the ribosomal binding site. Resistance of *S. aureus* to erythromycin generally is indicative of resistance to azithromycin and clarithromycin

as well. The Enterobacteriaceae produce an esterase that hydrolyses the drug.

### Pharmacokinetics

The macrolides and azolides are largely water insoluble and are unstable in the acidic gastric environment.<sup>274</sup> However, each of the macrolides is available as an oral preparation. Erythromycin also is available as a topical and ophthalmic preparation. Erythromycin base preparations generally are coated to prevent gastric degradation. Oral absorption of enteric-coated or delayed-release products designed for humans may be unpredictable in animals.<sup>2</sup> Oral salts include the estolate and ethylsuccinate salts, which must be de-esterified after oral absorption, and the stearate (octadecanoate) and phosphate salts. The former (and possibly the latter) dissociate in the duodenum to be absorbed as the free base. The disposition of selected erythromycin salts has been described in dogs.<sup>277</sup>

After oral administration, the erythromycin base is incompletely but adequately absorbed. Food may increase acidity and thus delay absorption. Esters (stearate, estolate, ethylsuccinate) improve stability and absorption but do not appear to increase PDCs. Among the salts, estolate appears to be best absorbed orally and minimally affected by food. For the azolides, clarithromycin is characterized by greater acid stability compared with erythromycin. Clarithromycin is more rapidly absorbed (in humans), but food delays absorption and first-pass metabolism (to an active metabolite) further reduces oral bioavailability of the parent compound to 50%. Azithromycin also is absorbed rapidly, but, again, food decreases bioavailability to 43% (in humans). Erythromycin is approximately 75% protein bound; binding is as high as 96% (in humans) for the estolate salt. Protein binding for clarithromycin is concentration dependent and ranges from 40% to 70%. Despite their large molecular size, macrolides are sufficiently lipid soluble that they diffuse through membranes, albeit slowly. With a Vd of 2 L/kg in dogs, erythromycin will reach effective concentrations in all tissues except the brain and CSF. In general, the macrolides act as weak bases and, as such, trapped in an acidic environment, including acidic intracellular organelles. Consequently, tissue concentrations will exceed plasma in many tissues. Although accumulation occurs in selected tissues (e.g., bile, bronchial secretions, phagocytic white blood cells), concentrations reach only 50% of plasma in the prostate and aqueous humor and less than 15% in the CSF. Concentrations in the middle ear will approximate 50% of those in plasma. Clarithromycin and its active metabolite are well distributed, achieving higher concentrations than erythromycin in both the middle ear and CNS. Among the macrolides, azithromycin distributes the most extensively, with a Vd that exceeds (in humans) 30 L/kg. Fibroblasts act as a reservoir, with transfer to phagocytic cells. Whereas erythromycin and azithromycin are eliminated principally in the bile, clarithromycin is extensively metabolized to an active (14 hydroxy derivative) metabolite. Excretion is primarily by biliary secretion into the feces; enterohepatic circulation of active drug might be anticipated. Urine excretion is not significant (3% to 5%), with concentrations in urine being low (approximately 50% of plasma); an

exception is clarithromycin, for which the active metabolite might achieve high concentrations in urine. The elimination half-life for azithromycin has been reported at 1 to 1.5 hours in dogs<sup>279, 280</sup> and cats.

The disposition of erythromycin as the estolate tablet and ethylsuccinate suspension and tablet has recently been described in dogs.<sup>277</sup> Intravenous administration revealed a Vd of 4.8 L/kg (see Table 7-1) and a clearance of  $2.64 \pm 0.84$  L/hr/kg. Oral absorption of all three products was poor: the ethylsuccinate tablets did not yield predictably detectable concentrations, whereas, based on mean AUC adjusted for differences in dose, the bioavailability of the estolate tablet was only 11% ( $T_{max}$  1.7 hr) and the ethylsuccinate suspension only 3% ( $T_{max}$  0.7 hr). Absorption of the suspension, in particular, was described by the authors as erratic. Peak concentrations did not reach MIC<sub>90</sub> for susceptible *Staphylococcus* spp. of 0.5 µg/mL (reported by the authors) for any of the oral preparations. The apparent efficacy of erythromycin, despite poor absorption, may reflect accumulation of drug in tissues such that higher concentrations are achieved at the site of infection.<sup>277</sup> All dogs vomited after dosing, regardless of route of administration, with vomiting apparent 5 to 10 minutes after intravenous administration, approximately 45 minutes after oral succinate preparations, and 1 to 2 hours after the estolate tablet administration.

