

6

Principles of Antimicrobial Therapy*

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JUDICIOUS ANTIMICROBIAL USE

"Even experienced practitioners may not realize that giving a patient antibiotics affects not just that patient, but also their environment, and all the other people that come into contact with that environment." Dancer's¹ statement, intended as a warning to practitioners of human medicine, emphasizes the importance of judicious antimicrobial therapy. It is understood that the goal of antimicrobial therapy is successful treatment of infection. However, the less judicious the approach taken to achieve that goal, the more likely a path to future failure is paved. The goal of antimicrobial therapy must be further modified to

include avoidance of resistance, a goal that is not necessarily accomplished with successful resolution of infection. Although it might be tempting to consider that human and veterinary medicine are differentially affected by antimicrobial resistance, in reality both are inexorably linked, and what affects one will affect the other. As early as 1998, the National Foundation for Infectious Diseases estimated the cost of antibiotic-resistant bacteria to be as high as \$4.5 billion annually and that they are responsible for more than 19,000 (human) deaths per year.² The impact is evident globally, nationally, in the community setting, in the hospital environment, and within the hospital, particularly with regard to at-risk patients (e.g., critical care).³ Any antimicrobial used to treat a patient ultimately must be excreted into the environment; the impact of this is just now being addressed scientifically.

Empirical antimicrobial selection may become an approach of the past. As medical communities struggle to assess impact,

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Box 6-1

Bergey's Classification of Medically Relevant Bacteria—cont'd

Endospore-Forming Gram-Positive Rods and Cocci	Corynebacterium
Bacillus	Propionibacterium
Clostridium	
Regular, Nonsporing Gram-Positive Rods	Mycobacteria
Erysipelothrix	Mycobacteriaceae
Kurthia	Mycobacterium
Lactobacillus	
Listeria	Nocardioforms
Renibacterium	Nocardia
Irregular, Nonsporing Gram-Positive Rods	Nocardioides
Actinomyces	Rhodococcus
Bifidobacterium	Saccharopolyspora

(From Krieger NR, Staley JT, Hedlund B et al: *Bergey's manual of systematic bacteriology*, ed 2, Volume 4: The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes, New York, 2010, Springer.)

Helicobacter and *Borrelia* spp.¹⁹ When collecting a culture sample for such organisms, extreme care must be taken to prevent its exposure to oxygen. **Aerotolerant** organisms are not affected by either the presence or the absence of oxygen.

KEY POINT 6-2 Antibiotics are natural antimicrobials secreted by one microorganism to inhibit another. A microbe that secretes antibiotics also carries the genes for resistance to that antibiotic.

The term **organism** refers to either the genus or the genus and species of a microorganism. Examples include *E. coli*, *Staphylococcus pseudintermedius* group (SIG), *Enterococcus faecalis*, and *Bacteroides fragilis*. For each of these organisms, multiple **strains** exist. An **isolate** refers to one **colony-forming unit** (CFU) of the resident population of that organism. This might be from any site, such as a lake, a feedlot, a surgical table, or the sample collection site of a patient. The cultured isolate is only one among what are likely to be thousands or hundreds of thousands of CFUs that make up the resident population, or **inoculum**, of the organism in the patient. Whether the inoculum in the patient represents a true infection rather than normal flora is based, in part, on the size of the inoculum—that is, how many CFUs of that organism are present in the animal.

KEY POINT 6-3 An infection is defined by the size of the inoculum, which varies with the tissue and method of culture collection.

The goal of antimicrobial therapy is to achieve sufficient concentrations of an appropriate drug at the site of infection such that the infecting organism is killed, while simultaneously avoiding side effects of the drug in the patient. In today's age of emerging resistance, the goal must be modified to include the avoidance of antimicrobial resistance. Therapeutic decisions concerning antimicrobial therapy for the infected patient are among the most challenging (Figure 6-1). Unlike most other drug therapies, antimicrobial therapy must take into account

microbe, drug, and patient factors (i.e., the chemotherapeutic triangle), many of which confound successful therapy to the point of causing failure (Figure 6-2). Antimicrobial therapy is most likely to be successful when the target (and thus spectrum of antimicrobial activity) is known such that pharmacodynamics (PD) of the infecting organism can be integrated with the pharmacokinetics (PK) of the drug in the patient.

IDENTIFYING THE NEED FOR ANTIMICROBIAL THERAPY

The first decision to be made regarding antimicrobial therapy is determining the need to treat (see Figure 6-1). The decision includes confirming, to the extent possible, the existence of infection; identification of the cause of the infection bacteria (or fungal, etc), the need for treatment of the infection; and, if treatment is deemed necessary, whether antimicrobials should be part of the therapy. This first decision is probably given the least consideration yet may be the most important if resistance is to be avoided. It also may be the most difficult to make. The presence of infections frequently cannot be confirmed for a variety of reasons, such as the lack of (infection-) specific clinical signs, location in an inaccessible site, and costs associated with accurate diagnosis. Infection is supported, but not necessarily confirmed, by clinical signs or laboratory tests indicating fever, inflammation, and organ dysfunction or structural changes detected by imaging techniques such as radiology, ultrasound, and magnetic resonance imaging. Culture may support, but does not necessarily confirm, infection. Newer detection methods based on molecular diagnostic techniques (e.g., polymerase chain reaction) may ultimately prove to be important tools in the rapid bedside diagnosis of infectious diseases, including multidrug-resistant bacteria.²⁰ However, simply documenting the presence of these microbes may not be a sufficient indication of cause and effect. These methods may not discriminate infection (reproducing, pathogenic organisms) and colonization (the presence, growth, and multiplication of the organism without observable clinical

symptoms or immune reaction), or pathogens from normal microflora. An exception can be made if cytology reveals organisms phagocytized by white blood cells, but the absence of phagocytosis does not eliminate infection.

KEY POINT 6-4 The first and most critical decision to be made regarding antimicrobial therapy is determining the need to treat. This includes confirming, as much as possible, the existence of infection; deciding if it must be treated; and, if so, whether antimicrobials should be part of the therapy.

Identifying the presence of infection is important in avoiding indiscriminate antimicrobial use. Increased risk of toxicity, cost, and inconvenience are obvious reasons that antimicrobial drugs should not be used indiscriminately. Less obvious reasons are an increased risk of superinfection and the potential emergence of resistant microbes. These latter reasons reflect, in part, the impact of antimicrobial therapy on normal flora.

Internal structures and organs (e.g., bone, heart, kidneys, the lower respiratory tract) are normally sterile. Sterility may be maintained, in part, by secretions, which constantly clean or clear the site. In addition to bulk flow, secretions may

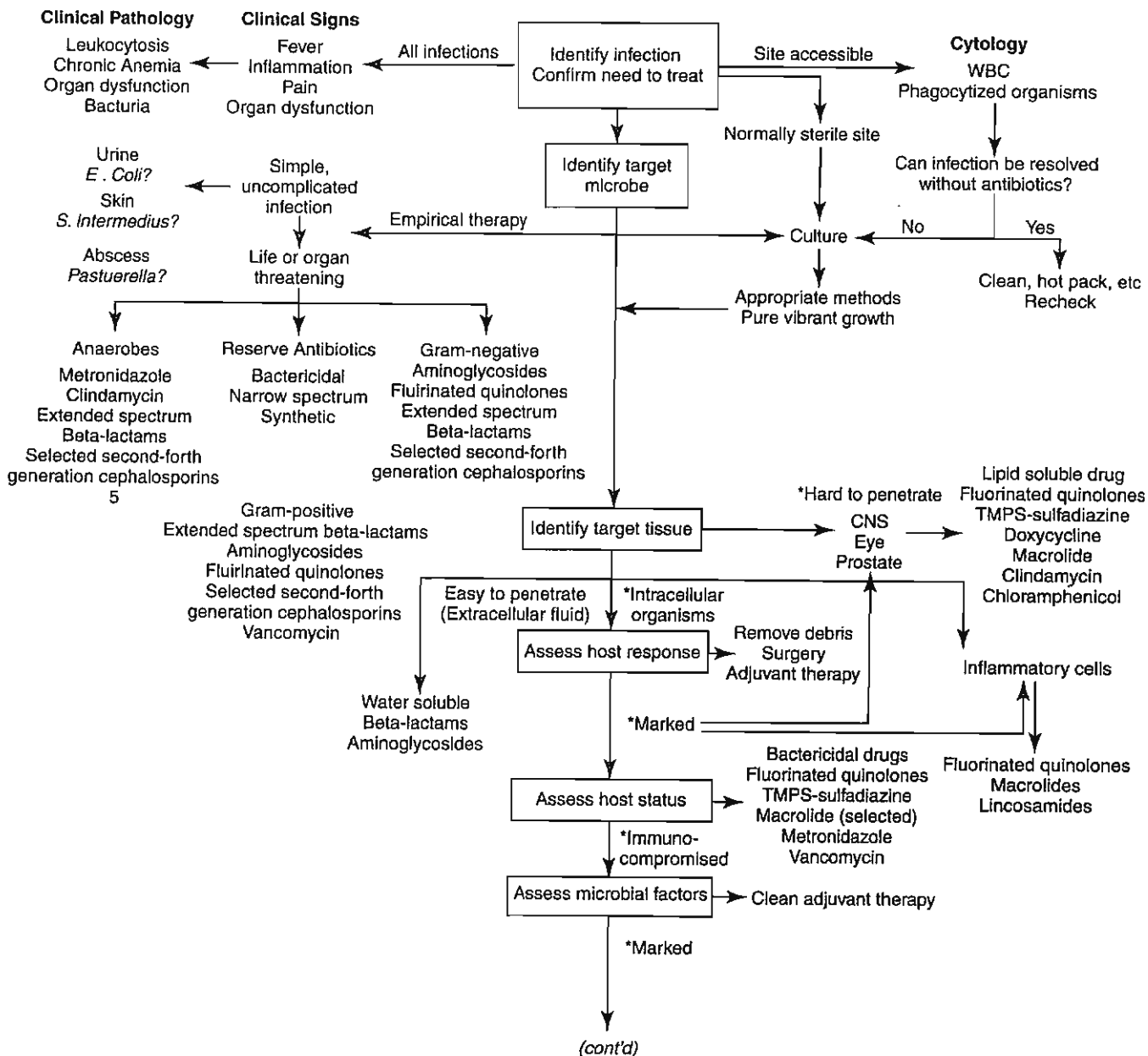


Figure 6-1 Therapeutic decision making for judicious antimicrobial therapy requires multiple steps. Antimicrobials should not be used indiscriminately; whenever possible, the most narrow-spectrum drug that targets the infecting organism should be used. Achieving adequate drug concentrations at the site of infection is critical to successful therapy. Dosing regimens should be modified for the patient; modifications should include changes in the dose and/or interval as is relevant. The asterisk at the 'Design Dosing Regimen' step refer to those indications previously encountered that should also lead to either a shortened interval or an increased dose, depending on whether the drug is concentration versus time dependent.

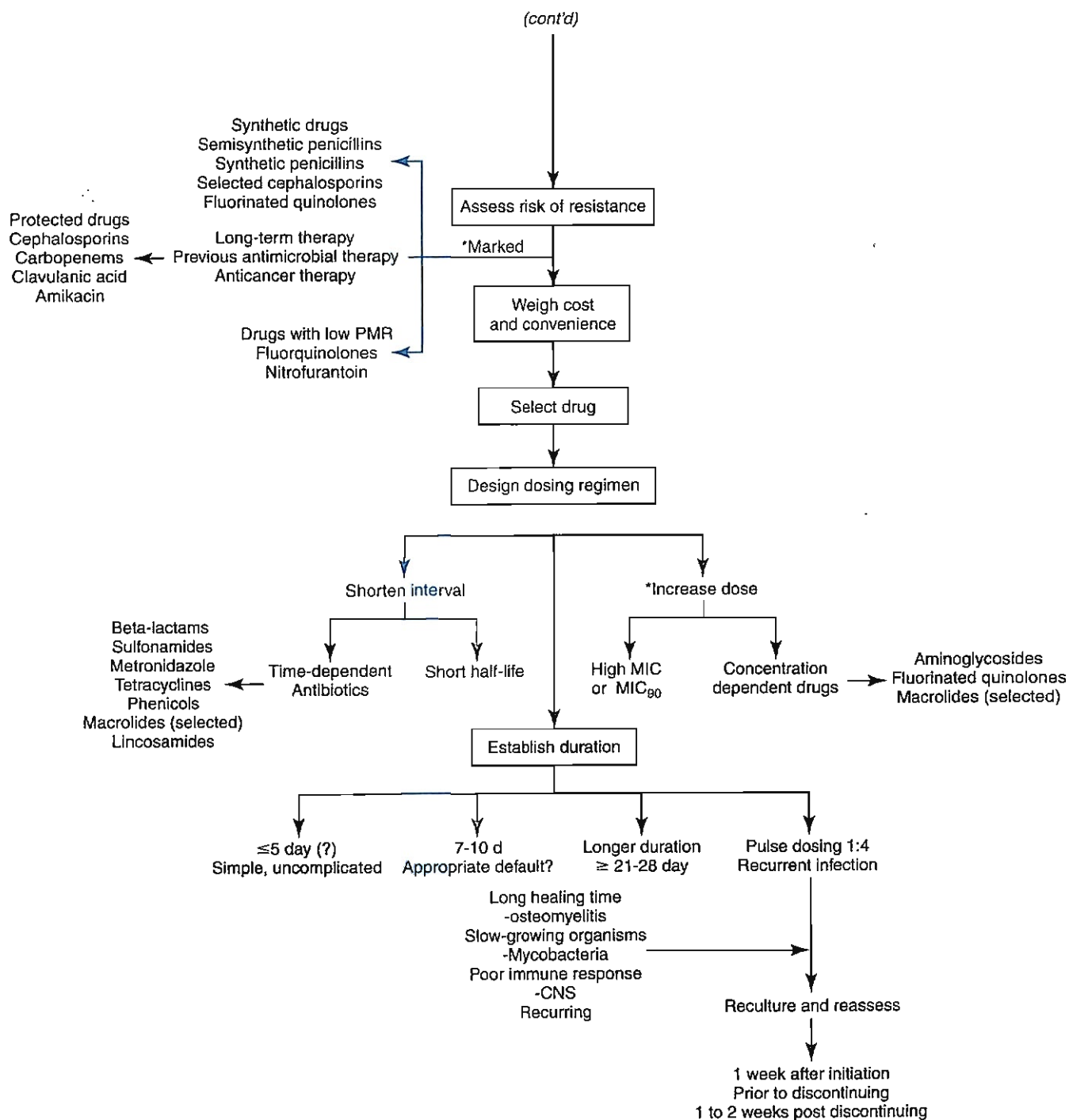


Figure 6-1, cont'd

contribute to sterility by the presence of endogenous antimicrobial compounds (e.g., tears, saliva, respiratory tract secretions, gastrointestinal acidity). However, in contrast, external (skin and conjunctiva of the eye) and internal (linings of the respiratory, digestive, and urogenital systems) surfaces are characterized by normal microflora. Normal flora may be further defined according to their contribution to host health or well-being. Most normal flora are **commensals** that appear to neither harm nor help the host. Some commensals, however, are also **opportunistic** in that they may become pathogenic, particularly if host health is impaired. A **pathogen** is a microbe

that is associated with and capable of causing host damage.²¹ Pathogens often reflect the normal flora of infected sites, with *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus* being common examples of opportunistic normal flora that can become pathogenic (Table 6-1). **Mutualistic** organisms help maintain microbial balance through host-microbe interactions. They provide beneficial effects such as producing acids that lower pH and blocking colonization by more dangerous microbes. Antibiotics secreted by mutualistic organisms help maintain the composition of aerobic and anaerobic commensal bacteria, resulting in a population that is most appropriate for host

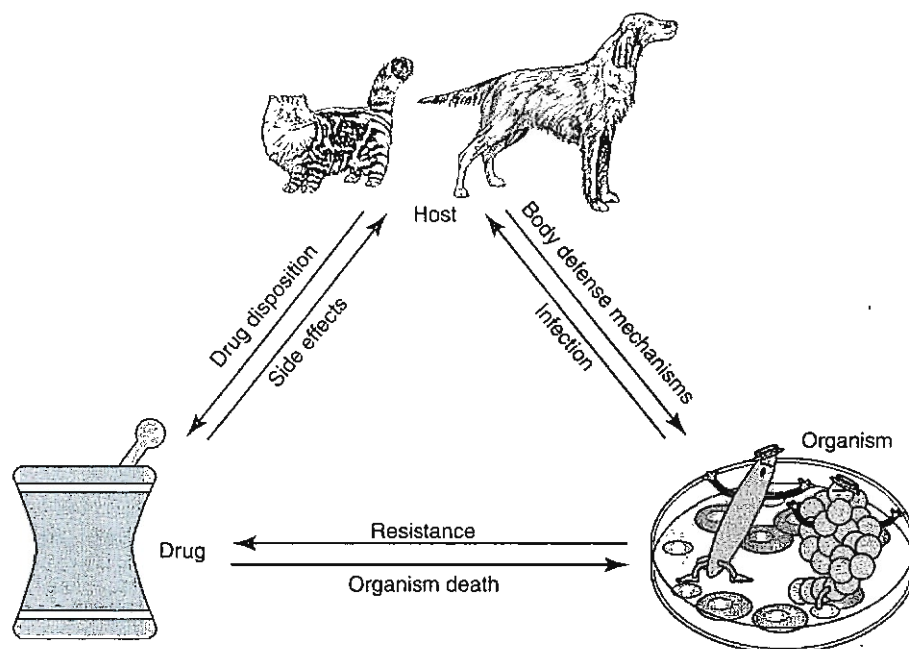


Figure 6-2 In contrast to other drug therapies, antimicrobial therapy involves not only the host and drug but also the microbe. Interactions among the three profoundly complicate successful antimicrobial therapy.

health and preventing colonization by pathogenic organisms. Opportunistic organisms may not originate from normal microflora but rather may be acquired from the environment (e.g., *Aspergillosis*, fungal organisms). **Nosocomial** organisms cause infections as a result of medical treatment, usually in a hospital or clinic setting. As such, a nosocomial infection is defined as one that arises 48 hours after hospital admission. Disruption of the environment, such as might occur with the use of antimicrobials that alter the anaerobic population, will also disrupt the balance of normal microflora, increasing the risk of infection (see the section on antimicrobial resistance). Not only will death of normal flora leave a void that can be filled in with more hardy and potentially pathogenic microbes, but the constant exposure of the microbes to antibiotics leads to ongoing development of mechanisms such that the microbes resist environmental drugs. Therefore the organisms are primed for resistance. Narrowing the spectrum of the chosen antimicrobial will help limit, although probably not prevent, the development of resistance.

KEY POINT 6-5 Discriminating among commensal versus pathogenic organisms is difficult and often cannot be determined simply by culture.

IDENTIFYING THE TARGET ORGANISM

Empirical Antimicrobial Therapy

After it has been determined that infection does exist and warrants medical management with antimicrobial drugs, identification of the target is the second critical decision to be made. Antimicrobial selection is probably most often made empirically—that is, on the basis of assumptions regarding

the infecting organism and its susceptibility to drugs. These assumptions are based on historic data that identify organisms most commonly associated with infections of various body systems (see Table 6-1).²² However, older data may not have discriminated between commensals and pathogens — indeed even today such discrimination often is not possible — which complicates the accuracy of prediction. More problematic, as resistance has emerged, the risk of incorrectly identifying the susceptibility pattern of an infecting microbe has increased. Thus the clinician should carefully balance the risk of therapeutic failure, including recurrence of infection with a resistant microbe, with the cost associated with more accurate diagnostic procedures.

The utility of Gram staining in the selection of an antimicrobial should not be overlooked as a means to narrow the spectrum of the chosen antimicrobial. Gram stain characteristics differ on account of differences in the layers penetrated by the Gram (purple) stain. The cell wall is many layers thicker in gram-positive organisms than in gram-negative ones thus rendering them more susceptible to some drugs that target the cell wall; further, the gram-positive isolates do not have an external lipopolysaccharide (LPS) covering that is present in gram-negative organisms (Figure 6-3). Whereas the LPS layer is the source of endotoxin responsible for the morbidity and mortality associated with many gram-negative infections, just as this external covering precludes stain movement into the cell wall, it also serves as a barrier to drug movement into the organism (see Figure 6-3).²³ Movement, particularly of water-soluble drugs, is generally restricted to outer membrane proteins that span the breadth of the covering (porins); however, changes in porin size and efflux pumps are mechanisms by which gram-negative organisms overcome drug movement through porins.

Table 6-1 Normal Flora and Clinically Significant Infections by Organ System (Dogs and Cats)

Organ or Site	Organism	Comment
Blood	<i>Staphylococcus intermedius</i> (D: 25%-35%)*, †, ‡ <i>Streptococcus</i> spp. (D: 18%-21%)† <i>Enterobacter cloacae</i> (D: 3%-8%, C: 7%)† <i>Escherichia coli</i> (D: 35%-45%*; D: 18%-71% & C: 14%)† <i>Klebsiella pneumoniae</i> (D: 25%-35%*; C: 14%)† <i>Proteus</i> (D: 14%)† <i>Pseudomonas aeruginosa</i> (D: 10%-20%) <i>Salmonella</i> (D: 11%-13%; C: 29%)† Obligate anaerobes (D: 10%-20%)	
Endocarditis†	<i>Staphylococcus intermedius</i> (D: 6%-33%) <i>Streptococcus</i> spp. (D: 12%-26%) <i>Escherichia coli</i> (D: 6%-30%) <i>Erysipelothrix rhusiopathiae</i> (D: 19%) <i>Corynebacterium</i> spp. (D: 19%)	
Respiratory		
Upper	<i>Staphylococcus intermedius</i> (D: 30%-35%)§, , ¶ <i>Streptococcus</i> spp. (15%-27%)§, , ¶ <i>Corynebacterium</i> spp, §, , ¶ <i>Escherichia coli</i> § (15%-29%)§, <i>Klebsiella pneumoniae</i> (D: 10%-15%) , ¶ <i>Moraxella</i> §, ¶ <i>Neisseria</i> §, <i>Pasteurella multocida</i> (D: 15%-34%; C: >50%)§, <i>Proteus</i> (C: <10%)§, <i>Pseudomonas</i> § (6%-34%)§, <i>Bacteroides</i> <i>Clostridium</i> spp. § <i>Fusobacterium</i>	Have been isolated from nasal swabs, tonsillar and pharyngeal swabs, or tracheal and lung swabs
Rhinitis, sinusitis	<i>Escherichia coli</i> <i>Pasteurella multocida</i> <i>Proteus</i> <i>Pseudomonas</i> spp.	
Tracheobronchitis*	<i>Bordetella</i>	
Lower		
	<i>Staphylococcus intermedius</i> (D: 10%-15%) <i>Escherichia coli</i> (D: 30%-40%; C: 15%-20%) <i>Bordetella</i> (D: 10%-15%) <i>Enterococcus</i> <i>Klebsiella pneumoniae</i> (D: 15%-20%; C: <10%) <i>Pasteurella multocida</i> (C: >50%) <i>Pseudomonas</i> <i>Proteus mirabilis</i> (D: <10%)	Normal bronchi and lungs sterile distal to first bronchial division
Pleuritis	<i>Actinomyces</i> , <i>Bacteroides</i> , <i>Corynebacterium</i> , <i>Fusobacterium</i> , <i>Nocardia</i> , <i>Pasteurella</i> , <i>Staphylococcus</i> , <i>Streptococcus</i>	
Gastrointestinal		
Oral cavity	Beta-hemolytic <i>Streptococcus</i> <i>Staphylococcus epidermidis</i> § <i>Acinetobacter</i> § <i>Escherichia coli</i> § <i>Moraxella</i> § <i>Neisseria</i> § <i>Pasteurella</i> § <i>Proteus</i> § <i>Pseudomonas</i> § Obligate anaerobes (80%-90%)	Isolates from healthy dogs§

Continued

Table 6-1 Normal Flora and Clinically Significant Infections by Organ System (Dogs and Cats)—cont'd

Organ or Site	Organism	Comment
Small intestine	<i>Escherichia coli</i> , <i>Klebsiella</i> †	Enteropathogenic bacteria in the stomach or small intestine associated with enterotoxin‡ or mucosal invasion
Enterobacteriaceae§	<i>Campylobacter fetus</i> ‡ <i>Moraxella</i> <i>Neisseria</i> <i>Proteus</i> spp. <i>Pseudomonas</i> spp. <i>Salmonella typhimurium</i> §, † <i>Shigella</i> § <i>Vibrio cholerae</i> ‡ <i>Vibrio parahaemolyticus</i> § <i>Yersinia enterocolitica</i> ‡ <i>Clostridium perfringens</i> (type A) † <i>Bacillus</i> §, †	
Large intestine	<i>Enterobacteriaceae</i> *	*Normal microflora; anaerobic make up 90% of microflora
Enterobacteriaceae§	Anaerobes	
Peritonitis		
Hepatobiliary		
	<i>Enterobacteriaceae</i> <i>Escherichia coli</i> <i>Enterobacter</i> <i>Klebsiella</i>	
Genital		
	<i>Staphylococcus intermedius</i> (D: 15%-25%)§ <i>Acinetobacter</i> § <i>Escherichia coli</i> (30%-35%)§ <i>Klebsiella</i> § <i>Moraxella</i> , § <i>Haemophilus</i> § <i>Pasteurella multocida</i> (10%-25%)§ <i>Proteus</i> sp.§ <i>Pseudomonas aeruginosa</i> (<10%) Obligate anaerobes (C: 10%-25%) <i>Mycoplasma</i> spp.§ <i>Ureaplasma</i> spp.§	Normal microflora of distal urethra and prepuce§
	<i>Staphylococcus intermedius</i> (D: 15%-25%)§ <i>Staphylococcus epidermidis</i> § <i>Streptococcus canis</i> , <i>S. faecalis</i> , <i>S. viridans</i> , <i>S. zooepidemicus</i> § <i>Corynebacterium</i> § <i>Acinetobacter</i> § <i>Citrobacter</i> § <i>Enterobacter</i> § <i>Enterococcus</i> § <i>Escherichia coli</i> (30%-35%)§ <i>Haemophilus</i> § <i>Klebsiella</i> § <i>Micrococcus</i> § <i>Moraxella</i> , § <i>Neisseria</i> § <i>Pasteurella multocida</i> (15%-25%) <i>Proteus</i> § <i>Pseudomonas aeruginosa</i> (<10%)§ Obligate anaerobes (C: 10%-25%) <i>Mycoplasma</i> § <i>Ureaplasma</i> §	Normal microflora of canine vagina§

Table 6-1 Normal Flora and Clinically Significant Infections by Organ System (Dogs and Cats)—cont'd

Organ or Site	Organism	Comment
Urinary Tract		
	<i>Staphylococcus intermedius</i> (D: <10%) <i>Enterococcus faecalis</i> (D: <10%) <i>Escherichia coli</i> (40%-50%) <i>Klebsiella pneumoniae</i> (10%-15%) <i>Pasteurella multocida</i> (C: 10%-15%) <i>Proteus mirabilis</i> (10%-15%) <i>Pseudomonas aeruginosa</i> (C: <10%)	
Central Nervous System		
	<i>Brucella</i> <i>Pasteurella</i>	
Ocular		
Conjunctiva	<i>Staphylococcus intermedius</i> §, ¶ <i>S. albus</i> ¶ Beta-hemolytic <i>Streptococcus</i> (C: 15%-25%)§, ¶ <i>Corynebacterium</i> §, ¶ <i>Escherichia coli</i> ¶ <i>Moraxella</i> § <i>Neisseria</i> § <i>Pasteurella multocida</i> (C: 10%-20%) <i>Pseudomonas</i> § <i>Proteus</i> <i>Bacillus</i> §, ¶ <i>Chlamydia psittaci</i> (C: 50%-75%) <i>Mycoplasma</i> ¶	Cultured from the conjunctival sac of clinically normal dogs or cats§¶
Eye	<i>Leptospira</i> <i>Brucella canis</i> <i>Clostridium tetani</i> <i>Mycobacterium bovis</i>	
Otitis externa	<i>Staphylococcus intermedius</i> (D: 25%-30%) <i>Escherichia coli</i> (D: 10%-20%) <i>Proteus mirabilis</i> (D: 20%-25%) <i>Pseudomonas aeruginosa</i> (D: 15%-25%)	
Skin	<i>Staphylococcus intermedius</i> (D: 60%-70%) <i>Escherichia coli</i> (20%-30%) <i>Pasteurella multocida</i> (C: >50%) <i>Proteus mirabilis</i> (<10%) <i>Pseudomonas aeruginosa</i> (D: <10%)	
Wounds, abscesses	<i>Staphylococcus intermedius</i> (D: 25%-50%) <i>Escherichia coli</i> (D: 20%-30%; C: 10%-20%) <i>Pasteurella multocida</i> (C: 30%-40%) <i>Proteus mirabilis</i> (D: 10%-20%; C: <10%) <i>Pseudomonas aeruginosa</i> (D: 10%-20%) Obligate anaerobes (25%-35%)	
Musculoskeletal		
Osteomyelitis	<i>Staphylococcus intermedius</i> (D: 40%-50%) <i>Staphylococcus aureus</i> <i>Escherichia coli</i> (D: 10%-20%) <i>Enterococcus faecalis</i> (D: 10%-20%) <i>Proteus mirabilis</i> (10%-20%)	

*Numbers in parentheses refer to probable percentages of infections in this tissue that are caused by the organism, as cited by Aucoin (1993). Unless noted otherwise, the percentages refer to both dogs and cats (D = dog; C = cat). Note that the probable percentage is likely to vary geographically and may be biased toward patients referred to a specialty service.

†Numbers in parentheses refer to probable percentages of infection in this tissue that are caused by the organism, as cited by Greene (1990).

‡Number in parenthesis reflects the range of percent cited by both Aucoin (1993) and Greene (1990).

§¶ For each tissue, the symbol is defined in the *Comment* column.

**Organisms that are cultured from clinically healthy animals may be difficult to distinguish from those that cause infection.

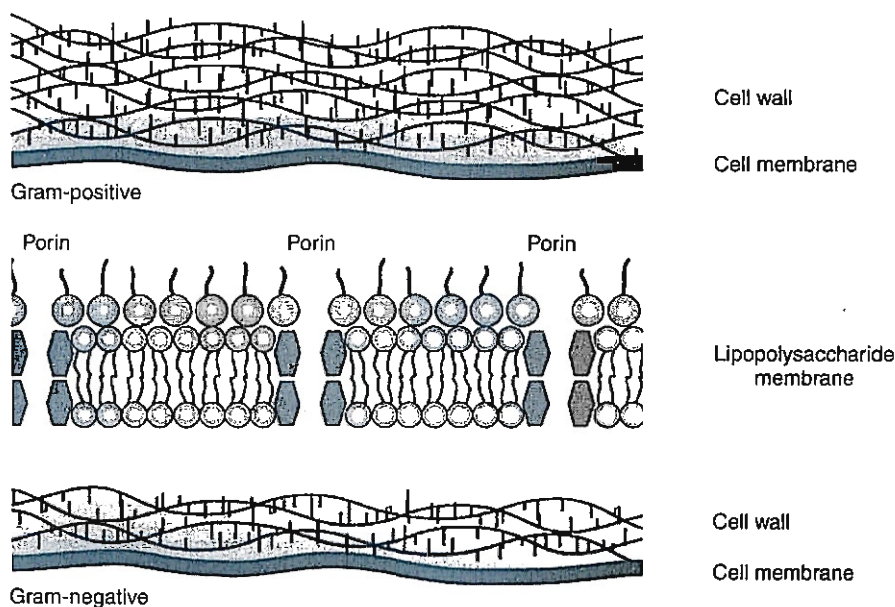


Figure 6-3 The gram-positive cell wall is thicker than the gram-negative cell wall, but the gram-negative cell wall is protected by an outer membrane including a layer of lipopolysaccharides. Endotoxin, derived from the lipopolysaccharides, contributes to the mortality and morbidity of gram-negative infections. The membrane also presents a challenge to drug movement. Although lipid-soluble drugs can diffuse through the membrane, movement of water-soluble drugs must occur through channels in outer membrane proteins called *porins*, which form aqueous channels that filter unwanted molecules. These porins are also associated with efflux pump proteins (the latter are also present in gram-positive organisms). Reduction in porin size or increased efflux pump activity are important mechanisms by which gram-negative organisms develop multidrug resistance.

In addition to Gram staining, determining the source of infection may help identify the microbe because some organisms are more likely than others to infect certain body systems. For example, genitourinary tracts are often infected with gram-negative aerobes, whereas abdominal infections generally are caused by gram-negative aerobes initially, followed by anaerobes after several days (see Table 6-1).^{24,25} Skin is most commonly infected with *Staphylococcus pseudintermedius* (to be referred to as *S. intermedius* group, or SIG), abscesses with anaerobes and *Pasteurella* spp., and the urinary tract with *E. coli*. Indeed, *E. coli* is one of the more common pathogens, infecting many tissues. One study of 674 *E. coli* isolates collected from dogs found the vast majority ($n=424$) associated with urinary tract infections (UTIs) ($n=424$); however, 61 were also collected from skin, respiratory tract (52), ear (43), female (42) and male (25) reproductive tracts, and other organ systems (23). However, although *E. coli* may indeed be the most common isolate associated with UTIs, it does not necessarily represent the majority of UTIs. In a study by the author, only 50% of UTIs were caused by *E. coli*, with the remaining 50% caused by *Staphylococcus* spp., *Enterococcus* spp., *Proteus*, and others. For critical patients, organisms generally represent the normal flora of the alimentary canal or a nosocomial organism.²⁶ Granulocytopenic or otherwise immunoincompetent patients also are more likely to be infected by aerobic gram-negative organisms.

