CHAPTER 94

GRAM-NEGATIVE INFECTIONS

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KEY POINTS

- Lipopolysaccharides are the major constituents of the outer membrane of most gram-negative bacteria.
- Endotoxin is a lipopolysaccharide and potent stimulator of the inflammatory response; it is believed to initiate the pathology of gram-negative sepsis.
- Immunosuppression, hospitalization, invasive procedures, and prior antimicrobial administration are suspected risk factors for colonization and infection with multidrug-resistant gram-negative bacteria.
- Increasing rates of antibiotic resistance among gram-negative pathogens threaten the effectiveness of empiric antibiotic therapy in veterinary medicine.
- Antimicrobial selection should be guided by culture and susceptibility results, especially for patients that do not respond to empiric therapy.
- Initial antimicrobial therapy for first-time gram-negative infections includes a β-lactam/β-lactamase inhibitor, alone or in combination with a fluoroquinolone.
- Therapy for life-threatening infections caused by resistant or nosocomial gram-negative pathogens should include a third-generation cephalosporin, extended-spectrum β-lactam/β-lactamase inhibitor, and/or an aminoglycoside
- Fourth-generation cephalosporins, aztreonam, and polymyxin are prescribed for select highly resistant gram-negative infections in human hospitals. Relevance to treatment in dogs and cats is not established and caution should be exercised.

Infections resulting from gram-negative organisms are a significant cause of morbidity and mortality in critically ill patients.[1-3] Important aerobic or facultatively anaerobic gram-negative infections are often due to an opportunistic invasion by commensal intestinal flora, including *Escherichia coli, Proteus* spp., *Pseudomonas aeruginosa,* and *Klebsiella* spp.[2,4] Less commonly, opportunistic infections are caused by environmental saprophytes that enter the body through wounds or the respiratory tract .[5]

GRAM-NEGATIVE CELL STRUCTURE AND PATHOGENICITY

In addition to having a cytoplasmic membrane and peptidoglycan layer similar to that found in gram-positive organisms, gram-negative bacteria possess unique factors that contribute to their ability to cause disease.[1,2,5] Among the bacterial products commonly implicated in the pathogenesis of gram-negative infections is endotoxin, a unique lipopolysaccharide (LPS) that accounts for 75% of the outer surface of the gram-negative cell membrane.[5,6] The role of LPS in triggering the cellular and physiologic host response is well established.[5-8]

Structurally, endotoxin consists of an outer polysaccharide chain that is bound to lipid A.[2] Although it is buried deep in the bacterial cell wall, lipid A is known to be the toxic moiety of endotoxin.[5,8] Lipid A induces a wide range of proinflammatory responses (i.e., release of cytokines and activation of the compliment cascade) and endothelial dysfunction.[5-9] During minor or local infections with small numbers of bacteria, small amounts of LPS are released, leading to controlled cytokine production. The cytokines released promote body defenses by stimulating inflammation, fever, and appropriate protective immunologic responses.[5,8] However, during severe systemic infections with large numbers of bacteria, increased amounts of LPS are released, resulting in excessive, and sometimes maladaptive, cytokine production by monocytes and macrophages (see Chapter 6).[2] Harmful effects of endotoxin include vasodilation, enhanced vascular permeability, tissue destruction, and activation of coagulation pathways.[5,7,9]

Gram-negative organisms also possess cellular structures that often are recognized as virulence factors.[2,5,6,9,10] Flagella are protein filaments that extend from the cell membrane and allow for locomotion. They undulate in a coordinated manner to move the bacteria toward or away from a chemical gradient, a process called *chemotaxis.* Pili (also called *fimbriae)* are straight filaments arising from the bacterial cell wall and most often serve as adherence factors, in which case they are referred to *adhesins*.[10] For many bacteria, adhesins are vital to their ability to cause disease. Capsules are protective walls, generally composed of simple sugar residues that surround the cell membranes.[5,10] Encapsulation enhances virulence by preventing bacterial phagocytosis by host neutrophils and macrophages.[10]

Failure to contain or eradicate gram-negative pathogens may result in further damage due to the inexorable progression of inflammation and infection.[8-10] Thus, of the many therapeutic interventions, early initiation of appropriate antimicrobial therapy is critical to ensure a favorable outcome.[1,11]

IDENTIFICATION OF GRAM-NEGATIVE BACTERIA OF MEDICAL IMPORTANCE

The classification of gram-negative bacteria is based on several criteria, including their appearance on selective media, use of carbohydrates (e.g., lactose), production of certain end products (e.g., acids and alcohols), and the presence or absence of specialized enzymes (e.g., oxidase).[5,10] Although the clinical relevance of these categories is a point for contention among clinicians, taxonomic schemes permit the microbiology laboratory to distinguish rapidly among commonly encountered bacteria.[5,10] For example, facultatively anaerobic oxidase-negative, gram-negative rods that grow on MacConkey agar are presumed to be members of the Enterobacteriaceae.[5,6,10] As more information accumulates, reclassification of bacteria among genera and species and the creation of new designations must be accepted as part of scientific progress.[3,11]

ENTEROBACTERIACEAE

Members of the family Enterobacteriaceae are the most frequently encountered gram-negative isolates recovered from clinical

specimens.[1,3,5] These commensal organisms are found in soil and water, on plants and, as the family name implies, within the intestinal tract of animals and humans.[2,5]

