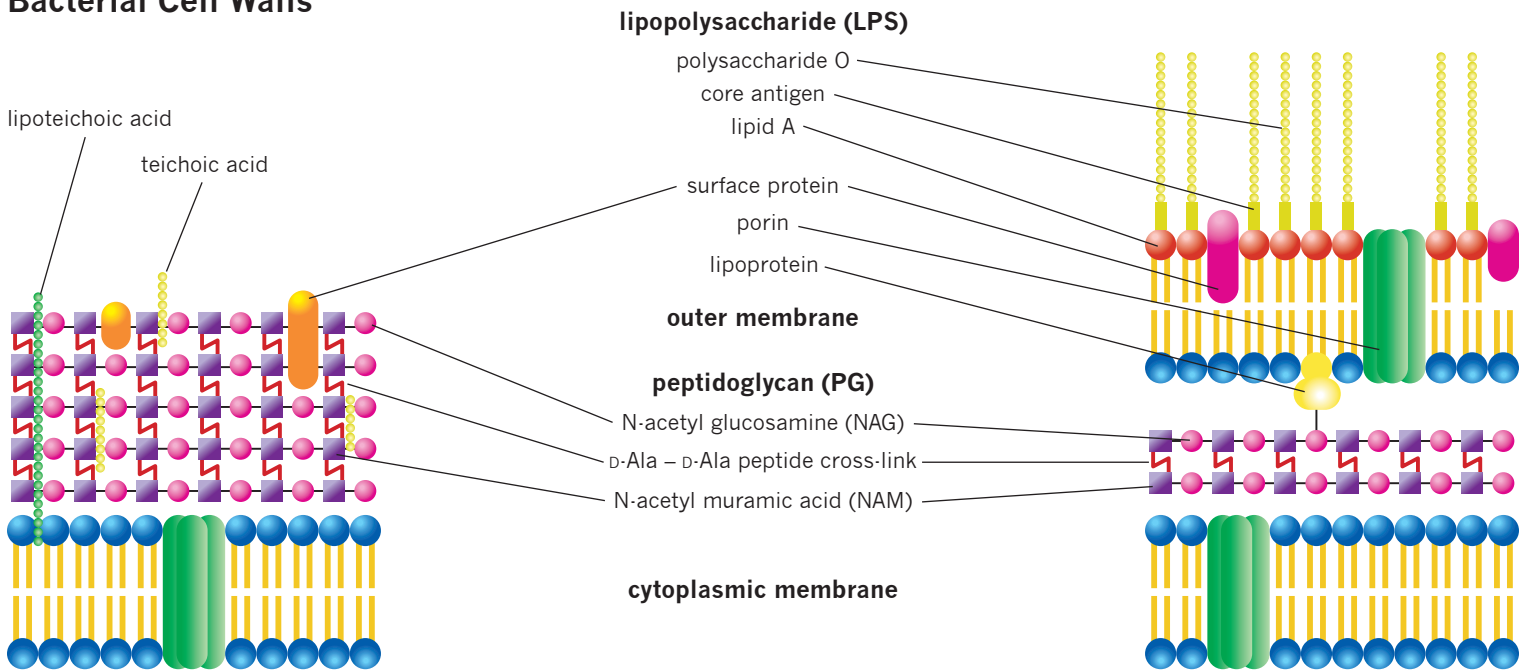


Antibacterials

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Bacterial Cell Walls



Gram Positive Cell Wall

Clinically important Gram positive bacteria include the cocci *Staphylococcus*, *Streptococcus*, and *Enterococcus*, and the rods *Bacillus*, *Corynebacterium*, and *Clostridium*. On the Gram stain, these organisms take up the crystal violet dye and appear purple on light microscopy.

The outer portion of the cell wall is made up of many interconnected layers of peptidoglycan subunits, NAM and NAG. Individual NAMs have side chains of 4 amino acids that terminate with a D-alanine residue, or D-Ala. This motif is recognized by enzymes that form a glycine bridge between the two side chains, linking the NAMs together. Lipoteichoic acids connect the peptidoglycan to the lipid bilayer of the cytoplasmic membrane, and teichoic acids within the layers help to give additional stability. Surface proteins include penicillin-binding proteins (PBPs), as well as bacterial virulence factors. These proteins, as well as peptidoglycan itself, are antigenic and initiate inflammatory pathways.

MICs and Kirby-Bauer Testing

Bacterial susceptibility of clinical isolates is frequently reported with a minimum inhibitory concentration (MIC) in $\mu\text{g}/\text{mL}$ and an interpretation – resistant, intermediate, or sensitive – based on *in vitro* data. The MIC represents the lowest concentration of drug that inhibits visible growth in liquid culture under standard growth conditions. (The minimum bactericidal concentration, or MBC, is the lowest amount of drug at which 99.9% of the original inoculum is killed.) An antibiotic's predictably achievable serum or tissue level (based on its pharmacodynamics) must reach and sustain the MIC for at least half the duration of its dosing interval in order for the drug to be effective at the site of infection. For example, if the reported MIC of penicillin is $\geq 16 \mu\text{g}/\text{mL}$ for a given isolate, the organism is probably going to be resistant *in vivo*, since the peak serum level for penicillin G is $20 \mu\text{g}/\text{mL}$ with normal kidneys – it would be nearly impossible to get a concentration of penicillin high enough to kill the bug. MICs can be estimated using automated “microdilution” assays in 96-well plates.

The most widely used method for reporting resistance to drugs is by disk diffusion assay, or the Kirby-Bauer interpretation method. Bacteria are inoculated evenly onto a plate, and then small paper disks impregnated with a fixed concentration of antibiotic are placed on the medium. As a gradient of drug diffuses out radially from the disk, the bacterial lawn may be inhibited by the drug. Based on manufacturer-specified guidelines, the zone of inhibition is interpreted; the MIC is inversely related to the radius of the zone. If the zone is greater than or equal to the defined diameter, the organism is susceptible. Synergism and antagonism of antibiotics can also be evaluated by examining the area between zones on the plate. The Kirby-Bauer method is qualitative, rather than quantitative in the microdilution assay.

Gram Negative Cell Wall

Clinically important Gram negative bacteria include the bacilli *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella*, *Moraxella catarrhalis*, *Haemophilus influenzae* and *Enterobacter*. On the Gram's stain, these organisms don't retain crystal violet and reveal the pink safranin counterstain instead.

For Gram negative organisms, a much thinner layer of peptidoglycan is sandwiched between two lipid bilayers. The semipermeable outer membrane is composed of lipopolysaccharide (LPS) molecules that face the environment, along with membrane-spanning porins and other proteins. LPS is highly antigenic and pro-inflammatory, much like peptidoglycan.

The “periplasmic space” between the outer and cytoplasmic membranes contains certain enzymes, including β -lactamases.