Even if the organism is correctly identified, the greater risk of failure associated with empirical treatment lies in the

inability to correctly predict susceptibility patterns. This is not a new concern: As early as 1996, a study of critical-care patients revealed that empirical selection of antimicrobials was incorrect, on the basis of cultures collected before antimicrobials were started, in nearly 45% of patients.²⁷ Further, isolates of four organisms collected between 1998 and 2000 (*P. aeruginosa*, *P. mirabilis*, *E. coli*, *Staphylococcus* spp.) widely considered to be susceptible to enrofloxacin (which had been approved for approximately 10 years) were characterized by a higher than expected incidence of resistance (28% for *E. coli*).²⁸ More recently, a high level of resistance was ascribed to drugs used empirically to treat otitis interna²⁹ and pyothorax.³⁰ Finally, our laboratory has demonstrated that more than 40% to 60% of *E. coli* associated with UTIs in dogs are characterized by resistance to first- and second-choice drugs (amoxicillin/clavulanic acid, cephalexin, potentiated sulfonamides, and enrofloxacin).³¹ These differences may be regional but the absence of a robust surveillance program for dogs and cats limits empirical antimicrobial selection. These studies suggest culture and susceptibility (C&S) testing will become increasingly important.

Culture and Susceptibility Testing

C&S data can be a powerful guide for judicious antimicrobial use. However, C&S testing is only one of several tools that should support antimicrobial therapy. Among the advantages of culture is facilitation of input from a veterinary diagnostic microbiologist. As such, it has multiple roles in antimicrobial therapy: identifying the potential pathogen, providing a list

of potentially effective drugs, offering guidance regarding the most effective drug, and serving as a basis for design of a dosing regimen of that drug through integration of pharmacokinetics (PK) and pharmacodynamics (PD).³²

KEY POINT 6-6 The more at risk a patient is for resistance to be present or emerge, the more important culture and susceptibility testing becomes in the support of drug selection and design of the dosing regimen.

To date, not all infections require C&S testing to be effectively treated. Indeed, basing treatment on C&S does not guarantee therapeutic success. However, C&S can be particularly prudent for at-risk patients. It is particularly important for patients that have been treated with antimicrobials in the past several months. Testing is important to critical patients; although empirical therapy will begin before its receipt, culture of blood, urine, respiratory secretions (collected by bronchoscopy) and other pertinent body fluids (i.e., pleural, peritoneal, or cerebrospinal fluid [CSF]) should be carefully sampled before antimicrobial therapy is begun. Testing is also critical if infection by nosocomial organisms is of concern because their complex resistance patterns often require more expensive and potentially toxic drugs.³³

Among the disadvantages of C&S testing is the time that often elapses between sample collection and receipt of results. Ideally, antimicrobial therapy will be withheld until the information is received and the accuracy of empirical choices is confirmed. The more a patient is at risk for developing resistance, the more important it may be to withhold therapy until results are received. However, treatment generally cannot be withheld. Still, if the data indicate that an incorrect choice may have been made regarding empirical antimicrobial selection, the data may no longer accurately reflect either the current infecting population or the susceptibility pattern. The clinician has several options, given that scenario (see Figure 6-1). If the patient has responded to therapy, the most prudent approach may be to stay the course, or perhaps add a second (nonantagonistic) drug to which the isolate is susceptible. If the patient has not responded sufficiently to therapy, therapy might be changed in light of the new data. However, the more prudent approach might be to reculture and wait until the new data arrive before changing course.

As with any tool, C&S data can be detrimental if misused. Contributing to improper use are the many pitfalls of testing, which begin with sampling, continue through the testing procedures and interpretation of results, and end with the design of the dosing regimen.

Culture data are only as good as the sampling methods of collection; the importance of proper culture techniques cannot be overemphasized (Box 6-2). For skin wounds the surface always contains commensals; normal flora, regardless of the site of collection, will cause background noise that must be filtered out. Swabs are often not ideal for sampling for a variety of reasons,³⁴ the most compelling of which is that only 3 out of 100 CFUs will actually make it to the culture stage. For anaerobes in particular, air between the fibers inhibits growth.

Despite the greater level of difficulty in sample acquisition, tissue is the preferred sample. This might be an aspirate of fluids or macerated tissues (the laboratory may prefer to perform the macerating). Cleansing before sample collection is indicated, particularly for contaminated sites. For the same reason, cystocentesis is the most acceptable sample for interpretation of bacteriuria; catheterized sample often contain microbes colonizing the catheter and associated biofilm. The properly collected obligate anaerobic sample is particularly difficult to achieve and the absence of anaerobes may simply reflect improper techniques. An anaerobic infection should be suspected if clinical signs are supportive (e.g., foul smell, adjacent to mucosal membranes or gas). Note that facultative anaerobes may be cultured and tested as susceptible under aerobic conditions but fail to respond to therapy as expected if the infection in the patient occurs under anaerobic conditions.

Even a properly collected culture may not confirm infection or identify the infecting pathogenic microbe. Cytology coupled with Gram staining should be considered when possible, with phagocytosis of the organism indicative of pathogenicity. Pathogenicity reflects virulence, which is often misconstrued as resistance. The chances of proper identification of the cultured isolate pathogen are greatest if vibrant growth is obtained in an otherwise sterile environment. However, for tissues characterized by a normal flora, culture may not be able to discriminate colonization and infection by normal opportunistic organisms that have become pathogenic. Most normal flora comprises commensals that are opportunistic, i.e., able to cause disease without the support of virulence factors. A population shift from colonization to infection by such organisms is more likely to occur in at-risk patients, such as the critical-care patient, or at sites for which local immunity is compromised. Infection generally reflects normal flora, such as *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. pseudintermedius*, although opportunistic organisms also may be acquired from the environment.

The culture may give some indication as to the quality of the sample based on evidence of contamination. If C&S data indicate contamination, the site should be resampled (tissue collection rather than swab) after proper cleansing. For example, selected organisms, such as *Bacillus* sp. and *Corynebacterium* spp. are common contaminants, and their presence in wounds may be indicative of contamination and thus, potentially, a poorly representative sample. The location of culture may also be important in identifying the organism as a contaminant. For example, whereas beta-hemolytic *Streptococcus* sp. (e.g., *S. canis*) collected from a wound may be important, it is a likely contaminant if cultured from the ear. *Streptococcus* sp. pathogenicity (i.e., the likelihood of infection) can be associated with its ability to hemolyze hemoglobin, with alpha designation (hemoglobin is simply reduced) being the least and beta (red blood cells disrupted) potentially the most hemolytic and pathogenic designation. Gamma hemolysis is actually the absence of hemolysis and is demonstrated by *Enterococcus* spp. (previously a subset of *Streptococcus* spp.). However, alpha-hemolytic also can be pathogenic under the right circumstances, such as in the patient that has undergone

Box 6-2

Techniques in Culture Sampling

Commonalities Regardless of Site*

Site preparation: Don sterile gloves. Clean wound. Do not culture purulent or necrotic debris. Thoroughly cleanse wound by removing excessive debris, flushing with saline, and blotting with sterile gauze. Change to sterile gloves before collection.

Tissue aspiration: Clean intact skin with antiseptic (e.g., 70% alcohol and 10% povidone-iodine). Allow to air dry (do not fan). Expel air from an appropriate-size syringe to which is attached a 22-Ga needle. Insert needle into intact skin at the deepest portion of the lesion. Aspirate approximately 0.5 mL fluid. Needle can be moved back and forth at different angles in skin. Remove needle with hemostat. Discard needle and recap syringe with blood-gas cap, or, particularly if anaerobes are suspected, transfer fluid to transport vial.

Swab techniques: Swab techniques are acceptable only for eye, ear, and uterus cultures. Note that only 3 of 100 colony-forming units collected in a swab are likely to be successfully cultured. Use a swab in appropriate carrier medium. The swab should be moistened with sterile, preservative-free solution if wound is not moist. Sample should be collected without touching the edge of the wound or skin. Rotate swab over 1-cm area of open wound for 4 seconds. Place swab aseptically in transport sleeve, making sure tip comes into contact with liquid transport medium (break ampule if present). One swab should be collected for each sample type (i.e., aerobic, anaerobic, and a third if cytology is of interest).

Skin or Wound Biopsy

Swab is strongly discouraged; aspirate is acceptable, but macerated tissue is preferred. Clean intact skin with antiseptic and allow to air dry (do not fan). Collect biopsy aseptically. Place in transport tube containing liquid medium. Clinical microbiology laboratory will macerate.

Bone

Place in transport system. Moisten with sterile physiologic saline as necessary.

Drain Tube Site

Treat as a contaminated wound. Care must be taken not to culture the biofilm associated with the foreign body. The drain tube should be removed, the site surgically cleaned and flushed, and the area cultured. Ideally, tissue will be collected at the presumed site of infection.

Respiratory Tract

Bronchoscopy specimens include bronchoalveolar lavage, bronchial washing, bronchial brushing, and transbronchial biopsy specimens. The bronchoscope should be passed transorally in nonintubated patients or through the endotracheal tube in intubated patients. Bronchial wash or bronchoalveolar lavage specimens should be obtained before brushing or biopsy to minimize blood in the recovered fluid.

- For lavage, sterile nonbacteriostatic 0.85% NaCl should be injected from a syringe through a biopsy channel of the bronchoscope. Recovered fluid should be placed in the transport vial.

- Bronchial brush specimens should be collected through a telescoping double catheter plugged with polyethylene glycol at the distal end (to prevent contamination of the bronchial brush) through the biopsy channel of the bronchoscope. The sample should be transported in a sterile container with a small amount of sterile nonbacteriostatic 0.85% NaCl.
- Lung aspirations should be placed in transport vial for laboratory submission.
- Pharyngeal samples are not acceptable.
- Nasal samples are of limited value. Culture requests should be limited to pathogen-specific samples, e.g., *Bordetella bronchiseptica*.

Urine

Samples should be collected by cystocentesis only. Catheterized samples generally are not preferred; if there is no alternative, collect from a fresh catheter, or (less ideal) discard the initial 5 to 10 mL of urine before collecting sample. Catheter tips or urine from a collection bag are not acceptable. Sample can be submitted in a red-top serum collection tube. Samples should be kept cold by submitting on ice and ensuring that samples are received by the laboratory within 24 hours of submission. Consider a urine paddle collection system supplied by some commercial diagnostic laboratories.

Blood Culture

Liquid medium is indicated. Volume is critical to maximize recovery. Bacteremia may consist of less than one colony-forming unit per mL of whole blood. Prepare the collection site using aseptic methods, and for blood cultures, note that at least three collections at three time points are indicated. Collection during a fever spike is recommended. For blood, the volume should be 1 part blood to 10 parts broth.

Cerebrospinal Fluid or Joint Fluids

Use blood culture bottle, and add entire sample aseptically to broth.

Other Body Fluids

See discussion of tissue aspiration.

Ocular

In general, instill 1 or 2 drops of topical anesthetic. Organisms are more readily detected in scrapings than from a swab.

- For conjunctival scrapings, scrape the lower tarsal conjunctiva with a sterilized spatula and place material directly into medium. Alternatively, use a calcium alginate swab or a cotton-tipped applicator to swab the inferior tarsal conjunctiva (inside surface of eyelid) and the fornix of the eye.
- Consider collecting a conjunctival sample first, which might help you assess the possibility of contamination. Using short, firm strokes in one direction, scrape multiple areas of ulceration and suppuration with a sterilized spatula. The eyelid should remain open, and care should be taken to avoid eyelashes. Multiple scrapings are recommended because the depth and extent of viable organisms may vary. Inoculate each scraping directly to appropriate medium.

Box 6-2

Techniques in Culture Sampling—cont'd

- Intraocular fluid should be collected using needle aspiration. Aspirate should be used to directly inoculate appropriate medium, with immediate transport to the laboratory in an anaerobic transport system.

Gastrointestinal Tract

Fecal specimens are submitted primarily for the detection of *Campylobacter*, *Shigella*, and *Salmonella* spp.; *Clostridium difficile*; and, in certain cases, to detect *Yersinia*, *Vibrio*,

and *Aeromonas* spp. and enterohemorrhagic *Escherichia coli*. Care should be taken to ensure that the sample is not contaminated with urine. Fecal white blood cells should be ordered on liquid stools to indicate degree of inflammation. Stool specimens should be mixed with transport medium to maintain viability of pathogens that may be present. Sample should be collected digitally wearing a sterile glove or using a sterile fecal loop.

*The laboratory to which the sample will be submitted should be consulted before collection to ensure that its recommendations are followed. These general guidelines are offered in the absence of specific guidance.

invasive procedures such as intubation (e.g., *S. pneumonia* in humans). *Enterococcus* also has expressed beta hemolysis. The laboratory may choose not to implement susceptibility testing for those isolates considered nonpathogenic, with the interpretation of pathogenicity by the microbiologist depending on the host circumstances, including sampling site. Such decision making can only be improved with effective communication between clinician and microbiologist.

The number of organisms may be helpful in identifying the cause and effect of microbial presence and infection. Isolation of multiple organisms from a site that is easily contaminated by normal flora may represent floral colonization rather than a polymicrobial infection.²⁴ In contrast, pure growth generally indicates infection and the potential need for therapy. For example, *Pasteurella* as one of several organisms collected from a nasal swab may not be relevant, but if cultured as a pure isolate, it is probably indicative of infection. A related indicator of infection is the intensity of growth. For countable tissues, the number of CFUs per mL of tissue should be considered when assessing whether the inoculum represents an infection (see previous discussion of inoculum size). Vibrant growth of a single organism generally is indicative of infection by a pathogen, even in an environment that is easily contaminated. If multiple organisms are cultured and the culture was improperly collected, cleansing of the site (if possible) and reculture may facilitate correct identification of the pathogen. If the culture was a properly collected sample, those isolates characterized by lighter growth might be deemphasized in favor of organisms with significant growth. Controlling the heavier growth may facilitate the patient's capacity to eradicate the less dense population. For example, *E. coli*, SIG, or alpha-hemolytic *Streptococcus* are rapid growers, and if present together, the organisms with the greater growth might be targeted. However, *P. aeruginosa* is an example of a slow grower that is easily overwhelmed by other organisms. The impact on different growth rates exemplifies the importance of post-collection sample handling (e.g., the need to refrigerate). The presence of slow-growing organisms in a properly collected sample generally indicates the need for treatment. Specialized procedures may be necessary to identify growth in tissues normally sterile (e.g., blood culture, cerebrospinal fluid, or well-collected

bronchial alveolar lavage). Thus as few as two colonies of *Pseudomonas* sp. cultured from a properly collected bronchial alveolar lavage might be considered significant, whereas the need for antimicrobial therapy might be reconsidered if growth is less than 10⁵ CFUs from a site that is easily contaminated (e.g., wounds, clean-catch or catheterized urine). Patient health also should be considered: whereas, up to 10³ CFU/mL of urine collected by cystocentesis may not be significant in normal dogs, it may be indicative of infection in a patient that is not concentrating urine (e.g., because of renal disease, diuretic or fluid therapy).

Although the susceptibility patterns of an isolate may offer clues as to pathogenicity of the cultured isolate, care must also be taken with this approach. Contaminants are often characterized by patterns of susceptibility rather than resistance. However, such an isolate may yet be a pathogen, particularly in a patient with no previous history of antimicrobial exposure. Complex patterns of resistance may suggest the isolate is an infecting pathogen rather than a colonizing commensal. This is exemplified by nosocomial organisms associated with medical treatments (arising within 48 hours of hospital admission). However, *Stenotrophomonas* and *Serratia* are common contaminants of antiseptics or disinfectants that are characterized by complex patterns of resistance. Multidrug resistance (discussed later) must also be considered in the context of the inherent susceptibility of the organism, being relevant only if expressed toward drugs to which the organism should be susceptible. For example, *P. aeruginosa* may be tested toward drugs to which it is inherently resistant, yielding results that appear to suggest the isolates as multidrug resistant. However, multidrug resistance should not be considered unless expressed toward ticarcillin, carbapenems, or aminoglycosides.

The clinical microbiologist can be a powerful ally in determining the significance of isolates yielded from a sample culture. The microbiologist that is trained in veterinary medicine will be of most benefit in providing guidance regarding the relevance of the isolated microbe. However, the contributions of the clinical microbiologist will be markedly curtailed if an insufficient history of the patient from which the sample was collected is provided.

Interpreting Culture and Susceptibility Test Results

The *in vitro* data generated by C&S testing eventually must be applied to *in vivo* patient conditions. Testing methods themselves may influence results such that the data are misinterpreted. The complex nature of C&S procedures mandates standardization and a well-documented quality control program. The Committee on Laboratory Standards Institute (CLSI; previously the National Committee for Clinical Laboratory Standards [NCCLS])³⁵⁻³⁹ validates method protocols, guidelines, and interpretive standards for C&S and molecular testing; one of its subcommittees promulgate veterinary-specific standards.^{37,38} These standards and guidelines, which are applicable throughout the nation, and are often used internationally, reflect careful and exhaustive review of PD (microbial response to drug) and PK (host handling of drug) data. Because microbial populations are dynamic, standards and guidelines addressing their culture and susceptibility are likewise dynamic. Intermittent re-examination results in new guidelines and adjusted criteria, as is appropriate for changing microbial trends. CLSI publishes its findings so that clinical microbiological laboratories can access and implement the standards. An important caveat to C&S testing is that manufacturers supplying materials to the laboratory may not implement recommended changes in their materials in a timely fashion. Further, some veterinary diagnostic microbiological laboratories do not necessarily adhere to these standards but rather generate their own guidelines. Yet only CLSI standards undergo national peer review assessment and discussion among unbiased experts representing government, industry, academia, and clinical practice.

CLSI has generated guidelines for a variety of C&S testing methods. The PD information varies with the susceptibility procedures, with disk diffusion (Figure 6-4) and broth dilution (Figure 6-5) offering excellent examples of contrasts in advantages.⁴⁰ It is the latter that provides the minimum inhibitory concentrations (MICs) necessary for comparison among drugs and design of dosing regimens. The data generated from culture and susceptibility testing represents the PD portion of PK-PD integration in that it indicates what is needed to target the microbe.

KEY POINT 6-7 The veterinary clinical microbiologist is a powerful ally in the interpretation of culture and susceptibility testing data.

Disk Diffusion Versus Broth Dilution Techniques

Both methods of susceptibility testing require rapid growth of organisms and therefore may not be available for all organisms. Broth dilution data are particularly dependent on rapid growth, and for some organisms disk diffusion may be the only available means of obtaining data. The disk diffusion method (e.g., Kirby-Bauer) involves disks that contain a known amount of the drug of interest. The agar is streaked with a standardized inoculum of the isolated organism, and the disks are placed in standardized positions on the inoculated gel. Drug diffuses from the disk into the agar at a known rate (see

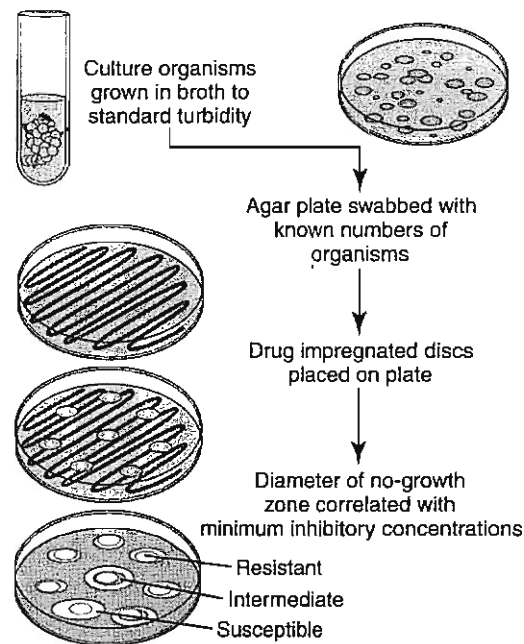


Figure 6-4 The disk diffusion method of culture and susceptibility testing. Drug diffusion from the disk results in concentrations that are higher close to the disk and gradually decrease as the diameter of the zone surrounding the disk increases. Resistant organisms can grow close to the disk despite high drug concentrations in the agar, whereas susceptible organisms will be inhibited at a standard distance from the disk. Concentrations in the agar correlate with the minimum inhibitory concentration (MIC) of the drug.

Figure 6-4),⁴⁰ such that, at a standard time, the concentration in the agar correlates with the minimum inhibitory concentration (MIC) of the drug as would be determined by the broth dilution procedures (the most common method serving as a gold-standard to other methods). At the prescribed time (i.e., as specified by CLSI³⁵⁻³⁷), a zone of no microbial growth (in mm) is measured around the disk. Because the concentration of the drug decreases with the distance (zone) diameter from the disk, the larger the zone, the lower the concentration of drug necessary to inhibit the growth of the organism and the more likely effective drug concentrations will be achieved at the site of the infection. A susceptible ("S") designation is given if the zone is sufficiently large. Growth up to the designated zone indicates that the concentration of drug necessary to inhibit the organism is too high to achieve in the patient, leading to a resistant ("R") designation. Intermediate ("I") designation is provided for some drugs. Zone sizes necessary for an organism to be considered susceptible as opposed to resistant to a specific drug are variable and are very sensitive to disruptions in protocols, which underscores the importance of following standards. An advantage to the disk diffusion method is that multiple drugs might be simultaneously tested on one plate. This is in contrast to the more tedious and costly, yet more informative, broth dilution methods. Because disk

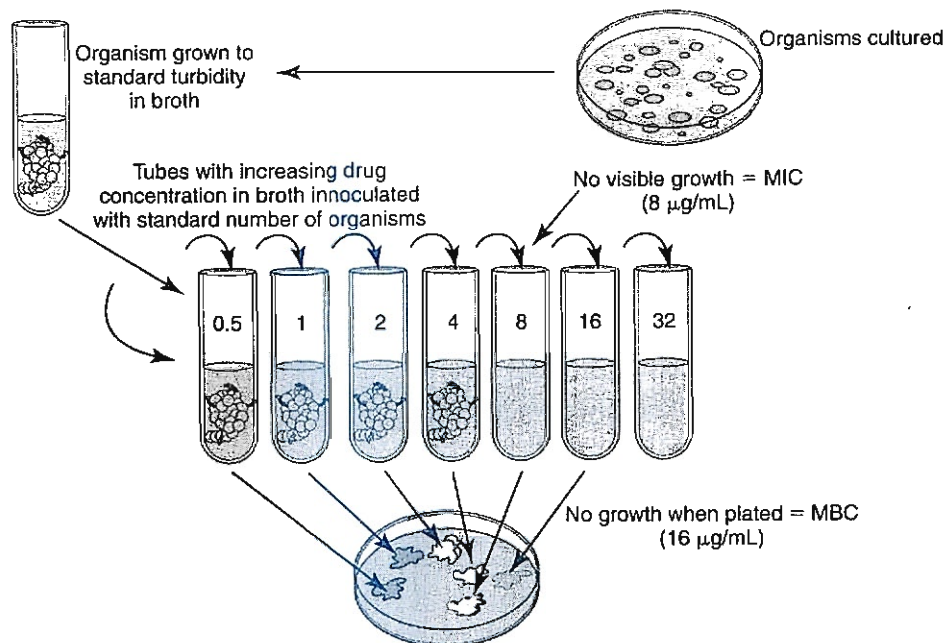


Figure 6-5 The broth dilution method of susceptibility testing provides a drug concentration to be targeted in the patient. Tubes containing serially increasing concentrations of drug are inoculated with a standard amount of the bacterial organism. At the proper time, tubes are observed for evidence of growth. The first tube (i.e., the one with the lowest concentration) that shows no evidence of growth contains the minimum inhibitory concentration (MIC) of the drug. The MIC can be used to evaluate relative drug efficacy and development of resistance and to calculate dosing regimens. This method is also one means by which the minimum bactericidal concentration (MBC) of a drug is determined. If the tubes exhibiting no growth are then used to inoculate solid agar, those tubes that yield no bacterial growth contained sufficient drug to kill, rather than simply inhibit, bacteria. The test tube that contains the lowest concentration of drug that yields no growth contains the MBC. If the MBC approximates the MIC, then the drug can be considered bactericidal.

diffusion results are reported as S, I, or R, it is described as semiquantitative.

In contrast to the disk diffusion method, the broth dilution method provides quantitative data regarding the amount of drug necessary to inhibit microbial growth (see Figure 6-5).⁴¹ For each drug of interest, tubes of liquid media are spiked with concentrations of the drug of interest, with the highest concentration generally being that just below the CLSI threshold of susceptibility (resistant MIC breakpoint). Subsequent test tubes containing serially diluted (by half) concentrations of the drug. As such, MICs are generally reported out as logarithmic fractions or multiples of 1 µg/mL (i.e., from lowest to highest 0.0312, 0.0615, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512; see Figure 6-5). Each drug to be tested must involve multiple test tubes or wells. The low and high range of concentrations tested for each drug will vary depending on concentrations achieved in tissues (including blood) when administered at recommended dosing regimens to the target species. For example, the ranges tested for ticarcillin would be expected to be much higher than the concentrations tested for enrofloxacin because the maximum concentration achieved in serum after administration of a recommended dose will be much higher for ticarcillin than for enrofloxacin (see Chapter 9). Occasionally, the MIC for some drugs deviates from the aforementioned tested concentrations; generally, these are drugs marketed as combinations (e.g., trimethoprim/sulfonamide combination). PD data generated for package inserts

or scientific reports also may incorporate dilutions other than those delineated by CLSI. It is important to remember that CLSI guidelines are intended only to support clinical microbiological laboratories that provide direct support for patient care.

The tubes that contain broth (standardized type and amount) of the appropriate dilutions of the drug of interest must be inoculated with a standard number of the isolated bacterial organism during the logarithmic phase of growth. Microbial growth continues under standardized conditions for the standardized period (as set by CLSI³⁵⁻³⁷). At the end of the incubation period, each tube is observed for evidence of growth. Evidence of growth is determined visually or using computer systems that allow miniaturized automation (see Figure 6-5 and Figure 6-6) The tube with the lowest concentration of drug that exhibits no detectable growth contains the MIC (in µg/mL), or the minimum amount of antimicrobial necessary to (in vitro) inhibit the growth of the organism cultured from the patient.^{23,41} Because of the complexities of the procedures, laboratories that provide clinical C&S testing may find MIC results on the same isolate that vary, even if CLSI guidelines and interpretive standards are followed. Generally, variations within 1 broth dilution are not considered significant. Laboratories ensure that quality standards of testing are met by performing drug MIC determination for control isolates (i.e., obtained from American Testing Cell Culture: e.g. *E. coli* ATCC 25922, *Staphylococcus intermedius* ATCC 45222).⁴¹

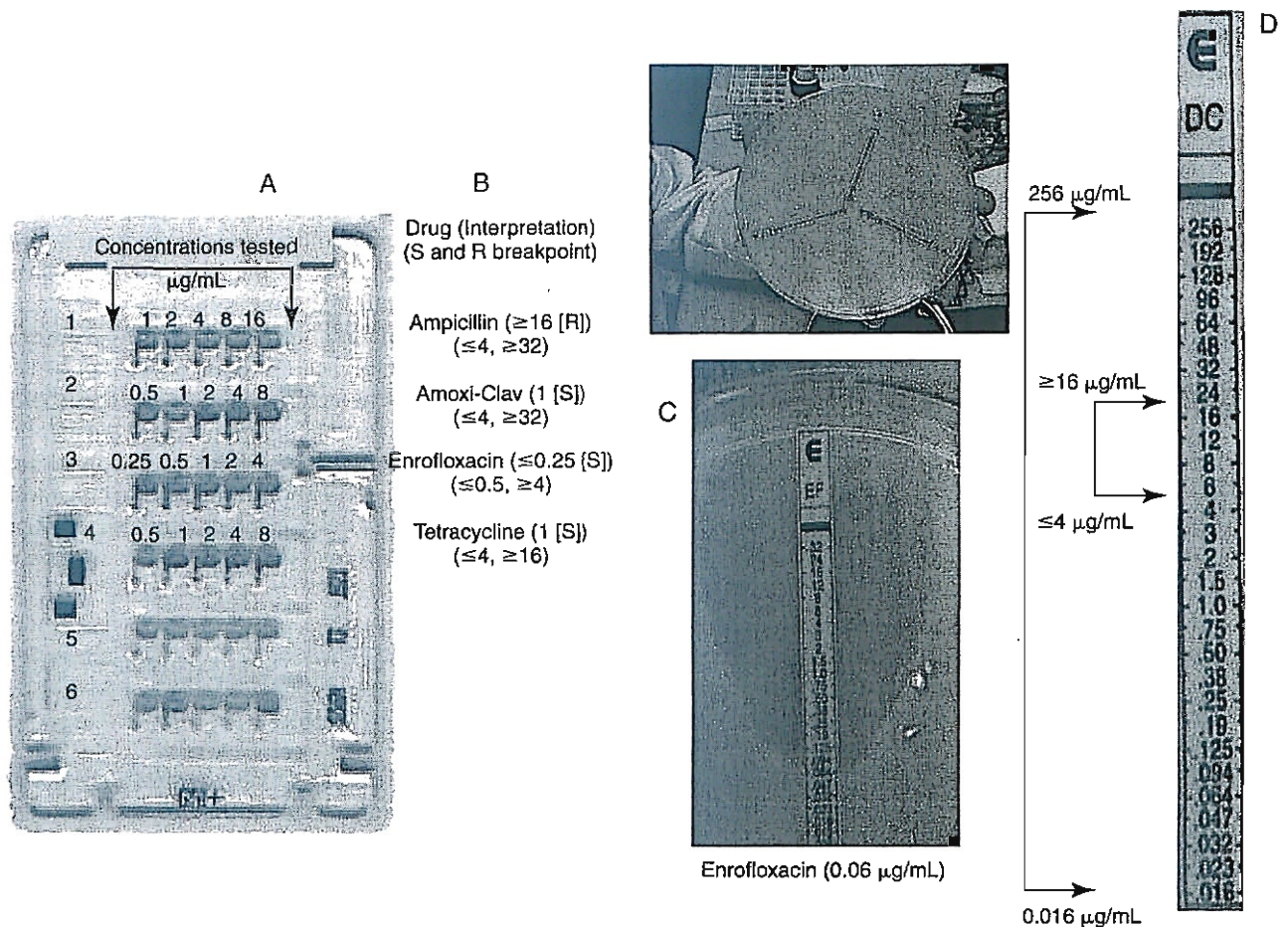
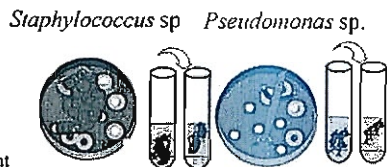


Figure 6-6 A, A commercially available antibiogram card and an E-test (C-D) with interpretation. The commercially available antibiogram card is a miniaturized broth dilution procedure that generates minimum inhibitory concentration (MIC) using a microwell design. Generally, one card is made for gram-negative isolates and another for gram-positive isolates. The size of the card limits testing of the number of drugs and the range of concentrations, with concentrations approximating the susceptible and resistant breakpoints (indicated under each drug). Some cards test only the susceptible and resistant breakpoints. Growth is indicated by a color change (all wells had growth in row 1, indicating resistance to ampicillin, but no wells had growth in row 3, indicating susceptibility to enrofloxacin). The ranges tested (above the wells) and interpretations (to the right of each drug) are provided for four of the drugs tested on the card. None of the isolates tested intermediate. A limitation of the cards is ability to indicate *how* susceptible an isolate is. This limitation is largely overcome with the E-test system (C; strip is enrofloxacin). Each strip releases the drug into the medium at logarithmic rates. Growth in susceptible isolates follows a tear-shaped pattern, with the point of the tear indicating the MIC. Advantages of the E-test include a very broad range of test concentrations (over 16,000-fold) indicated by outer bracket (see Table 6-3), exceeding both the susceptible and resistant breakpoints (indicated by inner bracket) by several magnitudes, thus allowing assessment of how susceptible or resistant the isolate might be to the drug of interest. The MIC of this isolate is 0.06 $\mu\text{g/mL}$; for comparison, the lowest concentration that would be tested on the antibiogram. The E-test suggests that this isolate is moderately susceptible to doxycycline. The differences in the MIC between the microwell dilution and the E-test may reflect subtle differences in methodology but also the lipophilic nature of doxycycline (better penetrability), thus highlighting a caveat of susceptibility testing: model drugs do not always represent the drug of interest well. Another advantage of the E-test is the smaller increments of change, and thus greater precision provided compared with tube dilution procedures. A more precise dosing regimen can thus be designed. For example, with standard tube dilution, concentrations increased from 8 to 16, whereas with E-testing, concentrations increased from 8 to 12 to 16 $\mu\text{g/mL}$. Finally, the individual nature of the test strips allows a “pick and choose” approach to individualizing drug therapy. This also, however, is a disadvantage in that costs are higher when multiple drugs are tested.

Research publications that address bacterial PD likewise should demonstrate adherence to CLSI guidelines, including quality-control procedures.

