ANTIBACTERIAL AGENTS

General Principles of Therapy

The use of antibacterial agents is critical to the successful management of ocular diseases, however several principles should be followed when instituting an antimicrobial therapeutic regimen. The basis of these principles requires that the clinician have a solid understanding of each individual’s condition, enabling him or her to determine whether antibiotic therapy is indeed indicated, and by what route of administration. If prophylactic use is indicated (i.e., simple superficial corneal ulcer), considerations should include spectrum of the drug relative to organisms of concern, potential for development of resistance, and potential for adverse reactions/toxicities. If therapeutic use is indicated (i.e., infected corneal ulcer), every attempt should be made to determine the causative organism(s) to enable targeted therapy with the most effective antibiotic agent available, as well as the potential for development of resistance and potential for adverse reactions/toxicities.

The mechanisms of action of antibiotic agents are based on differences between bacterial organisms and mammalian cells. First among these is the presence of a cell wall in bacterial organisms, which is absent in mammalian cells. One essential component of the cell wall, the peptidoglycan, is targeted by antibiotics, which impair the integrity of the cell wall and lead to cell death. Targeting the bacterial cell membrane is also a possibility, however the structural similarities to mammalian cell membranes decrease the overall selectivity of most antibiotics, thus increasing their toxicity to the recipient. An additional target is the bacterial ribosome, which differs in size and shape from those of mammalian cells, enabling selective inhibition of bacterial protein synthesis. Folic acid serves as an additional target in bacterial cells, as bacteria synthesize their own folic acid (mammalian cells do not). Inhibition of the folic acid synthetic pathway provides selective targeting of bacterial organisms. A final difference is the presence of DNA gyrase and topoisomerase IV in bacterial cells, two enzymes absent in human cells and thus selectively targeted in bacteria.

Mechanisms of Resistance

Bacterial resistance to antibiotics occurs by one of three general processes: chemical modification of the antibiotic by the bacteria; bacterial prevention of antibiotic from reaching the target; or bacterial modification of the target itself.

Modification of the antibiotic or its target may involve various methodologies, such as production of antibiotic-inactivating or altering enzymes, chemical alterations (such as acetylation or phosphorylation) to the antibiotic or its binding site, or chromosomal or DNA changes that induce protein structural changes to the target site. Prevention of the antibiotic from reaching its target site potentially involves a more complex series of events that either prevent intracellular drug accumulation or allow the bacteria to bypass the adverse effects associated with antibiotic exposure. Alterations in outer membrane porin channels may decrease drug influx, while development of efflux pumps may increase extracellular movement of drug. Phenotypic alterations in cell physiology induced by antibiotic effects may ultimately decrease the susceptibility to an antibiotic, such as changes in cell wall components that occur intrinsically as a response to altered nutrients or growth rate, and allow that strain to successfully propagate even with continued antibiotic exposure. Bacteria may also develop bypass mechanisms, such as the development of a second enzyme system in response to interrupted function on the first enzyme system, performing the same function as the first without the antibiotic susceptibility. Finally, alteration of the bacterial target may involve direct structural modifications or chromosomal alterations that reduce the affinity of the antibacterial agent for the target.

Bacteria are capable of transmitting resistance-conferring genes among related and unrelated bacterial strains in processes involving horizontal gene transfer. Conjugation (plasmid exchanged mediated by cell-to-
cell contact), transformation (DNA uptake from the environment), and transduction (virus-mediated DNA transfer) have each been implicated in propagation of bacterial resistance.

**Evaluating Antibacterial Efficacy**

Antibacterial efficacy is measured both in vitro and in vivo, with correlations drawn between the two scenarios in an effort to improve understanding of potential therapeutic efficacy. The minimum inhibitory concentration (MIC) is an in vitro measure of the intrinsic activity of an antibiotic versus an organism. This is a static assessment, and does not account for in vivo impacts on drug disposition or efficacy. The MIC is determined utilizing uniform lab standards dictated by the Clinical and Lab Standards Institute and the European Committee on Antimicrobial Susceptibility Testing. Incorporating the pharmacodynamics (PD), or in vivo pharmacologic effect of a drug (what the drug does to the body) with the pharmacokinetics (PK) of the drug (what the body does to the drug) provides a potentially more applicable assessment of the clinical effect of a drug. The PK/PD data provides clinical susceptibility breakpoints using commonly evaluated indices. The most commonly used are:

1. $C_{\text{max}}/\text{MIC}$ = maximum plasma concentration relative to the MIC
2. $AUC/\text{MIC}$ = plasma concentration time curve (duration of exposure) relative to the MIC
3. $T > \text{MIC}$ = % time antibiotic concentration is greater than the MIC

These measures are determined using various animal models, either utilizing immunocompromised or immunocompetent individuals, to provide information on both PK and PD parameters for comparison.

**1. Drugs That Inhibit Bacterial Cell Wall Synthesis**

Drugs affecting cell wall synthesis include the two classes penicillins and cephalosporins, as well as the individual drugs bacitracin and vancomycin.

**Penicillins**

Penicillins are bactericidal, via inhibition of bacterial cell wall synthesis. Formation of the cell wall component peptidoglycan is obstructed through penicillin-mediated inhibition of transpeptidase enzymes, which are responsible for forming peptide cross-linkages between the polysaccharide chains that compromise peptidoglycan. The resulting absence of a complete cell wall leads to bacterial cell death. Penicillins themselves are comprised of a thiazolidine ring and $\beta$-lactam ring connected to a side chain. The side chain determines pharmacokinetic parameters, spectrum activity, and susceptibility to destruction by gastric acid or $\beta$-lactamase enzymes, while the $\beta$-lactam ring itself is responsible for biologic activity.

Division of penicillins based on their spectrum of activity identifies those effective against Gram-positive bacteria, those resistant to penicillinases, those with extended spectra of activity, and those effective against *Pseudomonas* spp.

**Agents with Gram-Positive Activity**

The two primary members of this group are penicillin G (inactivated by gastric acid and thus administered parenterally) and penicillin V (may be given orally). Unfortunately, these drugs are highly susceptible to organisms that produce $\beta$-lactamases, which hydrolyze the $\beta$-lactam portion of the drug, thus inactivating it. Bacteria known to produce $\beta$-lactamases include *Staphylococcus aureus* and *Staphylococcus epidermidis*, thus these infections are not effectively treated with either penicillin G or penicillin V. Additionally, *Streptococcus pneumoniae* and viridans streptococci are acquiring increasing resistance through altering their intrinsic transpeptidases, the target of penicillins.

