

Introduction to Antimicrobials

Lecture Aim: To provide a brief introduction to antibiotics. Future lectures will go into more detail.

Major Learning Objectives:

- 1) Learn the different classes of antibiotics.
- 2) Learn the mechanism of action of each antibiotic class.
- 3) Understand the MIC (minimum inhibitory concentration). Know how to measure the MIC and what the MIC indicates about antibiotic susceptibility.
- 4) Conceptualize the major mechanisms of antibiotic resistance.
- 5) Have a basic understanding of antibiotic pharmacokinetics/dynamics.

What are antimicrobials?

Antimicrobials are drugs that destroy microbes, prevent their multiplication or growth, or prevent their pathogenic action. They differ in their physical, chemical, and pharmacological properties. They also differ in their antibacterial spectrum of activity and their mechanism of action. This lecture focuses on the different classes of antibiotics and their mechanisms of action.

Antibiotic classes and their mechanisms of action:

There are several major ways antibiotics kill microbes or inhibit their growth. Antibiotics generally target basic bacterial structures/functions necessary for life and/or replication.

Different **mechanisms of action** include:

- 1) **Inhibition of cell wall synthesis**
- 2) **Inhibition of protein synthesis**
- 3) **Alteration nucleic acid metabolism**
- 4) **Inhibition of folate metabolism**--necessary for production of DNA and other essential metabolites.
- 5) **Miscellaneous mechanisms** include disruption of the cell membrane and production of free radicals that damage DNA.

An '**antibiotic class**' refers to a group of antibiotics with a very similar chemical structure. Because of their similar chemical structure members of an antibiotic class have the same basic mechanism of action. Generally, within a class, there is the same core nucleus critical to function, while differing side chains modify the drug's toxicity, spectrum, pharmacokinetics, etc. For example, penicillins and quinolones are different antibiotic classes. Ampicillin and piperacillin are two different kinds of penicillins (both inhibit cell wall synthesis but have different antibiotic spectrums) while levofloxacin and ciprofloxacin are different kinds of quinolones (both alter nucleic acid metabolism, but are dosed once daily and twice daily, respectively). A few antibiotic classes are

represented by only one antibiotic. One example is linezolid, which is the only oxazolidinone currently available.

Below, the different antibiotic classes are grouped by their mechanism of action:

Inhibit cell wall synthesis:

- Penicillins
- Cephalosporins
- Monobactams (aztreonam)
- Carbapenams
- Glycopeptides (vancomycin)

Inhibit Protein synthesis:

- Aminoglycosides
- Tetracyclines
- Glycylcycline (tigecycline)
- Macrolides
- Lincosamides (clindamycin)
- Streptogramins (quintapristin/dalfopristin)
- Oxazolidinones (linezolid)
- Phenicols (chloramphenicol)

Alter nucleic acid metabolism:

- Rifamycins (rifampin)
- Quinolones

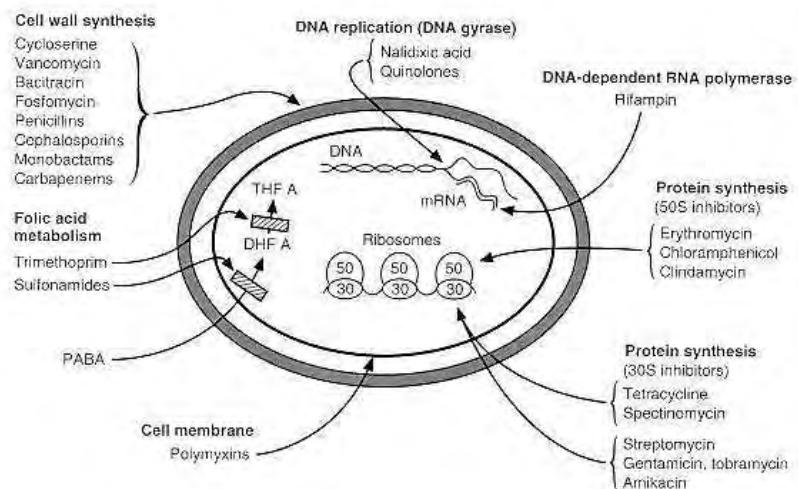
Inhibit folate metabolism:

- Trimethoprim
- Sulfonamides

Miscellaneous:

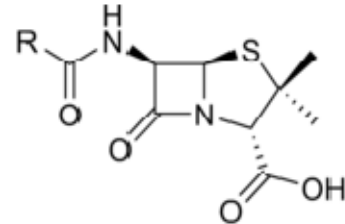
- Metronidazole
- Lipopeptides (daptomycin)
- Polymyxins

Figure demonstrating antibiotic targets:

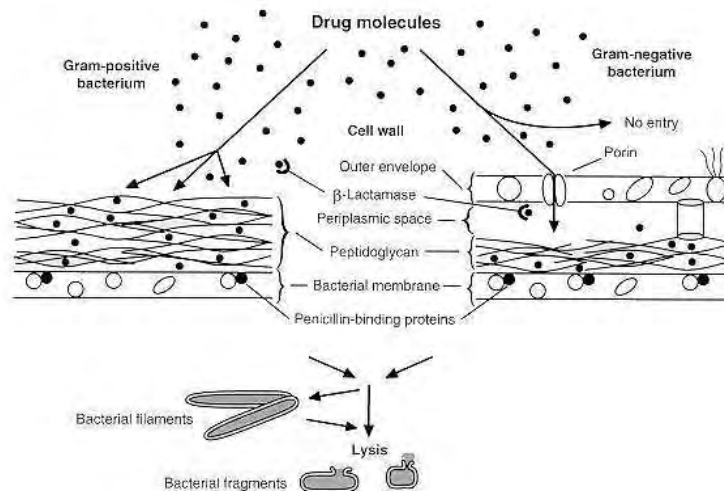


Antibiotics that inhibit cell wall synthesis:

Beta-lactam antibiotics (penicillins, cephalosporins, monobactams, carbapenems): beta-lactam antibiotics refer to several classes of antibiotics (listed above) that all share the same beta-lactam ring consisting of 3 carbon atoms and 1 nitrogen atom. The beta-lactam ring is the square at the center of the penicillin nucleus shown to the right. The different classes of beta-lactams differ from each other by structures (such as side chains/rings) apart from the beta-lactam ring.

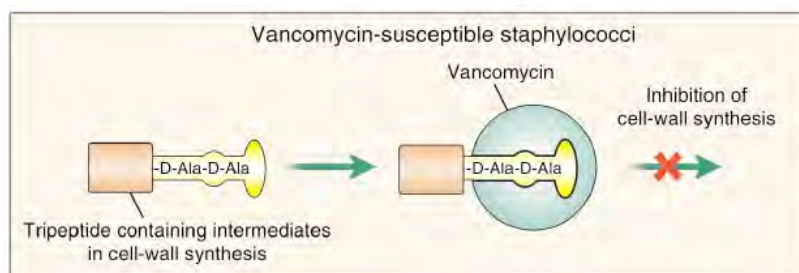


Beta-lactam antibiotics all inhibit cell wall synthesis by blocking the action of transpeptidases (aka penicillin binding proteins, PBPs) which are membrane bound and produce peptidoglycan, the major cell wall component. These antibiotics cause dividing bacteria to lyse and die as shown in the diagram below.



Some bacteria are able to produce a beta-lactamase which lyses the beta-lactam ring and disables the beta-lactam antibiotic. Beta-lactamase production is one mechanism of resistance that may be employed against these antibiotics. This is why some beta-lactam antibiotics are co-formulated with a beta-lactamase inhibitor. Examples are amoxicillin-clavulanate and piperacillin-tazobactam (beta-lactamase inhibitors are underlined).

Vancomycin (a glycopeptide antibiotic) Vancomycin also inhibits cell wall



synthesis by interfering with the production of peptidoglycan (but it is not a beta-lactam antibiotic). It does this by binding to the

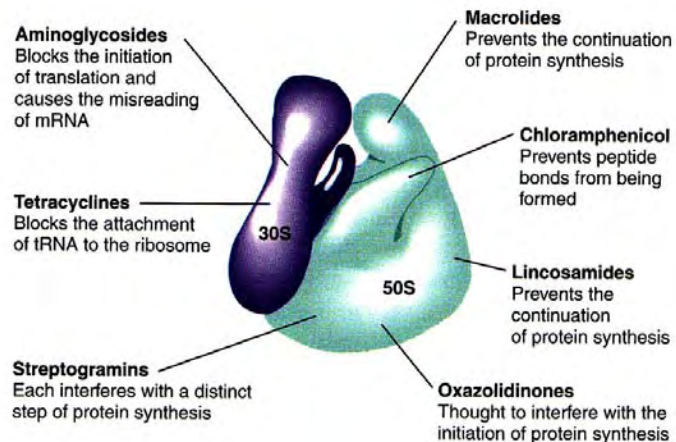
D-Ala-D-Ala terminals of peptidoglycan precursors on the outer surface of the cell membrane. As a result, the precursors cannot incorporate into the peptidoglycan matrix. This process causes dividing cells to lyse and die. With a rare exception, vancomycin is only active against Gram positive bacteria. One mechanism of resistance against vancomycin is alteration of the bacterial D-Ala-D-Ala target.