Broth dilution reports provide both the MIC (a concentration, reported in $\mu\text{g/mL}$) and (assuming CLSI procedures

are followed), the CLSI interpretation of S, I, or R for that MIC. The basis of the interpretation (S, I, or R) for broth dilution procedures is addressed later in this chapter. The MIC for selected drugs may be accompanied by a “ \leq ” or “ \geq ”. Using Figure 6-7 as an example, *Pseudomonas* have an MIC for



	MIC breakpoint					
	Susceptible	Resistant				
Amikacin	(≤ 16)	(≥ 64)	S	≤ 4	S	≤ 4
Ampicillin	(≤ 0.25)	(≥ 0.5 1)	R	≥ 0.5	R	≥ 1
Amox/Clav	($\leq 0.25/0.06$)	($\geq 1/0.25$)	S	0.25	R	≥ 1
Cephalothin	(≤ 2)	(≥ 8)	S	2	R	≥ 8
Enrofloxacin	(≤ 0.5)	(≥ 4)	S	0.25	I	1
Gentamicin	(≤ 4)	(≥ 16)	I	8	S	4
Penicillin G	(≤ 0.12 [8])	(≥ 0.25 [32])	R	≥ 16	R	≥ 32
Trim/Sulf	(≤ 2)	(≥ 4)	S	1	R	≥ 4

Figure 6-7 An example of a C&S report for broth dilution. The breakpoints have been added in parentheses in this report; for ampicillin and penicillin, a second breakpoint in [brackets] is for the gram-negative organisms (see text and Table 6.2). The relative in vitro efficacy of antibiotics can be evaluated by comparing the minimum inhibitory concentration (MIC) of the organism. For *Staphylococcus*, the resistant MIC breakpoint to MIC ratio is 64/4, whereas that for gentamicin is 16/4 or two tube dilutions from the breakpoint. Although the isolate is considered susceptible to both drugs, amikacin presumably would be more effective (although neither generally should be used alone to treat *Staphylococcus*). Differences greater than one tube dilution should be considered significant. For the beta-lactams, effective treatment of *Staphylococcus* with cephalothin (the model drug for cephalexin) may be more easily achieved (ratio of 8/2) compared to amoxicillin clavulanic acid (ratio of 0.25/0.25). However, as time-dependent drugs, elimination half-life of both drugs would need to be considered. All values (concentrations) are in μg per mL. S = susceptible; R = resistant; I = intermediate.

amikacin of $\leq 4 \mu\text{g/mL}$. The \leq indicates the absence of growth in the lowest concentration of amikacin tested by this laboratory ($8 \mu\text{g/mL}$); this lowest concentration may be different among laboratories that use different systems. However, often the lowest dilution tested is at or just below the lower threshold of susceptibility (the susceptible MIC breakpoint; see later definition) set by CLSI. Testing at concentrations at or very close to the susceptible breakpoint of a drug is a major disadvantage of current susceptibility testing methods: either isolate may be very susceptible to amikacin such that their actual MIC may be several tube dilutions below the lowest concentration tested (see below). As such, the closest approximation to the actual MIC for either isolate will be the concentration below the lowest dilution tested by the laboratory (i.e., $\leq 4 \mu\text{g/mL}$ or $< 8 \mu\text{g/mL}$ both indicate the same result). The isolate will be accompanied by an "S" designation, indicating susceptibility because the MIC is at or below the susceptible breakpoint determined by CLSI (Table 6-2). At the other end of the testing range, an MIC accompanied by \geq indicates that growth was present in the highest concentration tested by the laboratory.

Generally, for most automated procedures, the highest concentration tested is 1 tube dilution below the upper threshold of susceptibility (the resistant MIC breakpoint) set by CLSI for each drug (Table 6-2). For example, for cephalothin (the model drug for cephalexin in this example), the upper threshold of susceptibility (the resistant MIC breakpoint) set by CLSI is $8 \mu\text{g/mL}$. Thus for *Pseudomonas*, growth was present in the well containing $4 \mu\text{g/mL}$, indicating that the actual MIC is equal to or higher than $8 \mu\text{g/mL}$ ($\geq 16 \mu\text{g/mL}$ or $> 8 \mu\text{g/mL}$ both indicate the same result). However, again the testing range limitations of the current procedures emerges in that level of resistance of the isolate cannot be assessed. The isolate may be characterized by low-level resistance, although this is unlikely for *P. aeruginosa* and first-generation cephalosporins (indeed, testing of *P. aeruginosa* toward cephalothin is not appropriate). However, unless the range tested extends beyond the resistant breakpoint, all that is known is that the isolate is resistant, and the MIC will be accompanied by an R designation.

KEY POINT 6-8 Culture and susceptibility testing ideally is performed following the guidelines of the Clinical Laboratory Standards Institute (CLSI).

Among the pitfalls of C&S testing are the stepwise dilutions and the range of concentrations tested for each drug. The twofold dilutions at which MIC are tested affect the design of dosing regimens at the higher MIC. Precision in the design of dose would be facilitated if MICs could be determined between the tube dilutions. For example, the dose to target $64 \mu\text{g/mL}$ would be substantially cheaper and potentially safer than that necessary to target $128 \mu\text{g/mL}$. The limited range of concentrations tested for each drug negatively affects the ability to identify the drug to which the isolate is most susceptible (see Figure 6-6).⁴¹ Ideally, concentrations tested by broth dilution procedures should span the range of drug concentrations that characterize the range of MICs established in a sample population of isolates of the organisms, with the highest concentration being at least one dilution above the highest drug concentration achieved in target biological fluids.⁴¹ However, automated systems test in a very narrow range. As previously noted, because the lowest concentrations are at or just below the lower limit of susceptibility, isolates that are very susceptible to the drug of interest cannot be identified (see Table 6-2). Therefore standard antibiograms are more indicative of resistance rather than susceptibility.

A third testing system approved by the Food and Drug Administration (FDA) offers advantages to the standard commercial broth dilution card. The "E test" (Epsilon test) combines the simplicity of disk diffusion with the informative nature of broth dilution, but goes beyond standard broth dilution procedures. (see Figure 6-6). In general, MICs generated by the E-test correlate well with MICs generated from broth dilution procedures.⁴² A disadvantage of the E-test is that the length of the test strip limits the number of drugs that can be tested on a large plate (three strips for a large plate, one for a small plate), which contributes to the cost of the testing.

Table 6-2 Interpretive Standards for Disk Diffusion Equivalent Minimum Inhibitory Concentration Breakpoints for Selected Antimicrobials

Drug	Breakpoint µg/mL Susceptible ¹	Breakpoint MIC (µg/mL) Resistant ¹	Drug	Breakpoint µg/mL Susceptible ¹	Breakpoint MIC (µg/mL) Resistant ¹
Amikacin	≤16	≥64	Gentamicin*	≤4	≥16
Amoxicillin with clavulanic acid*	≤0.25/0.12/≤8/2*	≥1/0.5	Imipenem/ cilastin	≤4	≥16
Ampicillin ^{4*}	≤0.25 ^{2,9} ≤0.25 ³	≥0.5 ≥1	Kanamycin	≤16	≥64
Azithromycin	≤4	≥8	Levofloxacin	≤2	≥8 ⁹
Carbenicillin	≤16	≥64	Linezolid	≤4 ² ≤4 ¹⁵	≥8
Cefazolin ⁷	≤8	≥32	Lincomycin	≤0.5	≥4
Cefotaxime	≤8	≥64	Marbofloxacin	≤1	≥4
Cefoxitin	≤8	≥32	Meropenem	≤4	≥16
Cefpodoxime	≤2	≥8	Metronidazole	≤8	≥32
Ceftazidime	≤8	≥32	Nitrofurantoin	≤32	≥128
Ceftiofur ^{10*}	≤2	≥8	Orbifloxacin*	≤1	≥8
Ceftizoxime	≤8	≥32	Oxacillin ⁶	≤2	≥4
Ceftriaxone	≤8	≥64	Penicillin G	≤8 ³ ≤0.12 ²	≥16 ≥0.25
Cefuroxime	≤4	≥32	Piperacillin	≤16 ² ≤64 ⁵	≥128 ≥128
Cephalexin*	≤2	≥8	Rifampin	≤1	≥4
Cephalothin ⁷	≤2	≥8	Sulfadiazine	≤2	≥4
Chloramphenicol	≤8 ≤8 ⁹	≥32 ≥16	Tetracycline ¹⁴	≤4 ≤2 ⁹	≥16 ≥8
Ciprofloxacin ¹⁶ (see also enrofloxacin)	≤1	≥4	Ticarcillin	≤64 ⁵ ≤16 ⁴	≥128 ≥128
Clarithromycin	≤1 ≤8	≥8 ≥32	Ticarcillin with clavulanic acid	≤64/2 ⁵ ≤16/2 ³	≥128/2 ≥128/2
Clindamycin ^{8*}	≤0.5	≥4	Trimethoprim/ Sulfamethoxazole ¹¹	≤2/38 ¹³ ≤0.5/9.5 ⁹	≥4/76 ≥4/76
Difloxacin*	≤0.5	≥4	Vancomycin	≤4 ¹⁵ ≤1 ⁹ ≤4	≥32 ≥32
Doxycycline	≤4	≥16			
Enrofloxacin*	≤0.5	≥4			
Erythromycin	≤0.5 ≤0.25 ⁹	≥8 ≥1			
Florfenicol ^{10*}	≤2	≥8			

MIC, Minimum inhibitory concentration.

*Old breakpoints replaced by Clinical Laboratory Standards Institute (CLSI) for amoxicillin-clavulanic acid for all organisms were, for *Staphylococcus* ≤4/2 = S, and ≥ 8/4 = R and for non-staphylococci, ≤8/2 = S and ≥ 32/16 = R. The provision of a separate breakpoint of ≤8 µg/mL for UTI is new.

¹Clinical Laboratory Standards replaced by CLSI for cephalexin were ≤ 8 = S, and ≥ 32 = R. The new breakpoints were becoming official at the time of publication. Institute Interpretive standards that are based on animal pathogens are designated by an asterisk.

²When testing *Staphylococcus* organisms

³When testing gram-negative enteric organisms

⁴Ampicillin is used to test amoxicillin

⁵When testing *Pseudomonas* organisms

⁶Oxacillin is used to treat methicillin, cloxacillin

⁷Cephalothin is used to test all first-generation cephalosporins. Does not represent cefazolin, which should be tested separately if a gram-negative organism.

⁸Clindamycin is used to test lincomycin, which is less susceptible to *Staphylococcus*.

⁹When testing *Streptococcus* (*S. pneumoniae* for levofloxacin)

¹⁰When testing pathogens associated with food animal respiratory disease

¹¹Trimethoprim-sulfamethoxazole is used to test trimethoprim-sulfadiazine and ormetoprim-sulfadimethoxine

¹²For urinary tract infections

¹³For soft tissue infections

¹⁴Used to test chlortetracycline, oxytetracycline, minocycline, doxycycline

¹⁵When testing Enterococcus organisms

¹⁶A human criteria deemed relevant to dogs and cats. Note reduced oral bioavailability (mean of 40%) in dogs and negligible (0%-20%) in cats.

Table 6-3 Examples of Minimum Inhibitory Concentration Ranges Covered by the E-test

Drug Class	Antimicrobial Drug	CLSI Breakpoints (S, R)	MIC range ($\mu\text{g/mL}$) of E-test
Penicillins	Ampicillin	$\leq 8, \geq 32$	0.5-256
	Amoxicillin-clavulanic acid	$\leq 8/4, \geq 32/16$	0.25-512
Cephalosporins	Cefpodoxime	$\leq 2, \geq 8$	0.12-128
Fluoroquinolones	Enrofloxacin	$\leq 0.5, \geq 4$	0.06-128
Tetracyclines	Doxycycline	$\leq 4, \geq 16$	0.25-512
Aminoglycosides	Gentamicin	$\leq 4, \geq 8$	0.12-256
Potentiated sulfa	Trimethoprim-sulfamethoxazole	$\leq 2, \geq 8$	0.06-128

CLSI, Clinical Laboratory Standards Institute; MIC, minimum inhibitory concentration; R, resistant; S, susceptible.

However, because the drugs can be chosen for each patient, the method lends itself well to expanded susceptibility testing in the presence of a multidrug-resistant isolate. Although E tests are tedious and expensive, the wider range of concentrations tested (up to 1600-fold differences; Table 6-3) includes MICs well below the lower and higher thresholds of susceptibility, thus allowing identification of very susceptible isolates. Further, isolates with low-level resistance might be identified, potentially justifying the use of the drug, albeit at a higher dose or in combination with another drug.

KEY POINT 6-9 The E-test, but not current broth dilution procedures, allows identification of very susceptible isolates and isolates with low-level resistance.

Because of the inherent risks of inaccuracy associated with any C&S testing procedure, results yielded from procedures that are not based on CLSI standards should be interpreted with caution. Aspects subject to variability include pH; cation content and osmolality of the media; inoculum size; media volume; temperature and duration of incubation; humidity; and, for broth dilution, the method of observing growth.⁴¹ Accordingly, in practice, culture methods should be considered less than ideal unless CLSI protocols are followed. Further, preliminary data, quick “snap” tests, or other methods intended to generate rapid results must be interpreted cautiously; the role of the organisms in causing infection and the susceptibility of the organisms (unless identified as a multidrug-resistant microbe) may require full C&S testing. Whereas the FDA is responsible for approval of diagnostic tests for human medicine, such a pathway is not required for veterinary diagnostic tests.

Population Pharmacodynamic Statistics

Agar Gel Versus Broth Dilution Pharmacodynamics

A nonquantitative but helpful summary of PD data is an antibiogram that indicates the proportion of isolates that are susceptible (or resistant) to the drug of interest (Figure 6-8). Although it does not provide information regarding the level of susceptibility, it can provide direction regarding empirical drug selection by indicating the likelihood that an organism is susceptible to the drug of choice. The antibiogram might be generated for each practice on the basis of cumulative data summarized on an annual basis.

Population statistics generated from MICs can provide even more useful information. They are particularly helpful if MIC data is not available for an isolate infecting a patient. Population MIC statistics can be generated from a sample population of the same organism; ideally, at least 100 isolates will be collected from different patients. Pertinent PD (MIC) statistics that describe the population distribution include the range (lowest and highest MICs recorded for any isolate representing the organism), mode (the most frequently reported MIC), median (the middle MIC, the 50th percentile or MIC₅₀), and the MIC₉₀ (or the 90th percentile MIC; the MIC at which 90% of the organisms are inhibited (see Figures 6-9 to 6-11)). The two-fold dilution nature of MIC determination mandates that the geometric mean (converted to account for the non-continuous nature of MIC) be reported rather than arithmetic mean. If an MIC is not available for an organism infecting a patient, the MIC₉₀ (or even more ideally, the MIC₁₀₀) of a drug for an organism is the preferred surrogate indicator of “what is needed” by the author. For example, if *S. pseudintermedius* is a known or suspected cause of pyoderma in a patient and the drug to be chosen empirically is cephalexin, the MIC₉₀ of *S. intermedius* for cephalexin⁴³ can be used as an indicator of “what is needed”—that is, the PD target for therapy in the patient. PD information can be found on many package inserts scientific literature^{43,44} or textbooks (veterinary for animal drugs, human if not), and other resources (see Table 6-4 and Chapter 7). However, the dynamic nature of microbes in response to the presence of antimicrobials may render some population data obsolete even within several years of collection. In addition to the species, a number of host factors are likely to affect the sample population statistics and its applicability to the patient. Among the more important factors is previous exposure to antimicrobials, which is likely to be associated with higher MICs compared with MICs of isolates collected from antimicrobial-naïve animals (i.e., not pathogens). Ideally, separate statistics might be promulgated for isolates collected from animals not previously exposed to antimicrobials.

KEY POINT 6-10 Recent population pharmacodynamic data such as the MIC₉₀ can serve as a reasonable surrogate for patient data.

Klebsiella	No. of Isolates	Amikacin	Amoxicillin/CA	Ampicillin	Cefazolin	Cefoxitin	Ceftiofur	Cephalothin ^b	Chloramphenicol	Clindamycin	Enrofloxacin	Erythromycin	Gentamicin	Marbofloxacin	Orbifloxacin	Oxacillin	Penicillin	Tetracycline	Ticarcillin	Ticarcillin/CA	Trimethoprim/Sulfa
<i>Enterococcus faecalis</i>	30		97	97					83		40	28 (29)		40 (15)	10		93	63			
<i>E. faecium</i>	13		15	13					100		0	15		0 (11)	0		15	23			
<i>Escherichia coli</i>	120	98	63	48	69	65	76 (103)	46	81		63		83	65	62 (119)			68	52	64	67 (119)
<i>Klebsiella pneumoniae</i>	30	80	70	0	70	70	63	70	80		77		77	77	73			73	0	70	77
<i>Proteus mirabilis</i>	32	100	97	94	94	97	100	94	94		97		91	100	91				94	100	81
<i>Pseudomonas aeruginosa</i>	61	100							0		64		97	76 (50)	25				97	98	
<i>Staphylococcus aureus</i>	8	100	50	13	50	50	29 (7)	50	100	50	75	50	88	75	75	50	13	88			88
<i>S. intermedius</i>	102	92	66	17	64	64	60 (89)	64	97	43 (101)	55	43	87	63	60 (99)	72	18	42			60 (101)
<i>S. schleifen ss coagulans</i>	20	95	65	45	65	65	59 (17)	65	100	95 (19)	30	100	70	50	40	65	50	95			100
<i>S. pseudintermedius</i>	4	75	50	0	50	50	33 (3)	50	100	50	75	50	75	75	75	50	0	50			75

Figure 6-8 A cumulative antibiogram generated for the target species can be helpful in identifying drugs to which acquired resistance has emerged. The data will be specific to the facility (i.e., hospital). The number in each cell refers to the number of tested isolates designated as susceptible to the drug. When present, the number in parentheses in each cell refers to the number of isolates tested for that drug; otherwise, the number of isolates tested is indicated in the second left-hand column. Note that the data indicate that one species in a genera may not be well represented by another species in the same genera, particularly for *Enterococcus* and *Staphylococcus* genera.

Comparing PD data of a drug reveals differential susceptibility among organisms toward each drug. For example, using the antimicrobial package insert, comparison of MIC₉₀ among different organisms reveals that *P. aeruginosa* tends to be susceptible (if at all) only at high concentrations compared with the more susceptible *Pasteurella multocida* (see Figure 6-11). The MIC₉₀ of *P. aeruginosa* more often than not approaches or surpasses the upper threshold of susceptibility for most drugs. Thus achieving effective antimicrobial concentrations is more likely to be difficult in the patient infected with *P. aeruginosa* compared with one infected with *Pasteurella*. A review of the antimicrobial package insert reveals other differential susceptibilities.

The distribution of the MICs of organisms for drugs can help identify emerging resistance. For example, the distribution of *E. coli* for several drugs (see Figure 6-10) is bimodal, representing two different populations. The majority of isolates in the first population are characterized by an MIC well below the susceptible threshold of susceptibility (i.e., susceptible MIC breakpoint). This data demonstrates that even isolates considered susceptible are characterized by MIC that are close to the susceptible breakpoint. Further, a substantial portion of the population is higher than the upper threshold of susceptibility—that is, the MIC₉₀ exceeds the resistant MIC breakpoint. It is very possible that the second population, characterized by higher MICs, probably represents

isolates previously exposed to antimicrobials; as such, culture would be prudent for those animals previously exposed to antimicrobials. Finally, detecting increasing MICs determined from sequential cultures of the same organisms in a patient with recurrent infections might indicate emerging resistance, likewise, comparison of the MIC₉₀ of a sample population of an organism across time can reveal emerging resistance.

The Minimum Inhibitory Concentration: Determining Susceptibility Versus Resistance

Susceptibility data based on broth dilution procedures that are reported for a patient will include the MIC, as well as a susceptible, intermediate, or resistant (SIR) interpretation.

KEY POINT 6-11 Simplistically, the MIC is the pharmacodynamic target of antimicrobial therapy, indicating the minimum concentration to be achieved at the site of infection. However, it is only a starting point.

The clinical microbiology laboratory provides the interpretation on the basis of CLSI interpretive criteria. The criteria for broth dilution procedures are presented as thresholds or breakpoint MICs (MIC_{BP}) whose values will also be in terms of the concentrations tested for each drug (i.e., multiples or

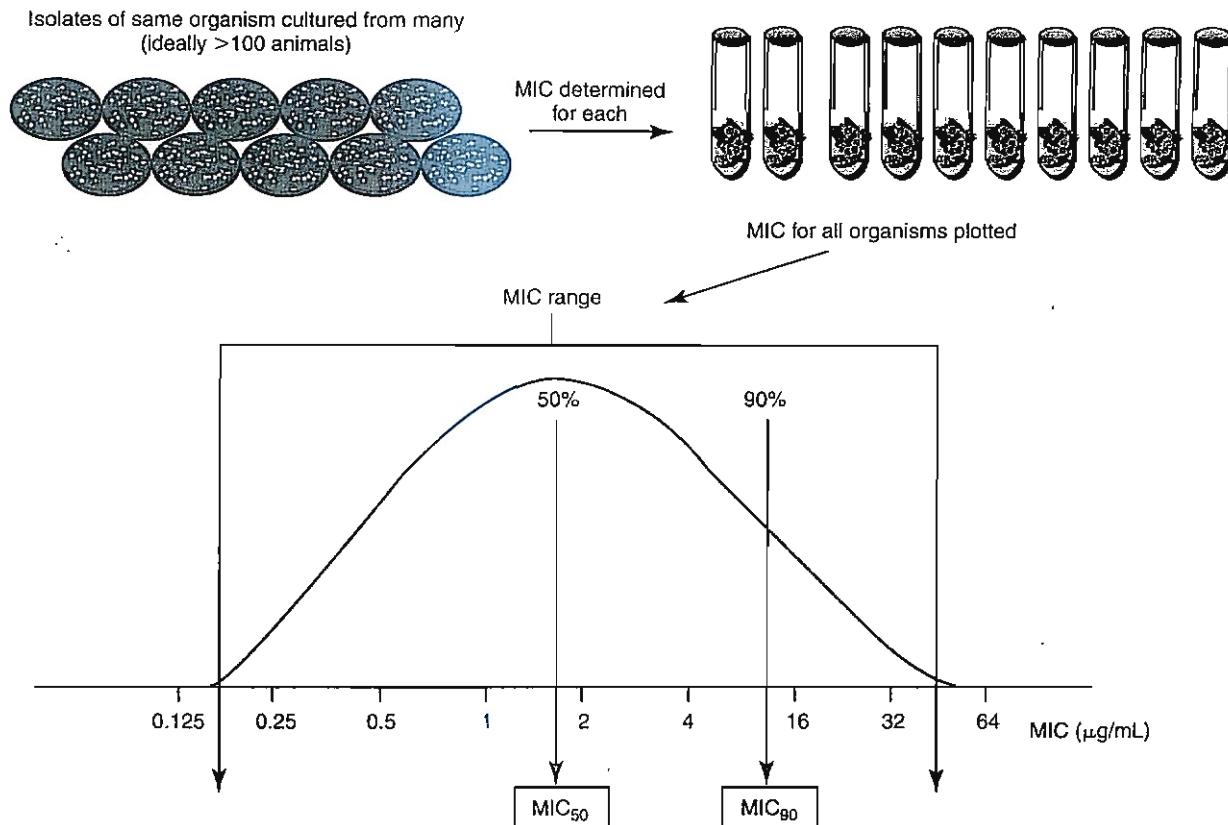


Figure 6-9 Population pharmacodynamic data. Each sample collected from a different animal (same species) yields an isolate of the organism of interest. Ideally, at least 100 representative isolates will be tested. The minimum inhibitory concentration (MIC) of each isolate is determined, and all are plotted in a distribution curve. The range represents the lowest and highest MIC determined for the isolate; the mode would be the most frequent MIC reported and the median represents the middle MIC or the 50th percentile (which, for normally distributed data, also represents the MIC). The MIC₉₀ is the 90th percentile MIC. A representative package insert demonstrating the presentation of population pharmacodynamic data can be found in Figure 6-11.

fractions of 1 µg/mL) (see Table 6-2). Two breakpoints are provided for each drug. An isolate inhibited at a concentration at or below the lower threshold or susceptible MIC breakpoint will be designated “S,” whereas an isolate that is able to grow after *in vitro* exposure to a drug concentration that equals the upper threshold or the resistant MIC breakpoint will be designated “R.” The susceptible breakpoint is at least one broth dilution below the resistant breakpoint for all drugs; for some drugs the susceptible breakpoint is 2 or more broth dilutions below the resistant breakpoint, allowing for an intermediate, or “I,” designation (see Figure 6-6). For example, for enrofloxacin, the susceptible and resistant MIC_{BP} are ≤0.5 and ≥4 µg/mL, respectively. Thus an isolate whose growth (under *in vitro* conditions specified by CLSI) is inhibited with as little as 0.5 µg/mL or less will be designated as “S.” On the other hand, if growth is present in the well that contains 2 µg/mL, then 4 µg/mL (the next broth dilution) or more will be necessary to inhibit the growth of the isolate, and the isolate will be designated as “R,” or resistant to enrofloxacin. An additional broth dilution occurs between 0.5 and 2 µg/mL. Isolates that are inhibited by enrofloxacin at 1 µg/mL will be designated as intermediate, or “I.” An isolate with an “I” designation has developed some level of resistance, and

such isolates should be treated with that drug only cautiously, at higher doses, or in combination with a complementary antimicrobial drug. The more prudent approach would be to consider “I” isolates as “R” for that drug. Use might also be considered in circumstances in which the drug accumulates in active (i.e., unbound) form at the site of infection such that concentrations exceed that achieved in plasma. Examples might include urine (produced by the normally functioning kidney) or accumulation in phagocytic white blood cells (selected drugs). Note, however, that such concentrations may yet be insufficient.

KEY POINT 6-12 Interpretive criteria for susceptibility testing by CLSI is dynamic, changing across time as microbes acquire resistance. However, once generated, the data is applicable for all laboratories in the United States.

CLSI determines the thresholds of susceptibilities—that is, the lower (susceptible) and upper (resistant) breakpoint MIC_{BP} for each drug after exhaustive evaluation of both PK data in the target species and PD data for the drug of interest toward the microbes of interest. For animal drugs approved

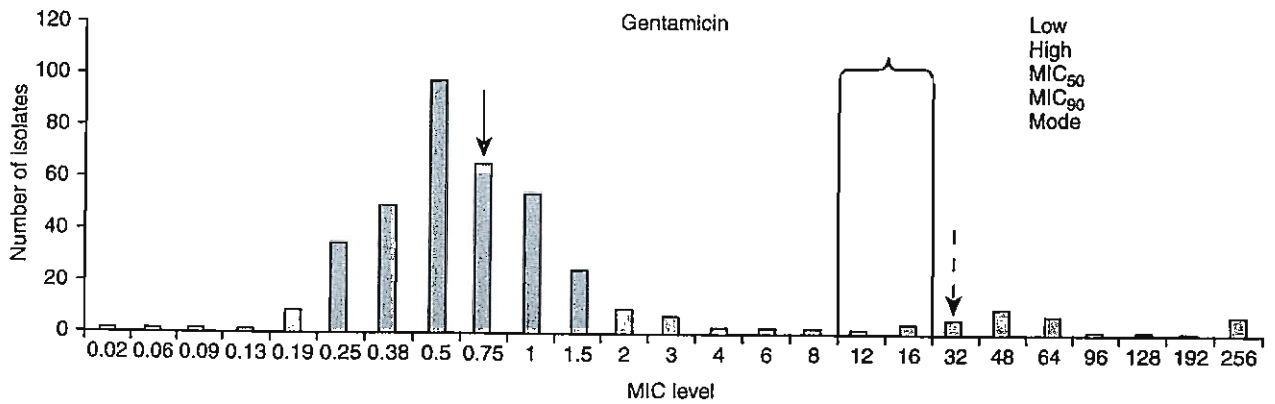
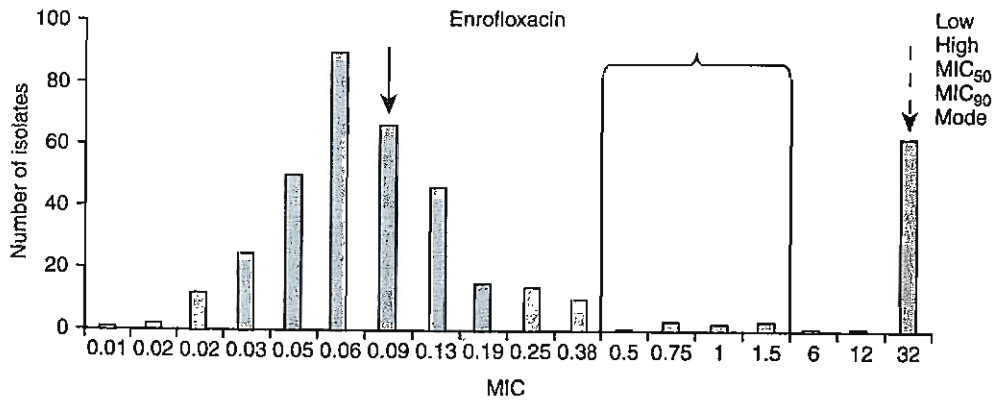
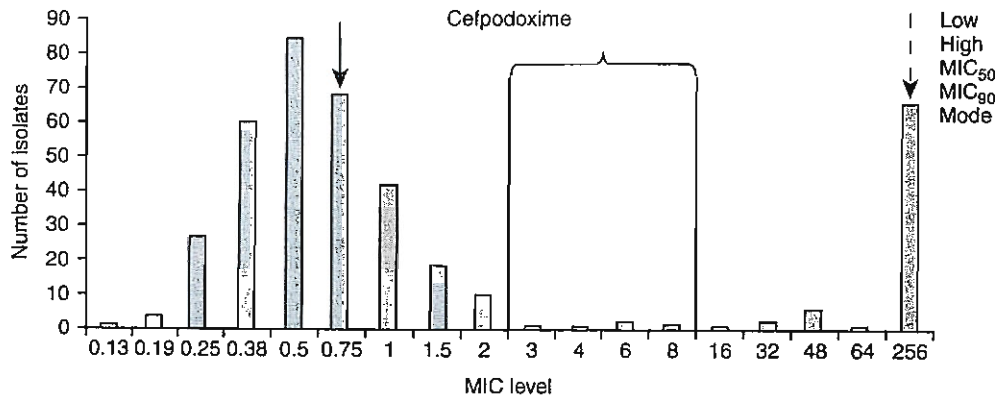
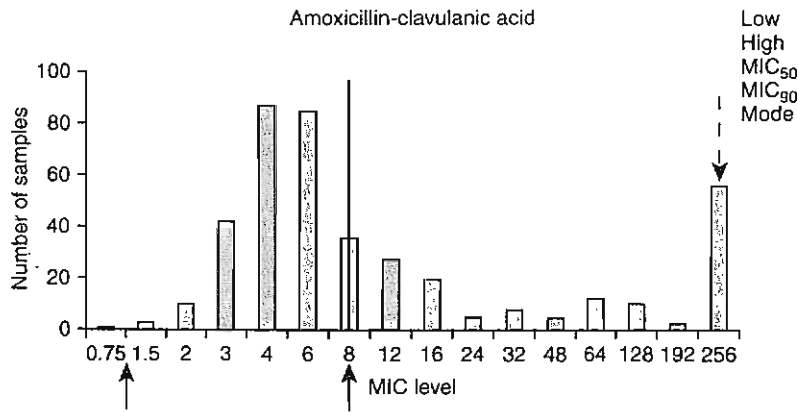


Figure 6-10 Population distributions of canine and feline *Escherichia coli* pathogens' minimum inhibitory concentration (MIC) based on E-testing for two time-dependent drugs (amoxicillin-clavulanic acid, and cefpodoxime, upper plots) and two concentration-dependent drugs (enrofloxacin, and gentamicin, lower plots). The susceptible (left) and resistant (right) breakpoints, as identified by Committee on Laboratory Standards Institute, are indicated in brackets and lines except for amoxicillin. CLSI's new breakpoints are indicated by arrows ($\leq 8 \mu\text{g/mL}$ for the susceptible breakpoint for isolates collected from the urinary the resistant breakpoint for other isolates; indicated by arrows below x axis). The distribution is bimodal for all drugs except gentamicin, as is indicated by a second distribution of isolates with an MIC well above the resistant breakpoint. This second population of isolates will cause the MIC₉₀ (dashed arrow) to exceed the breakpoint. The range is represented by the lowest and highest MIC recorded (either may be limited by the range tested), the median is the 50th percentile (or MIC₅₀) (solid arrow) the MIC₉₀ is the 90th percentile, and the mode is the most common MIC reported for that isolate and drug. Because an E-test was used, the MIC tested are not limited to two-fold dilutions. Because these isolates are pathogens that have been cultured from dogs or cats with spontaneous disease, they may represent isolates already exposed to antimicrobials which may explain the bimodal distribution (i.e., these isolates may have undergone stepwise mutations). The population distribution of drug-naïve only isolates is likely to be somewhat lower.

Fluoroquinolone A

Parameter For oral use in dogs and cats only	Dose		
	Dog Mean \pm SD* (2.5mg/lb) n=6	Dog Mean \pm SD* (5.5mg/lb) n=6	Cat Mean \pm SD* (5.5mg/lb) n=7
Time of maximum concentration, T _{max} (h)	1.5 \pm 0.3	1.8 \pm 0.3	1.2 \pm 0.6
Maximum concentration, C _{max} , ($\mu\text{g/mL}$)	2.0 \pm 0.2	4.2 \pm 0.5	4.8 \pm 0.7
AUC _{0-inf} ($\mu\text{g}\cdot\text{h/mL}$)	31.2 \pm 1.6	64 \pm 8	70 \pm 6
Terminal plasma elimination half-life, t _{1/2} (h)	10.7 \pm 1.6	10.9 \pm 0.6	12.7 \pm 1.1

Organism	Number of Isolates	MIC ₅₀	MIC ₉₀	MIC Range
<i>Staphylococcus intermedius</i>	135	0.25	0.25	0.125-2
<i>Escherichia coli</i>	61	0.03	0.06	0.015-2
<i>Proteus mirabilis</i>	35	0.06	0.125	0.03-0.25
Beta-hemolytic <i>Streptococcus</i> , (not Group A or Group B)	25	1	2	0.5-16
<i>Streptococcus</i> , Group D enterococcus	16	1	4	0.008-4
<i>Pasteurella multocida</i>	13	0.015	0.06	\leq 0.008-0.5
<i>Staphylococcus aureus</i>	12	0.25	0.25	0.25-0.5
<i>Enterococcus faecalis</i>	11	2	2	0-4
<i>Klebsiella pneumoniae</i>	11	0.06	0.06	0.01-0.06
<i>Pseudomonas</i> spp.	9	**	**	0.06-1
<i>Pseudomonas aeruginosa</i>	7	**	**	0.25-1

Table: MIC Values* ($\mu\text{g/mL}$) of FQA against pathogens isolated from skin, soft tissue and urinary tract infections in dogs enrolled in clinical studies conducted during 1994-1996.