The low lipophilicity of penicillin G restricts its passage through the blood-ocular barriers, thereby limiting its efficacy in treatment of intraocular infections. Administration of penicillin G (and other penicillins) topically or subconjunctivally has been reported in the management of keratitis in cows and horses, achieving variable residence times and clinical effect.\(^1\)

**Penicillinase-Resistant Agents**

These agents, which include methicillin, oxacillin, cloxacillin, dicloxacillin, and nafcillin, are able to resist bacterial $\beta$-lactamases due to alterations to their chemical structures, thus making them effective against
*Staphylococcus aureus* and *S. epidermidis* infections, resistant to penicillin G and penicillin V. These organisms have begun to acquire the alternate mechanism of resistance (modifications of intrinsic transpeptidases), which leads to the designation as a ‘methicillin-resistant’ strain. This designation implies resistance to all penicillins, not just methicillin, as well as cephalosporins, aminoglycosides, and macrolides.

**Extended-Spectrum Penicillins**

Ampicillin and amoxicillin are the two members of this group, produced by alterations to the basic structure of penicillins. While they have a broader spectrum than penicillin G or penicillin V, primarily to include more gram-negative rods, they are inactivated by β-lactamases. Addition of clavulanic acid to amoxicillin and sulbactam to ampicillin imparts resistance to β-lactamases, as those two compounds irreversibly inactivate the bacterial enzymes, in turn increasing the ability of these penicillins to treat *Staph. aureus* and *Staph. epidermidis* infections.

**Antipseudomonal Penicillins**

Carbenicillin, mezlocillin, piperacillin, and ticarcillin are effective against *Pseudomonas aeruginosa* and some *Proteus* and *Enterobacter* species, gram-negative organisms resistant to most other penicillins.

**Side Effects**

Penicillins are primarily known for inducing hypersensitivity reactions (types I, II, III, or IV) in susceptible individuals. Once such a reaction occurs, which may manifest in the dermatologic, hematologic, urogenital, or other systems, no further use of the offending penicillin or any other penicillin is advised. Penicillins may also alter the enteric microflora, producing gastrointestinal upset (vomiting, diarrhea, nausea).

**Contraindications**

Prior hypersensitivity to any penicillin or a cephalosporin is the primary contraindication to use of any other penicillin.

**Cephalosporins**

Cephalosporins, like penicillins, interfere with the cross-linking necessary to formation of a stable peptidoglycan cell wall through activity of a β-lactam ring. Rather than a thiazolidine ring, cephalosporins have a dihydrothiazine ring. Cephalosporins are also inactivated by β-lactamases, however they are able to resist inactivation by some β-lactamases, which are thus more specifically termed ‘penicillinases’. Cephalosporins are not inactivated by the β-lactamases produced by *Staph aureus*, however they are inactivated by β-lactamases produced by some gram-negative bacteria. Cephalosporins are divided into generations based upon side chain modifications that alter their spectrum of activity.

**First-Generation**

Cephalexin, cefadroxil, cefazolin, and cephradine are first-generation cephalosporins, all with good efficacy versus gram-positive organisms and only marginal efficacy versus gram-negative organisms. These cephalosporins are generally effective against methicillin-sensitive *Staph aureus*, as well as *Streptococcus* spp. Cefazolin (100 mg/ml diluted to 50 mg/ml) in particular is used in the treatment of corneal ulcers due to its good Gram-positive spectrum, however *Strep. pneumoniae* and viridans streptococci are developing increasing resistance in such infections, which is a cause for concern. Following subconjuntival injection in humans and rabbits and intravenous administration in dogs, cefazolin reaches therapeutic anterior chamber concentrations.1

**Second-Generation**

Cefaclor, cefprozil, cefuroxime, cefoxitin, and cefotetan are members of the second-generation class of cephalosporins, which have increased gram-negative activity relative to those in the first generation.

**Third-Generation**

Cefixime, cefdinir, ceftriaxone, and ceftazidime are third-generation cephalosporins with increased activity versus enteric gram-negative bacteria. Ceftazidime is used in human ophthalmology via intravitreal injection as treatment for endophthalmitis, due in part to excellent activity versus *Pseudomonas aeruginosa*. Use of these medications in veterinary ophthalmology is rarely justified.
**Fourth-Generation**
Cefepime has a broad spectrum versus both gram-positive and gram-negative organisms.

**Side Effects**
Hypersensitivity reactions are the most common side effect following administration of cephalosporins. Cross-hypersensitivity is more likely to occur between penicillins and first-generation cephalosporins, so patients with a known hypersensitivity to penicillins may be able to safely take a later-generation cephalosporin. Alterations in the enteric microflora may lead to gastrointestinal upset (vomiting, diarrhea, nausea), as well as vitamin K deficiency and subsequent bleeding abnormalities. Renal impairment may also develop in patients with a predisposition (i.e., concurrent aminoglycoside administration, previous renal disease).

**Contraindications**
The primary contraindication to use of a cephalosporin is known hypersensitivity. Additionally, administration in patients with previous vitamin K deficiency or renal impairment should be performed cautiously.

**Bacitracin**
Bacitracin also inhibits bacterial cell wall synthesis, however its mechanism of action relies upon inhibiting the movement of a peptidoglycan precursor from the cytoplasm through the cell membrane. Its spectrum of action is primarily toward gram-positive organisms. Bacitracin is a common component of triple antibiotic ophthalmic ointments (it is unstable in solution). Its combination with neomycin and polymyxin B (both having greater gram-negative efficacy) produces a good broad-spectrum preparation commonly used for prophylaxis (corneal ulcers) or therapy (nonspecific ocular surface infections). Bacitracin has no appreciable transcorneal penetration, and therefore is of limited value in deep corneal or intraocular infections.

**Side Effects and Contraindications**
The primary side effect of potential concern is a local hypersensitivity reaction, and patients with a history of such a reaction should not receive preparations containing bacitracin.