Before the advent of antimicrobials, chemotherapy, and immunosuppressive measures, the infectious diseases caused by the Enterobacteriaceae were relatively well defined and typically characterized by diarrhea and other gastrointestinal syndromes.[2,10] However, members of the Enterobacteriaceae now are incriminated in virtually any type of infectious disease and may be recovered from any tissue or fluid specimen submitted to the laboratory.[4-6,10] By definition, commensal organisms colonize an individual without causing disease. However, in a vulnerable host, these "pathogenic commensals" have the capacity to produce disease.[2,3,11] Generally, enhanced bacterial virulence factors or damage to the mucosal barrier or immune system of the host is required for infection to occur.[2,5,6,9,10] Critically ill and immunocompromised patients are susceptible to hospital-acquired infections after colonization with environmental strains or invasive procedures such as catheterization, endoscopy, and surgery.[9-11]

E. coli is the most commonly encountered bacteria in clinical microbiology laboratories and is thought to be the most important of the facultative aerobic gram-negative species that comprise the normal flora of the alimentary tract in most dogs and cats.* Most strains of *E. coli* are of low virulence, but they may cause opportunistic infections in extraintestinal sites.[1,2,6,10]

Pathogenic *E coli* have been classified based on their virulence properties into pathovars (e.g., enterotoxigenic, enteroinvasive, and uropathogenic strains). Such molecular typing may be performed to differentiate among different isolates.[2] However, this is not offered routinely by commercial veterinary laboratories and seldom warranted for *E. coli* isolates causing extraintestinal infection. *E. coli* organisms were previously susceptible to select drugs. However, multidrug-resistant (MDR) *E. coli* have emerged as a cause of opportunistic infections in companion animals.[2,4,12] The proportion of *E. coli* resistant to aminopenicillins, fluoroquinolones, and cephalosporins is increasing in human and veterinary medicine.[4,11,13,16] Indiscriminate use of antimicrobials, inadequate hygiene, and extended hospital stays are among the proposed reasons for resistance to these commonly used agents (see Chapter 175).[11-14]

Infections with serovars of *Salmonella enterica* are uncommon in dogs and cats. *S. enterica* can survive for relatively long periods in the environment, and transmission through food, water, or fomites contaminated by fecal material likely plays a role in disease pathogenesis.[15] The diagnosis of salmonellosis is traditionally made based on isolation of the organism in fecal samples, in conjunction with clinical signs and assessment for risk factors, such as hospitalization, age, and antibiotic exposure. Importantly, the prevalence of *Salmonella* spp. in canine fecal samples varies and does not correlate with clinical disease. Young dogs are more susceptible to infection and clinical illness.[15] Factors that increase susceptibility to salmonellosis include poor nutrition, anesthesia, overcrowding, concurrent disease, and prior or current antibacterial therapy. The severity of signs varies from none to subacute diarrhea and septic shock. Fever, lethargy, and anorexia may be followed by abdominal pain, vomiting, hemorrhagic diarrhea, and dehydration. Central nervous system (CNS) signs, polyarthritis, and pneumonia may be seen.[15] Only a small proportion of infected animals (less than 10%) die during the acute stages of salmonellosis. Recovered animals generally shed organisms for up to 6 weeks.[15] Salmonellosis is a zoonotic disease. Although most cases in humans are associated with food-borne outbreaks, dogs and cats are recognized as vectors for infection. Aggressive supportive care is the cornerstone of therapy. The use of plasma, probiotics, and polyclonal antiserum is controversial. Antimicrobial therapy is generally not advocated for uncomplicated *Salmonella* enteritis, as fecal shedding may be prolonged with injudicious antibiotic use. Antimicrobial therapy only is recommended for animals with severe extraintestinal signs. Aminopenicillins, chloramphenicol, and potentiated sulfonamides are generally effective, while aminoglycosides and carbapenems are reserved for immunosuppressed animals or those with overwhelming sepsis.[15]

Among the 16 species included in the genus *Enterobacter, Enterobacter aerogenes* and *Enterobacter cloacae* are the species most commonly encountered in clinical infections. *Enterobacter* species cause severe infections that can originate in virtually any body compartment. MDR *E. cloacae* have been recovered from the urinary tract, respiratory tract, and surgical wounds of veterinary patients.[2] *E. cloacae* strains are inherently resistant to amoxicillin, amoxicillin-clavulanate, narrow-spectrum cephalosporins, and cefoxitin. In addition, *E. cloacae* may acquire resistance to broad-spectrum β-lactams, especially when they are subjected to antibiotic pressure.[1] Carbapenems and fourth-generation cephalosporins (e.g., cefepime) are the most reliable antimicrobials for severe *Enterobacter* infections. Aminoglycosides, fluoroquinolones, and the potentiated sulfonamides are frequently, although less predictably, effective.[1] Third-generation cephalosporins frequently show in vitro activity against these organisms, but *Enterobacter* spp are known to develop resistance to these drugs during therapy. Thus third-generation cephalosporins are not considered drugs of choice. Because of the inherent resistance of *Enterobacter* strains to many antibiotics, and their propensity for resistance to develop during treatment, prompt submission of samples for culture and susceptibility testing is especially important for therapeutic success.