Fluoroquinolone B

Bacteria Name	Number of Isolates	MIC ₅₀	MIC ₉₀	MIC Range
<i>Enterobacter</i> spp.	9	0.11	3.66	\leq 0.05-3.66
<i>Escherichia coli</i>	28	\leq 0.05	0.11	\leq 0.05-7.3
<i>Klebsiella</i> spp.	8	0.11	0.11	0.11-0.23
<i>Pasteurella</i> spp.	8	\leq 0.05	\leq 0.05	\leq 0.05
<i>Proteus</i> spp.	15	0.92	1.83	0.11-1.83
<i>Pseudomonas</i> spp.	5	0.11	0.92	\leq 0.05-0.92
<i>Staphylococcus</i> spp.	193	0.23	0.46	\leq 0.05-1.83
<i>Streptococcus</i> spp.	56	1.83	3.66	0.11-7.3

Table: MIC values* ($\mu\text{g/mL}$) of FQB for bacterial pathogens isolated from skin and soft tissue infections and urinary tract infections in dogs enrolled in clinical studies conducted during 1991-1993.

Pharmacokinetic Measure	Mean Value
Peak plasma concentration (C _{MAX})	1.8 $\mu\text{g/mL}$
Time to reach C _{max} (T _{MAX})	2.8 hours
Elimination half-life (T _{1/2})	9.3 hours
Area under the plasma curve (AUC _{0-∞})	14.5 $\mu\text{g}\cdot\text{hr/mL}$
Total body clearance/F ^a (CL/F)	375 mL/kg/hr
Steady state volume of distribution/F ^b	3.8 L/kg
Volume of distribution (area)/F ^c	4.7 L/kg

Table: Plasma pharmacokinetics following administration of FQB tablets (5 mg/kg body weight) to dogs (n=20).

Figure 6-11 Package insert information two fluoroquinolones, FQA (top) and FQB (bottom). Comparison of MIC₉₀ among isolates for FQA suggests that *Pasteurella* sp. should be more easily treated compared with *Escherichia coli* for both drugs. Integration of pharmacokinetic data (C_{max} for these concentration-dependent drugs) and pharmacodynamic data (MIC₉₀) can be used to identify which drug is best used to treat each microbe and which dose might be used to treat the microbe. For example, for FQA at the low dose of 2.5 mg/kg, when treating *E. coli* (and no patient-specific MIC is available), the C_{max} is 2 $\mu\text{g/mL}$ and the MIC₉₀ is 0.06 $\mu\text{g/mL}$, resulting in a C_{max}/MIC₉₀ ratio of 25. For *Proteus*, the ratio is 2/0.125, or 16. For concentration-dependent drugs, the target ratio is ≥ 10 , suggesting the low dose may be effective for both, but the large dose might be considered for *Proteus* if the patient is considered at risk. The number of isolates of *P. aeruginosa* is not sufficient to represent the population. If the process is repeated for FQB with a C_{max} of 1.8 $\mu\text{g/mL}$ at the low dose, the MIC₉₀ for *E. coli* is 0.11 $\mu\text{g/mL}$, resulting in a ratio of 16. For *Proteus*, the MIC₉₀ is 1.8 $\mu\text{g/mL}$, resulting in a ratio of only 1. Although the dose might be sufficient for *E. coli*, the target ratio could not be reached even at the higher dose for *Proteus*. Note that the number of organisms on which the data are based for each organism often does not reach the ideal target of 100. The smaller the sample size, the more caution is indicated when extrapolating this data to the general population. (From Pfizer, Package Insert and Fort Dodge, Package Insert)

Table 6-4 Integration of Population Pharmacodynamic (PD) and Pharmacokinetic (PK) Data and Its Role in the Design of Dosing Regimens

Time-Dependent Drugs (T > MIC 50%)					Current Dosing			Calculated Dosing		
Drug	Organism	MIC ₉₀ * (µg/mL)	Route	Interval (hr)	Dose (mg/kg)	C _{max} (µg/mL)	Half-life (hr)	Half-lives T > MIC	Interval (hr)	
Amoxi-clav	<i>St. pseud</i>	≤ 0.5	PO	12	12.5	5.5	1	3.46	3.46	
	<i>St. aureus</i>	4						0.46	0.46	
	<i>E. coli</i>	32						NR	NR	
Cephalexin	<i>St. pseud</i>	2	PO	12	22	20	1.3	3.32	4.32	
	<i>St. aureus</i>	8						1.32	1.72	
	<i>E. coli</i>	32						0.32	0.42	
Cefovecin	<i>St. pseud</i>	0.25	SC	168	8	4.2	133	4.07	541.48	
	<i>St. aureus</i>	2						(unbound)	1.07	142.39
	<i>E. coli</i>	1						2.07	275.42	
Cefpodoxime	<i>St. pseud</i>	0.5	PO	24	5	8.2	5.6	4.04	22.60	
	<i>St. aureus</i>	NA						NA	NA	
	<i>E. coli</i>	0.5						4.04	22.60	
Meropenem	<i>St. pseud</i>	NA	SC	12	20	26	0.75	NA	NA	
	<i>St. aureus</i>	0.25						6.7	5	
	<i>E. coli</i>	0.5						8.8	7	
	<i>P. aerug</i>	2						3.7	3	

Concentration-Dependent Drugs (C _{max} /MIC > 10-12)					Current Dosing			Calculated Dosing	
Drug	Organism	MIC ₉₀ (µg/mL)	Route	Interval (hr)	Dose (mg/kg)	C _{max} (µg/mL)	C _{max} /MIC		
Enrofloxacin	<i>St. pseud</i>	0.25	PO	24	20	7.1	28		
	<i>St. aureus</i>	64					(plus cipro)	0.11	
	<i>E. coli</i>	64					0.11		
	<i>P. aerug</i>	0.5					14		
Marbofloxacin	<i>St. pseud</i>	1	PO	24	5.5	4.2	4.20		
	<i>St. aureus</i>	64					0.07		
	<i>E. coli</i>	64					0.07		
	<i>P. aerug</i>	0.5					8.40		
Orbifloxacin	<i>St. pseud</i>	2	PO	24	2.5	2.3	1.15		
	<i>St. aureus</i>	64					0.04		
	<i>E. coli</i>	64					0.04		
	<i>P. aerug</i>	16					0.14		
Ciprofloxacin	<i>St. pseud</i>	0.125	PO	24	20	2.8	22		
	<i>St. aureus</i>	0.25					11		
	<i>E. coli</i>	64					0.04		
	<i>P. aerug</i>	2					1.40		
Gentamicin	<i>St. pseud</i>		IM	24	3	27	NR		
	<i>St. aureus</i>	1					27		
	<i>E. coli</i> (7)	2					13.50		
	<i>P. aerug</i>	4					6.8		
Amikacin	<i>St. pseud</i>		SC	24	10	14	NR		
	<i>St. aureus</i>						NR		
	<i>E. coli</i>	8					1.75		
	<i>P. aerug</i>	8					1.75		

NA, Not available; NR, not reached at cited dosing regimen.

in the last several decades (only since then have MIC become standard testing procedures), it is likely that some of the data were collected by the drug manufacturer during the approval process. However, both PK and PD data may be drawn from peer-reviewed literature or other sources, particularly for drugs not approved for use in the target species, or for drugs whose susceptibility thresholds are being re-evaluated by CLSI.

Three criteria must be met for CLSI to establish an MIC_{BP} for each drug. The primary and initial consideration is the population distributions, with a focus on both the statistics as well as the type (i.e., modal or bimodal; see Figure 6-10). Obvious patterns of low versus high MICs can be used to identify susceptible “cutoffs” or breakpoints. Statistics will be compared among different strains of the same species being tested. Note that some organisms may be much more susceptible to the drug of interest—that is, they have MIC_{50} and MIC_{90} that are much lower compared with other organisms (e.g., *pasteurella* and *pseudomonas*). However, CLSI generally provides only one set of criteria for all susceptible isolates.

The second consideration upon which criteria are based is, the clinical pharmacology of the drug, ideally in the target species. Among the more important PK parameters evaluated by CLSI are the peak and trough plasma drug concentrations (C_{max} and C_{min}), area under the curve (AUC) for a 24-hour dosing period, and the drug elimination half-life (see later discussion of PD indices) (Figure 6-12).^{41,45-51} Volume of distribution; protein binding; and, when available, tissue (including urine) concentrations are also considered. Presumably, the MIC of an isolate considered susceptible should be below the peak plasma or tissue drug concentrations or C_{max} of a given drug when administered at a recommended dose. Indeed, selected resistant breakpoints correlate with C_{max} , as is demonstrated for amikacin when administered to dogs at 22 mg/kg. The C_{max} of 65 $\mu\text{g}/\text{mL}$ (see Chapter 7) is similar to the resistant breakpoint for amikacin. However, for some breakpoints, the correlation does not exist, as is exemplified by amoxicillin/clavulanic. The C_{max} of the labeled dose of 13.5 mg/kg, administered orally, will generate a C_{max} of approximately 4 to 6 $\mu\text{g}/\text{mL}$ of amoxicillin in dogs, yet the resistant breakpoint for nonstaphylococcal organisms has been $\geq 32 \mu\text{g}/\text{mL}$, well beyond the concentrations that can be achieved in plasma at any reasonable dose. (In response to this disparity, CLSI has recently re-examined and readjusted the breakpoint for amoxicillin-clavulanic acid as is discussed below; see Table 6-2). Another limitation of setting breakpoints based on peak plasma drug concentrations is their lack of precision: breakpoints are limited to concentrations used for susceptibility testing and thus will be reported using twofold dilutions. As such, a resistant breakpoint concentration may be considerably higher or lower than the actual concentration achieved at the recommended dose. As such, the actual MIC reported for an infecting isolate should be compared to C_{max} reported in a sample population of the target species is an ideal default when selecting drugs (and the dosing regimen; see later discussion). The original veterinary fluoroquinolones were

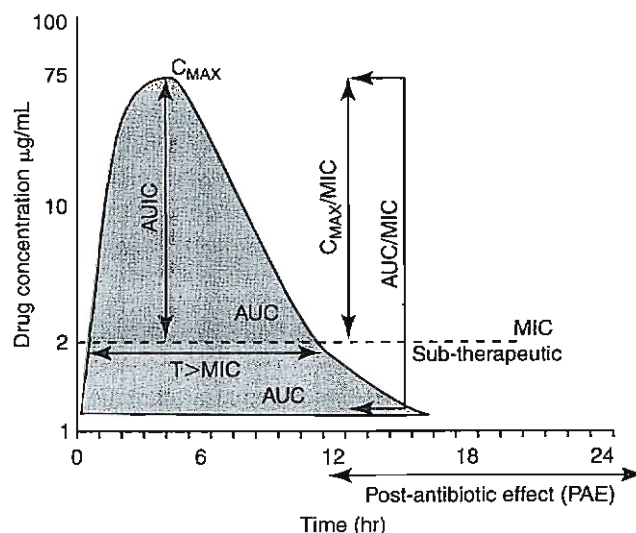


Figure 6-12 Pharmacodynamic indices (PDI) resulting from integration of pharmacokinetic (PK) data (from sample population of target or closely related surrogate species), represented here by C_{max} ($\mu\text{g}/\text{mL}$; target of 10 to 12) or area under the curve (AUC) ($\mu\text{g}\cdot\text{hr}/\text{mL}$; target of 100–125), and microbial or pharmacodynamic (PD) data, represented here by minimum inhibitory concentration (MIC). Note that the PDI are based on (Mouton 2005⁴⁷ and Amsterdam 2005⁴¹). Note that activity should be based on free (unbound) drug. The pertinent PK parameters for this hypothetical drug and infecting microbe would be as follows: C_{max} of 75 $\mu\text{g}/\text{mL}$, half-life of approximately 2 hours and AUC of 159 $\mu\text{g}\cdot\text{hr}/\text{mL}$. The PD parameter, or MIC of the infecting organism, is 2 $\mu\text{g}/\text{mL}$. The PDI for this drug and microbe combination would be as follows: $C_{max}/MIC = 37.5$ (surpasses target for a concentration-dependent drug); $AUC/MIC = 80$ (insufficient for a gram-negative organism if a fluorinated quinolone but potentially sufficient for a gram-positive organism); and $T > MIC = 8$ hours, which would allow a 12, 16, or 24- (32 hr is not a reasonable interval) hour dosing interval for a time-dependent drug if the target $T > MIC$ were 75%, 50%, or 25%, respectively. The AUIC (area under the inhibitory curve) is the integrated area of the curve above the MIC. Although somewhat similar to AUC/MIC , its use among investigators has caused confusion, leading experts in the field to focus on AUC/MIC as the area-based PDI of choice.

approved with “flexible labels” (multiple doses); for those drugs, the susceptible and resistant breakpoints reflect the C_{max} resulting from the lowest and highest labeled doses of the drug.

The third criteria that must be met as CLSI determines thresholds of susceptibility is one of clinical relevance. The MIC_{BP} must be clinically relevant—that is, the microorganisms defined as susceptible should respond clinically to the drug, and in vitro data must correlate adequately with in vivo findings.^{46,51} The MIC_{BP} values established by CLSI for each drug are generally not included in the susceptibility reports. However, having this information would be helpful because the MIC of the infecting organism might then be compared with the MIC_{BP} of the drug, allowing an assessment

of "level" of susceptibility of the isolate to the drug of interest (discussed in more detail later). Breakpoints set by CLSI are based on isolates collected from across the country and accordingly are relevant for any clinical microbiology laboratory that uses CLSI protocols. As such, CLSI breakpoints generally can be obtained by any microbiology laboratory that uses CLSI criteria for its testing procedures (the preferred choice). Interpretive standards generally also are delineated on package inserts (including sources such as *Physicians' Desk Reference* for human-marketed drugs or similar veterinary compendiums).

In order to simplify the use of interpretive criteria, when possible, CLSI breakpoints are generally inclusive to organisms whose spectrum is included in the drug, regardless of the site of infection. However, notable exceptions occur for both organisms and tissues. Organism exceptions generally are those either very susceptible to the drug or for organisms that easily develop in vivo resistance. For example, lower or more stringent penicillin breakpoints have been promulgated for *Staphylococcus* spp. because their beta-lactamases are particularly destructive toward penicillins compared to cephalosporins. Because destructive activity decreases the amount of drug at the site, a second set of lower breakpoints are provided. A higher breakpoint has been established for other susceptible isolates (e.g., gram-negative organisms; see Table 6-2). In contrast to *Staphylococcus*, *P. aeruginosa* is particularly susceptible to ticarcillin; accordingly, while another coliform is considered resistant if it is inhibited at 16 µg/mL *P. aeruginosa* is still considered susceptible even if, in vitro, it grows in the presence of 64 µg/mL (see Table 6-2). Another consideration regarding inclusivity is the generation of tissue specific breakpoints. Interpretive criteria are based on plasma drug concentrations. However, renally excreted drugs achieve much higher concentrations in urine compared to plasma. New interpretive criteria for ampicillin and amoxicillin-clavulanic acid includes a separate breakpoint that is tissue dependent: a higher breakpoint (≤8 µg/mL) has been set for *E. coli*-associated urinary tract infections, compared to much lower breakpoints for other tissues. However, caution is recommended when selecting a drug for treatment of a UTI when the "S" designation for a urinary isolate is based on breakpoints that differ from plasma (see Chapter 8) as it assumes, among other considerations, that infection occurs only in the bladder, that urine is concentrated by the patient, and the duration of exposure fulfills the needs of a time-dependent drug (see later discussion). Just as resistance can be detected in an infecting organism collected from a patient, across time, statistics may also indicate resistance (i.e., the MIC and MIC statistics [mode, median, MIC₅₀, and MIC₉₀]). Because CLSI reviews new data intermittently, their criteria for interpretive standards should result in new MIC_{BP}, and laboratories will implement these changes. The sequelae of increasing MICs in certain populations should result in an increasing number of isolates designated as "R" for drugs to which the organisms traditionally have been considered susceptible. An example is amoxicillin-clavulanic acid, whose susceptible breakpoint was recently decreased by CLSI (see Table 6-2).

Integration of Pharmacokinetics and Pharmacodynamics: How Much Is Needed Versus How Much is Achieved?

The information provided on the C&S report can be used effectively beyond the simple identification of "S" drugs. The MIC is an indicator of what is needed to target the organism and thus provides the PD information for the infecting isolate. Often, an organism will be designated as susceptible to several drugs. One advantage to the broth dilution method compared with the disk diffusion method of C&S is the ability of the

KEY POINT 6-13 The closer the MIC (or MIC₉₀) of an organism is to the C_{max}, the greater the risk of therapeutic failure and the more important the need to modify the dosing regimen for the patient.

former to rank the drugs according to relative efficacy based on MIC. However, the relative efficacy of antimicrobials designated as "S" against a specific pathogen should not be determined by directly comparing MICs among different drugs, even drugs in the same class. The MIC varies among the drugs for a number of reasons beyond susceptibility. These include, but are not limited to, differences in molecular weight (one drug is simply heavier than another), the ability of the drug to penetrate the organism, the number of molecules necessary to "neutralize" the target, and differences in the mechanisms of action. Further, each drug achieves different concentrations in the patient as a result of differences in disposition. Thus antimicrobials differ in potency and MIC.⁴⁷ Rather than direct comparison to an isolate MIC for one drug to an MIC for another drug, one should also consider the concentration of drug that will be achieved in the patient when the drug is administered at the recommended dose (i.e., what is achieved; C_{max}). A less than ideal method of standardizing MIC is to compare how far the MIC of the drug of interest is from the resistant breakpoint (i.e., ratio of resistant MIC breakpoint of the drug to MIC of the organism). This is less ideal, as was previously discussed, because the resistant breakpoint does not always equate with the C_{max}.

The limitations in using MIC breakpoint as an indicator of what will be achieved in the patient reflect the serial concentration used for susceptibility testing. If a drug achieves a C_{max} of 24 µg/mL at the recommended dose, this concentration falls between 16 and 32 µg/mL serial dilutions. A susceptible breakpoint of 16 µg/mL might underestimate while a resistant breakpoint of 32 µg/mL would overestimate what will be achieved at the recommended dose. Thus, a more relevant choice among the susceptible drugs might be based on comparing what is needed to what is achieved—in plasma (e.g., the C_{max}) when the drug is administered at the recommended dose. The ratio of C_{max} to MIC, however, requires that PK information be available for the drug of interest, ideally in the species of interest. This information often is available on package inserts for animal-approved drugs but must be collected from the literature for other drugs (see Chapter 7). The United States Pharmacopoeia antimicrobial monographs published

by the Journal of Veterinary Pharmacology and Therapeutics (2007) offers a compilation of PK information for a variety of antimicrobials and animal species. The integration of PD and PK is the first step in selecting a drug. However, this step is preliminary in that it does not take into account other factors that affect tissue concentrations, including host and microbial factors.

The relationship between the MIC of the infecting organism (what is needed) and the C_{max} also should be used as a basis for designing a dosing regimen.⁴⁸⁻⁵⁰ The closer the MIC (or MIC_{90}) to the C_{max} (or the MIC_{BP}), the higher the dose and for time-dependent drugs, the shorter the interval that is indicated. For a more specific design of a dose, the MIC can serve as the targeted drug concentration (see Enhancing Antimicrobial Efficacy). If MIC data is not available from the isolate collected in the patient, population PD [MIC_{90}] data might be used as a surrogate PD indicator of how much is needed; (see Figure 6-11).

Caveats to Culture and Susceptibility Interpretation

Despite the usefulness of C&S testing, the information nonetheless reflects in vitro testing that must be applied to in vivo situations.^{40,41} Results can be misleading or misinterpreted, despite ideal sampling and C&S techniques. Some of the pitfalls reflect the limitations presented by practicalities in testing (e.g., economics, technology), whereas others reflect limitations of applying in vitro data to an in vivo system. Examples include the following:

1. The limitations presented in the number of drugs and range of concentrations tested were previously addressed. The hazards of interpreting an "S" designation without knowing *how* close the MIC is to the susceptible breakpoint may facilitate emerging resistance for those isolates whose MICs are approaching the breakpoint of the drug or the C_{max} of the drug given at the recommended dose. For example, an isolate whose MIC for enrofloxacin is 0.5 $\mu\text{g}/\text{mL}$ is likely to already have undergone the first step toward resistance, compared to an isolate whose MIC is 0.06 $\mu\text{g}/\text{mL}$.

KEY POINT 6-14 Culture and susceptibility testing is an in vitro procedure and as such, cannot accurately mimic the conditions to which the data is applied—that is, the infected patient.

2. CLSI does not provide interpretive standards for all drugs; as such, these drugs do not appear on C&S testing, and MIC_{BP} are not available. This may be a decision on the part of the manufacturer of the drug not to pursue CLSI validation, the lack of adequate data for CLSI to determine criteria, or failure of the data to correlate with patient response. For such drugs population PD and PK data as reported in the literature, for example, may be the only reasonable approach to assess antimicrobial efficacy (see later discussion).
3. For some drug classes, CLSI has established criteria for a model drug that serves to reflect patterns of susceptibility

for other members in the same class. In some instances cross-susceptibility and resistance justify this approach (e.g., fluorinated quinolones might represent all veterinary fluorinated quinolones, ampicillin accurately predicts amoxicillin, and sulfamethoxazole/trimethoprim appears to predict other potentiated sulfonamides). However, exceptions to the relevance of model drugs to other members in the class occur. For example, ampicillin-sulbactam serves as a model for amoxicillin-clavulanic acid, but several diagnostic microbiologists find the latter to overestimate the efficacy of the latter. Generally, CLSI has indicated the exceptions in its interpretive guidelines (many are summarized in Table 6-2), and the veterinary diagnostic laboratory should indicate these exceptions in the C&S report. For example, cephalothin (which is no longer available) represents first-generation cephalosporins, yet cefazolin generally is less effective against *S. aureus* and more effective against *E. coli*. The spectrum of third- and fourth-generation cephalosporins is markedly disparate, and thus the class cannot be well represented by a model drug. Among the newer aminoglycosides, gentamicin is generally more effective than tobramycin against *Serratia* spp. and more effective than amikacin against *Staphylococcus*, whereas tobramycin and amikacin are more effective than gentamicin against *P. aeruginosa*.

4. Limitations in extrapolations of susceptibility data are not restricted to spectrum but also may reflect a mismatch between in vitro and in vivo response. For example, despite in vitro evidence of susceptibility, aminoglycosides should not be used to treat *Enterococcus* spp. or as sole agent to treat *Staphylococcus* spp. Potentiated sulfonamides are not considered by CLSI to be clinically effective toward enterococci, despite in vitro susceptibility. However, recent reports in the literature challenge this assessment, supporting the importance of continued surveillance of the data by CLSI. Generally, laboratories will not test drugs against organisms for which clinical efficacy has not been demonstrated. (see Figure 6-8). This is most obviously exemplified by gram-negative versus gram-positive susceptibility panels, with the drugs tested against the isolate being grouped according to anticipated efficacy for the type of organism (e.g., gram-negative isolates will not be tested against clindamycin or erythromycin; gram-positive isolates generally are not tested against ticarcillin, which was developed for gram-negative infections; anaerobes will not be tested against aminoglycosides; methicillin-resistant *Staphylococcus* should not be tested against any beta-lactam, and *Pseudomonas* generally is not tested against a variety of drugs to which it is consistently resistant). A more recently recognized limitation of susceptibility testing is detection of acquired resistance that is rapidly induced by the presence of the drug. This might be best exemplified by gram-negative organisms that produce extended-spectrum beta-lactamases (ESBLs). These enzymes destroy selected third- and fourth-generation cephalosporins but are induced at the site of infection by the presence of the drug.⁵² Therefore ESBLs generally are not expressed by the

isolate culture in vitro. Their detection may require additional testing of the isolate in the presence of cefpodoxime and ceftazidime alone or in combination with clavulanic acid, which is not susceptible to ESBL. A fourfold or greater reduction in cephalosporin MIC when it is combined with clavulanic acid versus when present as the sole drug has been interpreted as indicative of the presence of ESBL. At the time of publication, the criteria and need for special testing of ESBL was under scrutiny. Newer ESBLs are constantly emerging as resistance evolves. For example, an ESBL produced by *K. pneumoniae*, which targets carbapenems, was recently identified, thus highlighting the need for rapid incorporation of appropriate testing procedures into microbiology testing labs.⁵³

5. For any C&S method, generally only the parent drug is included in the interpretive standards, yet an active metabolite may contribute markedly to activity. For some drugs (e.g., ceftiofur), interpretive criteria include the metabolite, but for others, activity of the metabolite is not addressed. For example, most animals metabolize enrofloxacin to its de-ethylated metabolite, ciprofloxacin. Because the drugs act in an additive fashion, up to 40% to 50% of the C_{max} or area under the plasma bioactivity curve for enrofloxacin may be represented by ciprofloxacin, as has been demonstrated in dogs (see Chapter 7).⁵⁴ Consequently, efficacy of enrofloxacin may be underestimated by C&S methods, particularly because ciprofloxacin tends to be more potent than enrofloxacin toward gram-negative coliforms. PK of antimicrobial drugs characterized by activity of both parent and metabolite must be based on either bioassays, or analytic techniques that include the activity contributed by metabolites
6. As organisms are exposed to microbes, MICs increase across time. CLSI reevaluates and adjusts interpretive criteria to address these changes when possible. However, new criteria depend on the generation of new data. Current antimicrobial resistance surveillance systems focus on human medicine and thus largely address food animals (e.g., National Antimicrobial Resistance Monitoring System). Thus the lack of new data needed by CLSI to promulgate new guidelines may prevent timely reassessment. Clearly, a coordinated surveillance system for monitoring antimicrobial resistance of companion animal pathogens is needed. Additionally, the relevance of population PD data provided on labels and through scientific literature will decline with the passage of time, and caution might be taken when basing the use of a drug on population data that are more than a decade old.
7. Ideally, as MICs change, drug dosing regimens also should change. However, modification of dosing regimens cited on labels of approved drugs requires reapproval by the FDA, and manufacturers are not likely to pursue modification because of the cost associated with reapproval. Data necessary for dose modification may not be available for CLSI review. Thus modification of dosing regimens is likely to depend not only on generation of PD data but also on PK data by independent sponsors. Without CLSI

direction, manufacturers of commercial antibiogram materials are unlikely to adjust the range of concentrations. Laboratories and manufacturers of C&S materials also have been slow to incorporate the new standards into their interpretations.

8. Ideally, both PK and PD data on which CLSI bases MIC_{BP} should be collected from and promulgated for the target species to be treated. However, much of this data simply does not exist for the target species. For drugs approved for use in animals, assuming the manufacturer supplied the data, CLSI interpretive standards often do exist and are published separately³⁸ from those established for human medicine.³⁹ However, some of these standards published in veterinary interpretive criteria are actually human standards that CLSI has deemed relevant to animals (see Table 6-2). For other human drugs, human interpretive standards are used but have not been evaluated for relevancy in animals. Although the standards may be equivalent among species for some drugs, for others, PK data and possibly PD data may be substantially different among species. Data should be interpreted cautiously for such drugs. Drugs that are water soluble ($Vd \leq 0.3$ L/kg) may be most applicable among species (see Chapter 1), whereas added caution is indicated for lipid-soluble drugs ($Vd \geq 0.6$ L/kg). Amikacin offers an example of a water-soluble drug for which interpretive standards might be similar between animals and humans. Ciprofloxacin offers an example of the need for caution. Although oral bioavailability of ciprofloxacin is 80% to 100% in humans, oral bioavailability averages 40% to 60% in dogs (information courtesy of Bayer Animal Health) and is 0% to 20% in cats (see Chapter 7). Accordingly, C_{max} will be about 40% to 60% lower at equivalent doses in dogs. Drugs with variable (particularly low) oral bioavailability, a large Vd (≥ 0.6 L/kg), and clearance by the liver are less likely to behave similarly among species than are drugs characterized by close to 100% oral bioavailability, a Vd indicative of extracellular distribution and renal clearance. As such, greater caution should be taken when extrapolating human interpretive criteria to animals for lipid-soluble versus water-soluble drugs.
9. The greatest caveat to the use of C&S data as a basis for drug selection and design of the dosing regimen is the disparity between the controlled environment of the in vitro test system and the dynamic in vivo environment of the host. Once the list of susceptible drugs has been narrowed down, host, drug, and microbial factors must be considered when making the final selection, as well as the design of the dose.

DRUG FACTORS THAT AFFECT ANTIMICROBIAL EFFICACY

The conditions of C&S testing cannot mimic conditions of in vivo drug behavior. Most notably, drug concentrations in the host are not static, as occurs in the in vitro system, but are dynamic, with duration of exposure dependent on elimination half-life. The importance of the PK of the drug will

be addressed with discussion of concentration- and time-dependent drugs. In addition to the static exposure to drugs, *in vitro* systems currently do not take into account binding of drug to circulating proteins (e.g., doxycycline, cefovecin). Because only unbound drug is free to enter the microbe, and protein is not present in culture media, MICs generated from C&S should be compared with unbound, not total C_{max} . Finally, the *in vitro* system cannot take into account a variety of host (e.g., immunoglobulins, cytokines, secretory proteins, etc) or microbial (e.g., biofilm or other virulence factors) activities oriented toward defense.

Bactericidal Versus Bacteriostatic Antimicrobials

The MIC is a drug concentration that inhibits but does not necessarily kill the target microbe. The MIC is a reasonable clinical outcome target because the success of antimicrobial therapy usually depends on host defenses that sequester and ultimately kill the microbial population after its inhibition by the drug. Antibacterials are frequently classified according to their ability to kill (bactericidal) rather than inhibit (bacteriostatic) microbial growth. Whereas bacteriostatic activity is indicated by the MIC, bactericidal activity of a drug is indicated by its minimum bactericidal concentration (MBC). However, this classification is based on *in vitro* methods. The MBC can be determined in several ways. For example, those test tubes in which no visible sign of growth was observed following the broth dilution procedures can be reinoculated on nutrient-rich agar plates (see Figure 6-5). Those test tubes that yield no growth contained concentrations that killed, rather than inhibited, the microbe. Thus the test tube with the lowest concentration that yielded no growth contained the MBC of the drug. All antimicrobial drugs are characterized by an MBC; however, those drugs whose MBC approximates the MIC (e.g., within one broth dilution) might also be considered bactericidal. The MBC is most appropriately determined based on killing curves, which measure the number of surviving bacteria after exposure to fixed concentrations of drug; the concentrations are based on those achieved in serum at defined time intervals.⁴¹ For organisms noted as "S" to bactericidal drugs, achieving sufficient drug at the site to kill, rather than simply inhibit, the infecting pathogen is possible. For bacteriostatic drugs achieving the concentration necessary to kill the organisms without causing harm to the patient is much more difficult.^{23,41} Exceptions might occur for drugs that are accumulated (in an unbound state) at the site of infection (e.g., urine or phagocytic white blood cells); in selected instances bactericidal concentrations of a bacteriostatic drug can be achieved.

KEY POINT 6-15 Bactericidal and bacteriostatic activities are defined according to *in vitro* conditions, which may not necessarily translate to the patient.

Categorization of static versus cidal activity of a drug can be associated with its mechanism of action (Table 6-5). In general, drugs that target cell walls (beta-lactams, glycopeptides),

Table 6-5 Bactericidal Versus Bacteriostatic Drugs

	Target	Drug or Class	Drug
Bacteriostatic	Ribosomes	Tetracyclines	
		Phenicols	
		Macrolides*	
		Lincosamides*	
	Metabolic pathway	Sulfonamides	
		Trimethoprim	
	Ormetoprim		
Bactericidal	Cell wall inhibitors	Beta-lactams	Penicillins
			Cephalosporins
			Vancomycin
	Cell membrane	Polymyxin	
		Colistin	
	DNA	Fluorinated quinolones	
		Metronidazole	
	Ribosomes	Aminoglycosides	
		Macrolides*	
		Lincosamides*	
RNA	Rifampin		
Metabolic pathway	Trimethoprim-sulfonamides		
	Ormetoprim-sulfonamides		

*Accumulation in white blood cells may allow achievement of bactericidal concentrations.

cell membranes (polymyxin B, colistin), or DNA (fluorinated quinolones) tend to act bactericidal *in vitro*. Ribosomal inhibitors that target more than one subunit (i.e., 30s and 50s; or 70s) also tend to be bactericidal. In contrast, drugs that target a single ribosomal subunit (tetracyclines, macrolides, lincosamides) or metabolic pathway (sulfonamides) tend to act bacteriostatic. Combinations of two bacteriostatic antimicrobials that act in an additive or synergistic fashion may also result in bactericidal effects (e.g., a sulfonamide combined with a potentiating dipyrimidine). However, the distinction between

bactericidal and bacteriostatic effects of a drug depends on the concentration; a bactericidal drug can be rendered non-bactericidal if concentrations sufficient to kill the organism are not reached at the site of infection the site of infection, or under conditions that slow the growth of the target organism (e.g., hypoxic environment or if used in combination with drugs that antagonize bactericidal actions). In such instances, the bactericidal drug will act in a bacteriostatic fashion. On the other hand, drugs classified as bacteriostatic may act bactericidal if high enough concentrations can be achieved, as might occur if the drug is accumulated in an unbound form (e.g., WBC or other tissues).