**Vancomycin**
Vancomycin inhibits bacterial cell wall synthesis by inhibiting production of the mucopeptide portion of the peptidoglycan. Its activity versus gram-positive organisms is strong, particularly versus *Staphylococcus* spp and *Streptococcus* spp, including those classified as ‘methicillin-resistant’, as well as is its activity versus *Clostridium difficile*. Previously susceptible enterococcal organisms (which are resistant to ß-lactam antibiotics) are now resistant to vancomycin. Due to its spectrum and its associated toxicities (see below), the use of vancomycin is restricted to patients with resistant infections, or with known hypersensitivities to more effective antibiotics.

Ophthalmic use of vancomycin has included topical administration in infectious keratitis, as well as bacterial endophthalmitis. Evaluation of various concentrations of vancomycin in experimental MRSA keratitis in rabbits demonstrated efficacy at 0.3% or 1.0% concentrations (versus 0.03% or 0.1%). Experimental injection of vancomycin microparticles into the anterior chamber of rabbits demonstrated good prophylactic activity with minimal signs of corneal or retinal toxicity.

**Side Effects and Contraindications**
The primary toxicities associated with parenteral, long-term vancomycin therapy include ototoxicity and nephrotoxicity, which may produce a permanent deafness or fatal uremia. Vancomycin is contraindicated in patients with a known hypersensitivity to it, and should be used judiciously and with close monitoring in patients with pre-existing hearing or renal impairment.

2. **Drugs That Affect the Bacterial Cell Membrane**
As bacterial and mammalian cell membranes are quite similar, few antibiotics are able to selectively and effectively target this cellular component without inducing patient toxicity. Polymyxin B and gramicidin are the two most readily used.

**Polymyxin B**
Polymyxin B is a cationic surfactant (detergent) that disrupts cell membrane integrity by interacting with the phospholipids, thus increasing cell permeability and leading to cell death. Its spectrum is limited to gram-negative organisms (thus making it useful in combination with the gram-positive spectrum of bacitracin). Following topical ocular administration, local hypersensitivity reactions may occur, and patients exhibiting such a reaction should not receive the drug subsequently.

**Gramicidin**
Gramicidin also alters cell membrane permeability, however its spectrum is directed toward gram-positive organisms. Its stability in solution enables its substitution for bacitracin in triple antibiotic ophthalmic preparations.

### 3. Drugs That Affect Bacterial Protein Synthesis
This group of antibiotics includes aminoglycosides, tetracyclines, and macrolides, as well as chloramphenicol.

**Aminoglycosides**
Neomycin, gentamicin, tobramycin, kanamycin, and amikacin are common aminoglycoside antibiotics, with bactericidal effect. Their mechanism of action involves inhibition of bacterial protein synthesis via inhibition of the 30S bacterial ribosome. The spectrum of activity is strong versus gram-negative organisms (*Pseudomonas aeruginosa*, *Proteus*, *E. coli*, *Enterobacter*, etc.), with the exception of neomycin (generally considered inactive versus *Pseudomonas aeruginosa*). In contrast, their efficacy versus gram-positive organisms is restricted primarily to *Staphylococcus aureus* – *Streptococci*, enterococci, and methicillin-resistant *Staph aureus* are poorly susceptible or resistant to aminoglycosides. Aminoglycosides are also inactive versus anaerobes. An additive or synergistic effect may be achieved when an aminoglycoside is used in combination with a β-lactam antibiotic, resulting in increased efficacy versus aerobic gram-negative bacilli and aerobic gram-positive cocci. In combination with vancomycin, aminoglycosides may also have an additive or synergistic effect versus aerobic gram-positive cocci.

While in vitro patterns may not mimic the response in clinical settings, some discrepancies do exist between recent reports in the veterinary literature and generally accepted susceptibility trends. Consistent with general trends are the relatively high resistance patterns noted for β-hemolytic *Strep* and *Strep equis* isolates from dogs and horses, respectively, toward neomycin and tobramycin, however they demonstrated variable susceptibility to gentamicin. *Staph intermedius* isolates from dogs demonstrated good susceptibility to all three drugs, consistent with expected in vitro trends. Inconsistent with expected results however, was the moderate to good susceptibility of *Pseudomonas aeruginosa* isolates to all three drugs in both dogs and horses.

Resistance to aminoglycosides on the part of gram-negative bacilli occurs through bacterial production of enzymes that inactivate the drugs. Certain enzymes inactivate certain aminoglycosides, and the production of the enzymes is individualized to each strain of each pathogen, thus making definitive statements about overall resistance patterns to aminoglycosides difficult, further supporting the need for targeted therapy based on appropriate diagnostic procedures.

Aminoglycosides are poorly absorbed following oral administration, indicating their use is restricted to topical or parenteral routes. It is extremely important to be aware of the fact that penicillins and cephalosporins inactivate aminoglycosides if administered in a combination preparation, thereby emphasizing the need for individual preparations and appropriate time lapse between dosings.

**Neomycin**
Neomycin is a component of common ophthalmic triple-antibiotic preparations. Its primary use is for prophylaxis in superficial corneal ulceration, or for nonspecific treatment of ocular surface infections. Its primary side effect is contact hypersensitivity, and therefore is contraindicated in patients with a history of such a reaction. It has limited to no corneal penetration in the presence of an intact corneal epithelium.
**Gentamicin**

Gentamicin, administered topically or subconjunctivally, is used in the treatment of infectious keratitis, particularly that caused by *Pseudomonas aeruginosa*. The commercially available concentration (3 mg/ml) is generally too weak to be therapeutically effective, so solutions with greater concentration (13.6 mg/ml) may be utilized in the presence of infection. The ability of gentamicin to provide prophylactic protection is limited due to its gram-negative spectrum of activity, while primary agents of concern from the ocular surface tend to be gram-positive organisms.

Reports vary significantly regarding development of resistance to gentamicin, either indicating increased resistance of *P. aeruginosa* and *Streptococcus equi* isolates from horses with infectious keratitis, or indicating no increased resistance. Gentamicin achieves limited transcorneal penetration in normal corneas, however therapeutic levels are more readily reached in the presence of infection. Subconjunctival injection may augment ocular drug levels, however systemic absorption has been reported, emphasizing the need for awareness of possible confounding systemic conditions, particularly in smaller patients.