Protein Synthesis Inhibitors

The protein synthesis inhibitors interfere with different aspects of translation. Some classes act on the 30S ribosome while others act on the 50S ribosome as demonstrated below. Most protein synthesis inhibitors cause a reversible inhibition of protein synthesis and many are

bacteriostatic (prevent bacterial growth but don't kill them). An exception are aminoglycosides which bind irreversibly to the 30S ribosome and are generally bacteriocidal (cause cell death). (Note that an antibiotic's bacteriostatic or bacteriocidal 'status' may not be set in stone—it may depend on the antibiotic's

concentration and the organism). Aminoglycosides are also known for having a post-antibiotic effect where bacterial growth is inhibited for a time even after blood drug levels become undetectable.



Antibiotics that affect bacterial nucleic acid metabolism:

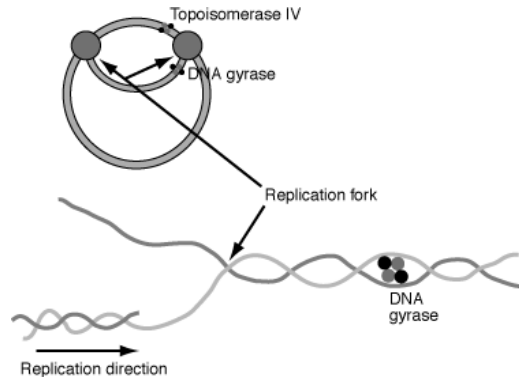
Rifamycins

Rifamycins include several antibiotics including rifampin (aka rifampicin). They inhibit mRNA synthesis (transcription) by binding to the bacterial DNA-dependent RNA polymerase. Resistance may occur as a result of a single-step mutation (usually a missense mutation) within the gene that encodes the β -subunit of the RNA polymerase. Because resistance can easily occur, rifampin is almost always used in combination with other antibiotics. One exception is for *N. meningitidis* prophylaxis.

Quinolones

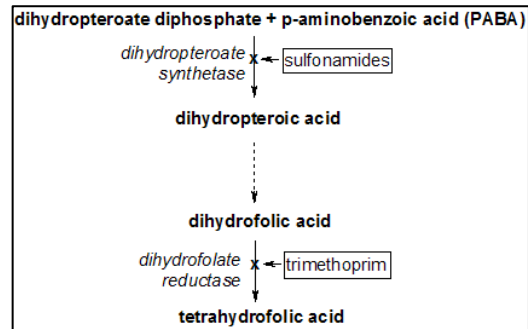
Quinolones inhibit DNA synthesis and cause cell death. They do this by inhibiting the topoisomerases responsible for supercoiling DNA (DNA gyrase) or relaxing the supercoiled DNA (topoisomerase IV). Ciprofloxacin is a quinolone that became a household name during the anthrax scare.

Quinolones: mechanism of action



Inhibitors of folate metabolism

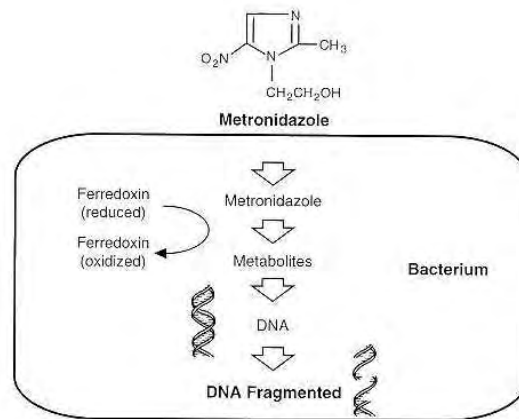
Both trimethoprim and sulfonamides inhibit folate metabolism. When folate metabolism is inhibited, formation of DNA precursors (e.g. purines) is reduced and ultimately, DNA synthesis is inhibited. Sulfonamides and trimethoprim act in different steps in folate metabolism and are often used together. Sulfonamides inhibit tetrahydroptericoic acid synthetase which inhibits PABA → dihydrofolic acid. Trimethoprim inhibits the conversion of dihydrofolic acid to tetrahydrofolic acid by inhibiting dihydrofolate reductase.



Miscellaneous mechanisms of action

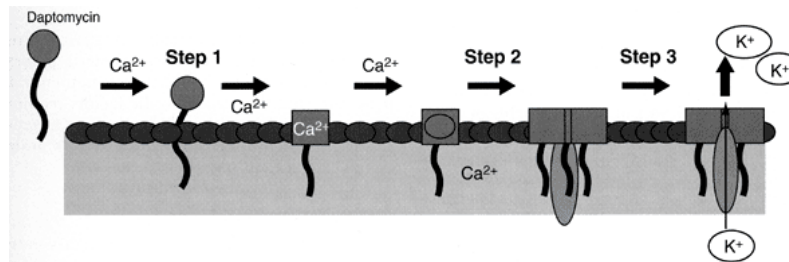
Metronidazole

Metronidazole is an antibiotic active against anaerobes and select parasites such as entamoeba, trichomonas, and giardia. It diffuses into the cell and is reduced (in anaerobes, it is reduced by ferredoxin, a mitochondrial electron transport protein). Metronidazole free radicals then cause breakage of organism DNA causing cell death.