Because host defenses must be effective to kill those organisms whose growth is merely inhibited, achieving bactericidal concentrations of an antimicrobial drug are paramount to therapeutic success in immunocompromised hosts (e.g., viral infections, granulopoietic patients, use of immunohibiting drugs) or immunocompromised sites (septicemia, meningitis, valvular endocarditis, and osteomyelitis).^{23,26}

Integration of Pharmacokinetics and Pharmacodynamics: Pharmacodynamic Indices

Although the MIC of a (presumed) infecting organism offers a target concentration for antimicrobial therapy, simply achieving the MIC of the organism in plasma may not be sufficient to ensure efficacy. Among the relationships that affect efficacy is the PK/PD relationship—that is, the dynamic relationship between the drug concentration to which the organism is exposed throughout the dosing interval (PK) and the response of the infecting organism to the drug, as estimated, for example, by the MIC (PD).⁵⁵⁻⁵⁷ This relationship is affected by many host and microbial factors. Definitions of terms used to describe the integration of PK and PD (PD indices or PDI) are varied, depending on the author. For the purposes of this text, definitions will be drawn from Mouton.⁴⁷ It is important to note that many of the terms are based on parameters determined through *in vitro* testing. Therefore host and microbial factors still need to be considered. Further, most PDI are based on a 24-hr-dosing interval, thus modifications in dosing regimens should be based on a 24-hr period (Figure 6-12). The relevance of PDI to drugs with half-lives longer than 24 hr (e.g., azithromycin, cefovecin) is not clear.

Postantibiotic Exposure

Antimicrobials may continue to exert an effect even though the drug is no longer present at concentrations that exceed the MIC. The term *postantibiotic exposure* has been promoted to refer to the combined definitions that have emerged experimentally. Among the terms is the *postantibiotic effect (PAE)*, which has both an *in vitro* and an *in vivo* definition.⁴⁷ The PAE is exhibited by drugs, and is defined *in vitro* as the period of suppression of bacterial growth after a short exposure of the organism to the antimicrobial.⁴⁷ The PAE for a drug, is determined *in vitro* by exposing a standard inoculum to it, removing the drug and determining the time that elapses (in hours) before the culture CFUs increase by tenfold. *In vivo*, the PAE is the time it takes for the number of CFUs to increase tenfold

in treated animals after concentrations drop below the MIC at the tissue site.⁴⁷ Clinically, the PAE indicates the ability of a drug to inhibit bacterial growth after the drug is no longer present or is below the MIC of the infecting microbe.^{49,58-60} As such, it also takes into account an effect a drug might have at subinhibitory concentrations. The impact of the PAE on antimicrobial efficacy can be profound, particularly for concentration-dependent drugs. It is the PAE that allows some drugs to be administered at long intervals despite short half-lives.^{41,50, 52,59,61,62} The PAE may be absent for some organisms or some patients (e.g., some immunocompromised patients).⁴⁹ The duration of PAEs varies with each drug and each organism and the relationship between PDC and MIC (Table 6-6).⁶³ In general, concentration-dependent drugs appear to exhibit longer PAEs, with the duration of the PAE being proportional to the magnitude of the peak PDC (i.e., longer with higher PDC).⁶⁴ However, for each drug, and within drug classes, the PAE is markedly variable, depending on the organism.⁶⁵ Whereas beta-lactams exhibit a substantial PAE toward selected streptococci (i.e., thus making treatment less time dependent for streptococci), their PAE toward gram-negative organisms is minimal.⁶⁶ Applying information regarding the PAE to clinical patients is complicated by variable results (reflecting marked variability in methods) among investigators. The PAE is enhanced by combination antimicrobial therapy.⁶⁷⁻⁶⁹ The duration of the PAE should be included in estimates of doses or dosing intervals. Some antimicrobials also have been associated with a postantibiotic sub-MIC effect (PASE) that may further prolong the dosing interval^{70,71}; further, a postantibiotic leukocyte enhancement effect (PALE) has been described for some antimicrobials. These are incorporated in *in vivo* estimates of PAE. However, clinical relevance of measurements of PAE, PASE, and PALE based on *in vitro* observations is not clear.^{66,72} These studies do point out the reasons that some antimicrobials are effective at long intervals and indicate the need for a better understanding of the relationship of PDC, MIC, and PAE in the clinical patient.

KEY POINT 6-16 The postantibiotic effect is particularly important to the efficacy of concentration-dependent drugs.

Time- Versus Concentration-Dependent Drugs

The relationship among efficacy, MIC, and the magnitude and time course of PDC can be categorized, *in vitro*, as either *concentration-dependent* (sometimes referred to as *dose-dependent*) or *time-dependent* (sometimes referred to as *concentration-independent*) (see Figure 6-13; and Table 6-4).⁴¹ A third classification has emerged with characteristics from each of these classes. (e.g., as shown by fluoroquinolones). Although studies that categorize drugs are largely *in vitro*, the categorizations generally are supported by *in vivo* studies that include animal models and human clinical trials.⁵⁷ Concentration-dependent drugs, best represented by the fluoroquinolones and aminoglycosides, are characterized by efficacy that is best predicted by the magnitude of PDC (C_{max}) compared to

Table 6-6 The Duration of the Postantibiotic Effect Demonstrated by Selected Drugs Toward Selected Organisms^{63,65,66}

Organism	Drug	PAE/hr	Concentration Dependent*	Time Dependent
<i>Bacillus anthracis</i> †	Fluoroquinolones	4-5	Aminoglycosides	Beta-lactams
	Macrolides	1-2	Fluoroquinolones	Glycopeptides
	Beta-lactams	1-2	Metronidazole	Macrolides*
	Vancomycin	1-2	Azithromycin	Linezolid*
	Rifampin	4-5	Ketolides	Tetracyclines*
<i>Pseudomonas aeruginosa</i> ‡	Gentamicin	4-5		Tigecycline*
	Imipenem§	Good		Clindamycin*
<i>Staphylococcus aureus</i> ‡	Macrolides	3-4		
	Aminoglycosides	5-10		
<i>Escherichia coli</i> ‡	Ciprofloxacin	1-2		
	Amikacin	1-2		
	Beta-lactams	0.5		
<i>Klebsiella pneumoniae</i> ‡	Ciprofloxacin	1-2		
	Amikacin	1-2		
	Beta-lactams	0.5		
§Streptococci	Beta-lactams	Good¶		
Gram negative	Beta-lactams§	Minimal		

PAE, Postantibiotic effect.

*PAE depending on organism; efficacy enhanced by higher concentration

†Athamna 2004⁶³

‡Wang 2001⁶⁵

§O'Reilly 2005⁶⁶

¶Allows once-daily dosing despite short drug half-lives for aminopenicillin

the MIC of the infecting organism (see Figures 6-11 to 6-13)⁷³⁻⁷⁹ For such drugs the magnitude of C_{max}/MIC (or C_{max}/MIC_{90}) generally should be 10 to 12; for more difficult infections (e.g., *P. aeruginosa*, or infections caused by multiple organisms), the higher index should be targeted.⁵⁷ The time that PDC is above the MIC—that is, the duration of exposure, ($T > MIC$ or $T > MIC_{90}$)—is not as important as is the C_{max}/MIC ; in fact, efficacy may be enhanced (e.g., for the aminoglycosides) by a drug-free period (i.e., a long interval between doses; see Figure 6-13).^{61,73,74,80-82} This may reflect, in part, the phenomenon of adaptive resistance.⁸³ Adaptive resistance refers to a reversible refractoriness to the bactericidal effects of an antibacterial agent. This phenomenon has been documented particularly for gram-negative organisms and the aminoglycosides, but it appears to occur with the quinolones as well. The resistance appears to reflect a protective phenotypic alteration in the bacteria, such as reversible downregulation of aminoglycoside active transport. Adaptive resistance occurs rapidly (within 1 to 2 hours) of antimicrobial therapy; duration reflects the elimination half-life of the drug. In humans adaptive resistance to aminoglycosides may last for up to 16 hours after a single dose of aminoglycoside, with partial return of bacterial susceptibility at 24 hours and complete recovery at approximately 40 hours.⁸³

For concentration-dependent drugs, a dose that is too low is particularly detrimental. In a mouse model of *E. coli* peritonitis, the antibacterial efficacy of ciprofloxacin, but not

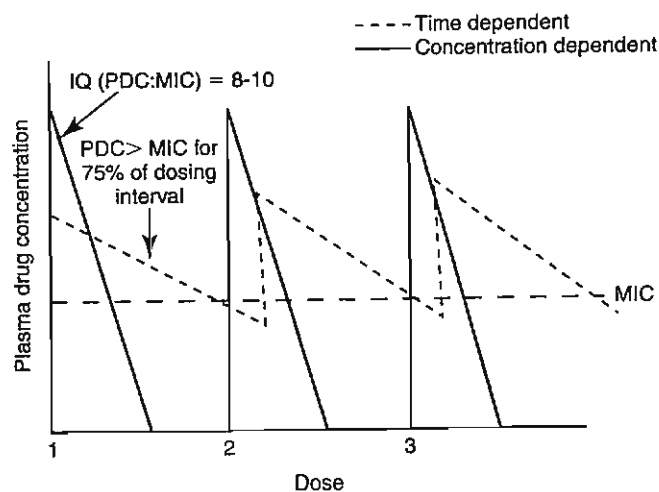


Figure 6-13 The relationship between plasma (tissue) drug concentration, and the minimum inhibitory concentration (MIC) of the organism may determine drug efficacy. The efficacy of concentration-dependent drugs (e.g., aminoglycosides; fluorinated quinolones; and, in some cases, azithromycin or other azalides) depends on a high C_{max} to MIC ratio. Doses might be increased to ensure sufficiently high plasma drug concentrations to achieve the target ratio. In contrast, for time-dependent drugs, such as beta-lactams, sulfonamides, and nonaminoglycoside ribosomal inhibitors, efficacy is maximized by ensuring that plasma drug concentration remains above the MIC for most (50% to 75%) of the dosing interval.

imipenem (or meropenem), was improved by doubling the dose. For some concentration-dependent drugs, efficacy may be both dose- and time-dependent, with the best predictor of efficacy being AUC/MIC. For example, efficacy of fluorinated quinolones can be predicted by both a C_{max}/MIC (target 10 to 12) or AUC/MIC (target 100 to 125) (see Figure 6-13).^{41,84-86} The AUC/MIC may be particularly predictive for fluoroquinolones when treating gram-positive isolates; adding a second full dose may be prudent for some infections. Otherwise, concentration-dependent drugs generally can be administered at longer intervals (e.g., once a day).

In contrast to concentration-dependent drugs, efficacy of time-dependent drugs (e.g., beta-lactams) is best predicted by the time that PDC remains above the MIC. For such drugs PDC should be 2 to 4 times the MIC of the infecting microbe and should be maintained above the MIC ($T > MIC$) throughout a significant portion of the dosing interval (see Figures 6-12 and 6-13). However, the recommended duration of $T > MIC$ varies from a low of 25% for carbapenems to 50% to 70% for extended-spectrum penicillins to potentially 100% for penicillin and aminopenicillins (an exception being treatment of streptococci).^{56,57,61,74,87,88} With time-dependent drugs, increasing the dose may be necessary to ensure that PDCs are above (ideally severalfold) the MIC.⁵⁷ Maintaining $T > MIC$ may be problematic for drugs with a short half-life unless the isolate is extremely susceptible (e.g., most beta-hemolytic streptococci for penicillins). Given that drug concentrations decrease by 50% every drug half-life, a C_{max}/MIC of 2 will result in a PDC that will reach the MIC in 1 half-life. The duration of the dosing interval then depends on the desired target duration (i.e., 25% to 100%) of $T > MIC$. If $T > MIC$ is 100%, then the dosing interval would be 1 half-life. If $T > MIC$ is 50%, the dosing interval would be 2 half-lives, and if $T > MIC$ is 25%, the dosing interval could be as long as 4 half-lives. For each additional half-life to be added to the duration that $T > MIC$, concentrations, and thus dose, must be doubled again (i.e., quadrupled if $T > MIC = 2$ half-lives, increased eightfold if $T > MIC = 3$ half-lives, and so on). Table 6-4 demonstrates the impact of C_{max}/MIC and half-life on time-dependent drugs. Although efficacy of time-dependent drugs requires $T > MIC$ for a sufficient time, efficacy might be enhanced by increasing the dose for drugs with a sufficiently long half-life, shortening the interval for drugs with a short half-life, or both. Constant-rate infusion,⁸⁹ or slow-release products,⁹⁰ might be ideal for time-dependent drugs with short half-lives. Drugs characterized by longer elimination half-lives might be preferred (e.g., cefovecin^{43,44}). Efficacy also should be enhanced for time-dependent drugs that persist to accumulate in the unbound state in selected tissues (i.e., macrolides, clindamycin,⁹¹ or drugs that accumulate in phagocytes.) The downside to using antimicrobial drugs with a very long half-life is that the time to steady-state concentrations and thus peak effects might be prolonged. Moreover, a "hit hard, get out quick" approach to therapy is difficult to implement with such antimicrobials.

The relationship between PDC, MIC, and time versus concentration-dependency might be explained, in part, by the mechanism of antimicrobial action. Efficacy of the

aminoglycosides or fluorinated quinolones depends on drug binding to the target (ribosome and topoisomerase or DNA gyrase, respectively); once sufficient binding occurs, protein synthesis or DNA activity, respectively, is prevented and does not re-initiate. However, beta-lactams substitute as a substrate for cell wall synthesis, and, as long as the organism is growing, it is synthesizing cell wall. Thus, the drug needs to be present as long as the organism is growing. The glycopeptides (e.g., vancomycin), which also target the cell wall, are also time-dependent drugs.

KEY POINT 6-17 Efficacy of concentration-dependent drugs can be enhanced by increasing the dose such that peak concentrations are 10 to 12 times the MIC of the infecting microbe ($C_{max}/MIC \geq 12$).

Increasingly, CLSI is using PDI as the basis for determination of breakpoint MIC. However, not surprisingly, the optimal relationship between PDC and MIC that determines efficacy of a drug is not so simple and varies with organisms and drugs. The optimal relationship between PDC and MIC and the parameter that best predicts antimicrobial efficacy (e.g., peak PDC; the ratio of area under the drug concentration versus time curve to the organism's MIC; duration of PDC above MIC) have not been established definitively for all antimicrobials.^{52,61,84,85} However, for drugs characterized by inhibition (bacteriostatic drugs), $T > MIC$ may best predict efficacy. For some fluoroquinolones, and particularly for gram-positive organisms, efficacy is best predicted by the ratio of AUC (which is influenced by both dose and interval) to MIC rather than simply the C_{max}/MIC . The optimal AUC/MIC also varies with the organism, ranging from as low as 30 to 40 for *S. pneumoniae* and levofloxacin to greater than 350 for *P. aeruginosa* and ciprofloxacin. The area under the inhibitory curve (AUC) reflects the integrated AUC above the MIC during the dosing interval. This parameter is similar to but varies from AUC/MIC in that it is the AUC that is above the MIC (in contrast, AUC/MIC involves the complete area). The AUC should exceed 125 for fluorinated quinolones to achieve bacterial killing; an AUC that exceeds 250 results in rapid killing.⁵⁷ Thus for treatment of some infections with concentration-dependent drugs, the dosing regimen might be designed to maximize both the C_{max}/MIC and the AUC/MIC. Some drugs (e.g., macrolides) are characterized by time-dependency for some organisms but concentration-dependency for others.

Table 6-4 offers examples of PDIs that are achieved using current recommended dosing regimens for selected drugs and selected PD data from selected pathogens that cause infection in dogs. The PD data are based on MIC_{90} obtained from package inserts of drugs approved in dogs or literature that provided PD data specific for canine pathogens that was more recent than package insert data. For some drugs PD was not available for canine pathogens, so PD from human pathogens was used. Doses were chosen from Table 7-1 and generally reflected the highest dose for which PK was available. When possible, nonintravenous routes were chosen

because C_{max} from intravenous data may not represent C_{max} following distribution. Table 6-6 is intended to demonstrate how PDI might be used to assess a dosing regimen. It is not unusual to find that the target C_{max}/MIC (>10) often is not reached for concentration-dependent drugs) or $T > MIC$ (50% for most) for time-dependent drugs. One could argue that the MIC_{90} is an unreasonable PD target; indeed, for some isolates it may be. The preferred PD statistic would be the MIC from the isolate infecting the patient; it can be substituted in this table for the MIC_{90} . Likewise, the 95th lower confidence interval is preferred to the mean C_{max} or half-life for the PK component of PDI. For concentration-dependent drugs, doses can be increased (whenever safety permits) to achieve the target C_{max}/MIC ; for time-dependent drugs, both the dose (increase) and interval (shorten) might be modified. Alternatively, or perhaps in addition to, combination therapy might be considered. Note that the PDIs are based on plasma drug concentrations (PDCs). For some drugs, PDC underestimates concentrations in extracellular fluid. However, for others, PDC frequently overestimates by 25% to 50% or more extracellular fluid drug concentrations. Doses may need to be increased by 25% to 50% to adjust for this difference. As important as PK/PD integration is to the design of the dosing regimen, its application to the clinical patient will be facilitated by an understanding of the microbial and host factors that influence response to the drug.

KEY POINT 6-18 Efficacy of a time-dependent drug can be enhanced by ensuring that concentrations at the site of infection are above the MIC ($T > MIC$) for most of the dosing interval.

MICROBIAL FACTORS THAT AFFECT ANTIMICROBIAL EFFICACY

Among the most obvious ways that microbes can affect antimicrobial efficacy is the advent of resistance. However, microbes can negatively affect antimicrobials through mechanisms that do not influence MIC. These effects are not as obvious to detect as resistance but nonetheless can profoundly affect therapeutic success.

Inoculum Size

The larger the bacterial inoculum at the target site, the greater the concentration (number of molecules) of antimicrobial necessary to kill the organism. Further, more CFUs are more likely to produce greater amounts of enzymes or other materials that can destroy the drug. The "inoculum effect" of ESBL resistance describes the increasing MIC of the organisms toward cephalosporins at a larger (10^7) compared with smaller (10^5) inoculum.⁹² In addition, the larger the inoculum, the greater the risk that spontaneous mutation will contribute to resistance or virulence. Note that resistance and virulence do not necessarily co-exist. In general, emerging resistance appears to be associated with *decreased* rather than increased virulence, although increasingly studies are identifying

exceptions. For example, community-acquired infections may be associated with increased virulence, but less resistance. For example, although hospital-acquired infections tend to be caused by nonvirulent organisms, community-acquired infections reflect virulent organisms that can infect even the overtly healthy patient. Concern regarding MRSA reflects, in part, its apparent acquisition of virulence factors that have facilitated its transition from a hospital to community-acquired infection.

Virulence Factors

The degree of pathogenicity of bacteria (virulence) will affect antimicrobial efficacy indirectly by facilitating infection. The ability of microbes to cause disease reflects the size of the inoculum, the effectiveness of host defense mechanisms, and the intrinsic pathogenicity of the microbes resulting from the presence of virulence factors. Like biochemical mechanisms of resistance, virulence factors generally involve proteins encoded by DNA of chromosomal or shared (e.g., plasmids, transduction) origin. Contributing to the negative impact of virulence factors is host response to their effects. Virulence factors facilitate adhesion to host cell surfaces, colonization (e.g., urease of *Helicobacter pylori*, which protects it from gastric acidity), invasion (facilitated by disruption of host cell membranes or stimulation of endocytosis), immunosuppression (e.g., antibody-binding proteins), or bacterial toxins that cause local, distant, or both (e.g., endotoxin) host damage. Pathogen attachment to host cells is a crucial early step in mucosal infections and is facilitated in epithelial tissues by bacterial adherence. Adherence is a specific two-phase process involving bacterial virulence factors called *adhesins* and complementary receptors of the host epithelial cells.^{93,94} Adhesins are generally found on the surface of microbes, (e.g., bacterial fimbriae) and along with other virulence factors facilitating infection, may be targets for alternative (to antimicrobial) therapy. Species differences exist among the types of receptors in the host epithelial cells. The predominant receptor type in humans is glycolipid in nature, and its presence varies with blood cell types, implying individual variation in susceptibility to bacterial adherence in several body systems. Bacterial adherence is discussed with regard to specific body systems in Chapter 8.

Another virulence factors that facilitate infection are *invasins*. Invasins are enzymes that damage physical barriers presented by tissue matrices or cell membranes, facilitating rapid bacterial spread. Examples include clostridial hyaluronidase, which is able to destroy connective tissue, and lecithinases and phospholipases of clostridial and gram-positive organisms. Bacteria have developed siderophores, which are specialized virulence factors that mediate the release or scavenging of iron critical for microbial virulence. Bacteria also have developed specialized transport systems that secrete toxic materials into the extracellular matrix. It is not clear whether the efflux proteins that transport toxins are related to those that transport drugs (see the discussion of resistance). Bacteria also facilitate invasion through materials (e.g., proteins, "slime") that prevent phagocytosis or, if the microbe is phagocytized,

preclude intracellular killing. Examples include lytic enzymes of gram-positive cocci or exotoxin A produced by *P. aeruginosa*. Toxins include both endotoxins (discussed in depth later) and exotoxins. Bacterial exotoxins are among the most potent toxins known, acting on either the cell surface (e.g., *E. coli* hemolysins, “superantigens” of *S. aureus* or *Streptococcus pyogenes*); membrane; or, once the membrane is penetrated, intracellular targets (e.g., A/B toxins).

KEY POINT 6-19 Virulence refers to the ability of the microbe to cause infection. However, a virulent organism often is not resistant.

Biofilm

Among the most effective and probably least appreciated protective microbial factors is biofilm. Bacteria exist in either a planktonic (free floating) or sessile (attached) state; while it is the former state that characterizes C&S testing, but it is the latter state that enables persistence of the resident population, as well as the formation of biofilm.⁹⁵⁻⁹⁷ Biofilm is defined as a biopolymer, matrix-enclosed bacterial population in which bacteria adhere either to one another or to a surface.⁹⁵ The outer layer of the biofilm may lose water such that it is hardened, thus providing better protection from the environment, including exposure to antimicrobials. The inner sactum of the biofilm is largely aqueous, composed of glycocalyx or slime (e.g., *Staphylococcus* spp.). In addition to passive diffusion, aqueous pores permeate the structure, allowing movement of nutrients and metabolic debris. Biofilm populations containing normal microflora in the skin or mucous membranes (e.g., urinary bladder) are lost with shedding of the skin (or bladder) surface or by the excretion of mucus; new cells and mucus are rapidly colonized by biofilm-forming bacteria. Microbes released from the surface may colonize new surfaces and subsequently produce new biofilms and new (e.g., persistent or recurring) infections. Bacterial communication during biofilm formation is sophisticated, involving quorum-sensing systems that ultimately may be targets of microbial therapy.⁹⁶ Biofilm may facilitate and protect growth of normal or pathogenic flora on foreign surfaces and can facilitate subsequent translocation of microbes to otherwise sterile tissues. Persistent, chronic bacterial infections may reflect biofilm-producing bacteria; persistent inflammation associated with immune complexes contributes to clinical signs. Dental plaque is a prototypic example of the impact that biofilm might have on preventing antimicrobial penetration. Cystic fibrosis associated with *Pseudomonas* is a disease in which biofilm contributes to mortality. Pathogens associated with biofilm in veterinary medicine include, but are by no means limited to, *Acinetobacter*, *Actinobacillus*, *Klebsiella*, *P. aeruginosa*, and *Staphylococcus (aureus and pseudintermedius)*.⁹⁵ Glycocalyx may contribute to protective mechanisms of other organisms as well (e.g., sulfur granules and *Nocardia*; Figure 6-14). Not all pathogens associated with biofilm cause infection (e.g., urinary catheters). However, because they ultimately may be

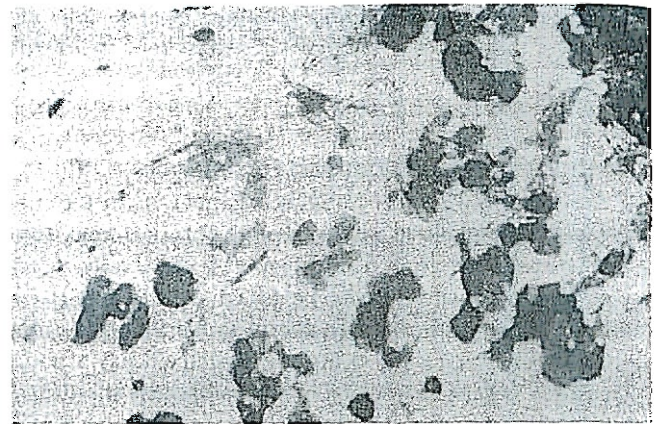


Figure 6-14 An example of combined host and microbial factors that negatively impacts therapy, *Nocardia* causes a marked inflammatory response by the host. Additionally, the organism causes secretion of calcium that combines with its biofilm, resulting in the formation of “sulfur” granules that protect the organisms from drug penetrations.

the source of infection, clinical resolution may not be possible until the biofilm is destroyed. Yet, its nature is difficult to predict based on the planktonic growth of individuals in cultures compared to the consortium that occurs *in vivo*.⁹⁷ Catheters (urinary or intravascular), orthopedic fixation devices, and materials used in wound management are examples of surfaces on which biofilm might develop.

KEY POINT 6-20 Biofilm can form on many foreign or natural surfaces and may profoundly decrease the likelihood of successful antimicrobial therapy.

Antimicrobial Resistance

The role of resistance in therapeutic failure of antimicrobials is well established.^{23,98} The use of antimicrobials increasingly is associated with emergence of resistance. For each class of antimicrobial drugs approved for use in human medicine, resistance generally has emerged within 1 to 2 decades of use. Clinically relevant resistance toward sulfonamides, the first class of antimicrobials approved in the United States (1930s) was documented by the 1940s. Penicillins, tetracyclines, streptomycin (aminoglycoside), and erythromycin (macrolides) were all approved within a 10-year span, with resistance documented within 5 years for methicillin versus approximately 10 years for streptomycin. Resistance to nalidixic acid, the progenitor of fluoroquinolones (approved in 1950), took 3 decades to emerge, perhaps convincing manufacturers that resistance to fluoroquinolones would emerge very slowly. However, resistance to norfloxacin, the first fluoroquinolone approved in the United States, took less than 3 years to emerge, despite the fact that the lack of plasmid-mediated resistance was among the attributes of this class. Resistance to extended-spectrum cephalosporins emerged within 4 years of approval and to amoxicillin-clavulanic acid, within 5 years. Resistance to vancomycin, specifically developed to treat MRSA, emerged in its second decade of use.

KEY POINT 6-21 Antimicrobial resistance increasingly will prevent the successful empirical selection of antimicrobial drugs.

Inherent Versus Acquired Resistance

Antimicrobial resistance might be inherent to the microorganisms or acquired, either through chromosomal mutations or transfer of genetic information.⁹⁹ Generally, spectrums of antimicrobials (listed on package inserts and elsewhere) reflect inherent resistance patterns rather than acquired resistance patterns. Examples include limited efficacy of aminoglycosides toward anaerobic organisms because the drugs must be actively transported into the cell (oxygen dependent) or the resistance of gram-positive organisms, which lack an outer cell membrane, to polymyxin B, which targets the same. Acquired resistance, on the other hand, generally renders a previously susceptible organism resistant. As such, it is not necessarily predictable and can occur during the course of therapy (leading to changes in a C&S pattern). More problematically, it is often shared among microbes.

Shared resistance among bacteria reflects the ability of bacteria to incorporate extrachromosomal DNA carrying the information for resistance from other organisms. Extrachromosomal DNA (including plasmids and bacteriophages) encode for resistance to multiple drugs and can be transmitted vertically (to progeny) or horizontally, across species and genera. Transposons are individual or clusters of resistance genes bound by integrons, which move resistance genes back and forth between chromosomes to plasmids. Consequently, bacterial resistance is extremely mobile and can spread rapidly.¹⁰¹ Among the mechanisms by which genetic resistance information is shared is (sexual) conjugation. Conjugation occurs particularly in gram-negative organisms and may be accompanied by genetic material that confers bacterial pathogenicity as well as altered metabolic functions. However, *Enterococcus* spp. and selected other gram-positive bacteria also transfer resistance to glycopeptides through conjugative transposons.¹⁰¹ Transduction, which requires a specific receptor, involves transfer of information by a bacterial virus (bacteriophage) and is implemented especially by *Staphylococcus* spp. Resistance, including methicillin resistance, can be transferred between coagulase-negative and -positive *Staphylococcus*.¹ Transformation involves transfer of naked DNA from one lysed bacterium to another; this mechanism of transfer tends to be limited (in humans) to pneumococcal meningitis.

Although present for eons, acquired antimicrobial resistance increasingly is becoming problematic. The impact of antimicrobial resistance can be extensive. In some human intensive care units, selected isolates are characterized by a resistance prevalence of 86%. The impact of resistance on the patient includes increased morbidity, mortality, and increased hospital costs.¹⁰⁷ Patterns of resistance have emerged in veterinary medicine, although differences appear to occur in the ability of organisms to develop resistance to an antimicrobial, varying with species and strain. Many organisms remain predictably susceptible to selected drugs (e.g., *Brucella*, *Chlamydia*),

whereas others are becoming problematic (e.g., *P. multocida*). Several organisms traditionally have developed resistance that can rapidly impair efficacy of new antimicrobials (e.g., *E. coli*, *K. pneumoniae*, *Salmonella*, *S. aureus*, *S. pneumoniae*). In general, these organisms have developed multidrug resistance (MDR). MDR is now considered the normal response to antimicrobials for gram-positive cocci pneumococci, enterococci, and staphylococci.¹⁰² Among these, *Staphylococcus* spp. is considered most problematic: it is intrinsically virulent, is able to adapt to many different environmental conditions, increasingly is associated with resistance to other classes of antimicrobials, and tends to be associated with life-threatening infections.^{102,103} In a veterinary teaching hospital the percentage of patients with *S. intermedius* susceptible to cephalexin and amoxicillin-clavulanic acid decreased from a high of 96% in 2005 to < 60% in 2007, a trend that appears to be emerging in other veterinary hospitals.¹¹⁰

KEY POINT 6-22 Acquired resistance can occur during the course of antimicrobial therapy.

E. coli is among the organisms that have developed multi-drug resistance.^{104,105} Fluoroquinolone-resistant *E. coli* emerged as early as 1998, little over a decade after the approval of enrofloxacin for dogs or cats.²⁸ Multidrug-resistant *E. coli* has emerged as a cause of nosocomial infections in dogs¹⁰⁸ and UTIs in canine critical care patients.^{104,109} The presentation is similar to the that in human critical care patients, with risk factors such as sex (males), hospital stay, and previous antimicrobial therapy being similar for both.

Factors Contributing to the Emergence of Resistance

Development of antimicrobial resistance is facilitated by several factors¹¹¹; among the most important is exposure to antimicrobials. In the individual patient, single-dose ciprofloxacin prophylaxis increased the prevalence of ciprofloxacin-resistant fecal *E. coli* from 3% to 12% in humans.¹¹² Ciprofloxacin treatment for prostatitis resulted in posttreatment fecal colonization with quinolone-resistant *E. coli* that was genetically distinct from the infection-causing strains after treatment in 50% of the patients.¹¹³ Our laboratory has demonstrated that standard doses of either amoxicillin or enrofloxacin given orally will cause close to 100% of fecal *E. coli* to become resistant to the treatment drug within 3 to 9 days of therapy; for enrofloxacin the isolates generally are multidrug resistant. As with MRSA or MRSI (*S. intermedius*), the advent of resistance by *E. coli* and other gram-negative organisms has been associated with increased cephalosporin use.¹

The gastrointestinal flora offers a natural environment that exemplifies the impact of antimicrobials on selection pressure. The normal flora of the gastrointestinal tract is extremely diverse, with anaerobes predominating. Among the aerobes, *E. coli* are the major gram-negative and *Enterococcus* the major gram-positive organisms.¹⁰¹ Environmental microbes maintain an ecologic niche through suppression of the competition

by either consumption of nutrients or secretion of antibiotics. Therefore commensal organisms are constantly being exposed to antibiotics, and are "primed" to develop resistance.¹⁰¹ However, the microbes producing the antibiotic, as well as surrounding normal flora, are resistant to the antibiotic. Thus genes for resistance develop along with genes directing antibiotic production.

Rapid microbial turnover in the gastrointestinal tract supports the development of resistance by ensuring active DNA replication and thus mutation potential (see previous discussion). Chromosomal (DNA) mutations (10^{-14} to 10^{-10} per cell division) are DNA mistakes that have been missed by bacterial repair mechanisms. These mistakes occur spontaneously and randomly, regardless of whether the antibiotic is present. If the mutation that confers resistance to an antimicrobial occurs in the presence of the antimicrobial when it is administered to the patient, the surviving mutant, reflecting its single-step mutation, confers a low level of resistance (see the discussion of mutant prevention concentration). The MIC of the organism is likely to increase. Further microbial turnover and continued therapy can lead to multistep mutations and rapid emergence of high-level resistance characterized by increasingly higher MIC. Stepwise mutations can lead to specific resistance such as that demonstrated toward fluorinated quinolones (stepwise mutation in the DNA gyrase gene). Nonspecific mechanisms of resistance, including that shared among organisms, are more likely to result in MDR. Microflora of the gastrointestinal tract can serve as a reservoir of resistance genes; a single drug, via integrons, plasmids, and transposons, facilitates the rapid transfer of MDR among organisms. The gastrointestinal environment exemplifies a pattern whereby resistance can emerge as a result of a combination of selection pressure and mutation. Clinically, similar mechanisms of emerging resistance are likely to occur at sites of infection.