**Tobramycin**

The spectrum of activity and indications for use of tobramycin are similar to that of gentamicin. It is available as a solution (0.3%) or an ointment (0.3%), and may be used in a fortified form with greater concentration (14.5 mg/ml) in cases of severe bacterial keratitis. Subconjunctival administration results in therapeutic intraocular drug levels, however systemic administration is generally not useful for ocular infections.

**Kanamycin**

Kanamycin has been used in cases of infectious bovine keratoconjunctivitis (IBK) caused by *Moraxella bovis*, with efficacy potentially being achieved with subconjunctival injection.

**Amikacin**

Amikacin is not available as a topical ophthalmic preparation, however its administration topically has led to therapeutic corneal levels which may be useful in treating patients with ocular infections caused by gram-negative bacilli that are resistant to gentamicin or tobramycin. Subconjunctival injection produces highly variable intraocular levels, and parenteral administration does not result in intraocular penetration. Of the aminoglycosides, amikacin is the least toxic to the retina, indicating its potential in the treatment of endophthalmitis.

**Side Effects**

Auditory and vestibular neurotoxicity, as well as nephrotoxicity, may result from administration of aminoglycosides systemically. Topical ocular toxicity includes corneal epithelial damage or conjunctival damage associated with topical administration of gentamicin or tobramycin. Hypersensitivity reactions may also occur in patients who have received topical aminoglycosides, with cross-reaction among agents possible. Intravitreal injection of gentamicin is toxic to the retina and the ciliary body, and in fact forms the basis for a treatment for end-stage glaucoma.

**Contraindications**

Aminoglycosides are contraindicated in patients with a hypersensitivity to them, and should be used cautiously and with extensive monitoring in patients with pre-existing renal disease.

**Tetracyclines**

Like the aminoglycosides, tetracyclines inhibit bacterial protein synthesis by interacting with the 30S ribosomal subunit. They may be classified as short-acting (tetracycline, oxytetracycline), intermediate-acting (demeclocycline), or long-acting (doxycycline, minocycline). While they have a broad spectrum of bacterial coverage, their clinically relevant spectrum of activity is relatively narrow, due in part to development of efflux mechanisms within bacteria that actively pump drug out of the cells to create resistant strains. Activity versus rickettsial organisms, *Borrelia burgdorferi*, *Mycoplasma* spp, *Chlamydophila* spp, and *Moraxella* spp is consistently strong, while *Staphylococcus* spp and *Streptococcus* spp are developing increased resistance.
*Pseudomonas aeruginosa* is generally considered resistant to tetracyclines, which has been supported by a recent evaluation of isolates from horses with ocular disease.\(^5\)

An additional pharmacologic benefit provided by the tetracyclines is their ability to inhibit matrix metalloproteinases (MMPs). MMPs are known to cause destruction of corneal collagen leading clinically to ‘melting’ ulcers. Oral therapy with tetracyclines may block the action of these collagenases, leading to improvement beyond that expected with their antibacterial spectra.

While topical formulations are available and indicated in appropriate cases, tetracyclines are also frequently administered orally in patients with ocular disease (i.e., *Chlamydophila felis* or *Mycoplasma* spp in cats, *Moraxella bovis* in cattle), even though the most significant clinical signs associated with these organisms are ocular surface lesions, particularly conjunctivitis.\(^7\) This is due in part to the potential for organisms to be sequestered in non-ocular (systemic) sites,\(^8\) as well as the ease of systemic versus topical administration in large populations of cattle. Orally administered tetracyclines are generally poorly absorbed from the gastrointestinal tract, with the exception of doxycycline.

In cats, oral doxycycline (5 mg/kg PO BID or 10 mg/kg PO q24h) has been shown to more effectively control chlamydiosis than topical administration of chlortetracycline,\(^8\) however it must be given in conjunction with water and/or food to avoid the potential for esophageal stricture.\(^9\) Additionally, long-term therapy is often necessary to eliminate organism (as determined by real-time PCR on conjunctival swab samples of experimentally infected cats).\(^10\)

In cattle, therapeutic drug levels were maintained in the tear film for up to 72 hours following a single subconjunctival injection of a long-acting oxytetracycline formulation, while an intramuscular injection was clinically successful in treating *M. bovis* ocular disease even in the absence of therapeutic tear film levels.\(^11\)

In horses, results are conflicting regarding the intraocular penetration of doxycycline following oral administration to normal horses. In one study, oral administration of doxycycline at a dose of 10 mg/kg every 12 hours for 5 days did not lead to detectable aqueous humor drug levels, and led to subtherapeutic (versus *Leptospira* spp) vitreous humor levels on the fifth day.\(^12\) A different study, also using normal horses, evaluated the aqueous humor levels following administration of 20 mg/kg PO BID for 5 doses, and revealed detectable aqueous humor levels.\(^13\) The differences could be associated with the doses and/or the detection methods used between the studies. Oral administration of 20 mg/kg of doxycycline once daily for 5 days to normal horses identified measurable tear film levels, however the effect of levels achieved (~10 µg/ml) in controlling collagenolysis is unknown.\(^14\)

**Side Effects**

Tetracyclines are known to induce photosensitivity reactions, gastrointestinal upset, and discoloration of teeth during development. They also may interact with other drugs or compounds, causing potentially significant variation in absorption and therefore effect. Administration with food may decrease signs of gastrointestinal upset, however that may significantly impact the absorption of tetracyclines, with the exception of doxycycline. Tetracyclines form complexes with divalent cations (iron, calcium, magnesium, aluminum), which markedly decreases absorption from the gastrointestinal tract – this is particularly important when considering mixing tetracyclines with molasses (very high iron content) for administration to horses.

**Macrolides**

Erythromycin, azithromycin, and clarithromycin are members of the macrolide antibiotic class. These antibiotics inhibit bacterial growth by binding the 50S bacterial ribosomal subunit, which prevents elongation of the peptide chain. Organisms may acquire resistance by altering their ribosomal RNA, which leads to poor binding of drug to the resulting ribosomal subunit. Bacteria developing such resistance patterns include *Staph aureus*, coagulase-negative staphylococci, and *Streptococcus* spp.