Cell-Wall Active: Penicillins

Penicillin was the first antibiotic, discovered in 1928 by Alexander Fleming after *Penicillium* mold accidentally contaminated a plate of *Staphylococcus* and created an area of clearing. Fleming didn't develop the drug, however – it wasn't until World War II that it was mass-produced for therapeutic use. The compound is based on a structure called the β -lactam ring. There are naturally occurring penicillins and four subsequent generations of synthetic derivatives. In general, each successive generation loses activity against Gram positive organisms and gains Gram negative activity.

Mechanism of Action

The β -lactams bind to and inhibit the penicillin-binding proteins, a group of transpeptidases in the cell wall that link the side-chains of NAMs together. Unable to cross link the monomeric subunits of peptidoglycan, the cell is vulnerable to osmotic changes and lyses. Early penicillins don't work against Gram negatives, because they can't cross the outer membrane.

Pharmacodynamics

All penicillins have short half-lives, so early generations are given as often as q4h for serious infections. Progressive generations lengthen this time. They achieve therapeutic levels in pleural, pericardial, peritoneal, and synovial fluids – and concentrate in bile higher than plasma levels. Most have poor CSF penetration in the absence of inflammation – but can be therapeutic in CSF during meningitis.

Naturals: Penicillin G and V

- Throat infections from *Streptococcus pyogenes*
- Enterococcal infections, when used with an aminoglycoside for synergy – a preferred regimen whenever possible. By itself, penicillin is only bacteriostatic against *Enterococcus*, not bactericidal
- Skin infections from most Gram positive rods, like *Clostridium*
- Oral abscesses and other dental infections from anaerobes
- Meningitis from *Neisseria meningitidis*, a Gram negative diplococcus
- Syphilis (*Treponema pallidum*) and leptospirosis (another spirochetal infection)

Anti-Staphylococals: Nafcillin, Oxacillin, Dicloxacillin

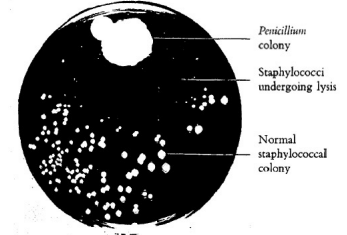
- Non life-threatening cellulitis from penicillinase-producing *Staphylococcus aureus*, but not oxacillin-resistant *Staphylococcus* (MRSA)
- Not really good for anything other than simple staphylococcal infections – they gain stability against *Staphylococcus*, but lose the other activities that natural penicillins have

2nd Generation/Aminopenicillins: Amoxicillin, Ampicillin

- Penetrate porins of Gram negatives, but are NOT β -lactamase stable
- Meningitis from *Listeria monocytogenes* (paired with an aminoglycoside)
- Upper respiratory and inner ear infections from *Moraxella catarrhalis* or nontypeable *Haemophilus influenzae* – in adults, only 15% of nontypeable *H. flu* has β -lactamase. (In contrast, “type b” *H. flu* causes more invasive disease, like meningitis and pneumonia.)
- Urinary tract infections from most *E. coli* and regular *Proteus*
- Gastroenteritis from *Salmonella* and *Shigella* (both Gram negatives)
- Both have identical spectrum, but amoxicillin is better absorbed orally and gets higher blood and urine levels

3rd Generation: Ticarcillin

- Penetrates Gram negative porins at high doses – but it's less active than its parent compound, ampicillin, on a weight-for-weight basis
- Carboxy group/side chain expands the spectrum and protects the ring from chromosomally-encoded β -lactamases
- Ticarcillin has especially good activity against *Pseudomonas aeruginosa*, but it's a disodium salt and can cause problems with CHF
- It may also cause platelet dysfunction, prolonging bleeding time



4th Generation: Piperacillin

- No added advantage over ticarcillin in terms of β -lactamase stability
- Also a derivative of ampicillin, with some notable differences from ticarcillin: greater activity on a weight-for-weight basis, slower killing of *Pseudomonas*, and no platelet dysfunction
- Works better than ticarcillin against *Pseudomonas* and the Gram negative enteric rods, aka Enterobacteriaceae (*Salmonella*, *Shigella*, *Serratia marcescens*, *Proteus mirabilis*, *Citrobacter*, *Enterobacter*, *E. coli*)
- Has limited activity against *Enterococcus* (a Gram positive coccus), *Klebsiella pneumoniae*, and *Bacteroides fragilis* (both Gram negative rods)

β -Lactamase Inhibitors

- Add anaerobic coverage to penicillins
- Inhibit plasmid-based β -lactamases by blocking their active sites, but generally don't work against chromosomally-encoded β -lactamases of highly resistant bugs
- *Clavulanate*, in amoxicillin/clavulanate (Augmentin) and ticarcillin/clavulanate (Timentin)
- *Sulbactam*, in ampicillin/sulbactam (Unasyn)
- *Tazobactam*, in piperacillin/tazobactam (Zosyn)

Carbapenems

- Broad-spectrum coverage of serious infections that might be polymicrobial, like necrotizing pancreatitis or bowel perforations
- Highly resistant to both plasmid and chromosomal β -lactamases
- Urosepsis from extended-spectrum β -lactamase (ESBL) producers like *Klebsiella pneumoniae*
- *Stenotrophomonas maltophilia* has, and *Bacteroides fragilis*, *Enterobacter cloacae*, and *Serratia marcescens* may have natural carbapenem-hydrolyzing chromosomal β -lactamases, so no activity against these organisms
- No activity against *Enterococcus faecium* or MRSA
- *Imipenem* is inactivated in the proximal tubule by human dihydropeptidase I, so it's paired with an inhibitor, *cilastatin* – it can achieve urine and kidney levels only with co-administration. Imipenem has prominent CNS toxicity (seizures), so can't use for meningitis
- *Meropenem* has a similar spectrum, it's stable by itself against the dihydropeptidase, and has a lower risk of seizure (OK for meningitis)
- *Ertapenem* has a narrower spectrum than either; better for anaerobes and Gram negative enteric rods, but poorer against Gram positives

Monobactams

- *Aztreonam* is a monocyclic β -lactam active only against Gram negative aerobes – no activity at all against Gram positives or anaerobes. Think of it like an aminoglycoside
- No cross-reactivity with pen-ceph allergic patients, unless allergic to ceftazidime

Cell-Wall Active: Cephalosporins

The cephalosporins are the other group of β -lactam antibiotics, originally isolated from *Cephalosporium* fungus. Just like penicillins, changing out side chains confers different pharmacodynamic properties on the drug. There are four generations of cephalosporins. In general, they follow the same pattern as the penicillins: subsequent generations gain Gram negative activity and lose Gram positive activity.

Pharmacodynamics

Drug penetrations are essentially identical to the penicillins; pleural, pericardial, peritoneal, synovial, and urinary levels are comparable to serum, and in bile they are concentrated. CSF penetration depends on the presence of inflammation.