Limited information is available for the second-generation macrolides in animals. Azithromycin and clarithromycin absorption is influenced by uptake transporters in the intestinal epithelium. Whereas efflux transporters, such as P-glycoprotein, decrease absorption, others (organic anion-transporting proteins) facilitate uptake.<sup>278</sup> Azithromycin has been studied in cats and dogs (see Table 7-1).<sup>279, 280</sup> Bioavailability in the dog is greater than 97%. Serum protein binding is less than 25%.

Clearance is 6.0 mL·min/kg. In dogs 67% of the drug is eliminated in the bile and 33% in the urine.<sup>279</sup> The majority of the drug (75%) is eliminated unchanged. The remaining portion is metabolized by cytochrome P450s into a number of metabolites, which, with one exception, are inactive. Tissue concentrations (based on homogenate) at 24 hours after 20 mg/kg orally were over 101, 20, and 39 µg/mL, respectively, for liver, kidney, and lungs. After 5 days of dosing, 23 µg/mL was achieved 24 hours after the last dose in the eye but only 1.2 µg/mL in the brain (at 30 mg/kg for 5 days). In cats the maximum drug concentration ( $C_{max}$ ) of  $0.97 \pm 0.65$  µg/mL occurs at  $T_{max}$  of  $0.85 \pm 0.72$  hr. Plasma concentrations (µg/mL) range from approximately 8 at 1 hour to 0.1 at 12 hours after intravenous administration of 5 mg/kg and approximately 1 µg/mL to 0.1 µg/mL during the same times after oral administration of 5 mg/kg. Although the elimination half-life is long, concentrations in plasma are below 0.1 µg/mL after 12 hours. However, concentrations of azithromycin approximate 0.75 to 1 µg/mL in the femur, skin, and muscle versus 10 µg/mL in tissues characterized by reticuloendothelial cells (liver, spleen, and to a lesser degree lung) and the kidney with concentrations persisting for 72 hours or more. Because tissue concentrations were based on homogenate, it is not clear how much

drug is available to interstitial fluid. Clearance is  $0.64 \pm 0.24$  L\*hr/kg. Oral bioavailability is  $52 \pm 22\%$ . The elimination half-life is 35 (range 29 to 51 hours).<sup>280</sup> The Clinical Laboratory and Standards Institute susceptible breakpoint for azithromycin (human pathogens) is 4 µg/mL. Because concentrations decline to less than 0.1 µg/mL by 12 hours, daily dosing should be considered in both cats and dogs; because time to steady state will approximate 3 to 5 days, a 15 mg/kg loading dose should be considered followed by once-daily dosing at a minimum of 5 mg/kg. Although cats do metabolize azithromycin, the unchanged drug is the predominant form in tissues. Biliary excretion is a major route of clearance in the cat.<sup>280</sup> Kinetics of clarithromycin become zero order (saturated) at higher doses. The large Vd of the macrolides contributes to their long elimination half-life. The half-life in cats exceeds 72 hours in some tissues.<sup>280</sup> In contrast to azithromycin, urinary concentrations of clarithromycin can be significant: up to 40% of the parent drug or its metabolite are eliminated in the urine. The mean half-life in plasma is 35 hours but varies in tissues from a low of 13 hours (fat) to a high of 72 hours (cardiac muscle).

### Adverse Effects

Side effects of the macrolides are limited. With injectable products, pain may occur with intramuscular injection and thrombophlebitis with intravenous injection. Reversible cholestatic hepatitis accompanied by jaundice has been reported in humans 10 to 20 days into erythromycin therapy, especially with the estolate preparation.

Gastrointestinal upset is the most common adverse effect of the macrolides. Up to 50% of animals treated with erythromycin may exhibit vomiting. Erythromycin is motilin-like in action and characterized by marked prokinetic effects on gastrointestinal motility. This effect is dose dependent in humans and may occur more commonly in younger animals. Abdominal cramping, epigastric pain, and increased gastric emptying resulting from increased gastric motility also may occur. However, because contraction is not coordinated, efficacy as a prokinetic is limited. Gastric emptying may decrease gastric maceration of ingested food, although the impact on digestion is not likely to be significant. Azithromycin and clarithromycin do not appear to have the same gastrointestinal side effects of erythromycin. In humans allergic reactions occur rarely and are manifested as fever or skin eruptions, which resolve once therapy is discontinued. Cholestatic hepatitis is an infrequent side effect in humans.