Klebsiella spp. are ubiquitous and may be regarded as normal flora in the alimentary canal, biliary tract, and pharynx in dogs and cats.[22] Of the pathogenic *Klebsiella*, *K. pneumoniae* is the most prevalent and clinically important; this is thought to be related to its large antiphagocytic capsule. Patients with *K. pneumoniae* infection frequently have predisposing conditions, including immunosuppression, indwelling devices, chronic respiratory disease, or extended hospital stays.[2,7] Although *K. pneumoniae* can cause severe pneumonia, it is more commonly the cause of hospital-acquired wound or urinary tract infections.[16] Antimicrobials with high intrinsic activity against *K. pneumoniae* include third-generation cephalosporins, carbapenems, and aminoglycosides. Fluoroquinolone susceptibility is less predictable. Extensive use of broad-spectrum antimicrobials in hospitalized patients has led to the development of resistant strains that produce extended-spectrum β-lactamases (ESBLs).[11,13] The bowel is the most common site of colonization, with secondary infection of the urinary tract, respiratory tract, peritoneal cavity, biliary tract, wounds, and bloodstream.[2,16]

Proteus includes five species. The most common clinical isolates are *Proteus vulgaris* and *Proteus mirabilis.* Both may be recovered from infected sites in immunocompromised hosts, and are associated with respiratory tract, wound, and genitourinary infections.[13] *Proteus* play an important role in urinary tract infections, utilizing fimbriae to mediate attachment to uroepithelium. *Proteus* organisms have the ability to produce urease and alkalinize urine by hydrolyzing urea to ammonia. This occasionally leads to precipitation of organic and inorganic compounds, promoting struvite stone formation. Most infections are caused by *P. mirabilis*. *P. vulgaris* is isolated less frequently, causing sporadic infections in hospitalized patients. The recovery of an indole-negative *Proteus* spp. can be identified presumptively as *P. mirabilis.* This is clinically important because different *Proteus* species differ in their susceptibility to different antibiotics. *P. mirabilis* isolates are typically more susceptible to antimicrobials

*References 2, 5, 6, 9, 10, 13.

than *P. vulgaris*. *P. mirabilis* is inherently resistant to tetracyclines, but unlike *P. vulgaris*, most strains are susceptible to aminopenicillins and first-generation cephalosporins. Susceptibility of *P. vulgaris* to fluoroquinolones is variable. Most veterinary isolates remain susceptible to sulfonamides and aminoglycosides. Rare strains of *P. vulgaris* possess inducible β-lactamases (not found in *P. mirabilis*) . Infections caused by these organisms may be treated with carbapenems and most third-generation cephalosporins.

Serratia species are widespread in the environment but are not common resident flora. *S. marcescens* is the primary pathogenic species responsible for surgical wound, urinary, and lower respiratory tract infections. Hospitalization is a well-described risk factor for infection. Mechanical ventilation and placement of intravenous and urinary catheters are known risk factors for *Serratia* infection in human ICUs, and *Serratia* bacilli have been linked to nosocomial infections in dogs and cats.[2,16] *S. marcescens* is naturally resistant to ampicillin, macrolides, and first-generation cephalosporins. The most reliable antimicrobials for *S. marcescens* infection are carbapenems and amikacin. There is increasing resistance to other aminoglycosides, including gentamicin and tobramycin. Fluoroquinolones are highly active against most strains. Definitive therapy should be based on results of susceptibility testing because MDR strains are common.

NONFERMENTING GRAM-NEGATIVE BACTERIA

The nonfermenting gram-negative bacteria are a group of aerobic, non–spore-forming bacilli that either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation.[2,5] Unlike the Enterobacteriaceae, the nonfermenting gram-negative bacilli do not fit conveniently into a single family of well-characterized genera, and the correct taxonomic placement of many of these organisms remains unresolved.[5,6]

Most often, gram-negative nonfermenters are niche pathogens that cause opportunistic infections in critically ill or immunocompromised patients.[2,11,16] Unlike the Enterobacteriaceae, gram-negative nonfermenters are intrinsically resistant to common antibiotics such as ampicillin, most cephalosporins, and macrolides.[2,11] These bacteria, most notably *Pseudomonas aeruginosa,* are also capable of rapidly acquiring resistance to other classes of drugs, and MDR is common.[3,11,16,17] Recent exposure to broad-spectrum antibiotics and invasive diagnostic procedures represent important risk factors for acquisition of these pathogens.[3,11,16]