First Generation: Cefazolin (IV), Cephalexin (PO)

- Simple skin or soft-tissue infections from oxacillin-sensitive *Staphylococcus* (MSSA) or *Streptococcus*. Works against penicillinase-producing *Staphylococcus*, but not MRSA.
- Works against *E. coli*, *Klebsiella*, and regular *Proteus mirabilis* – but no activity against “indole-positive” *Proteus* (meaning the organism can break down tryptophan to indole enzymatically, used to differentiate *Proteus* species from one another in the micro lab)
- No activity against *Enterobacter* or *Serratia*, which have chromosomal β -lactamases, or non-enteric Gram negative rods like *Acinetobacter* and *Pseudomonas*

Second Generation

- The second generation cephs have a little less activity against Gram positive cocci and a little more activity against Gram negative rods

Anti-Haemophilus: cefaclor (PO), cefuroxime axetil (IV)

- Induce *Enterobacter* and indole-positive *Proteus* chromosomal β -lactamases, which will degrade the antibiotic
- Cefuroxime is good against simpler *H. flu* and *M. catarrhalis* infections

Anti-Bacteroides: cefoxitin (IV), cefotetan (IV)

- Often used as peri-operative antibiotic in obstetrical-gynecologic and some general surgical procedures, because of expanded activity against *Bacteroides* and aerobic/facultative Gram negative rods
- Has *E. coli*, *Proteus mirabilis*, *Klebsiella* activity similar to first generation
- Resistant to most plasmid-based β -lactamases
- Like the anti-*Haemophilus* cephs, they induce *Enterobacter* and *Proteus* chromosomal β -lactamases
- Cefotetan has a side chain called N-methylthiotetrazole (NMTT) that can dissociate and competitively inhibit vitamin K, so it is coumadin-like and can increase the INR

Third Generation: Cefotaxime (IV), Ceftriaxone (IV), Ceftazidime (IV)

- Moderately serious infections in the lung, biliary tract, soft tissues, or urinary tract thought to be due to Gram negatives – less active against Gram positives in general, and without any activity against *Listeria*, *Enterococcus*, MRSA, or *Acinetobacter*
- Stable against the β -lactamases of common Gram negative rods
- Highly active against most Enterobacteriaceae (*E. coli*, indole-positive *Proteus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Serratia*)
- Works against *Neisseria meningitidis* and *gonorrhoeae*, as well as *H. influenzae* – ceftriaxone is the treatment of choice for meningococemia
- Fully penicillin-resistant *Streptococcus pneumoniae* will be resistant to third generation cephs
- Two subgroups: those with *Pseudomonas* coverage and those without

Poor pseudomonal coverage: cefotaxime, ceftriaxone

- Ceftriaxone can cause biliary sludging and pseudolithiasis with crystallized antibiotic.

Good pseudomonal coverage: ceftazidime

- Ceftazidime is stable to plasmid-encoded Gram negative β -lactamases and so it's good for the Enterobacteriaceae, *Neisseria* spp., *Pseudomonas*, and *H. flu* – it doesn't work against Gram positives, so reserve ceftazidime for infections thought to be due to *Pseudomonas*

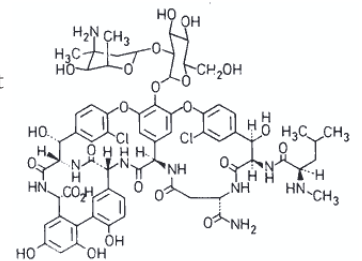
Fourth Generation: Cefepime

- Recommended for broad-spectrum empiric coverage of polymicrobial or multidrug-resistant infections among neutropenic patients and hospital-acquired pneumonia patients (since cefepime covers *Pseudomonas*)
- Similar to ceftriaxone and cefotaxime (third generations) against pneumococcus and MSSA – and it retains its activity against Gram negative enteric rods with inducible β -lactamases (*Enterobacter*, *Citrobacter*, indole-positive *Proteus*, and *Serratia*)

Cell-Wall Active: Vancomycin

Although it is a cell-wall active drug like the β -lactams, vancomycin (part of a naturally occurring group of compounds called glycopeptides) works in a much different way. The β -lactams inhibit the enzymes that are responsible for cross-linking the D-Ala side chains of NAM molecules with glycine bridges. Vancomycin fits around polypeptide intermediates terminating in D-Ala–D-Ala residues, sterically blocking both transglycosylases and transpeptidases that are critical to cross-linking the peptidoglycan subunits. This may promote cell lysis – without an intact peptidoglycan layer around the organism, it's vulnerable to osmotic pressure.

Vancomycin is a complex molecule and it's pretty much no surprise that it can't cross the outer membrane of Gram negative bacteria and only works in Gram positive infections. Orally administered drug isn't absorbed systemically.



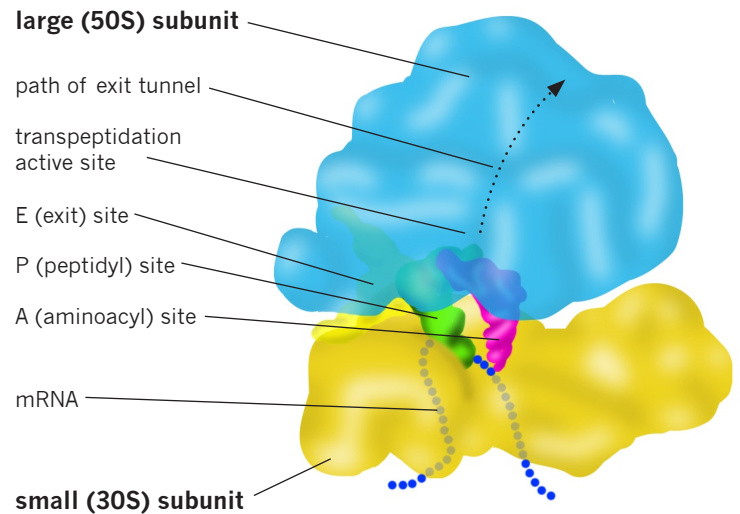
Common clinical uses for vancomycin include:

- empiric coverage of serious infections thought to be due to Gram positives (especially MRSA), such as line and port infections
- suspected pneumococcal meningitis, along with ceftriaxone (because of increasing incidence of cephalosporin-resistant *Streptococcus pneumoniae*)
- any confirmed MRSA infection – from skin to pneumonia
- oral administration for *Clostridium difficile*-associated colitis and diarrhea

Ribosomal Function

Two subunits make up the ribosome, creatively named large and small (and sometimes called 50S and 30S, respectively). To understand why antibiotics targeted at protein synthesis are effective, a quick look at the structure and function of ribosomes is helpful. (Incidentally, “S” refers to a unit of density called a “Svedberg unit,” a way of defining the sedimentation rate of macromolecules in an ultracentrifuge. It’s not additive; the combined ribosome is a 70S molecule.)