**Erythromycin**

Gram-positive organisms are susceptible to erythromycin, with the exception of enterococci. Organisms pertinent to veterinary ophthalmology effectively treated with erythromycin include *Mycoplasma* spp and *Chlamydophila* spp, along with *Borrelia burgdorferi*. Although staphylococcal and streptococcal organisms are developing resistance, erythromycin is still currently a reasonable prophylactic and potentially therapeutic option for disease caused by these organisms (i.e., staph blepharitis). In horses with bacterial keratitis, 16 of 17 *Streptococcus equi* isolates were sensitive to erythromycin.\(^5\)
Azithromycin

Azithromycin is a newer derivative of erythromycin that is rapidly absorbed following oral administration. Its spectrum of activity toward gram-negative organisms, in particular *Borrelia burgdorferi* and *Bartonella henselae*, is increased relative to that of erythromycin, and it concentrates in neutrophils and mononuclear phagocytes. Experimental inoculation of dogs with *Rickettsia rickettsii* followed by treatment with azithromycin (3 mg/kg PO q24h) documented a poorer clinical response than that achieved with systemic doxycycline or trovafloxacin therapy.15 It has been used in cats to treat infections with *B. henselae*, however a recent report indicates that azithromycin had lower efficacy versus *Bartonella* than pradofloxacin or enrofloxacin,16 and that resistance to azithromycin develops with relative ease.17 It has also been advocated as systemic treatment for disease caused by *Chlamydophila psittaci* in cats, however its efficacy versus that of doxycycline appears to be lower.18

Clarithromycin

Clarithromycin is a newer derivative of erythromycin and is not inactivated by gastric acid (as erythromycin is), thus making it a more viable option for oral administration in appropriate cases. A report of its use in a cat with ocular manifestations of systemic *Mycobacterium simiae* infection documented successful resolution in combination with enrofloxacin and rifampicin.19

Side Effects

Gastrointestinal irritation is the most common side effect reported in people receiving oral macrolides. Erythromycin has also been linked to cholestatic hepatitis in people, as well as hearing loss. Hypersensitivity reactions also may occur.

Chloramphenicol

Chloramphenicol inhibits bacterial growth by inhibition of the 50S ribosomal subunit. It is broad-spectrum, with activity versus gram-positive and gram-negative agents, as well as *Rickettsia, Chlamydophila*, and *Mycoplasma*. The primary restriction to its use is the development of two variants of hematopoietic disorders, which have occurred after both systemic and topical ocular administration of the drug. A dose-related toxic effect may lead to bone marrow depression associated with inhibited mitochondrial protein synthesis, and this may be reversed when therapy is discontinued. The more severe reaction is a fatal aplastic anemia, which is considered to be an idiosyncratic reaction, rather than a predictable toxicity. This may occur weeks to months following discontinuation of therapy.

*Staphylococcus* and *Streptococcus* isolates from dogs with bacterial keratitis were susceptible to chloramphenicol, while *Pseudomonas* isolates were resistant,4 a trend which was also present in isolates from horses with bacterial keratitis.5,6

4. Drugs That Alter Bacterial Folate Metabolism

These antibiotics include sulfonamides, pyrimethamine, and trimethoprim, and are generally considered bacteriostatic. They are frequently used in combination with one another due to an additive effect based on their mechanisms of action. Sulfonamides competitively inhibit the first step of bacterial folate synthesis (conversion of para-aminobenzoic acid [PABA] to dihydrofolic acid), while pyrimethamine and trimethoprim reversibly inhibit the second step via inhibition of the enzyme dihydrofolate reductase (reduces dihydrofolic acid to tetrahydrofolic acid). The first step in the pathway is particular to bacterial cells, leading to specific antibacterial effects with minimal host toxicity. Toxicity may occur with the second step however, as mammalian cells (as well as bacterial cells) also have the enzyme dihydrofolate reductase. The binding affinity of trimethoprim is much stronger for the bacterial form of the enzyme than for the mammalian form, limiting toxicity. Resistance mechanisms acquired by bacteria to sulfonamides are numerous, including overproduction of PABA, reduction in enzyme affinity to sulfonamides, reduction in bacterial permeability to sulfonamides, and inactivation of sulfonamides by bacteria.

The spectrum of activity generally includes gram-positive organisms with some gram-negative organisms, such as *Pseudomonas aeruginosa*. Two studies in horses however, documented 100% resistance of *Pseudomonas* isolates to trimethoprim.5,6 Pyrimethamine has also been used in the treatment of toxoplasmosis,
however this is In general, use of this class of antibacterials has decreased significantly due to availability of more effective alternative medications.

Transcorneal penetration of topically administered sulfonamides and intraocular penetration of systemically administered sulfonamides is highly variable among species and individual drug, based largely on the solubility characteristics and protein-binding of each drug (higher solubility and lower protein-binding agents more readily penetrate), as well as ocular disease condition (greater penetration with corneal ulceration or intraocular inflammation).

**Side Effects**
Systemic reactions may occur with the use of sulfonamides, including gastrointestinal disturbances, allergic skin reactions, renal complications, and blood dyscrasias, as may local reactions such as irritation and dermatitis.

In dogs, multiple sulfonamides are known to cause keratoconjunctivitis sicca postulated to be associated with a direct toxic effect on lacrimal acinar cells by the nitrogen-containing pyridine and pyrimidine rings. This reaction may occur in a dose-dependent or idiosyncratic manner, and is reproducible experimentally by structurally similar salicylic acid compounds. The estimated incidence is 15% to 25% in dogs treated with sulfas, and may occur with variable dosing levels and durations, developing potentially months to years following treatment. Frequently, dogs have other signs of toxicity (i.e., hepatopathy) as well, and if drug is discontinued early, had a chance of recovering adequate tear production.

Evaluation of the effect of long-term systemic trimethoprim-sulfadiazine therapy in horses did not identify a reduction in tear production in that species.

**Contraindications**
Sulfonamides are contraindicated in patients with a known hypersensitivity to any members of the folate metabolism inhibitor class of antibiotics, as well as in patients with a history of blood dyscrasias. While not necessarily contraindications to use of sulfonamides, it should be noted that any other drug that has PABA as part of its molecular structure may interact with and therefore decrease efficacy of sulfonamides, as may purulent diseases (purulent exudate contains PABA).