### Drug Interactions

Antacids decrease the rate (and thus peak) but not extent of absorption of azithromycin, whereas food decreases the extent by close to 50%. The macrolides may inhibit cytochrome P450 enzymes, and CYP 3A4 in particular, impairing the metabolism of other drugs.<sup>280a</sup> Among the macrolides, erythromycin followed by clarithromycin is most likely to be involved in significant drug interactions, although all three drugs inhibit drug-metabolizing enzymes. The effects of drugs metabolized by the liver, including selected anticonvulsants, cardiac drugs, and theophylline, are likely to increase. Drugs affected

in humans include glucocorticoids, digoxin, theophylline, and warfarin. The macrolide antimicrobials (clarithromycin, roxithromycin) also increase the risk of digoxin toxicity, although this effect may be more reflective of competitive interactions with P-glycoprotein transport proteins.<sup>281</sup> Azithromycin is a substrate; others may be as well.<sup>282</sup> Among the P-glycoprotein interactions with azithromycin in cats is cyclosporine; peak cyclosporine concentrations exceeded 4500 ng/mL in a cat receiving 5 mg/kg while being treated with azithromycin.

Because they are ribosomal inhibitors, care must be taken not to combine the macrolides with drugs whose efficacy requires rapid bacterial growth, unless scientific support exists, or “-cidal” concentrations of the macrolide are achieved at the target site for both drugs. For example, synergistic effects have been documented against *B. fragilis* when erythromycin is combined with cefamandole and against *Nocardia asteroides* when combined with ampicillin. The use of erythromycin in combination with other antimicrobials is limited in small animals. Erythromycin has been used in combination with rifampin to treat *Rhodococcus equi* in horses; a similar application has not been identified in dogs or cats. Synergistic antimicrobial actions also have been reported against *P. aeruginosa* for either azithromycin or clarithromycin when combined with sulfadiazine/trimethoprim or doxycycline. In humans azithromycin has been combined with antipseudomonadal drugs, particularly for treatment of cystic fibrosis-associated *P. aeruginosa* infections. This may reflect an apparent immunomodulatory effect of azithromycin or its ability to inhibit adherence of pseudomonad organisms to respiratory epithelium. Less commonly, synergism has been demonstrated for azithromycin when combined with ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, meropenem, imipenem, ciprofloxacin, trovafloxacin, chloramphenicol, or tobramycin.<sup>259</sup>

Although not included in their spectrum, the macrolides, like clindamycin, impair the ability of *P. aeruginosa* to adhere to tracheal epithelium, the first step in respiratory tract infection. The effect occurs at least at subinhibitory concentrations and reflects decreased ability to form pili.<sup>273</sup> Decreased adherence to human mucins also has been demonstrated for azithromycin.<sup>283</sup> Other proposed effects of azithromycin include decreased alginate formation and decreased biofilm. Azithromycin has been demonstrated to impede, but not prevent, biofilm formation by *Pseudomonas* spp.<sup>284</sup> These attributes have led to its long-term use for treatment of cystic fibrosis in humans, generally in association with some level of antipseudomonadal antimicrobials. Antiinflammatory effects have also been attributed to azithromycin's apparent long-term efficacy for treatment of cystic fibrosis.<sup>285,286</sup>

### Tylosin

Tylosin is classified as a macrolide, but it is structurally somewhat different from erythromycin, leading to differences in its mechanism and spectrum. Like erythromycin, it targets the 50s ribosomal subunit, but with different sequelae. It is stable in the gastric environment and does not require enteric coating for oral administration. Like erythromycin, tylosin is distributed well to most body tissues and is eliminated by

hepatic metabolism and biliary excretion. Approved for use in the United States for treatment of swine dysentery and other large animal syndromes, tylosin also has been used in small animals to treat infections of the gastrointestinal tract (associated with chronic inflammatory bowel disease) and bacterial pyodermas. Its spectrum is not clear but includes selected gram-positive and gram-negative organisms.

### Ketolides

Like the azolides, the ketolides are semisynthetic modifications of erythromycin designed to minimize barriers to penetration in gram-negative organisms.<sup>275</sup> Telithromycin is the first ketolide approved for clinical use in humans; the drug was developed specifically for treatment of upper and lower respiratory tract infections caused by organisms resistant to the macrolides.<sup>287</sup> Like the macrolides and azalides, the ketolides are well distributed into tissues, with concentrations being maintained in humans sufficiently long to allow a 24-dosing interval. Thus far, the ketolides have not been used or studied in veterinary medicine, perhaps because azithromycin currently meets the needs of infections that might otherwise be treated with ketolides.