Both subunits are made up of ribosomal RNA (rRNA) and different structural proteins. Small and large subunits must meet up properly in order for protein synthesis to occur. The small subunit is responsible for binding mRNA and holding it in an orientation that lets the proper aminoacyl tRNAs line up with the exposed codons. Binding sites for the tRNAs are along the surface of the small subunit. The large subunit has the active site for transpeptidation (peptide bond formation) to occur. Positioned together and bound to the small subunit, the tRNAs have their associated amino acids linked up to form the new polypeptide chain. This chain exits through a tunnel inside the large subunit. *Basically the small subunit handles the mRNA and the tRNA while the large subunit catalyzes peptide bond formation and extrudes the new polypeptide chain through its exit tunnel.*



Small Subunit Interference: Aminoglycosides (Gentamicin, Tobramycin, Amikacin)

Plain and simple, aminoglycosides used by themselves work best against aerobic Gram negative organisms. When used in combination with other antibiotics (usually cell-wall active drugs like the penicillins, cephalosporins, or vancomycin), they display synergy and broader coverage.

Mechanism of Action

They bind to small (30S) subunit and cause misreading of mRNA, inhibiting translocation (sliding of mRNA through the ribosome). The initial steps of peptide synthesis are uninterrupted (mRNA binding, “charging” of tRNAs with amino acids and association along small subunit), but elongation fails. So why are these bactericidal drugs, when other antibiotics like the macrolides and tetracyclines work in similar ways but are only bacteriostatic? Controversy exists, but the prevailing theory is that aminoglycosides also disrupt magnesium bridges between LPS molecules of the outer membrane of Gram negatives, and are then transported across the membrane in an energy-dependent way. Because they’re polar molecules, membrane penetration is pretty poor without assistance. The idea of “punching holes” in the outer membrane isn’t too far off, but that’s not considered their principal mechanism of action.

Pharmacodynamics

There are two terms that are helpful to understand with respect to aminoglycosides and other antibiotics: *post-antibiotic effect* (PAE) and *concentration-dependent killing* (CDK). The PAE refers to persistence of the drug’s effect after it has been removed *in vitro* or cleared *in vivo*. For aminoglycosides, this averages about 3 hours, but may be as long as 8 hours in patients with normal renal function. The ability of a higher concentration of drug (relative to the organism’s MIC) to induce rapid and complete killing is concentration-dependent killing.

Aminoglycosides get into serum, urine, and other body fluids because of weak protein-binding – but they have poor CSF, biliary, or bronchial penetration. About 99% of the dose is eliminated unchanged by the kidney. Because of a rare side effect of neuromuscular blockade, myasthenia gravis is an absolute contraindication to administration.

Drug Monitoring

Peak drug concentrations are seen 30 to 60 minutes after completion of a transfusion; troughs should be checked within 30 minutes of the next dose.

Clinical Uses

- Used empirically for serious conditions like sepsis, respiratory tract infections, complicated UTIs, intra-abdominal infection, and osteomyelitis – and are typically discontinued when the organism and its susceptibilities are identified
- It was thought that if you paired an aminoglycoside with a third generation cephalosporin (e.g. ceftazidime), you’d prevent the induction of β -lactamase among *Enterobacter*, *Citrobacter*, *Serratia*, and indole-positive *Proteus* – but this is not the case, the enzyme is still induced
- Only prophylactic use of aminoglycosides is with ampicillin or vancomycin in pre-procedural GI and GU patients with valvular heart disease, at risk of developing bacteremia with *Enterococcus*
- Minor variations in activity from drug to drug exist, such as gentamicin working better against *Serratia* spp., and tobramycin’s efficacy in *Pseudomonas* infections
- Resistance is infrequent, but when it does occur it’s due to drug-inactivating enzymes acquired from transposons or plasmids, or from altered membrane permeability and decreased uptake
- Enterococci are inherently resistant to low-to-moderate aminoglycoside levels, but synergism is seen with cell-wall active drugs
- *Enterococcus faecium* has inherent natural resistance to moderate-level tobramycin, so synergy won’t work with tobramycin – but the aminoglycoside-modifying enzyme only attacks tobramycin, not gentamicin or amikacin

Small Subunit Interference: Tetracyclines

In general, the tetracyclines (tetracycline, doxycycline, and minocycline) are bacteriostatic drugs, as they reversibly inhibit protein synthesis. Some derivatives are bactericidal in some organisms. A semisynthetic glycolcycline, called tigecycline, was approved in 2005 and has potent activity against resistant organisms like VRE, MRSA, and many multidrug-resistant Gram negative rods, except *Pseudomonas aeruginosa*.

Mechanism of Action

Although they've been around a long time, the mechanism of tetracyclines isn't well understood. The small subunit has a "high-affinity" binding site for tetracyclines that weakens the association of the tRNA with the subunit itself. They may interfere directly with tRNA binding.

Pharmacodynamics

Bacteria sensitive to tetracyclines have rapid uptake of the drug that's partially energy-dependent. Passive accumulation along the outside of bacteria is related to its binding of cell components like phospholipids and proteins. No influx pump has ever been identified, although long theorized. The generally accepted theory is that the drug passes across the outer membrane of Gram negative organisms via porins, driven by a voltage potential from the environment into the periplasmic space. The drug chelates a positively charged metal cation and this assists in moving it across. Once inside, it dissociates and because it's weakly lipophilic, moves across the cytoplasmic membrane.

Clinical Uses

- All tetracyclines have essentially same spectrum of activity, working as bacteriostatics in both Gram negative and Gram positive organisms, as well as mycoplasmal, rickettsial, and spirochetal infections – doxycycline is the tetracycline of choice for most prescriptions
- Community-acquired pneumonia, especially if an atypical organism like *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* (formerly *Chlamydia pneumoniae*) is suspected
- Rickettsial diseases like ehrlichiosis, Rocky Mountain spotted fever (*Rickettsia rickettsii*), and Q fever (*Coxiella burnetii*)
- STDs like chlamydia and lymphogranuloma venereum (*Chlamydia trachomatis*), or uncomplicated gonorrhea (*Neisseria gonorrhoeae*)
- Disorders from spirochetes, like Lyme disease (*Borrelia burgdorferi*)
- Because these drugs chelate cations, you can't administer them with antacids, as it will bind the drug and prevent absorption (just like the quinolones).