Mutant Prevention Concentration

Drlica and coworkers¹¹⁴ have hypothesized the *mutant selection window*, (see Figure 6-15) comprised of a lower threshold represented by the culture MIC of the infecting organism and an upper threshold or boundary, the MPC. Should a dose be designed such that drug concentrations fall within this window (i.e., between the MIC and MPC) at the site of infection, the mutant isolate is likely to emerge as a resistant colony. The practical application of the hypothesis explains the observed behavior of mycobacterium organisms toward fluoroquinolones (FQs). Increasing concentrations of the FQs inhibits the nonresistant (wild-type) organisms and colony numbers rapidly decrease. But this period of decline is followed by a plateau period of minimal or no growth. During this plateau phase, remaining resistant isolates recover and start to multiply again. The resistance of this emerging, second population presumably reflect a single-step (chromosomal or plasmid-mediated) mutation that resulted in an increase in the MIC to low-level resistance (e.g., MIC is close to the breakpoint). However, when these first-step mutants are exposed to even higher drug concentrations, a second rapid decline in numbers occurs, this time reflecting inhibition of

the mutated, resistant organisms. Again, once sufficient bacteria recover, a second plateau occurs as the first-step mutants mutate. This stepwise or multistep mutation confers high level resistance (MIC exceeds the breakpoint several fold) that can be overcome only by very high concentrations of the FQ. The mutant selection window, which is to be avoided with initial therapy, describes drug concentrations on either side of the initial plateau for the single-step mutants. The lower boundary is defined by those drug concentrations sufficiently high to remove the majority of the wild-type competitors (MIC), whereas the higher boundary (the MPC) is defined by the concentrations necessary to inhibit the least susceptible (most resistant) isolates (the single-step mutants).¹¹⁵ Above this concentration, a second mutation step (which is very rare) would be required for a population of resistant organisms to develop; the risk of this happening is reduced by preventing microbial turnover (i.e., killing all isolates).

KEY POINT 6-23 The mutant prevention concentration (MPC) is the highest MIC of any of the colony-forming units causing infection in the patient. Failure to achieve this concentration may allow resistant microbes to emerge, particularly in the at-risk patient.

On the basis of this observation, Drlica and coworkers contend that MIC-based strategies used to design dosing regimens readily select for resistant mutants.¹¹⁵ Their contention is based on the observation that only one resistant mutation is needed for bacteria to grow in the presence of an antimicrobial and that infections generally contain an adequate number of CFUs for several first-step resistant mutants to be present prior to treatment. They coined the term MPC as an *in vitro* measure of preferred antimicrobial concentration target. If the MPC (rather than the MIC) is achieved at the site of infection, the risk of resistance is minimized because isolates that exceed the MPC concentration must have undergone a second concurrent resistance mutation step prior to therapy. As such, the MPC, not the MIC, would be the concentration targeted at the site of infection in the patient. Indeed, simply achieving the reported MIC of the infecting microbe at the site of infection is probably the approach that is most likely to yield clinically resistant organisms. Accordingly, consideration should be given to assuring that "dead bugs don't mutate." If the least susceptible of the isolates is inhibited with the dosing regimen, then the recovering population should not be resistant.

Drlica¹¹⁵ has demonstrated that MPCs do not correlate to MICs. *In vitro*, the MPC would be defined *in vitro* as the (lowest) drug concentration (in the media) that yields no recovered organisms when over 10^{10} CFUs (mimicking bacterial load in the patient) are plated. Currently, determining the MPC is costly, requiring multiple testing steps and large numbers of cells; for example, standard culture procedures are based on 10^6 CFUs, whereas determination of the MPC requires at least 10^8 - 10^{10} CFUs. However, an MPC-based strategy to dosing clinically makes sense and should be an effective means of blocking the growth of first-step resistant mutants. Such a strategy

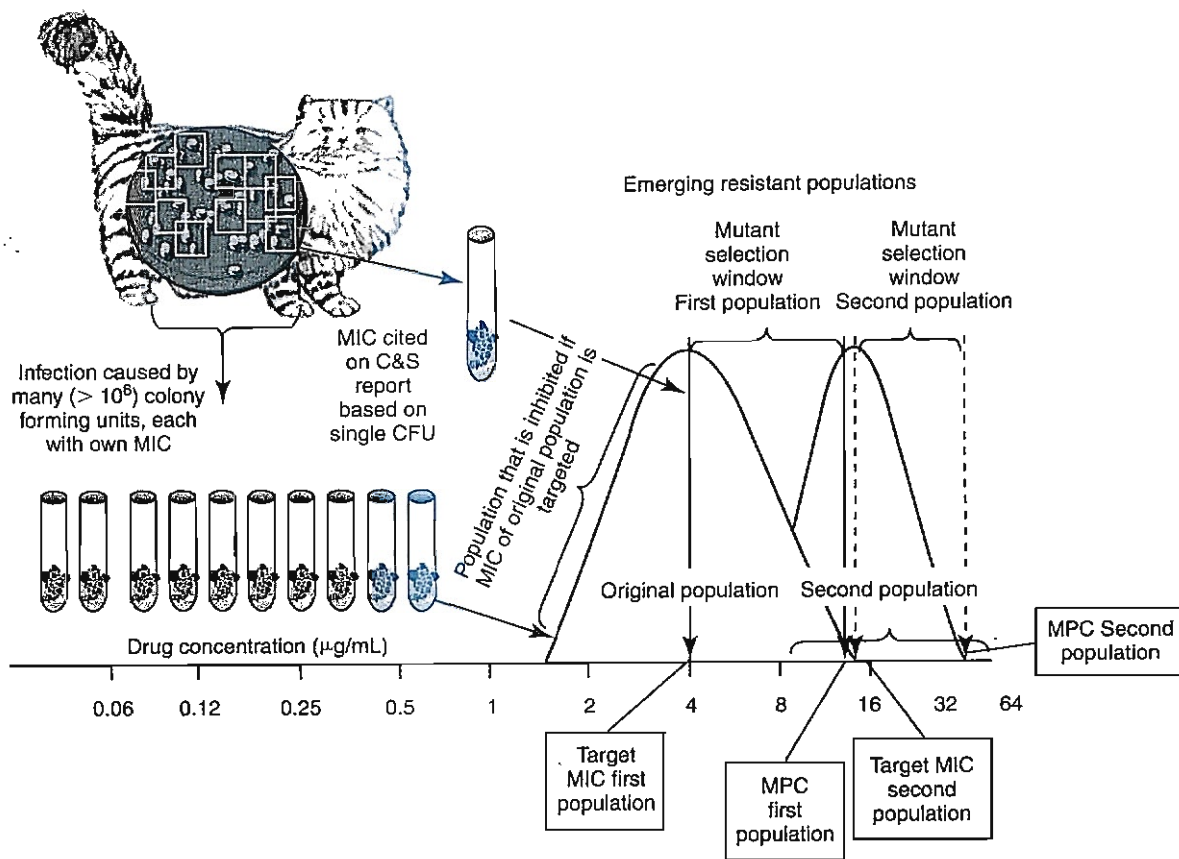


Figure 6-15 Stepwise mutation can emerge as a result of selection pressure induced by antimicrobial therapy that targets the minimum inhibitory concentration (MIC) of the infecting microbe. The mutant prevention concentration (MPC) is the concentration of drug that is necessary to inhibit first-step mutants, or the MIC of the least susceptible isolate in a resident population of pathogens. As the resident population or inoculum of wild (nonresistant) pathogen isolates reaches 10^8 - 10^{10} colony-forming units (CFUs), some isolates will spontaneously mutate such that resistance emerges to the drug of interest. However, when cultured, the MIC reported for the population is likely to represent the mode (the most commonly reported MIC), which in a normally distributed population, is also the MIC₅₀ for the population. In contrast, the MIC of the first-step mutant will be the high end of the population MIC range. This is the concentration that should be targeted to inhibit the entire population—that is, the MPC. If the dosing regimen is designed to target the the mutant selection window, that is, the MIC of the wild population rather than the MPC (the MIC of the first-step mutant)—treatment with the drug will inhibit all isolates at or below the MIC. The void in isolates will allow the remaining, more resistant first-step mutants to recover, particularly in patients not sufficiently healthy to suppress recovering microbes. As this new population expands, a second distribution curve emerges. If recultured, the MIC of the second first-step mutant population will be higher than the wild population. If the population reaches a sufficient size (e.g., 10^8 CFUs), a second, spontaneous mutation is likely to occur, resulting in a new, higher MPC. Targeting the MPC is particularly important when using drugs for which resistance emerges in response to mutations.

would force wild-type cells to acquire two resistance mutations for growth, an event that is rare. Experimental *in vitro* data¹¹⁴ have confirmed that MPC levels of an FQ do indeed inhibit strains that harbor first-step *gyrA* mutations (the mechanism of microbial resistance to FQ).¹¹⁶ Application of the MPC is most appropriate for drugs and organisms that develop resistance by chromosomally mediated point mutations (e.g., the fluorquinolones).¹¹⁷ However, the spirit of targeting the MPC might be assumed even for other drugs, in order to minimize the impact of selection pressure on emergent resistant populations. The mutant selection window can be narrowed if more than two bacterial sites are targeted, such as might occur with combination antimicrobial therapy, or with drugs that simultaneously target more than one site (e.g., the FQs).¹¹⁴

Biochemical Mechanisms of Resistance

Bacteria often respond to the presence of the antimicrobial by altering their physiology such that resistance occurs, often to multiple drugs. Microbes develop antimicrobial resistance by two primary mechanisms: modification of the target site or altered intracellular drug concentration. Methods by which intracellular drug concentration can be decreased include changes in porin sizes for gram-negative organisms (e.g., most drugs; see Figure 6-3). Porins are transmembrane proteins (e.g., OmpF) that form an aqueous channel that allows passive movement of large hydrophilic molecules. Porins are one of the few means by which drugs can gain access to intracellular targets. A change in porin size (i.e., by the addition of side chains that filter out drugs) or number increases antimicrobial

resistance, as is demonstrated by the loss of the OprD protein that imparts resistance to imipenem by *Pseudomonas* spp. Closely associated with the porin proteins are efflux proteins that pump drug out of the organism; the pumps often are associated with porin proteins (e.g., FQs and tetracyclines). Most of these pumps are fueled by energy associated with proton exchange, the most notable in gram negative organisms being of the RND (resistance nodulation division) family. The best characterized in this family is the Acr-AB/TolC system, which is a complex bacterial stress response system that allows bacteria to pump out toxic molecules.¹⁰¹ These and other pump systems are often characterized by a wide range of substrate specificities and, along with porins, are a common mechanism whereby an isolate can express multidrug resistance. In contrast, a number of microbes generate enzymes that destroy antimicrobials (e.g., aminoglycoside acetylases, beta-lactamases that destroy penicillins or cephalosporins, transferases that destroy chloramphenicol); in such instances resistance conferred by these mechanisms is generally limited to a single drug or drug class. Enzymatic inactivation is more likely for natural drugs to which microbes have previously been exposed (and thus presented with a greater opportunity to develop enzymes). In contrast, enzymes are less likely to destroy synthetic drugs.¹¹⁸ However, plasmid-mediated enzymatic destruction of FQs has recently been described, once again highlighting the resourcefulness of bacteria.¹¹⁹ Changes in target structure are another major mechanism of resistance. Example targets that have been modified include, but are not limited to, cell wall proteins (e.g., penicillin-binding proteins [PBs], particularly for MRSA [PB2] or *Enterococcus* [PB5]), or binding sites (i.e., on ribosomes, as for aminoglycosides, or DNA gyrase for FQs).^{120,121} Organisms often are characterized by more than one mechanism of resistance. Multiple mechanisms are well documented for some organisms against selected beta-lactams and have been described against FQs (e.g., altered DNA gyrase and increased efflux pumps) and others. Resistance can be induced, as is exemplified by beta-lactamase formation in *Staphylococcus* spp. which greatly increases in the presence of a beta-lactam antibiotic, or for fluoroquinolone, for which efflux pump activity is markedly upregulated. Discussion of specific mechanisms of resistance will be addressed with the appropriate drugs (see Chapter 7).

Avoiding Antimicrobial Resistance

Among the approaches to reducing resistance are pharmacologic manipulations and changes in antimicrobial use practices. Pharmaceutical manufacturers have been able to manipulate antimicrobial drugs in a variety of ways such that resistance is minimized, and these options can be selected in an attempt to minimize resistance. For example, bacterial resistance has been decreased by synthesizing smaller molecules that can penetrate smaller porins (e.g., the extended-spectrum penicillins ticarcillin and piperacillin); synthesizing larger molecules that force the microbe to develop more than one point mutation (e.g., later-generation FQs), "protecting" the antimicrobial from enzymatic destruction (e.g., with clavulanic acid, which diverts the beta-lactamase from the

Box 6-3

Reducing Transmission of Resistant Microbes (Decontamination)

- Treating infected patients in order of least-at-risk to most-at-risk
- Dedicated diagnostic or handling equipment
- Proper bandaging of infected sites
- Dedicated bandage areas
- Protection during animal handling (disposable gloves, masks, gowns, eyewear)
- Decreased contact with body fluids
- Dedicated disposal for contaminated materials
- Proper hand washing between patients
- Easy access to hand sanitizers (alcohol based)
- Strict asepsis during surgery
- Dedicated cleaning materials
- Clean in order of cleanest to dirtiest
- Proper disinfection of the following:
 - Exposed or at-risk rooms
 - Tables, doors, counter surfaces, floors, and so on
 - Equipment (stethoscopes, keyboards, pens, and so on)
 - Isolate carriers (not always necessary)

penicillin); modifying the compound so that it is more difficult to destroy (e.g., amikacin, which is a larger and more difficult to reach molecule than gentamicin and carbapenems, later generation cephalosporins); and developing lipid-soluble compounds that are more able to achieve effective concentrations at the site of infection (e.g., doxycycline compared with other tetracyclines). Increasingly, drug design-based tactics will be implemented to minimize emergent resistance. Increasingly the role of the practitioner is equally important. A three "D's" approach might reduce the risk of emergent resistance: **De-escalate** antimicrobial use, **design** a treatment regimen that minimizes resistance (dead bugs don't mutate), and **decontaminate** the environment through proper hygiene. These approaches are exemplified by strategies implemented by intensive care units to reduce antimicrobial resistance that often involve a multitiered approach (Box 6-3). Actions include the following:

KEY POINT 6-24 The goal of antimicrobial therapy is twofold: resolving clinical signs associated with infection and avoiding emergent resistance. The two goals are not mutually inclusive.

1. **De-escalate.** De-escalation begins with not using an antimicrobial when an alternate therapy (including no therapy) is more or perhaps equally effective. Enacting primary prevention by decreasing length of hospital stay, decreasing use of invasive devices, and implementing newer approaches (e.g., selective digestive decontamination and vaccine development).¹⁰⁷ De-escalation also includes setting limits on the duration of antimicrobial therapy (see later discussion) and rotating the use of antimicrobial drugs on a regular schedule.^{107,125} De-escalation might also refer to changing from a

higher to a lower tier category of drugs (following a “hit hard, get out quick”) in a critical patient.

2. **Design:** Improving appropriate antimicrobial use through proper dosing regimens includes selection of the most appropriate drug for the bug while narrowing the spectrum. This approach also should be applied to empirical antimicrobial therapy. Design of the dosing regimen should take into account the appropriate PDI for concentration or time-dependent drugs, and when possible, targeting the MPC. More controversial approaches to design include techniques implemented in hospitals include adhering to prescribed formularies or requiring prior approval for using certain antibiotics.
3. **Decontaminate:** Approaches intended to reduce bacterial exposure are among the most important to avoiding resistance. These include improving infection control through selective decontamination procedures, prevention of horizontal transmission through proper hand-washing technique, and use of gloves and gown, or prevention by reducing exposure to bandages or other contaminated materials by identifying proper work areas and disposal sites. Other approaches include, provision of soap alternatives, easy access to disinfectants (which should complement, not replace, hand washing) and improvement of the workload and facilities for health care workers.

Improved information systems technology also plays a role. Each proposed or implemented strategy has theoretical benefits and limitations, but good data on their efficacy in controlling antimicrobial resistance are limited.^{107,125} However, it is clear that decreased antimicrobial use is associated with a decrease in the advent of resistance.

Risk factors for emerging resistance in the hospital or community setting include but are not limited to increased antimicrobial use, host factors such as severity of illness and length of stay, and lack of adherence to infection control practices.¹⁰⁷ Consequently, among the de-escalation efforts implemented in human hospital and community environments is restricted antimicrobial use. In humans the increasing presence of drug-resistant bacterial infections among hospitalized patients is linked to the greater numbers of patients receiving inappropriate antimicrobial treatment.¹²³ A recent on-line report found that in human medicine, antimicrobials were often prescribed despite infection being an infrequent cause of the illness (i.e., pharyngitis). Further, the chosen antimicrobial often was inappropriate for those bacteria potentially causing infection in the treated body system.¹²⁴ Accordingly, reducing inappropriate antimicrobial use has become a priority in human medicine.

Among the more rational paradigms for antibacterial de-escalation, is an approach to empirical antimicrobial use in the hospital setting for patients with serious bacterial infections.¹²³ Such antimicrobial de-escalation attempts to balance the need to provide appropriate initial antibacterial treatment while limiting the emergence of antimicrobial resistance. The goal of de-escalation in this setting is to prescribe an initial antimicrobial regimen that will cover the most likely bacterial pathogens associated with infection while minimizing the risk of emerging antimicrobial resistance.¹²³ The three-pronged

approach includes narrowing the antimicrobial regimen through culture, assessing isolate susceptibility for dose determination, and choosing the shortest course of therapy clinically acceptable. Judicious antimicrobial use combined with restricted use of ceftazidime led to a decreased antimicrobial resistance to beta-lactams, in general, in a human teaching hospital environment.¹²⁶ Note that this strategy does not exclude the use of “big gun” antimicrobials. The approach of withholding use of high-impact drugs (e.g., meropenem or vancomycin) in patients whose need for effective therapy is critical to avoid emerging resistance that might limit drug use in later patients may not be rational or in the best interest of the patient. A more appropriate approach is to use the drug correctly. However, routine use of less powerful drugs is appropriate but only if these alternatives are just as effective. Regardless of the choice, once the decision is made to use an antimicrobial, attention must be paid to dosing regimens that minimize the advent of resistance by ensuring that infecting microbes are eradicated.

Another strategy to decrease the impact of antimicrobial use on resistance is a decreased duration of therapy (see the discussion of enhancing antimicrobial efficacy). One study in human critical care patients found that reducing the duration of antimicrobial therapy from 14 days to 10 days decreased the emergence of resistance.¹²⁷ Increasingly, clinical trials will focus on demonstrating efficacy of shorter (i.e., < 5 to 7 days) treatment regimens.

Rapid detection of the correct microbe and the presence of resistance would facilitate the proper design of a therapeutic regimen. Genetic changes (e.g., mutations) that result in resistance lend themselves to molecular detection. However, molecular tests are often limited to those mutations characterized by few polymorphisms (e.g., MRSA, potentially MRSIG, and *Enterococcus* sp.). Generally, these tests require culture conditions that are often designed to facilitate expression of the resistant gene and are based on amplification techniques. Yet, as with culture, although they are able to determine phenotypic expression, they do not necessarily document the isolate as the cause of infection. Further, they generally do not detect low levels of resistance that increase the MIC but do not render the microbe as “resistant” by susceptibility testing.¹²² Topical therapy should be considered when possible. Therapeutic drug monitoring may be helpful for some drugs (e.g., aminoglycosides). With at-risk patients in whom emergent mutants may not be sufficiently suppressed. Drugs inherently more resistant to bacterial inactivation should be selected (e.g., amikacin rather than gentamicin). Combination antimicrobial therapy (e.g., beta-lactamase-protected antimicrobial combinations; combination of beta-lactams with aminoglycosides) also reduces the incidence of resistance; for example, the use of an FQ reduced the advent of resistance to cephalosporins in one study.³³ Care should be taken in selecting a drug simply because of cost. Cost should be a factor only after other considerations have been taken into account. The cost of an excellent antimicrobial can be easily surpassed by the selection and use of several less expensive, but also less effective, antimicrobials.

HOST FACTORS THAT AFFECT ANTIMICROBIAL EFFICACY

Careful consideration must be given to host factors that can reduce concentrations of active drug at the site of infection.^{23,75,128} The impact of host factors on antimicrobial efficacy is often underestimated; such effects can be profound.

Among such host factors is distribution of the drug to the site of the infection (drug distribution is discussed under drug factors). Thus far, discussions on antimicrobial efficacy have been focused on achieving the MIC of the infecting isolate in the patient plasma. However, infections generally are not in plasma, and patients are not generally normal. The relationship between the MIC of the infecting organisms and drug concentrations achieved at the site of infection (both magnitude and duration) is so complex that predicting efficacy is difficult. Ultimately, mathematical models that integrate the major determinants of efficacy (bactericidal activity, relationship between PDC and MIC, duration of postantibiotic effect, and susceptibility versus resistance) may prove most predictive.¹²⁹ The determinants of this relationship and the influence of drug, microbial, and host factors on efficacy warrant further discussion.

The MIC_{BP} of a drug is based on plasma C_{max}, yet infections generally occur in tissues rather than plasma. More specifically, the site of infection generally is interstitial fluid. However, detection of drug in tissues is difficult, leading to PDC as the surrogate marker of tissue concentrations. In instances in which PDCs overestimate extracellular fluid, care must be taken to adjust doses. For such drugs C&S testing may overestimate efficacy of the drug (see the section on drug distribution). On the other hand, for some tissues, drug concentrations at the site may far exceed PDC (see below). Inflammation may profoundly alter drug efficacy (Table 6-7).^{23,128} Acute inflammation may initially increase drug delivery and drug concentration to the site of infection because of increased blood flow, increased capillary permeability, and increased protein release at the site (the latter effect increases the concentration of total, but not necessarily active, drug). However, chronic inflammation may do the opposite. Purulent exudate presents an acidic, hyperosmolar, and hypoxic environment that impairs the efficacy of many antimicrobials (Figure 6-16). Hemoglobin and degradative products of inflammation can bind antimicrobials.¹³⁰ Selected drugs, including aminoglycosides (and probably highly protein-bound drugs) are bound to and thus inactivated by proteinaceous debris that accumulates with inflammation. Some antimicrobials can inhibit neutrophil function. Accumulation of cellular debris associated with the inflammatory process can present a barrier to passive antimicrobial distribution. The deposition of fibrous tissue at the infected site further impairs drug penetrance and distribution (Figure 6-17).

KEY POINT 6-25 Although the host inflammatory response initially may facilitate therapeutic success, it can ultimately profoundly decrease the likelihood of success.

Table 6-7 Negative Effects of the Microenvironment on Antimicrobial Efficacy

Environmental Factor	Effect
Acidic pH	Penicillins inactivated at pH < 6.0
	Aminoglycosides and enrofloxacin more effective in alkaline pH
Hypertonicity/ hyperosmolarity	Impaired efficacy of beta-lactam antibiotics
Pus	Acidic pH
	Hypertonic
	Hyperosmolar
	Protein binding of selected drugs
Low O ₂ tension	Binding to sediment (aminoglycosides)
	Aminoglycosides inactive
	Growth of organisms slowed → decreased efficacy of bactericidal drugs
Large inoculum	Impaired phagocytic activity of leukocytes
	Greater concentration of antimicrobial inactivating enzymes
Leukocytes	Greater concentration of drug molecules required
	Impaired chemotaxis, phagocytosis, metabolism

Local pH becomes more acidic as degradative products such as lysosomes, nucleic acids, and other intracellular constituents from white blood cells accumulate. The efficacy of many antimicrobials can subsequently be impaired. In humans a pH level ranging from 5.5 to 6.8 can adversely affect both host defenses and antimicrobial activity. White blood cell oxidative bursts and phagocytosis are diminished in the presence of a low pH level. Some antimicrobials are inactive at a low pH level. Erythromycin loses all of its activity when pH is below 7. Similar effects have been reported for beta-lactam antibiotics. Although beta-lactam antibiotics are weak acids and therefore less ionized in an acidic environment, they are generally less effective at a pH 6. The activities of cefoxitin, piperacillin, and imipenem (or meropenem) are significantly less at pH 6 than at pH 6.5 with piperacillin being least affected. The activity of clindamycin is similarly decreased. In addition, the accumulation of some drugs in white blood cells that might otherwise facilitate efficacy is impaired in an acidic environment. Changes in pH also lead to changes in the concentration of un-ionized and thus active drug. Weak bases such as aminoglycosides and FQs are predominantly ionized in an acidic environment and are less effective than in a less acidic environment, in part because of impaired diffusibility.

Low tissue oxygen tension, which can accompany pus, reduces white blood cell phagocytic and killing activity; slows the growth of organisms, making them less susceptible to many drugs; and specifically prevents the efficacy

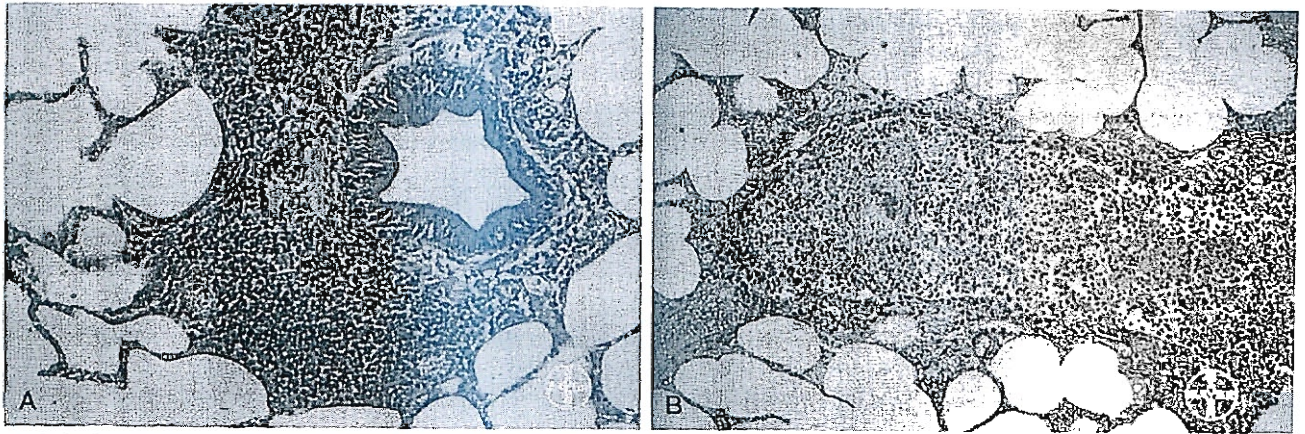


Figure 6-16 The inflammatory response to bacteria is intended to support the host in overcoming an infection. However, the response can become a confounding factor. For example, the inflammation of pneumonia or bronchitis dilutes the drug, presents a barrier to passive diffusion, and may bind and thus inactivate the drug. Local pH and thus drug ionization may impair drug action, and generation of a decreased oxygen tension further decreases drug efficacy. (Photo courtesy Bayer Animal Health.)

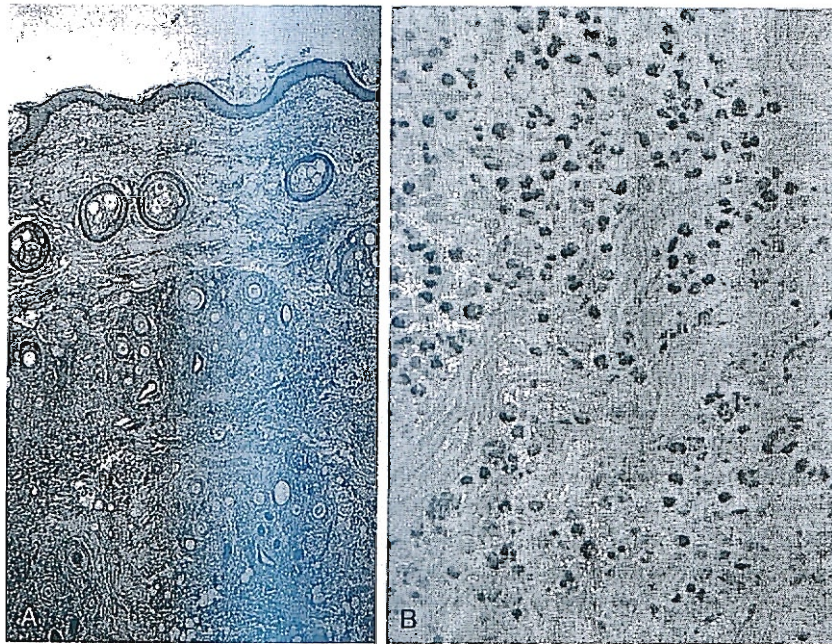


Figure 6-17 Deposition of fibrous tissue in deep pyoderma presents a barrier to drug penetration. (Photo courtesy Bayer Animal Health.)

of aminoglycosides, which depend on active transport into bacterial organisms. The aerobic component (i.e., facultative anaerobes) of a mixed infection may also be resistant to aminoglycoside therapy because the oxidative transport systems of such organisms (e.g., *E. coli*) may shut down in an anaerobic environment. Drugs that target cell walls, and beta-lactams in particular, are less effective in a hyperosmolar environment, which might occur as inflammatory debris accumulates and osmotic destruction of organisms is reduced.

Host response to infection and its impact on antimicrobial therapy may vary with the organ system infected. For example, in respiratory tract infections, mucus produced by the host can

directly interfere with antimicrobial therapy. Aminoglycoside efficacy may be decreased by chelation with magnesium and calcium in the mucus. Antimicrobials may bind to glycoproteins, and mucus may present a barrier to passive diffusion. In addition, some antimicrobials may alter the function of the mucociliary apparatus, either by increasing mucous viscosity or by decreasing ciliary activity (e.g., tetracyclines).

Changes in the health of the host can lead to changes in drug disposition that can result in lower than anticipated PDCs (see Chapter 2).⁵⁰ The volume to which a drug is distributed can be affected by the fluid compartments, which vary with age, species, and hydration status. Distribution to target organs can be affected profoundly by cardiovascular

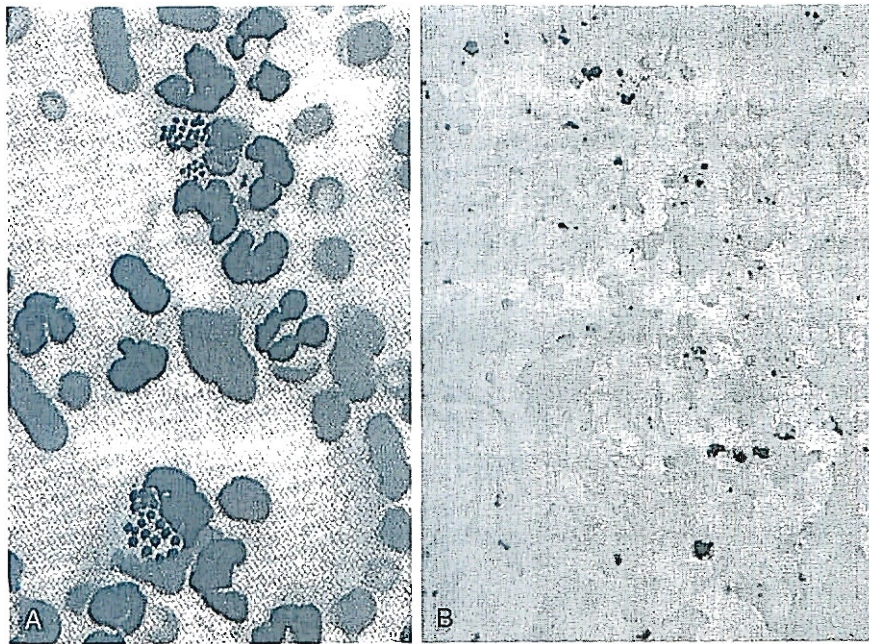


Figure 6-18 The intracellular location of organisms presents a barrier to drug penetration. Some organisms are obligate intracellular organisms, whereas others, such as *Staphylococcus* spp., demonstrated cytologically (A) and by special stain of infected skin (B), may survive phagocytosis, serving to reinfect tissue once the phagocytic white blood cell has died.

responses, particularly in the shock patient. Elimination of the drug must be considered when selecting antimicrobials for the critical patient. Changes in glomerular filtration cause parallel changes in renal excretion of drugs. Serum creatinine concentrations should be used to modify doses or intervals of potentially toxic drugs that are excreted renally (see Chapter 2).¹³¹ Likewise, severe changes in hepatic function may indicate selection of an antimicrobial drug not dependent on hepatic function for activation or excretion.

Host Factors That Facilitate Drug Efficacy

Host factors may also facilitate antimicrobial efficacy. Among the most important host factors are local and systemic defenses ranging from compounds that directly target microbes to healthy tissues that provide mechanical barriers and a competent immune system. The role of host defenses are beyond the scope of this chapter but cannot be underemphasized.