P. aeruginosa is an obligate aerobic organism that is ubiquitous in the environment, particularly in decaying soil and vegetation.[2,18] It can be cultured from normal tissues of healthy animals, including the alimentary tract, urethra, nasal cavity, mouth, tonsils, upper airways, and conjunctivae. Purulent exudates with a grapelike odor are characteristic of this bacteria. *P. aeruginosa* is the prototypic opportunistic pathogen in that it seldom causes disease in immunocompetent animals but can cause serious infection in almost any tissue when immune function is impaired.[12,16,18,22,23] *P. aeruginosa* expresses a variety of virulence factors that confer resistance to a broad array of antimicrobial agents.[11] *P. aeruginosa* also possesses a repertoire of exotoxins and enzymatic products designed to evade host defenses.[2,3,17,18] In animals, it has been incriminated as the causative agent of many infections, including otitis, urethrocystitis, endocarditis, surgical wound infections, conjunctivitis, pneumonia, intravenous catheter site colonization, endocardial valve infections, prostatitis, and osteomyelitis.[2,18] Most strains are resistant to chloramphenicol and retain susceptibility to aminoglycosides, particularly amikacin. The fluoroquinolones once were considered effective for *P. aeruginosa* infections, but strains are increasingly resistant to this drug class.[11,17] Indeed, exposure to fluoroquinolones causes *P. aeruginosa* to develop resistance more rapidly than occurs with other bacteria.[1,2,11] Many strains also retain susceptibility to enhanced spectrum β-lactam/β-lactamase inhibitors (e.g., piperacillin-tazobactam and ticarcillin-clavulanate) Of the third-generation cephalosporins, only ceftazidime is active against *P. aerugenosa*.[19] Amikacin, prescribed in combination with an antipseudomonal β-lactam, is effective for most infections.[1,10,19,22] The traditional approach of combining a β-lactam with an aminoglycoside to treat *P. aeruginosa* infections is fundamentally based on in vitro synergistic activity and the potential to prevent emergence of resistance. Although in vitro studies support the use of a β-lactam/fluoroquinolone combination for the treatment of *P. aeruginosa*, development of MDR phenotypes during fluoroquinolone therapy has been documented. The carbapenems (imipenem and meropenem) are reserved for serious infections resistant to other antibiotics.[17] The monobactams (e.g., aztreonam) and fourth-generation cephalosporins (e.g., cefepime) are effective, albeit last-line antipseudomonal agents.[19] The sporadic use of polymyxin E (colistin) in humans is relegated to therapy of *P. aeruginosa* infections that are resistant to all other antibiotics.

Acinetobacter spp. (principally *A. baumannii)* have emerged during the past few decades as one of the most difficult nosocomial microorganisms to control and treat.[1,11] *Acinetobacter* spp. colonize multiple sites and can persist on environmental surfaces for extended periods.[2,3,17,20] *A. baumannii* is a water organism and preferentially colonizes moist environments. *A baumannii* colonization is particularly common in intubated patients or in patients that have multiple intravenous lines, surgical drains, urinary catheters, and monitoring devices. Although colonization with *Acinetobacter* always precedes infection, colonization does not always result in infection. The recovery of the organism from a nonsterile body site (e.g., endotracheal secretions) does not indicate or imply an infectious pathogenic role. When infection occurs, it usually involves organ systems that have a high fluid content. *A. baumannii* is an important cause of nosocomial pneumonia, and it increasingly is associated with infections of the urinary tract and peritoneal cavity .[2,3,11,20] Infections occur predominantly in select patients with risk factors such as mechanical ventilation, extended intensive care unit (ICU) stays, and prior antimicrobial use.[2,3,11,17,20] *A. baumannii* is intrinsically resistant to several classes of antimicrobials, and it is able to develop and transfer resistance quickly.[2,11,16] Cephalosporins and penicillins have little or no activity against *Acinetobacter*. Most isolates of *A. baumannii* are effectively treated with carbapenems (particularly meropenem), amikacin, minocycline, and colistin. Although carbapenems and aminoglycosides are considered to be the most effective agents, *A. baumannii* strains that possess aminoglycoside-modifying enzymes and carbapenemases are reported increasingly in human ICUs, rendering these "last-line" antibiotics ineffective in such cases.[3,11] Every attempt should be made to isolate patients colonized with *Acinetobacter* in order to prevent transmission among hospitalized patients.

Other nonfermentative gram-negative organisms implicated in hospital-acquired infections in human and veterinary medicine include *Burkholderia cepacia, Aeromonas* spp., *Chryseobacterium* spp., and *Stenotrophomonas maltophilia*.[10,16] These opportunistic organisms are generally of low virulence but colonize and multiply in aqueous hospital environments such as intravenous fluids, irrigation solutions, respiratory tubing, and urine. They are generally contaminants and not considered primary pathogens. Antibiotic treatment of colonized patients is not always necessary and may be harmful unless or until infection is proven. Although infections are of low prevalence, these organisms survive in the environment for extended periods and are increasingly found to cause bacteremia, pneumonia, urethrocystitis, and surgical wound infections in humans and companion animals.[10,16] The increased incidence of infection is likely a

consequence of impaired host defenses, and the selective pressure caused by overuse of broad-spectrum β-lactams and fluoroquinolones.* Prospective epidemiologic studies of infections by these pathogens are lacking in veterinary medicine.