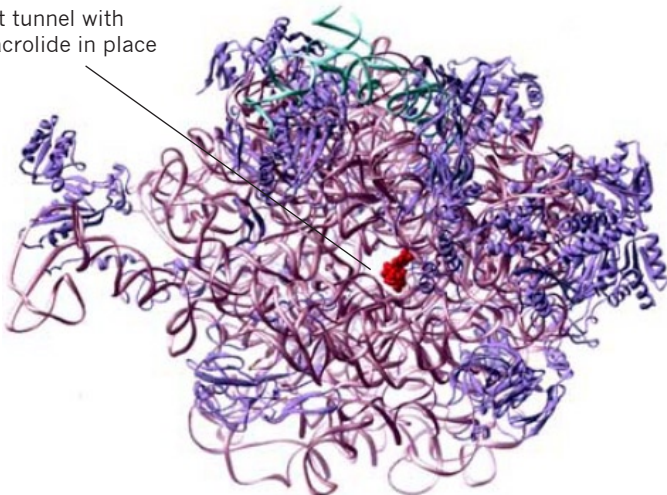
Large Subunit Interference: Macrolides (Azithromycin, Clarithromycin, Erythromycin, Telithromycin)

The macrolides are generally considered bacteriostatic, and have a fairly broad coverage across both Gram negatives and Gram positives. A "ketolide" antibiotic derived from the macrolides, telithromycin was approved in 2004 and has a somewhat enhanced activity against the same spectrum of organisms.

Mechanism of Action

Macrolides and ketolides share a unique mechanism that targets the large subunit's protein exit tunnel. Although it was once thought that the tunnel didn't do much other than protect the first part of the new polypeptide, studies have shown that it has an important role in post-translational modification and protein folding. Part way through the tunnel, it narrows – and this is where the macrolides bind. Sterically blocking the polypeptide from exiting the tunnel, they literally jam up the ribosome, and it can't continue with elongation. The ribosome loses the tRNAs bound to the small subunit, and eventually the whole ribosome may dissociate.

exit tunnel with
macrolide in place



Pharmacodynamics

Macrolides concentrate well in most tissues and especially inside polymorphonuclear lymphocytes (PMNs), so they're ideal for intracellular infections. In pulmonary secretions, levels can get as high as 100 times that of the plasma, so they have good efficacy in pulmonary infections with atypical organisms. Azithromycin achieves the greatest concentrations, followed by clarithromycin and erythromycin.

Clinical Uses

- Simple Gram positive infections with *Streptococcus* species, oxacillin-sensitive *Staphylococcus* (MSSA), and *Streptococcus pneumoniae* (pneumococcus)
- Gram negative respiratory infections with *Moraxella catarrhalis* and *Haemophilus influenzae*
- Community acquired pneumonia, including atypicals like *Chlamydia pneumoniae* (formerly *Chlamydia pneumoniae*) and *Mycoplasma pneumoniae*
- Bacterial sinusitis and acute otitis media
- Acute exacerbation of chronic bronchitis (AECB, also called "COPD exacerbation")
- Prophylaxis or treatment of disseminated *Mycobacterium avium* complex (MAC) infection
- Non-gonococcal urethritis and cervicitis from *Chlamydia trachomatis* (azithromycin, 1 gm PO once)
- Urethritis and cervicitis from *Neisseria gonorrhoeae* (azithromycin, 2 gm PO once)
- Erythromycin, clarithromycin, and telithromycin all inhibit cytochrome P450 3A4, so take caution with drug-drug interactions!

Large Subunit Interference: Clindamycin

Mechanism of Action

Clindamycin, a “lincosamide” antibiotic, binds to the large subunit of the ribosome and inhibits the formation of peptide bonds in the new polypeptide. This prevents chain elongation, similar to the macrolides and streptogramins – but clindamycin is structurally unrelated to either of these. It may make it easier for opsonization and phagocytosis of bacteria to take place, by disrupting synthesis of surface proteins. Staphylococcal toxin production associated with toxic shock syndrome is inhibited by clindamycin – important in treatment of necrotizing fasciitis and toxic shock.

Pharmacodynamics

Clindamycin has a significant post-antibiotic effect against some bacteria, and may have concentration-dependent killing for some species also. Aerobic Gram negative organisms like the Enterobacteriaceae, *Pseudomonas*, and *Acinetobacter* are resistant to clindamycin because it can't penetrate the outer membrane – but most anaerobic organisms, even if they're Gram negative, are susceptible. Bactericidal activity is seen with some staphylococci, streptococci, and anaerobes like *Bacteroides*. Bioavailability is good after oral administration, and is well distributed except into the CSF – even during meningitis. Clindamycin is concentrated in macrophages and neutrophils, so it usually gets into abscesses well.

Clinical Uses

- Suppression of staphylococcal toxin production in skin infections
- Osteomyelitis, because of excellent bone concentration
- Anaerobic infections from Gram positives or Gram negatives
- Abscesses in the lung or other tissues
- Caution with associated *Clostridium difficile* infection as a result of using clindamycin
- Not to be used if infection with a Gram negative aerobe is suspected



Necrotizing fasciitis at the elbow

Large Subunit Interference: Streptogramins (Quinupristin-Dalfopristin)

The combination of quinupristin and dalfopristin, marketed as Synercid, is in the group of drugs called streptogramins – part of a larger group that includes macrolides and lincosamides (clindamycin). Quinupristin is a derivative of streptogramin A, and dalfopristin is derived from streptogramin B. Although they are both streptogramins, they are structurally unrelated.

Mechanism of Action

Streptogramin A, or dalfopristin, binds to the large subunit of ribosomes and prevents elongation of peptide chains. It also causes a conformational change in the ribosomal subunit that increases affinity for streptogramin B. Quinupristin (streptogramin B) binds to the large subunit as well, further preventing peptide elongation. Incomplete polypeptide chains are released from the ribosome. So their function together is much like macrolides – but unlike their cousins, the streptogramins are generally bactericidal.

Pharmacodynamics

Synercid displays a significant post-antibiotic effect. Against vancomycin-resistant *Enterococcus faecium*, quinupristin-dalfopristin is bacteriostatic. It has no activity at all against vancomycin-resistant *Enterococcus faecalis*, so speciation of the organism is extremely important if you're considering the use of Synercid. For other Gram positives, like methicillin-sensitive *Staphylococcus aureus* (MSSA) and *Streptococcus pyogenes* the drug is bactericidal.

Clinical Uses

- The principal use is against vancomycin-resistant *Enterococcus faecium*. It does not work against vancomycin-resistant *E. faecalis* – only *E. faecium*. (Remember Synercid, faecium). *E. faecalis* is inherently resistant to quinupristin-dalfopristin.
- FDA-approved for complicated skin and skin structure infection (cSSSI) with oxacillin-sensitive *Staphylococcus aureus* (MSSA) and *Streptococcus pyogenes*.
- It may have activity against MRSA, and tests against VISA and VRSA are pending.

Large Subunit Interference: Oxazolidinones (Linezolid)

The first in a new class of totally synthetic antibiotics called the oxazolidinones, linezolid has activity against virtually all clinically significant Gram positive organisms.