## MISCELLANEOUS ANTIMICROBIALS

### Oxazolidinones

Oxazolidinones are a new group of synthetic antimicrobials effective against gram-positive bacteria, including methicillin- and vancomycin-resistant staphylococci, vancomycin-resistant enterococci, penicillin-resistant pneumococci, and anaerobes.<sup>288</sup> Linezolid is the first of this class of drugs to be approved for use in the United States (see Figure 7-4). Oxazolidinones inhibit the initiation of protein synthesis by binding at the P site of 50S ribosomal subunit; it also binds to the 70S subunit. Oxazolidinones compete with chloramphenicol and lincomycin for binding of the 50S subunit, which indicates that they have close binding sites, even though oxazolidinones do not inhibit peptidyl transferase as do chloramphenicol and lincomycin. Oxazolidinones may inhibit formation of the ribosomal initiation complex, similar to aminoglycosides. The mechanism is sufficiently different from other 50S binders that resistance to other protein synthesis inhibitors does not cross over to the oxazolidinones. Efficacy against *Staphylococcus* spp. is characterized by an MIC<sub>90</sub> between 1 and 4 µg/mL in humans; methicillin resistance does not appear to affect susceptibility. Linezolid also is effective against enterococci; even intermediate isolates appear to be susceptible at 1 µg/mL.<sup>288</sup> Streptococci also are susceptible. Anaerobic activity is comparable to clindamycin.<sup>289</sup> Linezolid is effective toward atypical mycobacterium<sup>290</sup> and both *Actinomyces* and *Nocardia* sp. Activity toward *S. pneumoniae* is generally bactericidal but bacteriostatic against staphylococci and enterococci.<sup>291, 292</sup> Antibacterial effects appear to be time dependent, with efficacy related to AUC/MIC. Resistance thus far is rare.

Disposition includes good oral absorption and good tissue penetration. Linezolid accumulates in bone, lung, vegetations,

hematoma, and CSF. Concentrations in sanctuaries are lower than those in plasma.<sup>293</sup> Linezolid has been approved by the FDA for treatment in humans of complicated skin infections or nosocomial pneumonia caused by MRSA, concurrent bacteremia associated with either vancomycin-resistant *E. faecium* or CA pneumonia caused by penicillin-resistant *S. pneumoniae*.<sup>288</sup> It has become the drug of choice for treatment of resistant gram-positive infections. The oxazolidinones have been minimally used in dogs and cats, and their use is discouraged unless warranted on the basis of C&S testing and until kinetic studies are available in the target species (e.g., cats).

Linezolid PK has been described in the dog after oral and intravenous administration (see Table 7-1).<sup>293</sup> Oral absorption is rapid and complete, allowing intravenous and oral dosing to be the same. The drug is minimally protein bound. Clearance is  $2.0 \pm 0.3$  mL·min/kg. The drug appears to undergo limited metabolism to inactive metabolites that are extensively enterohepatic recycled. Renal excretion occurs for parent compounds and metabolites. In humans renal disease causes accumulation of metabolites that may contribute to adverse effects.<sup>294</sup>

Linezolid appears to be well tolerated in humans. Myelosuppression has occurred in humans. Additionally, it is an inhibitor of monoamine oxidases, and care should be taken in patients also receiving serotonergic or adrenergic drugs or dietary supplements. Peripheral neuropathies have been associated with long-term use. Drug interactions involving cytochrome P450 do not appear to occur. Linezolid inhibits mitochondrial protein synthesis, causing hyperlactatemia in humans.<sup>294</sup> Linezolid may decrease intracellular movement of aminoglycosides, affecting rapid killing.<sup>94</sup> Based on in vitro killing curve studies, linezolid efficacy against MRSA was enhanced most by rifampin, compared with vancomycin or gentamicin; indeed, efficacy of the latter was reduced by linezolid, with activity antagonistic toward gentamicin.