RESISTANCE AMONG GRAM-NEGATIVE PATHOGENS

The frequency of antibiotic resistance is increasing dramatically, particularly among gram-negative bacteria, and antibiotics that were once formidable weapons are now commonly ineffective.[1-3,11,13,17] Resistance among gram-negative pathogens may be due to alterations of the target binding site on the bacteria, decreased penetration of the antimicrobial drug into the bacteria, and enzymatic degradation of the target antibiotic by enzymes such as β-lactamases, the single most common cause of gram-negative bacterial resistance to β-lactam drugs (see Chapter 175).[3,11,14,16]

Acquired resistance to antibiotics occurs by either a mutation in the bacterial chromosomal DNA or acquisition of new genetic material. Mutations are uncommon events but may result in the development of resistance during therapy in organisms that are initially susceptible. Of greatest concern is the development of resistance by acquisition of new genetic material. Genes mediating antibiotic resistance are found on transposons and plasmids. These mobile genetic elements may be transferred from organism to organism and even from one bacterial species to another.[1,11,12] This mechanism of resistance is best exemplified by the β-lactamase family of enzymes, which act by hydrolyzing the β-lactam ring of penicillins, cephalosporins, and carbapenems. There are hundreds of β-lactamase enzymes that may be distinguished by their substrate profiles and activities.[12]

Plasmid-mediated β-lactamases such as those exhibited by *E. coli, K. pneumoniae,* and *B. fragilis* generally can be overcome with β-lactamase inhibitors such as clavulanic acid, sulbactam, or tazobactam. *P. aeruginosa, Serratia* spp., and *Enterobacter* spp. have chromosomal β-lactamase genes and can increase β-lactamase production if induced by penicillins or cephalosporins.

Third-generation cephalosporins were developed to circumvent β-lactam hydrolysis, but *Enterobacteriaceae* that produce extended-spectrum β-lactamases (ESBL) have emerged. ESBLs are a heterogenous group of enzymes encoded by plasmid-borne genes. There is no consensus on the precise definition of ESBLs. A commonly used working definition is that ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillins; first-, second-, and third-generation cephalosporins; and aztreonam by hydrolysis of these antibiotics, and which are inhibited by β-lactamase inhibitors such as clavulanic acid.[1,3,12,13] The most common ESBL-producing organisms in human and veterinary medicine are strains of *E. coli*, *P. mirabilis,* and *K. pneumoniae.* [1,11]

Screening for ESBL-producing organisms is not challenging for microbiology laboratories, and veterinary diagnostic laboratories are encouraged to follow Clinical Laboratory Standards Institute (CLSI) guidelines.[23a]

Disk-diffusion and broth microdilution methods can be used to screen *Enterobacteriaceae* for ESBL production. Resistance to aztreonam, cefpodoxime, or ceftazidime should raise suspicion for ESBL production and is an indication that phenotypic confirmatory testing should be performed. According to CLSI guidelines, isolates with a positive confirmatory test should be reported as resistant to aztreonam, penicillins, and all cephalosporins regardless of results reported on standard antibiograms. Significantly, ESBL producing organisms exhibit co-resistance to many other classes, including aminoglycosides, chloramphenicol, sulfonamides, tetracyclines, and fluoroquinolones, often resulting in limited therapeutic options.[2,4] Although increased carbapenem use is associated with the development of resistant strains of bacteria, carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms.[3,11,12,17] Accordingly, empiric prescription of carbapenems for non–life-threatening infection is discouraged. Combinations of β-lactams and β-lactamase inhibitors may represent an alternative for treating infections resulting from susceptible ESBL-producing *Enterobacteriaceae* in dogs and cats. There may be poor correlation with susceptibility results when routine published break points are applied to ESBL-producing bacteria; thus veterinarians should ensure that their microbiology laboratory of choice is performing to the standards put forward by the CLSI subcommittee on veterinary susceptibility testing (VAST) to limit therapeutic failures in their patients.

Implanted materials have significant potential for incurring biofilm infection with gram-negative pathogens.[14] Biofilms are antibiotic-resistant colonizations of bacteria that attach to surfaces and form a slimelike barrier that acts as a formidable defense mechanism, protecting the bacteria from eradication.[14,16] The biofilm matrix offers bacterial protection and thereby increases resistance to humoral immunologic responses and the phagocytic activity of host neutrophils and tissue macrophages.[14,16] Significantly, biofilms provide a suitable environment for the spread of resistance to several antibiotics that encode for multidrug resistance.[14,16] In addition to rational prescription of antimicrobial agents in the ICU, emphasis should be placed on adequate infection control procedures to prevent transmission among hospitalized patients. ICUs must implement a thorough disinfection protocol and have in place a means of identifying and handling patients with MDR or nosocomial infections.[2,12,14,16]

THERAPY FOR GRAM-NEGATIVE INFECTIONS

Veterinarians are faced with the dilemma of selecting an antibiotic on two occasions during life-threatening bacterial infections. Initially the clinician must prescribe empiric antimicrobial coverage when the causative pathogen and its susceptibilities are unknown. Broad-spectrum antimicrobial therapy is advocated at this time for most critically ill patients with documented or suspected bacterial infections.[9,12,14] The second decision point occurs when the causative pathogen is identified; the transition should be made to treat the patient with the most narrow-spectrum agent once the pathogen's susceptibility profile is determined. These basic tenets of pharmacotherapy are expected to reduce selective pressure for resistance to the more extended-spectrum antibiotics (see Chapter 175).[12,14] The choice of antibiotics for most serious gram-negative infections is ideally based on culture and susceptibility testing. Although therapy is not withheld until results are reported, careful sampling of representative samples (e.g., urine, blood, respiratory secretions, macerated tissue) should be performed before antimicrobial therapy is begun. Among the disadvantages of culture and susceptibility testing is the time that elapses between sample collection and pathogen identification. The utility of Gram staining in the selection of an antimicrobial should not be overlooked. Direct Gram staining of relevant fluid or tissue specimens often allows rapid confirmation of bacterial infection and aids in the initial selection of antibiotics. When slides are stained appropriately and examined promptly, gram-positive bacteria will retain crystal violet dye and stain blue, whereas gram-negative bacteria stain pink or red. Although Wright-Giemsa stain is most often used to evaluate peripheral blood smears, it can also aid in the diagnosis of bacterial infection. Giemsa-stained slides often reveal microorganisms and help classify bacteria based on morphologic characteristics. The observation of intracellular rods from an otherwise normally sterile site is supportive of infection with a gram-negative pathogen.