Mechanism of Action

As a class, the oxazolidinones inhibit protein synthesis in a completely unique way: by preventing association of the large and small subunits. The drug binds to the large subunit near where it interfaces with the 30S small subunit, preventing the ribosome from assembling at all. Resistance is slow to develop resistance in *in vitro* studies. Although it's technically a bacteriostatic agent, linezolid does display *in vitro* bactericidal activity – albeit slower than other cidal agents.

Pharmacodynamics

The drug has complete bioavailability like the quinolones, so IV and PO dosing is equivalent. Its half-life is long enough to allow for twice-daily administration. Protein binding is low and it has good penetration to tissues that have adequate vascular supply. Linezolid's greatest activity is against Gram positive organisms, and it has poor action against Gram negative enteric bacteria (Enterobacteriaceae)

and *Pseudomonas*. For mycobacterial species, including *Mycobacterium tuberculosis* and *Mycobacterium avium* complex organisms, linezolid has good *in vitro* activity. *Nocardia* species are susceptible as well *in vitro* studies.

Clinical Uses

- Both vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*
- Community acquired pneumonia from penicillin-sensitive *Streptococcus pneumoniae* and *Staphylococcus aureus* – but not as a first-line agent because of poor activity against *Haemophilus influenzae* and atypicals like *Mycoplasma* and *Legionella*
- Hospital acquired pneumonia from MSSA and MRSA
- Complicated skin and soft tissue infections from *Streptococcus pyogenes* and *Streptococcus agalactiae*
- Causes reversible but sometimes severe bone marrow suppression when given for >2 weeks

Nucleic Acid Interference: Quinolones

There are four generations of quinolones, and they sort of follow a reverse pattern from the penicillins and cephalosporins in terms of coverage. The first quinolones were best at Gram negatives, and with each passing generation they gain Gram positive coverage. The first generation drugs, like norfloxacin, aren't used much currently.

Mechanism of Action

Bacterial replication of DNA is targeted by quinolones. Remember that different enzymes are used to package or condense the DNA, and others are used to unwind it during replication or transcription. In Gram negative organisms, the unwinding is handled mostly by DNA gyrase, while in Gram positives, it's DNA topoisomerase type IV. Inhibiting these enzymes makes the DNA structurally vulnerable and prone to breakage as it twists in and out of supercoils.

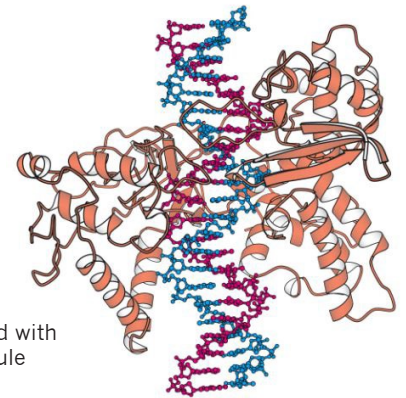
Pharmacodynamics

Considered bactericidal drugs, the quinolones display properties of concentration-dependent killing and a one-to-two hour postantibiotic effect. However, at higher concentrations they inhibit RNA and protein synthesis – since their killing ability is related to active cell division, this effect can make them paradoxically less active at high concentrations.

Serum levels are equivalent for PO and IV dosing, but they chelate cations and will have reduced bioavailability if given orally and taken with antacids or calcium supplements. Elimination is both renal and non-renal. Quinolones are very good at getting into macrophages and neutrophils. They get into tissues better than into fluids or plasma, so they work well in kidney infections. Bone and prostatic fluid penetration is equal to plasma levels (with no site-dependent concentration of drug). CNS penetration is poor in general; they achieve levels that are high enough only to cause nausea and dizziness. These medications can also cause QT prolongation.

Second Generation: Ciprofloxacin

Ciprofloxacin (Cipro) has expanded atypical and Gram negative coverage, but very limited for Gram positives. Aerobic Gram negative rods are the most susceptible. Although historically ciprofloxacin is considered the best for *Pseudomonas aeruginosa* coverage, almost half of



DNA topoisomerase complexed with a double-stranded DNA molecule

all isolates in 2003 at Rhode Island Hospital and The Miriam Hospital are resistant to the drug. Along with levofloxacin, ciprofloxacin has higher renal clearance and increased concentrations in the urine, so ciprofloxacin works well in complicated urinary tract infections.

Third Generation: Levofloxacin, Gemifloxacin

Levofloxacin (Lеваquin) retains ciprofloxacin's expanded Gram negative coverage and activity against atypical and intracellular organisms, but adds Gram positive activity – specifically, *Streptococcus pneumoniae* is covered. Gemifloxacin (Factive) was introduced in 2004.

Fourth Generation: Moxifloxacin, Gatifloxacin

The fourth generation quinolones have increased Gram positive coverage over the third generation, as well as increased anaerobic coverage – all while retaining the original Gram negative spectrum. Third and fourth generations work best against atypicals, like *Legionella*, *Chlamydia*, and *Mycoplasma*. An important warning came out in 2005 regarding gatifloxacin (Tequin), which can cause profound hypoglycemia in some older patients, especially diabetics.

Nucleic Acid Interference: Rifamycins (Rifampin, Rifabutin, Rifapentine)

Although rifampin is principally used as part of multidrug therapy for *Mycobacterium tuberculosis* (along with isoniazid, pyrazinamide, and ethambutol), it and its cousins rifabutin and rifapentine have broad-spectrum antibacterial action.

Mechanism of Action

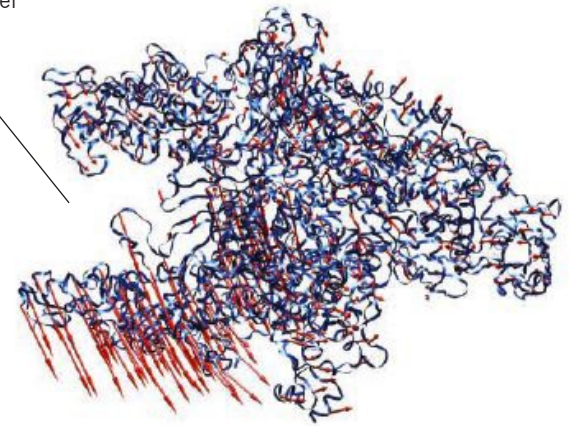
The rifamycins have a unique mechanism of action. Bacterial DNA-dependent RNA polymerase is responsible for transcribing DNA into RNA, and it has variable ability at proofreading (sensing an error in transcription and reversing direction to remove and replace a mismatched nucleotide base pair). Along one margin of the enzyme, there is a groove or channel that hugs the DNA and RNA as it is processed. The groove is flexible and the rifamycins bind there, physically blocking RNA elongation.