### MISCELLANEOUS ANTIBIOTICS

**Daptomycin.** Daptomycin is a lipopeptide derived from *Streptomyces* that was discovered several decades ago but has been reconsidered for treatment of vancomycin-resistant gram-positive organisms. Its spectrum includes gram-positive and anaerobic microbes. However, its mechanism involves binding to the cell membrane, and although bactericidal, daptomycin is associated with an increased risk of toxicity. It acts in a concentration-dependent fashion.<sup>80</sup> Vancomycin-resistant drugs require higher concentrations. Daptomycin is minimally orally bioavailable, requiring intravenous administration for systemic effects. It cannot be given intramuscularly because of direct toxicity. It is not involved in any clinically relevant drug interactions. Although largely renally excreted, it is approximately 92% bound to plasma proteins in humans. The result is a longer half-life that allows once-daily dosing in humans. Daptomycin causes skeletal muscle damage in dogs at doses that exceed 10 mg/kg and peripheral neuropathies at higher doses.<sup>80</sup> Disposition has been described for Beagles after once- and twice-daily dosing.<sup>295</sup> When given at 5, 25, or 75 mg/kg intravenously, peak serum concentrations were 58, 165, and 540 µg/mL, respectively (total drug); concentrations extrapolated from the terminal component of the curve approximated



30, 100, and 300  $\mu\text{g}/\text{mL}$ , respectively. The elimination half-life appeared to be between 2 and 3 hours, which may indicate that the drug is not as highly protein bound in dogs compared with people. All doses caused skeletal muscle damage, as indicated by serum creatine phosphokinase; damage, however, was worse with 8-hour administration of 25 mg/kg than with once-daily administration of 75 mg/kg.<sup>295</sup>

**Fusidic Acid.** Fusidic acid is a steroidlike antimicrobial that interferes with ribosomal translocation (peptidyl tRNA). Efficacy is limited to gram-positive bacteria. It is bactericidal at high concentrations against both coagulase-positive and coagulase-negative staphylococci. It is available as oral, intravenous, topical, and ocular preparations. In humans it is characterized by 90% oral bioavailability. Adverse reactions include granulocytopenia, rash, and hepatotoxicity; thrombophlebitis; and venospasm, which may accompany intravenous infusion. Resistance develops rapidly when used as a sole agent. Drugs with which it has been combined include the aminoglycosides, quinolones, rifampin, and vancomycin. However, combination therapy has not precluded development of MRSA.

### Topical Antimicrobials

The advantage of topical antimicrobials is achievement of very high concentrations at the site of infection and avoidance of side effects that otherwise might occur with systemic therapy.

#### Bacitracin

Bacitracin is a complex polypeptide isolated from *B. subtilis*. It inhibits peptidoglycan synthesis in bacteria by interfering with the enzyme responsible for movement of cell components through the membrane. Its spectrum of activity includes gram-positive and very few gram-negative organisms. Systemic use causes nephrotoxicity, and use is limited to topical administration. The drug is not absorbed after oral administration and can be used to treat gastrointestinal infections caused by susceptible organisms.<sup>5,297</sup>

#### Polymyxins

Polymyxins are a group of large acetylated decapeptides produced by *Bacillus* spp. At least six compounds have been identified, of which only two, polymyxin (polymyxin B) and colistin (polymyxin E), are used clinically. Polymyxins are cationic detergents that interact and interfere with the phospholipid of the bacterial cell membrane, resulting in increased permeability. The polymyxins are thus bactericidal. However, a number of compounds can interfere with their activity, including divalent cations, purulent exudate, fatty acids, and quaternary ammonium compounds. The spectrum of activity of the polymyxins includes most gram-negative organisms, including *P. aeruginosa*. Two exceptions include *Proteus* spp. and most *Serratia* spp. The drugs are weak bases ( $\text{pK}_a$  8 to 9) and are not orally bioavailable. As such, they have been used to "sterilize" the gastrointestinal tract.

Elimination is principally by way of the kidneys, which are also the primary sites of toxicity. Glomerular and tubular epithelial damage has limited their usefulness. Other side

effects include respiratory paralysis (after rapid intravenous administration), CNS dysfunction, fever, and anorexia. Use of the polymyxins is primarily limited to topical administration. However, pemphigus vulgaris has been reported in association with topical use for otitis externa in the dog.<sup>296</sup> Polymyxin protects against gram-negative endotoxemia by binding to the anionic lipid component of the lipopolysaccharide at concentrations much lower than those associated with toxicity. Relevance to treatment in dogs or cats is not established.