*References 10, 11, 14, 16, 17, 21.

Not all gram-negative infections require culture and susceptibility testing to be effectively treated. Many first-time or uncomplicated gram-negative infections in antibiotic-naïve patients are treated empirically, and are often successfully treated with amoxicillin, ampicillin, amoxicillin-clavulanic acid, or a fluoroquinolone, depending on regional resistance patterns and location of the infection. Infections in "hard to penetrate" tissues (e.g., eye, prostate, CNS), should be treated with a lipid-soluble drug such as a fluoroquinolone or trimethoprim-sulfamethoxazole (TMP-SMZ). Skin infections are commonly caused by β-lactamase producing strains of *Staphylococcus*. Thus amoxicillin-clavulanate, but not amoxicillin, would be a suitable choice in an otherwise healthy patient.

Life-threatening infections invariably require immediate and aggressive intravenous administration of antibiotics to achieve high concentrations of the drug at the site of infection. Subcutaneous or intramuscular administration of antibiotics is generally discouraged for managing infections in most critically ill patients. Parenteral antimicrobials used to treat aerobic or facultatively anaerobic gram-negative infections in dogs and cats include the cephalosporins, aminoglycosides, fluoroquinolones, and β-lactam/β-lactamase inhibitor combinations (e.g., ampicillin-sulbactam, piperacillin-tazobactam, ticarcillin-clavaulanic acid) (Table 94-1). The monobactams (aztreonam) and polymyxin (colistin) are viewed as drugs of last resort for treating MDR gram-negative pathogens. While both are life-saving drugs in human ICUs, dosing recommendations and indications for their use have not been established in veterinary medicine.

The first-generation cephalosporins (e.g., cefazolin) have a spectrum of activity that includes wild-type strains of some enteric bacteria (see Chapter 176). However, resistance among gram-negative bacteria develops easily, primarily by synthesis of β-lactamase enzymes capable of hydrolyzing the parent drug.[1,11,12] Until recently, first-generation cephalosporins were often effective for treating first-time, community-acquired skin, soft tissue, and lower urinary tract infections caused by *P. mirabilis* and *E. coli* in otherwise stable animals. However, resistance of most gram-negative enteric pathogens toward cefazolin and other members of its class has increased significantly and susceptibility is no longer predictable. Moreover, cefazolin is not active against *P. aeruginosa* or other opportunistic gram-negative organisms. Second-generation cephalosporins are characterized by enhanced activity toward *Enterobacter* spp., *Klebsiella* spp., *E. coli,* and *Proteus* spp., but they are similarly ineffective against *P. aeruginosa.* The cephamycins, cefoxitin and cefotetan, the most frequently prescribed members of this group in veterinary medicine, are very effective for most anaerobic organisms, including *Bacteroides* spp. Cefoxitin is often used to treat mixed aerobic/anaerobic infections, including abscesses and perioperative and postoperative management of intraabdominal sepsis associated with bacterial translocation or intestinal perforation. The intravenous third-generation cephalosporins (ceftriaxone, ceftazidime, and cefotaxime) are very active against most gram-negative bacteria. Cefotaxime is effective for anaerobic infections, whereas others (ceftazidime, ceftriaxone) are not. Among cephalosporins, only ceftazidime and cefepime (a fourth-generation cephalosporin) have activity against *P. aeruginosa.* There is little information on cefepime use in veterinary patients.

Despite their potential nephrotoxicity, the aminoglycosides remain the cornerstone of therapy for complicated or serious gram-negative infections. Gentamicin and amikacin are predictably effective against most gram-negative aerobic pathogens, including many MDR and nosocomial strains (see Chapter 177). Amikacin is more effective than gentamicin for many gram-negative pathogens and is used preferentially over other aminoglycosides to treat life-threatening infections with nosocomial gram-negative bacteria, including *P. aeruginosa* and many Enterobacteriaceae.[2] The prevalence of resistance remains relatively low compared with that of fluoroquinolones and cephalosporins, and emergence of resistance during treatment is uncommon. However, low-level resistance has been identified in MDR *P. aeruginosa*, *Burkholderia* spp., *Acinetobacter,* and *E. coli*.