5. **Drugs That Affect Bacterial DNA Synthesis**
Nalidixic acid and related fluoroquinolones (FQN) are bactericidal via inhibition of bacterial DNA through targeting DNA gyrase (topoisomerase II) and/or topoisomerase IV. Topoisomerase II is responsible for maintaining the supercoiling tension, while topoisomerase IV is responsible for appropriate segregation of daughter DNA strands into daughter cells. DNA gyrase tends to be the target for Gram-negative organisms, while topoisomerase IV tends to be the target for Gram-positive organisms. Resistance may develop as either low-level or high-level resistance – low-level resistance is achieved when bacteria alter genes encoding either topoisomerase II or IV, or genes involving efflux pumps or membrane permeability proteins; high-level resistance is achieved when genes encoding both topoisomerases II and IV are mutated.

**Side Effects**
Side effects that may occur with systemic administration of FQN include gastrointestinal upset, skin reactions, and headaches, dizziness, or drowsiness. A juvenile arthropathy, unique to animals and not documented in children, occurs after prolonged administration of high doses of drug. Tendonitis has also been reported following systemic administration.

In cats, systemic administration of enrofloxacin has been associated with irreversible retinal degeneration resulting in blindness.

Ciprofloxacin appears to be the topical preparation most frequently associated with side effects. These include irritation and foreign body sensation, as well as precipitation of drug within the cornea due to its relatively low pH and therefore tendency to precipitate out of solution. The side effects noted from systemic administration have not been reported to occur with topical administration.

**First Generation**
Nalidixic acid is considered the first-generation FQN and has a spectrum of activity relatively limited to gram-negative organisms, with weak activity versus *Pseudomonas aeruginosa.*
**Second Generation**

Second generation FQN generally include lomefloxacin, norfloxacin, enoxacin, ciprofloxacin, and ofloxacin. Their spectrum of activity is increased relative to that of nalidixic acid, particularly towards *Pseudomonas aeruginosa*, as well as some gram-positive organisms, however increasing resistance is developing, particularly to *Streptococcus* spp. *Staph* spp and *Strep* spp isolates from dogs with bacterial keratitis demonstrated 100% susceptibility to ciprofloxacin and enrofloxacin, while *Pseudomonas* isolates demonstrated 93% susceptibility to ciprofloxacin and 87% susceptibility to enrofloxacin. Another study confirmed the good susceptibility of *Pseudomonas* isolates from dogs to ciprofloxacin, as well as ofloxacin and norfloxacin. *Pseudomonas* isolates from horses were 100% susceptible to enrofloxacin, while 85% of *Strep* spp isolates were susceptible.

Comparison of the pharmacokinetics of topically applied ciprofloxacin and ofloxacin in dogs prior to cataract surgery identified that 1 drop every 15 minutes (8 drops total) of ciprofloxacin resulted in significantly lower aqueous humor concentrations than those resulting from the same treatment protocol for ofloxacin. The concentrations of ofloxacin achieved were much more likely to be therapeutic against potential ocular pathogens *E. coli*, *Bacillus* spp, and *Staph* spp than were the concentrations of ciprofloxacin.

**Third Generation**

Third generation FQN include sparfloxacin, gemifloxacin, and levofloxacin. Their use in human and veterinary ophthalmology is relatively infrequent.

**Fourth Generation**

Fourth generation FQN include gatifloxacin, moxifloxacin, and newer agents such as besifloxacin (considered by some to be fifth generation). Their gram-positive spectrum is increased relative to that of earlier FQN, however their efficacy versus *Pseudomonas* spp may be limited. Development of resistance to fourth-generation FQN is more difficult due to the necessity to achieve mutations in both topoisomerase II and topoisomerase IV enzymes – agents such as besifloxacin that have balanced targeting of both enzymes exhibit increased potency and decreased resistance even relative to other fourth-generation FQN agents. Moxifloxacin has consistently demonstrated superior intraocular penetration to other FQN.

In normal horses, moxifloxacin achieved greater AH concentrations than did ciprofloxacin following repeated topical ocular administration. Following experimental keratectomy in rabbits, moxifloxacin and gatifloxacin demonstrated no significant deleterious effect on epithelialization, relative to BSS control.

**Trends in FQN Resistance**

The incidence of resistance of Gram-positive organisms to older generation FQN (particularly ciprofloxacin) has as much as tripled in some cases, particularly involving MRSA and MSSA, while resistance to newer generation FQN has also increased in MRSA and MSSA, although to a lesser degree. Resistance of Gram-negative organisms to FQN has increased as well, particularly *Pseudomonas aeruginosa*, also to a lesser degree than other organisms.
ANTIFUNGAL AGENTS

General Principles of Therapy

Medically significant fungi exist as either yeasts (single cells, reproduce by budding), molds (cylindrical hyphae, reproduce by branching and apical extension, form multicellular filamentous mass in culture called mycelium), or as dimorphic forms (yeast phase in host tissues, mycelial phase in culture media). Fungi are eukaryotic, with a rigid outer cell wall composed of chitin and polysaccharides (glucans and mannans), inner cell membranes that create organelles, nucleic acids stored within a nucleus, and soluble cytoplasmic carbohydrates and storage compounds for energy. In contrast to bacteria, fungal organisms are more complex and share more structural commonalities with mammalian cells, making selective pharmacological targeting more difficult. Considering the variability, potential for greater toxicity to the patient, and more variable susceptibility patterns (as well as unclear correlations between in vitro and in vivo susceptibility profiles), antifungal medications are generally indicated only in the presence of confirmed infection.

Antifungal drugs fall into one of four classes – polyenes, pyrimidines, azoles, or echinocandins – all of which act primarily on the fungal cell membrane, with the goal being greater selectivity for the fungal organism versus the host.

1. Polyenes

The polyenes (amphotericin B, nystatin, and natamycin) target the fungal cell membrane component ergosterol, binding to it and forming a polyene-sterol complex, which ultimately induces leakage of vital intracellular constituents (i.e., K⁺). Their action is concentration-dependent, with the likelihood of fungicidal activity present at higher concentrations.