Other host factors that facilitate therapy include the accumulation of the drug in active form at the site of infection, which may facilitate antimicrobial efficacy and decrease the risk of resistance. Obvious examples include drugs that undergo renal or biliary excretion. For such drugs urine or bile concentrations (respectively) may exceed PDC thirtyfold to several hundredfold (see the discussion of treatment of urinary tract infections, Chapter 8). Another site of drug concentration is the phagocytic leukocyte (WBC), both in peripheral circulation and at the site of inflammation. Active concentrations of some antimicrobials (e.g., macrolides, lincosamides, and FQs) may increase concentrations 20 to 100 or more times the PDC.^{28,132-137} Phagocytic accumulation may facilitate

treatment of intracellular infections (e.g., *Brucella* spp., cell wall-deficient organisms, intracellular parasites, and facultative intracellular organisms such as *Staphylococcus* spp.). Thus drugs that achieve only bacteriostatic concentrations in plasma may become bactericidal inside the cell, particularly against organisms that locate and survive inside cells (Figure 6-18). Additionally, accumulated drug released by dying phagocytes at the site of infection may increase concentrations to which the infecting microbe is exposed. Accumulation of drug inside WBC has been assumed as an explanation of the disconnect of azithromycin efficacy in pulmonary infections despite low PDCs.⁹¹ Note, however, that drug accumulation does not necessarily enhance drug efficacy. Often, the accumulated drug is sequestered into subcellular organelles, where it cannot reach the organism. In addition, the drug may become otherwise inactivated once inside the cell. The different mechanisms of action of these drugs may not occur in an anaerobic environment, and concentrations by the WBCs might be impaired in an anaerobic environment. The FQs are an example of a class of drugs whose uptake by WBCs is facilitated in an acidic environment; these drugs are distributed throughout the cytosol, where they remain active. The drug will leave the WBCs and enter a drug-free environment and thus may facilitate drug concentrations at the site of infection. Phagocytic WBCs with accumulated enrofloxacin delivered drug to inflamed tissue cages in dogs, demonstrating that accumulation may increase therapeutic response.¹³⁷ Drugs that do not accumulate in WBCs include the beta-lactams, aminoglycosides, and metronidazole. Drugs that are moderately accumulated in WBCs include chloramphenicol (onefold to fivefold) and selected sulfonamides (threefold to fivefold).¹³⁸

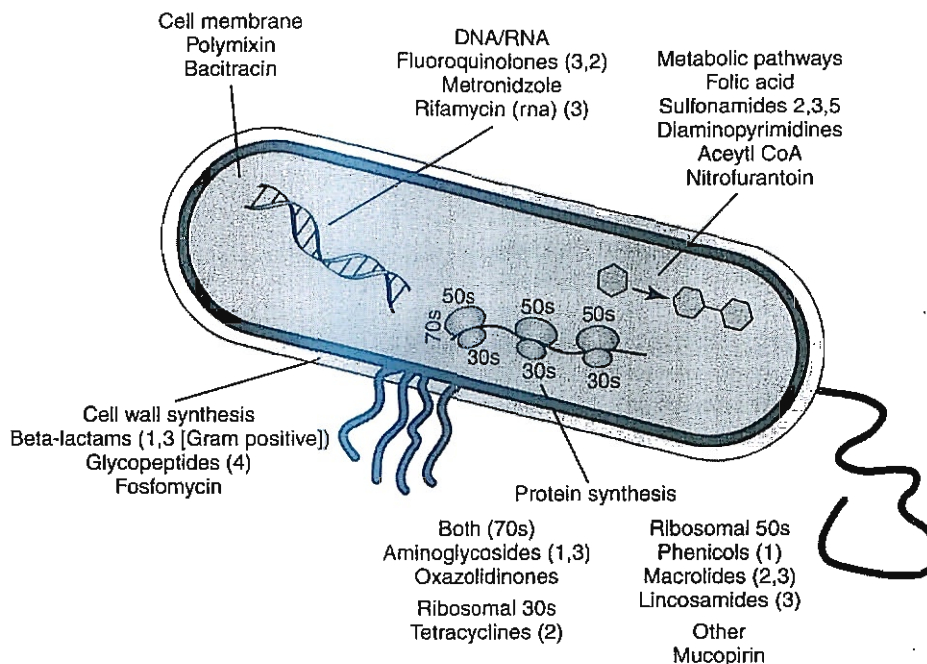


Figure 6-19 Targets of antimicrobial actions for the different classes of antimicrobial drugs. The number in parentheses refers to the major mechanism(s) of acquired resistance (other mechanisms also exist; see Chapter 7): 1 = enzymatic destruction (e.g., beta-lactamases for beta-lactams, acetylases for phenicols); 2 = increased efflux pump activity (may be associated with altered porin influx in gram-negative isolates); 3 = altered targeted site (e.g., mutations in DNA gyrase for fluoroquinolones or penicillin-binding proteins for gram-positive isolates); 4 = interfering protein and 5 = increased production of targeted metabolite. Decreased porin size is a common mechanism of resistance associated with increased efflux pump activity for many gram-negative isolates.

Another potential facilitating host factor is infection at a site that is topically accessible. In such situations several 1000-fold concentrations of the MIC may be reached with topical administration. The rationale for collecting C&S data for such infections might be controversial, but identification of the organism and some indication of susceptibility is prudent, particularly if initial therapy fails.

DRUG FACTORS THAT AFFECT ANTIMICROBIAL EFFICACY

Mechanisms of Drug Action

Knowledge of the mechanism of action (see Figure 6-19) of a particular antimicrobial is important for several reasons:

1. The mechanism of action of a drug determines whether the antimicrobial can act in a bactericidal or bacteriostatic manner (assuming proper concentrations are achieved at the tissue site; see previous discussion). Drugs that are capable of bactericidal effects at therapeutic doses are listed in Table 6-7.
2. The mechanism of action may determine whether or not the drug is concentration-dependent or time-dependent, which will impact the design of the dosing regimen.
3. The therapeutic efficacy of some antimicrobials can be impaired by host factors that alter the mechanism of action of the drug. Knowledge of the mechanism of action will facilitate anticipation of therapeutic failure.
4. The mechanism of antimicrobial action often reflects the mechanism of resistance. Identifying mechanisms by

which resistance might be avoided or minimized requires an appreciation of these mechanisms of action.

5. Understanding or anticipating selected host toxicities associated with antimicrobials can be improved by understanding their mechanism of action.
6. Understanding antimicrobial mechanisms of action provides a basis for the selection of antimicrobials to be used in combination. Such drugs should be selected on the basis of mechanisms of action that complement rather than antagonize one another (see Combination Antimicrobial Therapy section).

The cell wall is an important target for several antimicrobials, protecting the hypertonic intracellular environment of the organism from the hypotonic extracellular environment.²³ A variety of proteins located in the cell wall (penicillin-bound proteins) are important in the formation of the cell wall during division of growth of the organisms. These proteins are the target of several antimicrobial agents. Destruction of the peptidoglycan layer, which provides support to the cell wall, increases the permeability of the cell wall to the hypotonic environment, resulting in osmotic lysis of the cell. Intracellular structures are also major targets for various antimicrobial agents. Binding of ribosomes, the site of protein synthesis in the cell, can either inhibit protein formation or result in the formation of faulty proteins that eventually prove detrimental to the organism. The nuclear material of microbes is another target: Interference with cellular DNA inhibits cellular division, as well as initial cellular functions. Generally, impaired DNA synthesis results in cell death. Other intracellular targets

include selected metabolic pathways such as folic acid synthesis, which, when interfered with, prevents formation of materials vital to the microorganism.

Drug Disposition

Absorption

Care must be taken when selecting the antimicrobial that the disposition of the drug meets the needs of the patient (see earlier discussion of host factors). The availability of drug preparations determines drug selection in many instances because not all drugs are available for administration by all routes. To maximize plasma and thus tissue drug concentrations, intravenous administration is the preferred route for critically ill patients or difficult-to-penetrate tissues, with intramuscular and subcutaneous administration being second and third choices, respectively. Oral administration of antimicrobials, however, is preferred for long-term use, for nonhospitalized patients, and when drug therapy is targeting the gastrointestinal tract.

Note that although a drug may be 100% bioavailable after oral administration (i.e., the drug is completely absorbed), the rate of absorption may be sufficiently slow that the peak effect is minimized (although the duration of drug in circulation may be prolonged). Efficacy may be impaired, particularly for organisms with a high MIC or for concentration-dependent drugs. Slow-release preparations, either orally or parenterally administered, should be used cautiously because prolonged absorption (controlled rate of release) may be so slow that therapeutic concentrations are not achieved. The risk of resistance may be increased in such situations. Although slow-release products might improve compliance for time-dependent drugs, their use may also preclude shorter duration therapy. Topical administration is the sole route for drugs that are too toxic to the host to administer systemically. Care must be taken, however, with drugs applied to skin whose surface has been damaged. Sufficient drug absorption may occur to render the patient at risk of developing toxicity. Drugs applied to the ear canal may be ototoxic, particularly in the presence of a perforated tympanic membrane.

Distribution

Once in circulation, the antimicrobial must distribute well to target tissues (i.e., the site of infection). The principles determining drug distribution to and from tissues are discussed in Chapter 1, and movement of each antimicrobial is discussed in Chapter 7. Whereas sinusoidal capillaries, found primarily in the adrenal cortex, pituitary gland, liver, and spleen, present essentially no barrier to drug movement. Fenestrated capillaries such as those located in kidneys and endocrine glands contain pores (50 to 80 nm in size) that facilitate movement between plasma and interstitium. Because the ratio of capillary surface area to interstitial fluid volume is so large, unbound drug movement from plasma into the interstitium occurs very rapidly in these tissues.^{139,140} Continuous capillaries, such as those found in the brain, CSF, testes, and prostate, present a barrier of endothelial cells with tight junctions.¹³⁹ Muscle, lungs, and adipose tissue also contain continuous

capillaries.¹³⁹⁻¹⁴¹ Therapeutic antimicrobial failure in a number of body systems in humans has been associated with failed drug penetration, including soft tissue infections, osteomyelitis, prostatitis, otitis, endocarditis, ocular infections, peritonitis, and sinusitis.¹⁴¹

KEY POINT 6-26 Interstitial fluid concentrations often parallel plasma concentrations for tissue with fenestrated capillaries. The same is not likely to be true for other tissues or in the presence of host or microbial factors that impair effective drug movement.

Models for detection of drugs in tissues focus, appropriately so, on interstitial (extracellular) concentrations.^{141,142} Methods that measure concentrations in tissue homogenates (including both intracellular and extracellular fluid) do not accurately represent interstitial concentrations. Extracellular fluids can be collected by a variety of methods, although a major limitation is the volume of fluid that can be collected. Detection of drug in fluids is often based on methods that require at least 1 mL or more of fluid. Of these models, those that are based on ultrafiltration techniques appear to be most accurate representations of extracellular fluid in the normal animal.¹⁴³ Tissue cages that contain an inflammagen are reasonable methods to study the impact of inflammation on drug distribution.¹³⁷ Determination of drug in tissues protected by specialized barriers is difficult, generally requiring anesthesia.¹⁴⁴ If concentrations are compared with plasma, data must be based on the entire time versus concentration curve (i.e., AUC, C_{max}) rather than single-point comparisons because drug does not distribute immediately into tissues. Care must also be taken to address the impact of protein binding, as can be demonstrated for cefovecin, a drug that is 90% to 99% bound to serum protein. Total serum concentrations are markedly higher than that in extracellular fluid because the latter contains less protein.⁴⁶

Doses for drugs generally should be higher when treating infections in tissues with continuous capillaries, particularly for water-soluble drugs. Comparison of MIC data with tissue drug concentrations may be useful when designing dosing regimens for such tissues.

Examples of different distribution patterns might be predicted somewhat based on V_d (Box 6-4; see also the section on antimicrobial drugs in Chapter 7). Although the V_d of a drug does not indicate to which tissues drug is distributed, it can be used to approximate likelihood of tissue penetration in that a lipid-soluble drug is more likely than a water-soluble drug to move beyond extracellular fluid. Urine and the central nervous system (CNS) offer two divergent examples of tissue penetration. Urine is easy to target by drugs that are renally eliminated. Other components of the urinary tract, such as the kidney and particularly the prostate, can, however, be more difficult to penetrate. Antimicrobial therapy of the CNS is very difficult, although success may be facilitated by inflammation, which enhances drug penetration. However, once

Box 6-4

Tissue Distribution Pattern of Selected Drugs

Drugs Distributed to Extracellular Fluid ($V_d \leq 0.34$ L/kg)

Beta-lactams
Aminoglycosides

Drugs Distributed to Total Body Water ($V_d \geq 0.6$ L/kg)

Chloramphenicol
Clindamycin
Doxycycline/minocycline
Erythromycin
Fluorinated quinolones
Sulfonamides/trimethoprim

Drugs Concentrated in Urine

Beta-lactams
Aminoglycosides
Fluorinated quinolones
Sulfonamides/potentiated sulfonamides
Vancomycin

Drugs Concentrated in Bile

Clindamycin
Doxycycline/minocycline
Macrolides (erythromycin)
Rifampin

Drug Penetration of the Blood-Brain Barrier**Drugs that readily enter the cerebrospinal fluid (CSF)**

Chloramphenicol
Doxycycline/minocycline (unbound)
Fluorinated quinolones (for some organisms)
Metronidazole
Rifampin
Sulfonamides/trimethoprim

Drugs that enter the CSF in the presence of inflammation

Penicillins
Selected cephalosporins (e.g., cefotaxime, ceftriaxone, ceftazidime)
Fluorinated quinolones
Vancomycin

Drugs that do not enter the CSF

Aminoglycosides
Carbenicillin
Cephalothin
Cefazolin
Cefotetan
Clindamycin
Erythromycin
Tetracycline

Drugs that Accumulate in White Blood Cells

Clindamycin
Erythromycin (macrolides)
Fluorinated quinolones
Rifampin

inflammation resolves, drug distribution may again decrease. The blood-brain or CSF barrier represents a particularly challenging site because it not only prevents movement of antimicrobials into the CNS but also actively transports out or

destroys some antimicrobials (i.e., penicillins and selected cephalosporins) (see Box 6-4). Care must be taken even with tissues normally characterized by excellent blood flow. For example, distribution of beta-lactams, aminoglycosides, and selected sulfonamides into bronchial secretions is generally <30% of that in plasma (see Chapter 8).^{130,145,146}

Lipid-soluble antimicrobials should be used for infections that are more difficult to treat, including those associated with tissue reaction or those caused by intracellular organisms, and when the site of infection presents a distribution barrier. Tissue distribution of aminoglycosides and most beta-lactam antimicrobials is limited to extracellular fluid; in contrast, many other antimicrobials (e.g., FQs, macrolides, and trimethoprim/sulfonamide combinations) are distributed well to all body tissues, including the prostate gland and eye. Enrofloxacin approximates or surpasses unity with plasma in many tissues.¹⁴⁴ Imipenem (or meropenem), trimethoprim/sulfonamide, and FQs can achieve bactericidal concentrations for some infections in the CNS (particularly organisms with a low MIC); chloramphenicol will achieve bacteriostatic concentrations.¹⁴⁷ Accumulation of antimicrobials in WBCs facilitates treatment of intracellular infections.¹³²⁻¹³⁷

Protein binding of a drug to plasma proteins may affect antimicrobial efficacy both in the patient and in vitro as data supporting drug selection and dose design are generated. Only unbound drug is pharmacologically active (see impact on ceftiofexin).⁴⁵ In vivo, bound drug is retained in the vasculature; once in the interstitial fluids or inside the cell, the drug may again be bound and inactivated. In vivo C&S testing and determination of MIC occur in the absence of protein. Further, PK on which MIC_{BP} is based (C_{max} being a major consideration) frequently is based on total drug, rather than the fraction of unbound. For a drug insignificantly protein bound, this disconnect is generally not significant. However, as the fraction of bound drug increases, C&S testing may markedly overestimate efficacy by the proportion of drug that is bound (i.e., a drug that is 50% protein-bound will actually yield an "active" C_{max} that is 50% of the total). Clearance and V_d may be underestimated. Attempts should be made to base therapeutic decisions on unbound drug.^{130,148}

Drug movement into bacteria must also be considered. The roles of drug pK_a and the environmental pH of a target tissue on drug efficacy have already been addressed. Ionization may impair drug movement through the LPS for drugs that passively move through this layer.

Drug Elimination

The route through which the drug is eliminated is an important consideration for two reasons. First, if the site of infection is also a route of elimination for that drug, higher drug concentrations can be expected at the site. Second, if the drug is toxic to an organ of elimination, use of the drug should be avoided if the organ is already diseased. Also, if the drug is toxic to any tissue, the drug should be used cautiously in the presence of disease of the organ of elimination or dosing regimens should be appropriately modified.

Nonantimicrobial Effects of Antimicrobials

A number of antimicrobials influence various aspects of the immune system. The phagocytosis of drugs (e.g., macrolides, lincosamides, and FQs) was previously discussed.^{23,132-134,138} In addition to accumulation in WBCs, antimicrobials can influence WBC function. However, the effect can be variable. The negative effect of antimicrobials on phagocytic function has been well established, although the clinical relevance of this effect is less clear.¹⁴⁹ Functions that are targeted include chemotaxis (increased, decreased, or unchanged by clindamycin, erythromycin, chloramphenicol, and lincomycin and decreased or unchanged by gentamicin), phagocytosis (increased by erythromycin and chloramphenicol and decreased by tobramycin and polymyxin B), oxidative burst (increased by clindamycin, cefotaxime, and quinolones and decreased by cefotaxime, trimethoprim/sulfonamides, chloramphenicol, and erythromycin), bacterial killing (increased by cefotaxime and decreased by sulfonamides and aminoglycosides), and cytokine production or activity (interleukin 1 [IL-1] increased by cefotaxime and cefaclor and IL-10 by erythromycin; IL-1 and tumor necrosis factor decreased by cefoxitin, erythromycin, and ciprofloxacin).¹³⁸ Apoptosis of neutrophils may be accelerated.¹⁵⁰

The clinical relevance of these potentially beneficial effects on phagocyte function is not clear, but relevance is supported by some studies. For example, long-term use of azithromycin appears to improve lung function in children with cystic fibrosis and is increasingly being included in its therapeutic regimen; the disease appears to progress more rapidly if azithromycin is not added to therapy. This effect of macrolides appears to target inflammation, because the effect occurs at concentrations below the MIC of the infecting organisms. Potential mechanisms include a reduction in IL-1 β , IL-8, and neutrophils in bronchoalveolar lavage fluid.^{151,152} In addition to the antiinflammatory effects, macrolides appear to decrease *Pseudomonas* virulence by reducing the number of pili, thus altering adherence to tracheal epithelium, altering membrane proteins, and decreasing alginate formation.^{153,154}

KEY POINT 6-27 Selected antimicrobials facilitate therapeutic success through immunomodulation or their ability to decrease virulence of the infecting microbe.

Antimicrobial Effects of Nonantimicrobial Drugs

Antimicrobial effects have been described for a number of nonantimicrobial drugs at plasma concentrations achieved when the drug is used for noninfective indications. For example, a number of phenothiazines, including those with antihistaminergic effects, are antibacterial. Because these effects occur both in vitro and in vivo, the effects cannot be attributed simply to immunomodulation. Chlorpromazine is antimycotic at concentrations much higher than can be achieved safely in plasma, but its accumulation over a hundredfold in macrophages containing phagocytized pathogens facilitates effective therapy at recommended doses.¹⁵⁵ The less psychotically active thioridazine enhances the antimycotic activities of

rifampin and streptomycin; between 2 and 3 months of use has been promoted as adjuvant therapy. Trifluoperazine and prochlorperazine inhibit *S. aureus* at concentrations of 10 to 50 $\mu\text{g}/\text{mL}$ and selected other microbes (*Shigella*, *Vibrio*) at the same or higher concentrations and have demonstrated inhibitory effects in an animal model.^{156,157} Selected cardioactive drugs, including oxyfedrine and dobutamine, exhibit antimicrobial effects, again toward selected microbes.¹⁵⁸ Amlodipine has broad antibacterial efficacy at concentrations as low as 5 to 10 $\mu\text{g}/\text{mL}$, with *S. aureus* being the most susceptible and gram-negative organisms (*E. coli*, *Klebsiella*, and *Pseudomonas*) requiring higher concentrations.¹⁵⁹ Other drugs with demonstrated antimicrobial effects include the antispasmodic drug dicyclomine¹⁶⁰ and selected nonsteroidal antiinflammatories.¹⁶¹ Among the dietary supplements with recognized antibacterial effects are the flavones. Flavone dietary supplements exhibited antibacterial activity to a variety of microbes in a mouse infection model.^{162,163} Chitosans have demonstrated efficacy toward a number of bacterial organisms, particularly gram-negative isolates at concentrations as low as 0.05 $\mu\text{g}/\text{mL}$.¹⁶⁴ Several antifungal drugs have antibacterial properties, which are addressed in Chapter 9.

Adverse Drug Events and Antimicrobials

Actions that minimize host toxicity enhance therapeutic success. However, host cells are eukaryotic, whereas the bacteria are prokaryotic. As such, targets of antibacterial therapy are sufficiently different from mammalian cells that, as a class, antibacterials (but not antifungals) tend to be safe. For example, beta-lactam antibiotics are among the safest antimicrobials because they target cell walls, a structure not present in mammalian cells. Often, even if cellular structures are present in both microbe and host, differences in the structure will result in different antimicrobial binding properties. For example, sulfonamides and FQs tend to be safe because the antimicrobials have a much greater affinity for the bacterial target enzymes than the mammalian enzymes. As with other drugs, the incidence of predictable (type A) drug reactions to most antimicrobial therapy correlates with maximum or peak PDC. However, aminoglycoside-induced nephrotoxicity and ototoxicity are an exception; toxicity tends to be related to duration of exposure and is more likely if minimum or trough PDCs are above a maximum level.^{76,165,166} Occasionally, toxicity of antimicrobials does reflect their mechanism of action, if the microbial target occurs in mammalian cells and is structurally similar (see Chapter 7). For example, colistin and polymyxin target both microbial and host cell membranes. Administration of either drug is associated with a high incidence of nephrotoxicity (probably because drug is concentrated in renal tubular cells), and subsequently their use generally is limited to the topical route of administration. Drugs that inhibit protein synthesis by binding to ribosomes (e.g., tetracyclines, chloramphenicol) may cause (limited) antianabolic effects in the host at sufficiently high doses. For most antimicrobial drugs, host toxicity may occur through mechanisms unrelated to its mechanism of action, but as a result of targeting structures in host cells. Aminoglycosides cause nephrotoxicity and

ototoxicity, not because of their ribosomal inhibition (their antibacterial mechanism of action) but because they actively accumulate in renal tubular (or otic hair) cells (as they do in bacterial organisms) and in lysosomes causing lysosomal disruption. Topical application is more likely to cause ototoxicity with aminoglycoside and other drugs (see Chapters 4 and 7). FQs cause retinal degeneration in cats, through mechanisms yet to be defined. Tilmicosin causes (potentially lethal) beta-adrenergic stimulation; the caustic nature of doxycycline can cause esophageal erosion in cats. Allergies are a less common adverse reaction caused by antimicrobials. Some drugs cause anaphylactoid reactions as a result of direct mast cell degranulation. True allergic reactions should be differentiated from anaphylactoid reactions (more common with intravenous administration of FQs). The latter may occur with the first dose and may be dose dependent. Anaphylactoid reactions can be minimized by administration of a small first dose before therapy. In contrast, drug-induced allergies generally require previous administration or a duration of therapy sufficient to allow antibody formation to the drug, which acts as a hapten (generally 10 to 14 days). Few drug allergies have been documented in animals. Among the most notorious are reactions to the potentiated sulfonamides.

KEY POINT 6-28 Because antimicrobial targets are prokaryotic and hosts are eukaryotic, adverse events seldom reflect the mechanism of antimicrobial activity.

Among the adverse reactions associated with antimicrobial use are those associated with drug interactions. Those most clinically relevant involve drug metabolizing enzymes. Examples of drugs that inhibit the metabolism of other drugs are the macrolides; chloramphenicol; and for selected drugs, the fluoroquinolones. In contrast, rifampin is an inducer. Increasingly, drugs that alter drug metabolizing enzymes are emerging as drugs that compete for or alter drug transport proteins (e.g., P-glycoprotein). Drug interactions involving antimicrobials are discussed with each class (see Chapter 8).

Adverse reactions to antimicrobials may reflect their antimicrobial success. Many orally administered drugs cause disruption of normal gastrointestinal microflora (see previous discussions). For example, the author has detected emergence of *Clostridium perfringens* in dogs treated with fosfomycin. *Streptococcus* spp. are generally associated with opportunistic infections. However, infections caused by members of this genus (*S. pyogenes* in humans and *Streptococcus canis* in animals) are associated with streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis (NF).¹⁶⁷ These syndromes appear to reflect the presence of lysogenic bacteriophage-encoded superantigen genes encoded in the bacterial organisms.¹⁶⁷ The superantigen genes are powerful inducers of T-cell proliferation; the presence of the superantigens then causes release of host cytokines in quantities that may be sufficient to cause lethal effects. In one study a bacteriophage-encoded streptococcal superantigen gene was identified in the majority of *S. canis* isolates. Induction of these genes can lead

to bacterial lysis and subsequent release of proinflammatory and other destructive cytokines. Indeed, use of the FQs has been associated with STSS and NF in dogs (see Chapter 7).¹⁶⁸

Release of endotoxin is another example of seeming therapeutic success potentially leading to therapeutic failure (Figure 6-20). However, the clinical relevance of endotoxin release may be species dependent. Endotoxin release is a side effect of antimicrobials that occurs with therapeutic success, and it may influence antimicrobial selection for the patient infected with a large number of gram-negative organisms.⁸⁴ Endotoxins cause further release of cytokines and other mediators of septic shock (see Chapter 8). Most of these effects are mediated by the inner lipid A component of the LPS molecule that becomes exposed after antimicrobial therapy. In human patients suffering from endotoxic shock, outcome of antimicrobial therapy has been related to plasma endotoxin levels. A number of antimicrobials cause release of endotoxin from gram-negative organisms. Attempts have been made to correlate the amount of endotoxin released to the class of antimicrobial and specifically to its mechanism of action.

Continued bacterial growth or rapid cell lysis and death have been suggested as important criteria for endotoxin release after antimicrobial therapy. In contrast, the rate of bacterial killing and antimicrobial efficacy do not appear to be related to the rate and amount of endotoxin release. The amount of endotoxin release varies among the antimicrobial classes and even within the classes. Release can be related to mechanism of action. Among the drugs traditionally used to treat septicemia, aminoglycosides have been associated with the least and beta-lactams with the greatest endotoxin release (with imipenem or meropenem causing the least amount of endotoxin release among the beta-lactams).¹⁶⁹ The different amounts of endotoxin released by beta-lactams may reflect different affinities of the drugs for different penicillin-binding proteins. In vitro studies indicate that those beta-lactam antibiotics that specifically bind penicillin-binding protein (PBP)-3 are associated with endotoxin release, whereas those that bind PBP-2 cause little to no endotoxin release.¹⁷⁰ The difference may reflect the fact that PBP-3 appears to form a complex with PBP-1, 4, and 7;¹⁷¹ binding of PBP-3 might thus affect a larger component of cell wall synthesis compared to binding of another PBP. The release of endotoxin by quinolones varies depending on the study. However, in a study of mouse *E. coli* peritonitis, imipenem (or meropenem) and ciprofloxacin caused less endotoxin release than did cefotaxime.⁸⁴ Selected third-generation cephalosporins also appear to be associated with less endotoxin release: In a study of septicemic patients with acute pyelonephritis, the amount of endotoxin released did not differ among cefuroxime, ciprofloxacin, or netilmicin and each was deemed safe in the septicemic patient.¹⁷²

KEY POINT 6-29 As a class, aminoglycosides are associated with the least and beta-lactams (excluding carbapenems and selected later generation cephalosporins) the most endotoxic release.

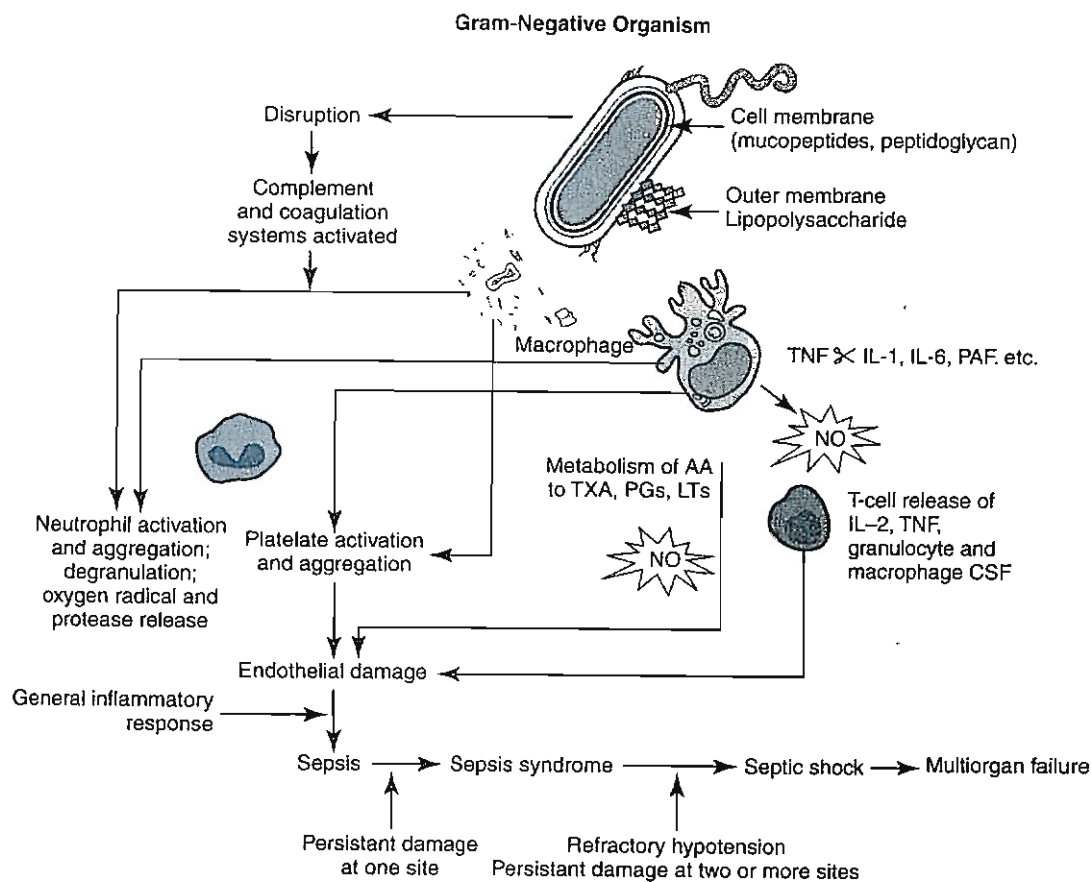


Figure 6-20 Among the adverse reactions of antimicrobial therapy is release of bacterial toxins. The risk of damage to the host is greater with a large inoculum. In this example, rapid death of gram-negative organisms can result in rapid release of endotoxin. Drugs whose mechanism results in osmotic lysis (e.g., penicillins) are more likely to be associated with sufficient endotoxin release to cause harm to the patient.

The release of endotoxin may also be dose (concentration) dependent. For example, endotoxin release is greater at half the recommended dose of ciprofloxacin (3 mg/kg versus 7 mg/kg ciprofloxacin) according to the previously described model.⁸⁴ Actions that might minimize the sequelae of endotoxin release after antimicrobial therapy have not been established. Presumably, administering a dose more slowly may decrease the rate of endotoxin release. Binding and subsequent inactivation of endotoxin by antimicrobials have been documented, particularly for cationic antimicrobials (e.g., quinolones, aminoglycosides, and polymyxin).^{84,173}

ENHANCING ANTIMICROBIAL EFFICACY

Selecting the Route

Drugs may be selected on the basis of their route of administration. Not all drugs are available for parenteral or oral administration. Parenteral, and particularly intravenous, administration is indicated for life-threatening infections or whenever tissue concentrations must be maximized. Parenteral drugs are also indicated for the vomiting animal. Oral drugs are indicated for long-term use, outpatient therapy, and treatment of gastrointestinal tract illness. Topical therapy may be selected to enhance drug delivery while minimizing

toxicity. Topical therapy with lipid-soluble drugs might, however, best be limited to situations in which systemic therapy of the same drug is implemented, thus preventing development of subtherapeutic drug concentrations in tissues other than the site of topical application, as might occur if topical administration alone is implemented.

Designing the Dosing Regimen

Antimicrobial therapy must be implemented in a timely fashion. An effective dose of antimicrobials administered at the first appearance of a clinical infection has a much greater therapeutic effect than therapy initiated a week later; in critical care patients, hours can mean the difference between patient recovery or death. Dosing recommendations printed on the label generally might be followed for recently approved drugs; however, exceptions occur, particularly for older drugs as we learn more about optimizing antimicrobial therapy and identify changing patterns of susceptibility. In general, to maximize efficacy, doses should be increased particularly for serious or chronic infections, tissues that are difficult to penetrate, or infections associated with detrimental changes at the site of infection. Product labels may not reflect new findings regarding antimicrobial efficacy because pharmaceutical companies may choose not to incur the costs associated with

gaining approval for a new label that reflects the new dosing regimen. Dose modification beyond that on the label should be based on C&S data, current literature, and clinical signs of the patient. Adverse reactions also should be considered. Although antimicrobials are safe as a class, several are associated with dose- or duration-dependent adversities, and client counseling with informed consent is indicated when off-label dosing presents potential harm to the patient.

The approach taken to determine a dosing regimen for a patient depends on the information that is available—that is, how much is needed (PD) and how much is achieved (PK) (Table 6-4). In each instance it is assumed that patient factors are well known.