Aminopenicillins (e.g., ampicillin and amoxicillin) are active against some gram-negative pathogens, including *Salmonella*, *Proteus,* and *E coli.* The aminopenicillins are β-lactamase susceptible and monotherapy with ampicillin or amoxicillin is inadequate for treating serious gram-negative infections. Combination with a β-lactamase inhibitor (e.g., clavulanic acid or sulbactam) broadens the spectrum to include organisms that have acquired resistance through β-lactamase production, including strains of *E. coli, Klebsiella, Proteus,* and *Bacteroides* spp. However, many pathogenic *E. coli* are no longer susceptible to amoxicillin-clavulanate. *Pseudomonas* spp. and other gram-negative organisms remain resistant. The affinity of clavulanic acid toward β-lactamases is much greater than that of sulbactum. Thus an organism resistant to amoxicillin-clavulanate is always inferred to be resistant to ampicillin-sulbactam. Ampicillin-sulbactam is available in the United States and most countries. Injectable formulations of amoxicillin-clavulanic acid are available and widely used in many countries outside the United States. The extended-spectrum penicillins, piperacillin and ticarcillin, generally have greater activity than aminopenicillins against gram-negative bacteria due to their enhanced penetration through the cell wall of susceptible pathogens. Their major advantage is their excellent activity toward *P. aeruginosa*. Like other penicillins, they contain a β-lactam ring that can be cleaved by β-lactamase enzymes. Piperacillin and ticarcillin are manufactured and sold as fixed combinations with a β-lactamase inhibitor (tazobactam and clavulanic acid, respectively) that protects the parent drugs from degradation and expands their utility. Imipenem and meropenem, members of the carbapenem class of β-lactam antibiotics, are among the most broadly active agents available against gram-positive and gram-negative bacteria.[12] As a result they are often used as "last line" agents in patients infected with highly resistant bacteria. They are prescribed

Table 94-1 Intravenous Antibiotics for Gram-Negative Infections in Dogs and Cats

Drug	Recommended Dosage
Amikacin	15 mg/kg IV q24h (dogs) 10 mg/kg IV q24h (cats)
Ampicillin-sulbactam	22 mg/kg IV q8h
Azithromycin	5 to 10 mg/kg IV q24h
Cefazolin	22 mg/kg IV q6-8h
Cefotaxime	25 to 50 mg/kg IV q6-8h
Cefotetan	30 mg/kg IV q8h
Cefoxitin	30 mg/kg IV q6-8h
Ceftazidime	30 mg/kg IV q6h
Enrofloxacin	15 to 20 mg/kg IV q24h (dogs) 5 mg/kg IV q24h (cats)
Gentamicin	10 mg/kg IV q24h (dogs) 6 mg/kg IV q24h (cats)
Imipenem-cilastatin	5 to10 mg/kg IV q6-8h
Meropenem	8 to 12 mg/kg IV q8-12h
Piperacillin-tazobactam	40 mg/kg IV q6h
Ticarcillin-clavulanate	50 mg/kg IV q6h
Trimethoprim-sulfamethoxazole or trimethoprim-sulfadiazine	15 to 30 mg/kg IV q12h

for mixed bacterial infections and for organisms that are not susceptible to other antibiotics, including most nonfermenting gram-negative organisms and the Enterobacteriaceae. Meropenem is slightly more effective than imipenem against the *P. aeruginosa.* Both agents are active against almost all anaerobic bacteria, including *B. fragilis.* Imipenem administration may be associated with CNS toxicity (e.g., seizure activity). In contrast, meropenem has a greater margin of safety. In addition, meropenem is more water-soluble and thus can be administered more flexibly, as short infusions or bolus injections. Ertapenem and doripenem are newer members of this drug class approved for use in humans. Classified as a monobactam, aztreonam is a β-lactam drug with a spectrum of activity restricted to gram-negative aerobic bacteria. It has few known side effects and has been used extensively in human medicine for more than 20 years. It is often active against MDR gram-negative pathogens including nonfermenting gram-negative bacteria as well as many Enterobacteriaceae. It may be a therapeutic consideration in rare circumstances when fluoroquinolones or aminoglycosides are ineffective or relatively contraindicated. Dosages are not established in veterinary medicine, and reports of its use for managing clinical infections in dogs and cats have not been published.

The fluoroquinolones are considered "broad-spectrum" drugs when referring to their gram-negative spectrum. Organisms particularly susceptible include *Klebsiella* spp., *E. cloacae*, *P. mirabilis,* and *S. marcescens*. While *E. coli* and *P. aeruginosa* are included in their spectrum, fluoroquinolone-resistant strains are occasionally retrieved from critically ill animals and from pets with a history of recent antibiotic therapy. Enrofloxacin is the only approved parenteral fluoroquinolone for use in companion animals. Although it is administered intravenously to dogs and cats, enrofloxacin is licensed for intramuscular injection in dogs only (see Chapter 178). Intravenous ciprofloxacin is marketed for use in human medicine, but clinical use of this formulation has not been reported in animals. Enrofloxacin is metabolized partially to ciprofloxacin, which may account for 30% to 40% of the peak fluoroquinolone concentration.[22] Fluoroquinolones display largely concentration-dependent kill characteristics, and inappropriately low dosing of fluoroquinolones is associated with the emergence of resistant bacterial strains, particularly *P. aeruginosa*.[17,23]

Polymyxin E (colistin) is a polypeptide antibiotic (see Chapter 181). In addition to its ability to neutralize endotoxin, it is active against many gram-negative pathogens, including *P. aeruginosa, E. coli, Acinetobacter* spp., and *Stenotrophomonas maltophilia.*[23,24] Because of its nephrotoxicity, its use was abandoned as less toxic antimicrobials became available, but the emergence of MDR pathogens has prompted reevaluation of its utility in human ICUs.[7,23,24] Relevance to treatment of clinical infections in dogs or cats is not established.