Pharmacodynamics

Intracellular penetration is excellent, so pathogens contained within other cells are good targets. The rifamycins are bactericidal for most strains of *Mycobacterium tuberculosis*, but resistance to isoniazid and rifampin tends to travel together (by definition, this would be multidrug resistant tuberculosis, or MDR-TB). Oral dosing provides very good absorption from the GI tract, and is widely distributed to most tissues – including the CSF. Within polymorphonuclear lymphocytes (PMNs, or segmented neutrophils), concentrations can be up to five times that of the serum level.

There are multiple drug interactions, as *rifampin ramps up* the cytochrome P450 system (specifically, isoenzyme 3A4). This is important, as co-administration of rifampin can lower levels of other drugs like oral contraceptives, phenytoin (Dilantin), warfarin (Coumadin), azole antifungals (e.g., fluconazole), cyclosporine, β -blockers, and the protease inhibitors of highly active antiretroviral therapy (HAART) in HIV treatment. Dose adjustments and monitoring are essential. Resistance to the drug develops rapidly when it's used as monotherapy – so unless it's being used as prophylaxis, rifampin is always given with other antibiotics.

DNA-RNA channel



Clinical Uses

- Treatment of active pulmonary or extrapulmonary tuberculosis
- Prophylaxis for close contacts in *Neisseria meningitidis* and *Haemophilus influenzae* outbreaks
- Adjunctive therapy for Gram positive infections with *Staphylococcus*, especially endocarditis, osteomyelitis, or deep-tissue infections with MRSA. Coagulase negative *Staphylococcus*, *Streptococcus pyogenes*, pneumococcus, and viridans group *Streptococcus* species are all affected. Activity has been demonstrated *in vitro* against many different Gram negative organisms, but rifampin is rarely used for these.

Metabolic Disruption: Metronidazole (Flagyl)

Flagyl is one of the drugs of choice for anaerobic infections and for pseudomembranous colitis and *Clostridium difficile*-associated diarrhea. It also can be used for infections with some protozoans, like *Trichomonas vaginalis*.

Mechanism of Action

Although metronidazole is an older drug, its mechanism isn't well understood. It is known to produce free radicals which damage the organism, and its activity is limited to anaerobic organisms, either facultative or obligate. An oxidoreductase enzyme in the organism's mitochondria acts on the compound, and the drug soaks up some of the electrons in the electron transport chain that would otherwise go toward making ATP. By-product free radicals spill out through the organism, damaging DNA and destabilizing other structures.

Pharmacodynamics

Both the parent drug and several metabolites have antibacterial activity *in vivo*. Concentration-dependent killing is seen with anaerobes, as well as the protozoans *Trichomonas vaginalis*, *Giardia lamblia*, and *Entamoeba histolytica*. High serum concentration is achieved with oral administration, with excellent tissue penetration. This includes the CSF, at about half of the serum level – making it useful for brain

abscesses. Protein binding is relatively low, so most of the drug is free in the circulation. In patients who have non-obstructed biliary disease, with preserved gallbladder functioning or at least a patent cystic duct, bile concentrations are approximately equal to serum. If there is obstruction with a stone, very little metronidazole makes it into the gallbladder bile.

Clinical Uses

- Anaerobic infections, essentially anywhere in the body
- Diarrhea due to *Clostridium difficile* – although initial response rates are better with oral vancomycin than with metronidazole
- The drug is bactericidal for most species of *Bacteroides*, *Clostridium*, *Gardnerella* (bacterial vaginosis), *Prevotella* (vaginal flora), and *Fusobacterium* (oral). About three-quarters of isolates from the genera *Propionibacterium* (skin anaerobe), *Actinomyces*, and *Lactobacillus* (vaginal flora) are resistant to metronidazole. *Mobiluncus*, another agent of bacterial vaginosis, has variable resistance.

Metabolic Disruption: Trimethoprim and Sulfonamides

Targeting a biochemical pathway that is present in bacteria but absent in humans is one way of achieving *selective toxicity*. The quintessential example of this involves folate metabolism in bacteria. For humans, dietary folate either diffuses into or is transported into cells – but in bacteria, their cell wall components limit this movement. They must synthesize their own folate *de novo*, and have evolved unique metabolic pathways to take para-aminobenzoic acid (PABA) and convert it into a folate precursor, tetrahydrofolate.

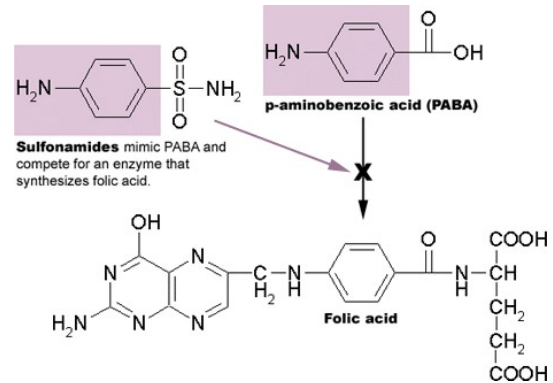
Mechanism of Action

Sulfonamide antibiotics like sulfamethoxazole are structural analogs of PABA and competitively inhibit the dihydropteroate synthase enzyme – significantly diminishing the pool of dihydropteroate available for conversion into dihydrofolate. When sulfamethoxazole is used in combination with trimethoprim – a potent inhibitor of dihydrofolate reductase (DHFR) – the limited supply of dihydrofolate is unavailable for metabolism at all. Independent of one another, each drug is bacteriostatic, but the combination is generally bactericidal.

Pharmacodynamics

Sulfonamides are usually given PO and have excellent absorption from the GI tract, with penetration into the CSF as well as peritoneal, pleural, and synovial fluids. Similarly, trimethoprim has almost complete GI absorption and distributes widely, with higher-than-plasma concentrations in most bodily fluids. Although the optimal ratio of trimethoprim (TMP) to sulfamethoxazole (SMX) is 1:20 *in vitro*, the fixed-dose combination in single-strength Bactrim or Septra is 1:5 (80 mg TMP:400 mg SMX). Renal adjustments are made based on the TMP component, expressed in mg/kg of TMP per day.

Sulfa drugs are known to cause a wide range of side effects, the most common being a pruritic rash, nausea, and vomiting. Drug-induced lupus, hemolytic anemia (sometimes related to glucose-6-phosphate dehydrogenase deficiency), agranulocytosis, and hypersensitivity syndromes like erythema nodosum, erythema multiforme, Stevens-Johnson syndrome, or toxic epidermal necrolysis are more rare but more serious adverse reactions. Pregnancy is an absolute contraindication to administration of either TMP or SMX, because they cross the placenta easily and may have deleterious effects on folate homeostasis in the fetus; remember that neural tube defects are related to folate deficiency.