#### Novobiocin

Novobiocin is derived from coumarin and is effective against both gram-positive and gram-negative organisms. The drug is particularly efficacious against *Staphylococcus* spp. Its mechanism of action is not certain but involves both cell membrane and cell wall synthesis. Novobiocin causes a number of toxic effects when used systemically, including bone marrow suppression, nausea, vomiting, and diarrhea. Its use is limited to topical application.<sup>5,297</sup>

#### Mupirocin

Mupirocin (pseudomonic acid) is a naturally occurring fermentation product of *Pseudomonas fluorescens*. It is available as a cream or ointment, and its use has been largely limited to topical application. Although it acts to inhibit protein synthesis, its mechanism is novel in that it prevents incorporation of isoleucine into proteins by binding to isoleucyl transfer-RNA synthetase.<sup>297</sup> Its unique mechanism precludes cross-resistance with other antibacterials. Resistance is unusual, low level, and generally overcome by higher concentrations. The spectrum of mupirocin includes aerobic gram-positive cocci (high efficacy toward *S. aureus*, *S. epidermidis*, and beta-hemolytic streptococci) and selected gram-negative cocci. An advantage to mupirocin is that it minimally affects normal flora. Its indications in human medicine include prophylaxis in ulcers, operative wounds, and burns and treatment of skin infections. In humans mupirocin has proved efficacious as an oral antibiotic. In addition, mupirocin has proven useful in the management of secondary pyodermas or superinfection of chronic dermatoses. Mupirocin is generally not associated with side effects; local burning, stinging, itching, or pain has been reported in about 1% of human patients.<sup>297</sup>

#### Silver Sulfadiazine

Silver sulfadiazine (see the discussion of sulfonamides) is approved for use in humans in a polypropylene glycol vehicle and in a water-soluble gel. It is approved for use in dogs combined with enrofloxacin as an otic preparation. The synergistic coupling of the silver with sulfadiazine results in efficacy against *P. aeruginosa* as well as a broad range of gram-positive and other gram-negative organisms. The silver component interferes with the cell wall. Silver sulfadiazine has been approved for use in the treatment of human burn patients, but other antimicrobials have proved more efficacious (e.g., iodophors; combinations of povidone iodine with neomycin, polymyxin, and bacitracin [Neosporin]; and silver

sulfadiazine-cerium nitrate cream). However, the low toxicity, low hypersensitivity, and low level of resistance warrants its continued use in veterinary patients.<sup>297</sup>

### Urinary Antimicrobials

**Nitrofurans.** The nitrofurans are synthetic compounds whose antimicrobial activity occurs through the 5-nitrofur group (see Figure 7-12).<sup>5,297</sup> Nitrofurantoin and furazolidone are examples. They are weak acids. These drugs block oxidative reactions necessary for formation of bacterial acetyl coenzyme A. They are bacteriostatic in action. The spectrum of activity of nitrofurantoin includes a number of gram-positive or gram-negative organisms, but its use should be based on C and S testing. The spectrum also includes selected protozoa. Nitrofurans are orally bioavailable but require an acidic environment to cross cell membranes. Use is limited to urinary tract infections, and ideally those associated with an acidic pH. Because 50% of nitrofurantoin is eliminated in urine in an active form, the drug is appropriate for treatment of urinary tract infections. Its use is, however, limited by gastrointestinal and systemic toxicity. Systemic toxicities in humans include peripheral neuropathy at therapeutic doses. The time to onset ranges from 3 weeks to over 12 months (median: 2 to 3 months). Although not common, peripheral neuropathy can be both severe and irreversible. Old age and renal disease increased the risk of toxicity.<sup>271</sup> Albeit rare, pulmonary pneumonitis and fibrosis have been associated with long-term (6 months or more) use in humans and may be insidious in onset. The use of nitrofurantoin is limited to infections of the urinary tract that are not susceptible to other drugs. However, a current advantage to this drug is limited resistance among those organisms considered susceptible, including *E. coli* and selected other organisms.

**Methenamine.** Methenamine (hexamine; hexamethylenetetramine is the name for commercial uses) is a chemical that releases formaldehyde and ammonia on hydrolysis (see Figure 7-12). It is usually sold as the hippurate salt. The degree of hydrolysis, and thus antibacterial efficacy, is pH dependent, requiring an acidic pH. The drug is bactericidal in an acid environment and bacteriostatic in a more alkaline environment. Therefore it is less effective in the presence of urease-producing bacteria that alkalinize the urine. Its spectrum of activity includes both gram-positive and gram-negative organisms. Methenamine is orally bioavailable and reaches high concentrations in urine.<sup>220</sup> The chemical is used primarily to treat urinary tract infections in dogs. Generally, it is used in combination with urinary acidifiers to enhance antibacterial actions. Its safety in cats could not be verified.

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