A target C_{max} can be calculated from MIC data that have been adjusted for time or concentration dependency. The dose of a drug administered intravenously is calculated as dose = target concentration $\times V_d$. For orally administered drugs, the V_d must be corrected for bioavailability (F): dose = target concentration $\times V_d/F$ (see Chapter 1). For antimicrobials the target concentration, or “what is needed,” is the MIC of the infecting microbe or a reasonable surrogate, such as the MIC₉₀, modified as needed to account for host, drug, or microbial factors. For a concentration-dependent drug, the MIC or MIC₉₀ must be multiplied tenfold to achieve the targeted PDI $C_{max}/MIC \geq 10$. Thus for amikacin, a concentration-dependent drug, the targeted PDI for a patient infected with an *E. coli* with an MIC of 4 $\mu\text{g}/\text{mL}$ is 40 $\mu\text{g}/\text{mL}$. If infection is in extracellular tissue and concentrations that are lower than in plasma are anticipated, the target C_{max} plasma may need to be multiplied by 2 or more to achieve the target in tissues. Thus the target becomes 80 $\mu\text{g}/\text{mL}$.

For amikacin the reported V_d in dogs is 0.23 L/kg. Assume an infection is in the lungs, where drug concentrations reach 50% of PDC. The dose of amikacin to target a microbe causing infection in the lungs then would be 4 $\mu\text{g}/\text{mL}$ (mg/L) $\times 10 \times 2 \times 0.23 \times \text{L}/\text{kg}$ or 18.4 mg/kg. If the drug is given by a route other than intravenous, the dose must be modified further for bioavailability. For example, if amikacin is generally about 70% bioavailable (F=0.7) following subcutaneous administration, the subcutaneous dose for *E. coli* would be (4 $\mu\text{g}/\text{mL}$ (mg/L) $\times 10 \times 2 \times 0.23 \times \text{L}/\text{kg}$)/0.7 = 27 mg/kg. As the MIC for this *E. coli* and amikacin was quite low, next consider the same approach for a *P. aeruginosa* with an MIC of 16 $\mu\text{g}/\text{mL}$. If the infection is in the upper respiratory tract (e.g., sinus of a cat), distribution will probably be <30% of that in plasma (multiply dose by 3). The calculated dose would be 16 mg/L $\times 10 \times 3.3 \times 0.23 \text{ L}/\text{kg}$ or 121 mg/kg. This is well beyond the recommended dose, and although it might be safe given once daily in a normal patient (drug concentrations would reach the target trough of 2 $\mu\text{g}/\text{mL}$ by 6 to 12 hours after dosing), the risk of adversity may outweigh the benefits of treatment with this dose. Combination therapy is indicated for this patient.

The design of a dosing regimen for a time-dependent antimicrobial is more complicated. For a time-dependent drug, the magnitude of C_{max} depends on how many half-lives are to elapse between doses. The ratio of C_{max}/MIC is important for determining the number of half-lives that can elapse before

PDC = MIC. A good start is to multiply the MIC fourfold ($C_{max}/MIC = 4$) to allow a duration of two half-lives for $T > MIC$ (each doubling of the ratio or dose provides another half-life of $T > MIC$). The duration of the dosing interval then depends on the desired duration of $T > MIC$. For $T > MIC = 50\%$, the duration of the dosing interval is twice the number of half-lives that $T > MIC$; in this case, ($C_{max}/MIC = 4$), the dosing interval will be 4 half-lives. Although this sounds adequate, in reality, it may not be for drugs with a short half-life. For example, if the drug of interest is amoxicillin (half-life = 1 to 1.5 hr), the duration of the interval is 4 to 6 hrs, assuming all drug in plasma makes it to the site of infection. Thus, the ratio of C_{max}/MIC may need to be higher for drugs with a short half-life if a convenient dosing interval is desired. Alternatively, a drug with a longer half-life can be chosen. Using cefpodoxime as an example, based on package insert data, the MIC₉₀ of *S. intermedius* is 0.5 $\mu\text{g}/\text{mL}$. Peak concentrations at 10 mg/kg approximate 15 $\mu\text{g}/\text{mL}$, yielding a ratio C_{max}/MIC of 30. The time that elapses before C_{max} and MIC reach unity is just under 5 half-lives (30 to 15 to 7.5 to 3.5 to 1.75 to 0.75). With a half-life of 4.5 hours, $T > MIC$ duration approximates 24 hours. Theoretically, if the target is $T > MIC = 50\%$, a dosing interval of 48 hours might be possible. However, the PDI upon which time and concentration dependency are based are limited to a 24 hr period, thus a 24-hr-dosing interval is prudent. This is particularly true if the drug is targeting tough-to-penetrate tissues or inflammatory debris: the concentration might then be reduced to 10 $\mu\text{g}/\text{mL}$, yielding a C_{max}/MIC of 10, or a duration of 2 half-lives, or 9 hours, for $T > MIC$. In this situation, a 24-hour dosing interval might be more appropriate; a 12-hour dosing interval might be prudent. Further, these calculations are based on a target of $T > MIC$ of 50%. Although this target is often recommended, $T > MIC$ of 75% to 100% might be better to minimize the risk of resistance, particularly in a patient at risk. Therapeutic drug monitoring can be used to establish or confirm a dose or interval for a drug for the individual patient and is ideally the basis of dose modification for critical patients. Unfortunately, few drugs (primarily the aminoglycosides and vancomycin) can be rapidly and accurately measured at a reasonable cost. The risks associated with these drugs, including the potential cost of using them at ineffective doses, however, may justify the cost.

Duration of Therapy

Among the most difficult decisions regarding antimicrobial therapy is the duration of administration. Generally, the duration of therapy should be 2 to 3 days beyond resolution of clinical signs. Indeed, if the dosing regimen is designed according to the saying “dead bugs don’t mutate,” then clinical signs of resolution should emerge rapidly. This is true, however, only if the clinical signs are discreet and able to respond rapidly. Such is not likely to be true in the absence of fever, or when radiographic resolution of inflammation or healing of inflamed skin are benchmarks. Not surprisingly, clinicians often adhere to the “longer is better” approach. However, emerging data in human medicine suggest a more pro-active approach to therapy duration reduction is prudent. Animal models have demonstrated that therapy beyond 5 days increases the intensity

Table 6-8 Examples of Synergistic Drug Combinations

Drug One	Drug Two	Organisms
Dicloxacillin	Ampicillin, penicillin, cephalothins	<i>Escherichia coli</i> , <i>Klebsiella</i> , <i>Pseudomonas aeruginosa</i>
β -Lactam: cephalothin, ampicillin, piperacillin, cefotaxime, cefamandole	Aminoglycoside: gentamicin, amikacin	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , enterococci, others
Chloramphenicol	Ampicillin	<i>Salmonella typhimurium</i> , <i>Staphylococcus aureus</i> (effect is bacteriostatic in nature)
Penicillin	Gentamicin	<i>Bacteroides melaninogenicus</i>
Imipenem	Vancomycin	<i>Staphylococcus aureus</i>
β -Lactam, vancomycin	Aminoglycoside	<i>Staphylococcus aureus</i>
Trimethoprim/sulfonamide	Imipenem, amikacin	<i>Nocardia asteroides</i> (effect is bacteriostatic)
Imipenem	Trimethoprim/sulfonamide, cefotaxime	<i>Nocardia asteroides</i> (effect is bacteriostatic)
Ethambutol	Rifampin, aminoglycosides, ciprofloxacin (enrofloxacin), clarithromycin	<i>Mycobacterium avium</i> (effect is bacteriostatic)

From Wiedemann B, Atkinson BA: Susceptibility to antibiotics: species incidence and trends. In Lorian V, editor: *Antibiotics in laboratory medicine*, Baltimore, 1996, Williams & Wilkins, pp. 900-1168.

of drug therapy necessary to prevent emergent resistance.¹⁷⁴ In human medicine a number of clinical studies have investigated the impact that reduced duration of therapy might have on efficacy and resistance. In general, the longer-is-better approach is not appropriate.¹⁷⁵ Five days of therapy has been suggested as the upper limit in selected populations, including intrabdominal infections,^{175,176} community-acquired pneumonia,¹⁷⁷ and other respiratory tract infections,¹⁷⁸ and 3 days for pneumonia characterized by a low likelihood of becoming nosocomial.¹⁷⁹ These studies demonstrate the increasing focus on the role of duration of therapy in the advent of resistance. However, their extrapolation to companion animals is not clear, in part because compliance differences might affect results. Exceptions for which duration of therapy might be longer include infection of sites characterized by poor local immunity (or the immunocompromised patient), tissues in which healing is prolonged, or in the presence of foreign bodies that facilitate antimicrobial growth. Exceptions also may apply to slow growing organisms.

KEY POINT 6-30. For uncomplicated infections the duration of therapy should be 5 to 7 days or less.

Combination Antimicrobial Therapy

Combination therapy can be used to achieve a broad antimicrobial spectrum for empirical therapy, treat a polymicrobial infection involving organisms not susceptible to the same drugs, reduce the likelihood of antimicrobial resistance, and reduce the risk of adverse drug reactions by minimizing doses of potentially toxic antimicrobials.^{23,24,26,137} Rational combination antimicrobial therapy may be the single most effective action taken to enhance antimicrobial efficacy for the chronic or serious infection. Primary reasons to avoid combination therapy include increases in risk of suprainfection, risk of toxicity (if both drugs are potentially toxic), high cost, and inconvenience to the patient.²⁴

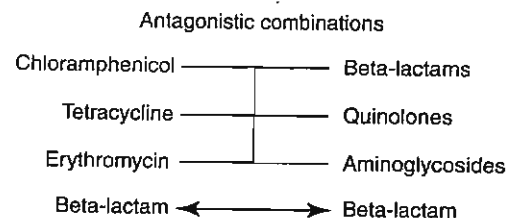


Figure 6-21 Combining antimicrobials can have different sequelae. Antagonistic antimicrobial combinations most commonly result when a drug that inhibits bacterial growth is combined with a drug whose action depends on rapid cell growth. Drugs that act at the same site may be antagonistic, additive, or synergistic (e.g., beta-lactams, depending which penicillin binding protein is targeted).

Synergism and Antagonism

Antimicrobials to be used in combination therapy should be selected rationally and based on target organisms as well as on mechanism of action (Table 6-8). Combinations might result in antagonistic, additive, or synergistic antimicrobial effects (Figure 6-21).¹⁸⁰ Generally, these effects are defined by in vitro systems; clinical relevance is more difficult to establish. Also, the combined effects of two or more antimicrobials are likely to differ with the organism. Avoidance of *antagonism* is particularly important for patients with inadequate host defenses.^{23,24,26,180} In general, bacteriostatic drugs that inhibit ribosomes and thus microbial growth (e.g., chloramphenicol, tetracyclines, erythromycin) should not be combined with drugs whose mechanism of action depends on protein synthesis such as growth of the organism (e.g., beta-lactams) or formation of a target protein. The bactericidal activity and continued degradation or destruction of the microbial target of beta-lactams and FQs depend on continued synthesis of bacterial proteins. Antagonistic effects have been well documented between beta-lactam antibiotics and inhibitors of ribosomal activity. The degree of antagonism between FQs and growth inhibitors is controversial; antagonism has been

reported with the use of ciprofloxacin and chloramphenicol,¹⁸⁰ but impaired efficacy was not detected in other studies.¹⁸¹ Antagonism between chloramphenicol and gentamicin has also been documented.¹⁸⁰ Occasionally, the combination of a bacteriostatic ribosomal inhibitor and a drug whose efficacy depends on rapid growth might enhance efficacy, even though the “-cidal” drug will act only in a “static” fashion. For example, chloramphenicol enhances the efficacy of ampicillin toward *Salmonella typhimurium* and *Staphylococcus* spp., presumably because it inhibits the production of beta-lactamases by the organisms that might otherwise destroy ampicillin.

KEY POINT 6-31 The appropriate combination of two drugs characterized by resistance may render the microbe susceptible.

Chemical antagonism is also possible between two or more antimicrobials (see Chapter 2).^{181,182} Aminoglycosides and quinolones are chemically inactivated by penicillins at sufficient concentrations. Ticarcillin has been used therapeutically to reduce the risk of toxicity in a patient overdosed with an aminoglycoside.¹⁸³ Chemical antagonism is unlikely in most clinical uses of these drugs. The risk of antagonism is increased, however, with simultaneous intravenous use of high doses of both ticarcillin and aminoglycosides, such as might occur if aminoglycosides are administered once daily. Potential chemical interactions between other antimicrobials should be identified before combination therapy. Certainly, antimicrobials should not be mixed in the same syringe or intravenous line unless a lack of antagonism has been confirmed.¹⁸²

Drugs that have the same mechanism of action may act in an additive or synergistic fashion. For example, chloramphenicol and clindamycin bind the same 50S ribosomal subunit and will antagonize each other. 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) if there is scientific support. One study indicates an *in vitro* synergistic effect of the combined use of doxycycline and azithromycin against *P. aeruginosa*.^{183a}

Additive effects probably occur when active metabolites are produced from an active parent compound, such as metabolism of enrofloxacin to ciprofloxacin.¹⁸⁴ Antagonistic effects might occur, however, if the drugs compete for a limited number of target sites (e.g., chloramphenicol and erythromycin). In contrast, synergistic actions might occur if the antimicrobial targets are subtly different. For example, a combination of different beta-lactams generally results in additive antimicrobial activity. If the two antimicrobials target different PBPs, however, their combined effect may actually be synergistic (“double beta-lactam therapy”).^{185,186} In contrast, combinations of other beta-lactam antibiotics (including combining selected cephalosporins) are antagonistic.¹⁸⁶ The different sequelae of combined beta-lactam therapy might be caused by the PBPs targeted by each drug.

Synergism between antimicrobials can occur if the two antimicrobials kill bacteria through independent mechanisms

or through sequential pathways toward the same target.^{180,187} The combination of trimethoprim and a sulfonamide exemplifies synergism resulting from sequential actions in the same metabolic pathway (see discussion of potentiated sulfonamides) (see Chapter 7). Clavulanic acid “draws” the beta-lactamase activity of the microorganism away, allowing the protective beta-lactam to impair cell wall synthesis. Synergism between beta-lactams and aminoglycosides exemplifies synergism resulting from killing by independent pathways. Synergism is expected because their mechanisms of action complement one another, but efficacy is enhanced further because aminoglycoside movement into the bacteria is enhanced by increased cell wall permeability induced by the beta-lactam (Figure 6-22).^{180,188} Indeed, aminoglycoside activity against enterococci is adequate only when used synergistically with a cell wall-active antimicrobial, such as beta-lactams and vancomycin. Synergism also has been demonstrated against 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 an MRSA, presumably because of induction of an aminoglycoside-modifying enzyme. Enhanced movement in a bacteria may occur with other drugs (e.g., potentiated sulfonamides, FQs) when combined with beta-lactams (see Figure 6-22). Rifampin is another drug for which combined use enhances antimicrobial efficacy of a number of drugs.

Combination therapy is a powerful tool for enhancing efficacy (Figure 6-23) as well as preventing resistance. Occasionally, the combination of drugs, which by themselves would not be expected to have efficacy against organisms not included in their spectrum, may exhibit efficacy against the organisms. For example, azithromycin and clarithromycin may exhibit synergistic effects with several other drugs against *P. aeruginosa*. When studied in patients with cystic fibrosis, the most active combinations demonstrating synergy were azithromycin combined with sulfadiazine/trimethoprim or doxycycline. Azithromycin occasionally demonstrated synergism against *P. aeruginosa* when combined with timentin, piperacillin/tazobactam, ceftazidime, meropenem, imipenem, ciprofloxacin, trovafloxacin, chloramphenicol, and tobramycin.¹⁸⁹ In the treatment of *S. aureus*, clindamycin inhibits early rapid killing of amikacin but acts synergistically with it at 24 to 48 hours.¹⁹⁰

Polymicrobial Infections

Combination antimicrobial therapy may be selected because of the presence of a polymicrobial infection (Figure 6-24).^{23,24,77,191} Aminoglycosides or FQs are often combined with beta-lactams, metronidazole, or clindamycin to target both aerobic gram-positive and gram-negative infections or infections caused by both aerobes and anaerobes. The combined use of selected antimicrobials may result in therapy effective against a given microbe when either drug alone was ineffective.

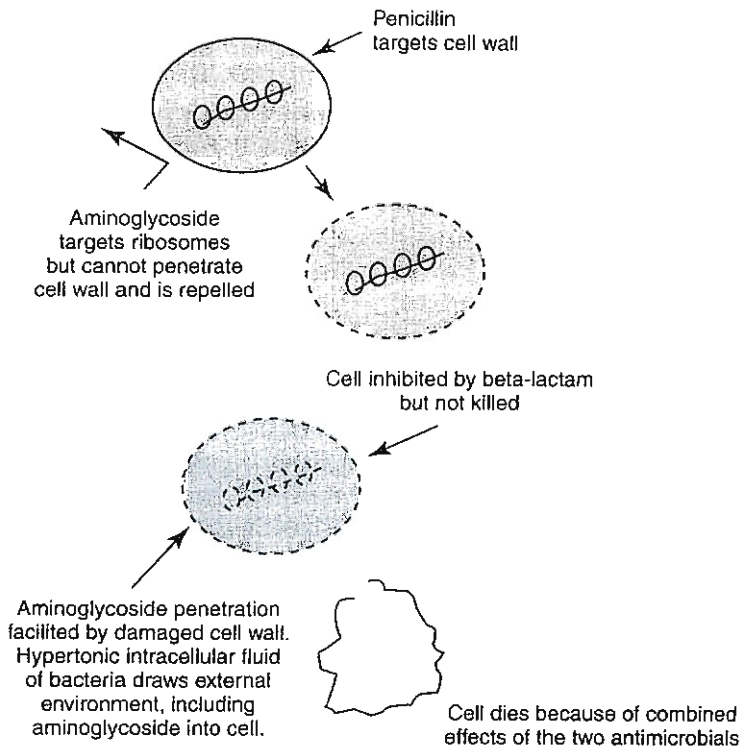


Figure 6-22 The combination of any number of drugs with a beta-lactam may result in synergistic antimicrobial effects. The prototypic example is a beta-lactam combined with an aminoglycoside, a class of water-soluble drugs whose movement through the cell to target ribosomes is limited. Changes in the cell wall permeability associated with the beta-lactam exposes the hypertonic (compared with the host) intracellular cytoplasm to the isotonic host, resulting in the influx of solutes into the organism. Intracellular access is thus facilitated for drugs also in the environment. Together, the two drugs are now more likely to kill the microbe. Such synergism has been documented *in vitro* between beta-lactams and a number of drugs, particularly those classified as bactericidal.

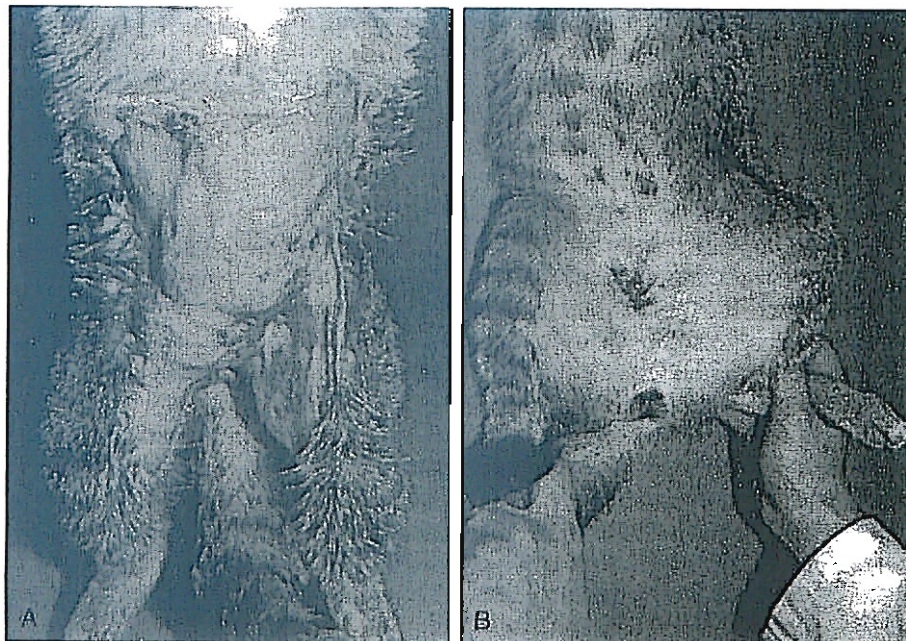


Figure 6-23 Atypical mycobacterium in a cat is associated with marked inflammation, including deposition of fibrous tissue. This cat was successfully treated with a combination of sulfadiazine/trimethoprim and enrofloxacin after 3 months of therapy.

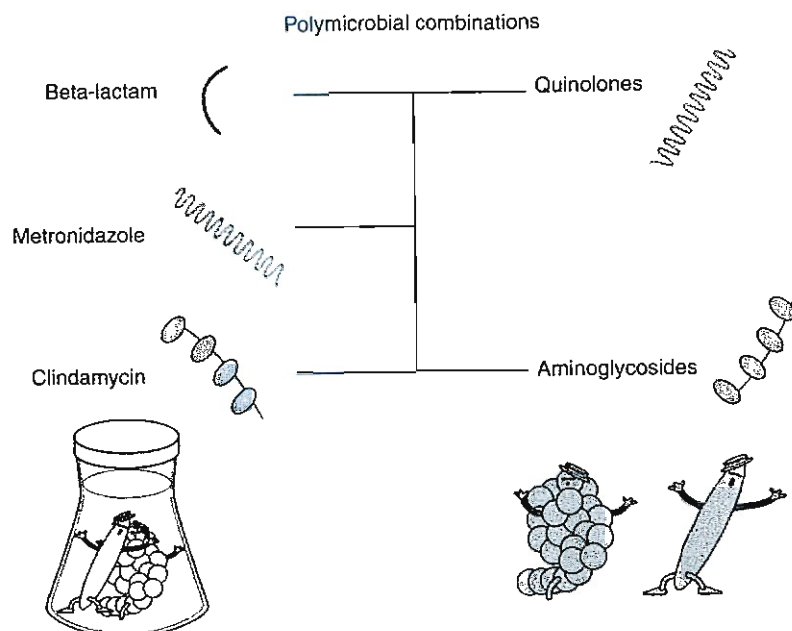


Figure 6-24 Polymicrobial infections may require combination therapy. The quinolones and aminoglycosides offer excellent aerobic gram-negative coverage; the beta-lactams (especially penicillins), metronidazole, and clindamycin offer excellent gram-positive and anaerobic coverage.

ANTIMICROBIAL PROPHYLAXIS

The prophylactic use of antimicrobials should be distinguished from treatment. The presence of infection or anticipated infection after bacterial contamination (e.g., an open fracture, contamination of abdominal contents with intestinal fluid) indicates the need for treatment rather than prophylaxis. If antimicrobial prophylaxis is to be implemented in anticipation of an invasive procedure (e.g., surgery), the following should serve as a basis for selection: The antimicrobial should target the most likely pathogenic organism, adequate concentrations of drug should be at the site of invasion before potential contamination, the antimicrobial should either have a long elimination half-life or be redosed during lengthy procedures, the least toxic drug should be selected, and the duration of therapy should be as short as possible.^{23,26}

Prophylactic antimicrobials should not be used indiscriminately in the immunocompromised animal. The granulocytopenic patient is particularly predisposed to the development of suprainfection. Suprainfection occurs in 10% to 20% of human granulocytopenic patients receiving empirical broad-spectrum antimicrobials. Prolonging therapy increases the chance that suprainfection will occur.²⁶ Prophylactic suppression of gastrointestinal flora is recommended in human patients who are profoundly granulocytopenic for more than 2 weeks. Traditional use of nonabsorbable antimicrobials effective against aerobic gram-negative organisms (e.g., neomycin) and drugs that target anaerobic organisms (e.g., metronidazole) are being replaced by use of trimethoprim/sulfonamide combinations or FQs.²⁶ Trimethoprim/sulfonamide combinations are more palatable and less expensive, yet they are equally effective in preventing infections when compared with more expensive drugs in human critically ill patients. FQs

allow persistence of anaerobic organisms in the gastrointestinal tract, thus reducing overgrowth of resistant gram-negative organisms and preventing rapid repopulation and overgrowth of aerobic gram-negative organisms as the antimicrobial is discontinued.

Other indications for medical prophylaxis include dentistry and prevention of recurrent, chronic infections (e.g., urinary tract, skin). The use of antimicrobials prophylactically for these conditions is discussed separately in the corresponding chapter.

SURGICAL PROPHYLAXIS*

Antimicrobial prophylaxis is defined as the administration of an antimicrobial agent in the absence of infection. The aim of antimicrobial prophylaxis is to reduce the number of viable bacteria present in the surgical wound to a level that normal host defenses can handle, thus preventing infection. Contaminating bacteria can enter the surgical wound from exogenous sources or the patient's endogenous flora. Exogenous sources include surgical equipment, the surgery room, and surgical personnel. Duration of the surgical procedure plays a role in the incidence of wound infections, especially for procedures that last longer than 90 minutes.

Endogenous bacterial sources probably play a greater role in postoperative infections than exogenous sources. Endogenous sources include skin and mucosal surfaces that are transected during surgery. Hematogenous spread of bacteria may result from overt or occult septic foci or dental manipulations.

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Such sources should be either eliminated before surgery by appropriate therapeutic antimicrobial agents or avoided by not combining dental manipulations with surgery of body cavities (abdominal or thoracic) or orthopedic procedures.

Antimicrobial prophylaxis is not a substitute for good surgical practices, which include aseptic technique and gentle tissue handling. Considerations in the use of antimicrobial prophylaxis are the type of surgery, potential pathogens encountered, host competence, and pharmacologic and antibacterial properties of the antimicrobial agent.

Type of Surgery

Surgical wounds are classified as clean, clean-contaminated, contaminated, or dirty. Clean wounds are made under aseptic conditions, are closed primarily, and are not drained. Prophylactic antimicrobial therapy is not warranted for most clean procedures because bacterial contamination is minor, and the patient's competence helps prevent wound infection. Possible indications for the use of antimicrobials in clean surgical procedures are when the consequences of infection would be catastrophic (e.g., total joint replacement) or when surgical implants are used.

Clean-contaminated wounds include those made in the gastrointestinal, genitourinary, or respiratory tract without significant intraoperative spillage. Also, clean procedures in which a break in sterile procedure occurred are considered clean-contaminated. Clean-contaminated wounds may benefit from prophylactic antimicrobial therapy, and consideration of the following factors seems appropriate when contemplating the use of perioperative antimicrobial therapy: number of resident bacteria encountered, amount of spillage expected, and impact of disease condition on bacterial colonization. Resident bacterial numbers vary depending on the site of the tract incised and the nature of disease. In the normal gastrointestinal tract, resident bacteria are numerous in the oropharyngeal cavity, distal ileum, and colon. Numbers are normally much lower in the distal esophagus, stomach, and most of the small intestine. The normal genitourinary tract above the distal urethra has low bacterial populations. The normal trachea and bronchi also have relatively sparse flora. Although amount of spillage cannot always be predicted preoperatively, prophylactic antimicrobials are probably indicated if the risk of intraoperative spillage seems high. Diseases, in general, tend to modify both bacterial numbers (usually increased numbers) and populations (usually more virulent forms).

Contaminated wounds include those in which there is acute, nonpurulent inflammation or those in which gross contamination from a hollow viscus occurs. Antimicrobial prophylaxis is generally warranted when surgery is performed on contaminated wounds. Also, the presence of extensive tissue damage or accumulation of blood within wounds may warrant prophylactic drug administration, because bacterial colonization is usually promoted.

Dirty or infected wounds benefit from irrigation with antiseptics. Chlorhexidine (0.05%) is an effective wound disinfectant for infected wounds. Use of antimicrobials (systemically, topically, or both) is generally indicated before surgery to treat

an infected or dirty wound. Such use is more appropriately termed *therapeutic antimicrobial therapy*.

Potential Pathogens Encountered

The most frequently encountered pathogenic bacterial contaminants of surgical wounds are *Staphylococcus* spp. and *E. coli*. The most common skin bacteria are *Staphylococcus* spp., although many other organisms may be present as transient, topical flora. The oropharynx has a mixed population of gram-positive organisms (especially *Staphylococcus* spp., *Streptococcus* spp., and *Actinomyces pyogenes*), gram-negative organisms (*Proteus*, *Pasteurella*, *Pseudomonas*, and *E. coli*), and anaerobic organisms. The stomach and small intestine have very few organisms normally present, whereas the distal ileum and large intestine have large numbers of gram-negative (especially *E. coli* and *Klebsiella*, *Pseudomonas*, and *Salmonella* spp.) and anaerobic organisms. Potential pathogens encountered in the genitourinary tract include both gram-positive and gram-negative organisms (especially *Staphylococcus* and *Streptococcus* spp., *E. coli*, and *Proteus* and *Pseudomonas* spp.). Pathogens of the respiratory tract (especially lower respiratory tract) include both gram-positive organisms (*Staphylococcus* spp., *Streptococcus* spp., and *A. pyogenes*) and gram-negative organisms (*Pseudomonas* spp., *E. coli*, and *Klebsiella*, *Pasteurella*, and *Enterobacter* spp.).

Host Competence

Host resistance may be compromised systemically or locally. Patients with systemic immunodeficiency often have chronic, recurrent, or partially responsive infections. Prophylactic antimicrobial therapy is probably indicated for such patients regardless of the surgical procedure to be performed. Secondary immunodeficiencies have been associated with a variety of diseases, including hepatic or renal failure, hyperadrenocorticism, diabetes mellitus, and neoplasia. Other factors that may affect systemic host competence include advanced age, severe malnutrition, obesity, immunosuppressive drugs, and splenectomy.

Local factors of importance in the maintenance of host competence include tissue perfusion and tissue trauma. The competence of local defense mechanisms may be affected adversely by obstruction, neoplasia, ulceration, and hemorrhage. For example, the bacterial flora of a stagnant loop of jejunum caused by intestinal obstruction resembles that of the normal distal ileum (i.e., large numbers of resident bacteria). For the purposes of selecting perioperative antimicrobials, the clinician should accurately assess host competence before the surgical procedure.

Pharmacologic and Antibacterial Properties

The primary goal to be achieved by administration of prophylactic antimicrobial agents is to produce adequate concentrations of antimicrobial at the surgical incision site at the time of wound contamination. Also important is the concept that the major risk of contamination is at the time of surgery until a fibrin seal develops between wound edges (approximately 3 to 5 hours postoperatively). Factors of importance in the use of

perioperative antimicrobials are absorption (timing and route of administration), distribution, and elimination characteristics. Absorption issues are of least concern with intravenously administered antimicrobials. For most antimicrobials distribution is relatively rapid and complete within 30 to 60 minutes after intravenous administration. The concentration of drug achieved in the tissue correlates with the concentration of free drug in the serum. Highly protein-bound drugs (i.e., little free drug in the serum) achieve lower tissue concentrations than do weakly bound agents (e.g., cefazolin, gentamicin, and ampicillin). Other factors such as lipid solubility, pH, and local environment may also influence tissue penetration of the drug. Elimination of most antimicrobials is principally by way of the kidneys. The rate of elimination determines the dosing interval that is selected. More rapidly eliminated drugs require more frequent administration. Cefazolin, for example, should be administered at 2-hour intervals during the surgical procedure to maintain adequate tissue and serum levels.

The following prophylactic antimicrobial regimen seems appropriate: an intravenous dose of drug given 30 to 60 minutes before incision (i.e., at anesthetic induction) and another dose given at the completion of the procedure. If the surgical procedure lasts longer than 3 hours, an additional intraoperative dose of antimicrobial should be given approximately 2 to 3 hours after the initial dose. There is no rationale for continuing antibiotic administration longer than 24 hours after surgery in the absence of documented infection. If infection is documented, therapeutic antimicrobial therapy is initiated.

The selected drug should be bactericidal for the pathogens that are most likely to contaminate the surgical site. First-generation cephalosporins (e.g., cefazolin) are generally as effective as and less expensive than second- and third-generation cephalosporins. Surgery of the lower gastrointestinal tract may require a more elaborate schedule of prophylactic drug administration, partly because of the presence of anaerobic organisms. A second-generation cephalosporin (e.g., cefoxitin) or an aminoglycoside/anaerobic combination (e.g., amikacin and clindamycin or gentamicin and amoxicillin) should be administered systemically. The use of oral antimicrobials for prophylaxis may not be prudent, in part, because peak concentrations are likely to be less than with intravenous administration, even if bioavailability is close to 100% (and many are not).

Inappropriate perioperative antimicrobial use has been shown to increase the incidence of complications. Examples of inappropriate perioperative antimicrobial use include use of antimicrobials for clean surgical procedures, initiation of prophylactic antimicrobials postoperatively, and continuation of antimicrobial administration for longer than 24 hours. Each of these actions risks the occurrence of one or more of the following complications: reduced efficacy, suprainfection, selection of resistant bacterial pathogens, greater client cost, and a potential for higher incidence of drug-associated complications.

Although surgical prophylaxis has been integrated into the perioperative surgical plans for veterinary patients, surprising little information supports its use. In one controlled study

of dogs ($n = 329$) and cats ($n = 544$) undergoing clean and clean-contaminated surgical procedures, the postoperative infection rate did not differ in placebo (9.4%) compared with the cephalexin-pretreated group (8.9%).¹⁹² In another study investigating the impact of flushing in dogs undergoing total ear canal ablation, organisms were characterized by a higher incidence of antimicrobial resistance to cefazolin,²⁹ suggesting that cefazolin may not be a rational choice in all presurgical candidates.

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