Amphotericin B

Amphotericin B is a broad-spectrum agent produced by the bacteria Streptomyces nodosus. Due to its poor water-solubility, the initial formulation of amphotericin B was available as a colloidal suspension with deoxycholate added as a solubilizer (Fungizone 50 mg/vial injectable), however this formulation is associated with significant systemic toxicities (renal, hematologic, hepatic toxicities). Recent formulations meant to enhance lipid solubility reduce these systemic toxicities without adversely affecting the spectrum of activity. These include liposomal (Ambisome, 50 mg/vial injectable), lipid complex (Abelcet 5 mg/ml injectable), and colloidal dispersion (Amphotec 50 mg/vial, 100 mg/vial injectable) formulations. No topical ocular formulation of amphotericin B is available. When reconstituting amphotericin B, sterile water should be used as amphotericin B is incompatible with saline, and the bottle should be refrigerated and protected from light.

Amphotericin B is highly effective against most Candida strains, and also against agents of systemic disease (i.e., Blastomyces, Coccidioides, Cryptococcus, Histoplasma), making it useful for invasive fungal infections involving the orbit, barring adverse systemic effects (oral bioavailability is extremely poor, and therefore parenteral administration is necessary in such cases). Its use for surface ocular or intraocular infections is relatively limited by more variable efficacy versus filamentous organisms common in ocular surface disease (i.e., Aspergillus, Fusarium, Mucor), minimal intraocular penetration following topical application (in the absence of corneal ulceration) or systemic administration, and ocular-specific toxic effects (conjunctival hyperemia, corneal edema, delayed healing of corneal ulcers).

In patients with keratomycosis, amphotericin B may be administered topically to reach acceptable corneal tissue levels in the presence of corneal ulceration, however concentrations greater than 0.15% are associated with greater toxicity. Injection of amphotericin B intrasmally has been reported in humans, and intravitreal injection has been used as well, however the risk for retinal toxicity is great in such cases. Subconjunctival injection has been utilized in horses with stromal abscesses non-responsive to aggressive antifungal therapy with good clinical outcomes (BC Gilger, personal communication; 0.2 ml of 5 mg/ml solution q48h x 3 doses), however the pharmacokinetics and pharmacodynamics, as well as local tissue toxicities, associated with this route of administration are unknown.

Natamycin (Pimaricin)

As the only approved topical ophthalmic antifungal agent (5% suspension), natamycin has an improved spectrum of action relative to that of amphotericin B, with better coverage versus organisms common in keratomycosis (i.e., Fusarium, Aspergillus, Curvularia, Acremonium). It is well tolerated and induces fewer
ocular toxicities following topical application, however it has historically poor transcorneal penetration, limiting its use in deep corneal or intraocular infections.

2. Pyrimidines

Pyrimidines block thymidine synthesis, and therefore DNA and RNA synthesis, leading to a fungistatic effect. The sole pyrimidine used as an antifungal agent is flucytosine (5-fluorocytosine), which is converted within the fungal cell to 5-fluorouracil, and then to 5-fluorodeoxyuridine monophosphate (inhibits DNA synthesis) and 5-FUMP (incorporated into RNA). Use of flucytosine is severely restricted due to the high likelihood of development of resistance, and is not indicated as an agent in monotherapy. Flucytosine is more effective versus yeast organisms, however combination therapy with amphotericin B has shown efficacy versus *Aspergillus* for systemic infections. Topical ocular administration of a 1% solution has been reported, however efficacy is lower than for other antifungal medications.

3. Azoles

The azoles are a large class of antifungal agents and, like polyenes, target the fungal cell membrane component ergosterol. Rather than binding to ergosterol however, they inhibit its synthesis through inhibition of the cytochrome P450-dependent enzyme 14α-sterol demethylase. Inhibition of this step in the synthesis of ergosterol leads to accumulation of toxic sterol precursors, inhibits fungal growth, and increases membrane permeability. Additionally, inhibition of mitochondrial oxidative and peroxidative enzymes leads to accumulation of other toxic intracellular products. These effects combine to result in fungistatic activity. Due to their action on the cytochrome P450 enzyme pathway, they also may exert inhibitory effects on other mammalian metabolic pathways utilizing that enzyme system, increasing the potential for drug interactions. Therefore, drugs that are metabolized through the P450 enzyme system may exhibit drug interactions with azoles, and caution should be utilized when combining such medications.

**Imidazoles**

Ketoconazole and miconazole are azoles based on the imidazole (C₃H₄N₂) parent ring structure.

**Ketoconazole**

Ketoconazole is a broad-spectrum azole, however its spectrum is more limited, potential hepatotoxicity is greater, and the onset of clinical effect is slow relative to that of other azoles, somewhat limiting its use. It is well tolerated when administered systemically, and has been administered topically (compounded formulation) and orally as treatment for keratomycosis in people. Topical ocular treatment with a 1% solution did not significantly impair wound healing or produce signs of ocular irritation in rabbits, and was able to penetrate the cornea and aqueous humor well.¹

**Miconazole**

Miconazole is a broad-spectrum azole frequently administered topically as a 1% compounded suspension (not commercially available). Alternatively, the 2% dermatologic or 2% vaginal creams are also administered to the ocular surface. When administered topically, miconazole is reported to have good intraocular penetration and exhibit minimal ocular toxic side effects.

**Triazoles**

Fluconazole, itraconazole, voriconazole, posaconazole, and ravuconazole are examples of azoles based on the triazole (C₂H₃N₃) parent ring structure.

**Fluconazole**

Fluconazole has a spectrum of activity limited primarily to yeast, such as *Candida* and *Cryptococcus*, with minimal efficacy versus mold forms frequently implicated in fungal keratitis in horses (i.e., *Aspergillus, Fusarium*). An advantage to the use of fluconazole is that it is well tolerated and highly bioavailable following oral administration, and also achieves measurable (and potentially therapeutic) levels in intraocular fluids.³¹ Measured aqueous humor fluconazole concentrations following oral administration to normal horses (14 mg/kg
once, then 5 mg/kg q24h x 10d) were 11.39 ± 2.83 µg/ml, comparable to synovial fluid and CSF concentrations (14.19 ± 5.07 and 14.99 ± 1.86 µg/ml, respectively).31

**Itraconazole**

Itraconazole has a broader spectrum of activity than does fluconazole, particularly toward filamentous fungal organisms, and is also well-tolerated following oral administration. Fusarium species are more resistant than other filamentous organisms.32 Its intraocular penetration however, is poor, with no drug detected in the aqueous humor of normal horses following administration of a single dose of 5 mg/kg itraconazole.33 The same study documented that absorption following oral administration of the commercially available solution was more consistent than absorption following oral administration of capsules with the contents dissolved in corn syrup. Topical administration of 1% itraconazole/30% dimethyl sulphoxide petroleum-based ointment resulted in high corneal tissue levels and minimal signs of ocular irritation.34