Clinical Uses

- Treatment of acute, uncomplicated cystitis without pyelonephritis
 - Infections with *Nocardia asteroides*, an uncommon Gram positive rod considered to be an opportunistic infection among immunosuppressed patients
 - Non-life-threatening skin and soft tissue infections with Gram positives or Gram negatives, including oxacillin-resistant *Staphylococcus aureus* (MRSA) and community-acquired MRSA (CA-MRSA)
 - Cerebral or chorioretinal toxoplasmosis from *Toxoplasma gondii*.
- Note that TMP-SMX is a second-line regimen to the combination of pyrimethamine (another DHFR inhibitor), sulfadiazine, and leucovorin (aka folinic acid, an antidote to toxicity from inhibition of human DHFR. Leucovorin is the same drug used to prevent toxicity from another well-known inhibitor of human DHFR used in chemotherapy regimens – methotrexate)
- *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*) pneumonia (PCP), with or without concomitant HIV infection
 - Chemoprophylaxis against *Toxoplasma* and *Pneumocystis*

Cell Wall Disruption: Daptomycin

The first of a new class of drugs called cyclic lipopeptides, daptomycin was discovered back in the 1980s but shelved by the manufacturer because of concerns over skeletal muscle toxicity. In 2004, the FDA approved the drug for use in complicated skin and soft tissue infections from *Staphylococcus aureus*, *Streptococcus pyogenes*, and vancomycin-sensitive *Enterococcus faecalis* (not VRE).

Mechanism of Action

Derived from *Streptomyces* fungi, the drug acts at the cytoplasmic membrane through a calcium-dependent insertion of its lipid tail into the bilayer. These drugs work only on Gram positive organisms, because it can't penetrate the outer membrane of Gram negatives. The theory of its action is that an ion-conducting structure forms from the drug's penetration of the membrane, causing K⁺ efflux and dissipation of concentration gradients used for metabolism. Death results from dysfunction of macromolecular synthesis (nucleic acids and proteins). Bacterial cells don't undergo lysis as a direct result of the drug's effect, but it is a cidal antibiotic.

Pharmacodynamics

Like the aminoglycosides and quinolones, daptomycin has rapid concentration-dependent killing for most susceptible organisms. Its

half-life is about 8 to 9 hours, so it allows for once-daily dosing (a significant post-antibiotic effect). It is highly bound to proteins because of its hydrophobic tail, and has a low volume of distribution because it can't cross biologic membranes.

Clinical Uses

- So far, its only indication is for skin and soft tissue infections.
- In drug testing, daptomycin didn't get into bronchial secretions, lung tissue, or broncho-alveolar fluid – so it's not indicated at all for Gram positive community or hospital acquired pneumonias.
- Efficacy testing for use with vancomycin resistant bacteria is pending.
- Because of reports of myopathy, weekly CPKs are recommended, and caution should be used when giving it along with HMG-CoA reductase inhibitors (statins).

Topical Antibacterials

Used for prophylaxis of infection in superficial wounds, topical antibiotics are formulated specifically for external use – either because the drug isn't absorbed much, or its systemic effects are toxic if given orally or parenterally.

Cell Wall Active: Bacitracin

Bacitracin is a derivative of a natural antibacterial compound in *Bacillus subtilis*.

Mechanism of Action

The drug complexes with a chaperone molecule involved in transporting polysaccharide, peptidoglycan, and lipopolysaccharide to the growing cell wall, thus inhibiting its synthesis.

Clinical Uses

- Component of Polysporin and Neosporin ointments
- Prevention of superficial wound infection from skin flora, including *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Clostridia* species
- Primary or adjunctive treatment of impetigo, although patients are usually at high risk for failure when it is used as monotherapy

Cell Wall Disruption: Polymyxin B

Bacillus polymyxa, a saprophytic (soil) Gram positive rod, produces the compound as a natural antibacterial.

Mechanism of Action

Polymyxin B is a cationic, branched compound that behaves like a detergent, disrupting the phospholipid membrane of Gram negative bacteria. Because Gram positives do not have an outer bilayer membrane, polymyxin has virtually no effect on them.

Clinical Uses

- Component of Polysporin, Neosporin, and Cortisporin ointments
- Bactericidal activity against Gram negative skin organisms, including *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Serratia marcescens*

Small Subunit Interference: Neomycin

An aminoglycoside antibiotic like gentamicin, neomycin is derived from naturally occurring compounds in *Streptomyces fradiae* fungus.

Mechanism of Action

Like other aminoglycosides, neomycin disrupts reading of mRNA at the small (30S) ribosomal subunit. (See aminoglycoside section above.)

Pharmacodynamics

Although not absorbed through intact skin, neomycin can diffuse across damaged tissue and cause sensitization or ototoxic effects similar to other aminoglycosides.

Clinical Uses

- Component of Neosporin and Cortisporin ointments
- Prevents infection from a wide variety of Gram positives like *Staphylococcus* species, and Gram negatives like *Serratia* species, *Escherichia coli*, *Haemophilus influenzae*, and *Proteus* species. *Pseudomonas aeruginosa* is usually resistant to neomycin, and its activity against *Streptococcus pyogenes* is unclear.

t-RNA Synthesis Inhibition: Mupirocin (Bactroban)

A totally unique agent, mupirocin is derived from a fermentation product of *Pseudomonas fluorescens*.

Mechanism of Action

Mupirocin binds to bacterial isoleucyl-tRNA synthase, the enzyme responsible for linking a specific tRNA molecule to the amino acid isoleucine. Without this amino acyl-tRNA available, isoleucine can't be incorporated into nascent polypeptide chains and protein synthesis halts.

Pharmacodynamics

At concentrations achieved from the topical 2% formulation, mupirocin is bactericidal for *Staphylococcus aureus*, including oxacillin-resistant *S. aureus* (MRSA). It is not absorbed systemically from topical administration if skin is intact, and the small amount that does make it into the bloodstream from application over damaged tissue is rapidly converted to a therapeutically inactive form and eliminated in the urine. The drug is highly protein bound, so if applied to a wound leaking significant amounts of serum, the activity of mupirocin may decrease.

Clinical Uses

- Superficial skin infections (such as folliculitis and impetigo) with Gram positives like *Staphylococcus aureus* and *Streptococcus pyogenes*. Studies have suggested mupirocin is more effective than other topicals, and at least as effective as anti-staphylococcal penicillins, cephalexin, or erythromycin in treating mild impetigo with a limited skin area affected.
- Primary or adjunctive treatment of secondarily infected dermatologic lesions such as burns, lacerations, ulcerations, and eczematous skin
- Elimination of nasal carriage of *Staphylococcus aureus*, including MRSA. (Especially useful in patients on contact precautions because of MRSA isolated from the nares, where mupirocin is applied twice daily to the inside of each nare for 5 days. Short-term eradication of the organism is excellent in general, but relapses are common.)

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