Empiric recommendations for managing infections with gram-negative bacteria vary based on geography and prior exposure to antimicrobials.[2] In general, most Enterobacteriaceae are not susceptible to chloramphenicol, tetracyclines, first-generation cephalosporins, aminopenicillins, or TMP-SMZ.[2,12] Thus appropriate initial therapy for animals with first-time and non–life-threatening infections caused by gram-negative enteric organisms may include amoxicillin-clavulanate, ampicillin-sulbactam, or a fluoroquinolone. Pathogen retrieval should be initiated preferably before the first dose of antibiotic is given, but should not delay therapy in critically ill patients. Empiric antibiotic therapy must be comprehensive and cover all likely pathogens. Definitive therapy should be based on results of susceptibility testing. Pending such results, patients with life-threatening infections presumptively ascribed to *Proteus*, *E. coli,* or *Klebsiella* may be treated with a third-generation cephalosporin, fluoroquinolone, extended-spectrum β-lactam/β-lactamase inhibitor or amikacin.[2] *P. aeruginosa* is notorious for its intrinsic resistance to multiple antibiotics. Initial recommendations for life-threatening infections with *Pseudomonas* and other nonfermentative gram-negative bacteria include ticarcillin-clavulanic acid, as a single agent or in combination with amikacin or a fluoroquinolone. Optimal treatment with aminoglycosides and fluoroquinolones requires achieving high plasma and tissue concentrations for a successful clinical outcome. Although the fluoroquinolones are generally safer than aminoglycosides, the latter are predictably more effective for the treatment of gram-negative pathogens and less likely to contribute to antibiotic resistance.* Nosocomial, recurrent, or recalcitrant infections with *P. aeruginosa, Acinetobacter* spp., or other opportunistic pathogen suggest acquired resistance and should be aggressively treated with a combination of two antipseudomonal agents. Carbapenems and ceftazidime are stable against hydrolysis by various β-lactamases and generally kept in reserve. However, therapy with either drug should not be delayed when cultures from initial specimens grow strains that are resistant to other antibiotics or when patients are deteriorating in the face of therapy with other broad-spectrum agents. Individualization of regimens based on prior antibiotic use may reduce the risk of inadequate therapy.

The escalating number of infections caused by MDR and ESBL producing strains of *E. coli*, *Klebsiella*, and other Enterobacteriaceae in veterinary and human hospitals is accompanied by rising rates of antibiotic resistance. Treatment options to meet this challenge are increasingly limited. Selection of an antibiotic lacking activity against the causative organism can have dire consequences for patients and underscores the need for early, appropriate antibiotic therapy. Empiric combination therapy with two drugs directed at gram-negative pathogens is a logical approach when selecting therapy for critically ill animals, particularly when there are risk factors for acquiring MDR organisms (e.g., prior antibiotic therapy, extended hospitalization, wounds, invasive devices or procedures such as mechanical ventilation, urinary catheters, or surgery). Combination therapy is also indicated for mixed infections or those of unknown etiology to ensure coverage of gram-positive and anaerobic pathogens. Ultimately, the clinical utility of combination therapy rests on reducing the likelihood of inappropriate treatment or microbiologic failure. There is, however, considerable debate over the role of monotherapy versus combination therapy when treating gram-negative infections, particularly those caused by *P. aeruginosa, A. baumannii,* and MDR Enterobacteriaceae.[1,11,12,21] Combination therapy has several theoretic advantages, including *in vitro* bacterial killing superior to the simple additive activity of each antibiotic alone, a phenomenon termed *synergism,* as well as reducing the emergence of subpopulations of microorganisms resistant to the antibiotics.[1,12,21] Interest in monotherapy has increased, particularly since the introduction of broad-spectrum β-lactam antibiotics effective against *P. aeruginosa.*. Although both fluoroquinolones and aminoglycosides demonstrate in vitro synergy with β-lactams, some of the proposed advantages of combining an aminoglycoside or fluoroquinolone with a broad-spectrum β-lactam agent for treating gram-negative infections are not substantiated in the clinical setting.[1,21] The importance of initial combination therapy is well established, although de-escalation to a single agent once susceptibility data are available is often recommended. Despite in vitro and animal models demonstrating a beneficial effect of continued combination therapy, convincing clinical data that demonstrate a need for combination therapy once susceptibilities are known are lacking. Similarly, superiority of combination therapy has not been prospectively examined in critically ill veterinary patients.[2,12,22]

*References 10, 11, 14, 16, 17, 21.

Newer classes of antimicrobial agents with activity against many MDR gram-negative pathogens include the glycylcyclines (e.g., tigecycline) and the monobactams (e.g., aztreonam) (see Chapter 181). To curtail the development of drug resistance among small animal patients, veterinarians are cautioned against prescribing these agents. If existing antibiotics are used appropriately for gram-negative bacteria, veterinarians may be able to avoid the expense of newer and highly valuable antimicrobials developed for use in humans. Consultation with a pharmacologist or veterinary microbiologist is recommended before concluding that a dog or cat requires therapy with these agents.

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