**Voriconazole**

Voriconazole is a derivative of fluconazole that has a significantly improved spectrum of activity (including filamentous organisms) that has replaced amphotericin B as the treatment of choice for invasive aspergillosis in people. Following administration of a single oral (4 mg/kg) or IV (1 mg/kg) dose to horses, it has excellent absorption and bioavailability and was well tolerated by all horses.35 A separate study involving normal horses documented therapeutic plasma levels within 10 minutes following a 10 mg/kg IV dose and within 20 minutes following a 10 mg/kg PO dose, remaining at potentially therapeutic levels for up to 24 hours.36 Additionally, likely therapeutic precorneal tear film levels (mean > 3 µg/ml) were reached following twice daily administration of 3 mg/kg orally for 10 days.

While in vivo clinical efficacy of antifungal agents is known to vary correlate with in vitro susceptibility results, and in vitro susceptibility results vary among isolates from different geographic regions, a few studies have evaluated isolates from equine clinical cases of fungal keratitis. Aspergillus and Fusarium isolates from Florida, Missouri, Georgia, and Tennessee consistently demonstrated significantly lower susceptibility to ketoconazole, fluconazole, and itraconazole, with consistently greater susceptibility to voriconazole and miconazole.37 Fusarium demonstrated greater susceptibility to natamycin than did Aspergillus. Another study evaluated the in vitro susceptibility patterns of Aspergillus, Fusarium, Penicillium, Cladosporium, and Curvularia isolates from equine ocular disease to natamycin, miconazole, ketoconazole, and itraconazole with and without a buffered chelating agent.38 The buffered chelator (composition unknown) reduced the MICs for all drugs to each fungal organism by 50% to 100%, with greater concentrations necessary for organisms with greater MICs.

4. **Echinocandins**

Echinocandins (caspofungin, micafungin, anidulafungin) are the most selective of the antifungal agents, targeting the fungal cell wall that is unique to fungal organisms (versus mammalian cells). Inhibition of glucan synthesis depletes the building blocks of the cell wall, weakening its structure and producing a uniquely fungicidal effect. Echinocandins do not affect the cytochrome P450 enzyme system either, producing fewer drug interactions or other systemic toxicities.

**Caspofungin**

Caspofungin is the first FDA-approved echinocandin, and is typically broad-spectrum with consistently strong activity versus Candida spp and Aspergillus spp, as well as against other azole-resistant organisms, with poorer efficacy versus Fusarium.32 It is a large molecule and therefore would be suspected to have minimal ocular penetration in the absence of inflammation. A study using rabbits with unilateral experimentally-induced uveitis (LPS) documented potentially therapeutic AH drug concentrations up to 8 hours following a single dose of IV caspofungin, along with potentially therapeutic corneal drug concentrations up to 24 hours following a single dose, in the eye with uveitis only,39 supporting the necessity of blood-ocular barrier breakdown for intraocular penetration, as well as the potential for the cornea to be an efficient drug reservoir. No detectable drug concentrations were achieved in the vitreous in inflamed or uninflamed eyes. In contrast, topical administration of a 7mg/ml solution of caspofungin to normal rabbit corneas did not achieve detectable
aqueous humor drug concentrations, while measurable (and potentially therapeutic) drug concentrations were reached in rabbits following creation of epithelial defects.\textsuperscript{40}

Treatment of experimentally induced \textit{Candida} keratitis in rabbits with 0.5\% caspofungin administered topically showed comparable clinical efficacy to treatment with 0.15\% amphotericin B, while 0.15\% caspofungin was inferior (but better than saline controls).\textsuperscript{41} Additionally, no signs of ocular toxicity were noted following the aggressive treatment protocol (q5min x 1hr, then q30min x 9h, stopped for 12h, then q1h x 12h). While the in vitro efficacy of caspofungin to \textit{Fusarium} isolates is limited, treatment of experimentally-induced \textit{Fusarium solani} keratitis in rabbits with 1\% caspofungin administered topically (q1h x 14h for 2 days, then q6h x 3 days) resulted in comparable clinical and microbiological efficacy to that of 0.15\% amphotericin B.\textsuperscript{42}

Clinical use of IV caspofungin in concert with IV voriconazole in four of five patients with endogenous \textit{Candida} endophthalmitis unresponsive to previous systemic therapy with fluconazole documented successful clinical resolution in all.\textsuperscript{43}

\textbf{Micafungin}

Micafungin is also effective versus \textit{Candida}, with some efficacy versus filamentous fungal organisms such as \textit{Aspergillus}. It is not effective against \textit{Fusarium}. Toxicological evaluation of topical ocular administration of 0.1\% micafungin to eyes of rabbits 13 times a day for 7 days documented no difference in IOP, endothelial cell density, or tear lactate dehydrogenase activity between treated and control (saline) animals, however a slight but significant reversible decrease in corneal thickness was recorded in treated animals.\textsuperscript{44} Intravenous injection of micafungin in rabbits identified retina-choroid tissue levels that were comparable to plasma levels, with minimal concentrations in the vitreous humor, indicating potential therapeutic efficacy for posterior fungal infections.\textsuperscript{45} Intravitreal injection of micafungin following intravitreal inoculation with \textit{Aspergillus} in rabbits documented prolonged preservation of the b-wave on ERG (relative to saline control), with the suggestion of lower toxic effects relative to amphotericin B or voriconazole.\textsuperscript{46}

Study of potential efficacy of subconjunctival injection of micafungin in rabbits with experimentally-induced \textit{Candida} keratitis showed superior clinical response to that of fluconazole, with no ocular side effects noted.\textsuperscript{47}

In clinical cases, topical administration of 0.1\% micafungin in one eye each of three patients with refractory fungal ulcers associated with \textit{Candida} spp or unidentified yeast resulted in satisfactory improvement when conventional antifungal therapy failed.\textsuperscript{48}

\textbf{Anidulafungin}

Anidulafungin is fungicidal and effective versus \textit{Candida} strains, as well as amphotericin B- and azole-resistant strains. No reports of efficacy in ocular disease exist at this time.

\textbf{References}

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