Lipopeptides (Daptomycin)

Daptomycin is the only antibiotic in the lipopeptide class. It binds to the cell membrane of Gram positive bacteria in a calcium-dependent process. Channels form causing ion leakage, depolarization of the cell, and cell death. Daptomycin is a useful agent for resistant staphylococcal and enterococcal infections; however, it can not be used for pulmonary infections as it is bound by surfactant.



Polymyxins

Polymyxins use a surface detergent-like mechanism to kill bacteria. They penetrate into cell membranes, interact with membrane phospholipids, and disrupt the membranes causing cell death. Polymyxins are broad Gram negative agents. They are old drugs that became mainly restricted to topical and oral use (the oral formulation is not absorbed) due to toxicity. However, with the emergence of multi-drug resistant Gram negative bacteria, these drugs are being used intravenously with increasing frequency.

How are antibiotics used?

Antibiotics may be used as **empiric therapy**, **definitive therapy**, or **prophylactic therapy**. Empiric therapy is used when the pathogen has not yet been identified; therefore, an antibiotic must be chosen that is effective against the most likely pathogens. Antibiotics used for empiric therapy are usually **broad spectrum**, meaning that they are active against a wide variety of pathogens. For example, carbapenams are active against a wide range of Gram positive and Gram negative organisms. Definitive therapy is used once the pathogen has been identified (or the diagnosis is clear). When possible, a broad spectrum agent should be changed to a 'narrower spectrum' antibiotic once therapy becomes definitive. For example, a patient with pyelonephritis and shock may be treated empirically with piperacillin/tazobactam which is a broad spectrum antibiotic. If a pan-sensitive *E. coli* is identified in blood and urine cultures, the regimen could be changed to kefzol, which is a cephalosporin with a narrower spectrum. Finally, antibiotics may be used as prophylactic therapy with the aim of preventing an infection or its recurrence. For example, postal workers exposed to *B. anthracis* spores received a quinolone antibiotic to prevent anthrax from developing.

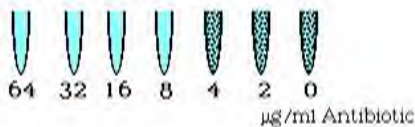
Will the antibiotic you choose be effective?

Before initiating antibiotics, you must decide whether or not the patient is infected. If you determine the patient is or may be infected and that antibiotic

therapy should be initiated, cultures should usually first be obtained from the appropriate sites, if possible (e.g. aspiration of joint fluid for culture if septic arthritis is suspected or blood cultures if endocarditis is suspected).

The microbiology laboratory will identify the pathogen and determine its sensitivities *in vitro* to a panel of antibiotics. Sensitivity is determined by the interpretation of the **minimum inhibitory concentration (MIC)**. The MIC is the lowest concentration of an antibiotic that prevents visible bacterial growth. 'Susceptible' means that the concentration of antibiotic in the serum is \geq MIC. 'Resistant' means that the organism will not be inhibited by achievable drug concentrations in the blood. 'Intermediate' implies that if a high enough dose of the antibiotic is used, the antibiotic may be effective. The National Committee on Clinical Laboratory Standards (NCCLS) provides cutoff values that are both organism and antibiotic specific for help interpreting an MIC as meaning sensitive, intermediate, or resistant. A key issue is whether or not concentrations above the MIC can be achieved at the site of infection (e.g. lung, brain, etc.)--this must occur in order for the organism to be truly susceptible to antibiotic therapy.

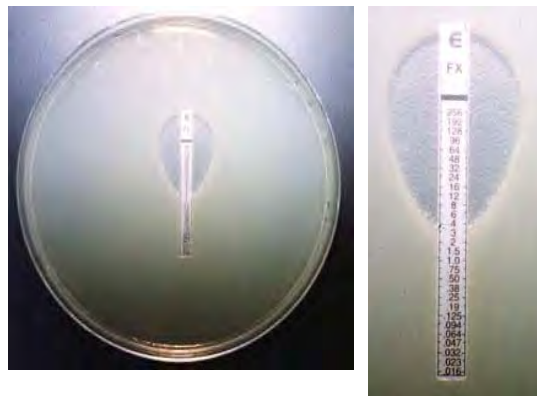
Susceptibility can be determined in several ways, including **broth dilution, E-test, and disk diffusion** (these will be demonstrated in the Lab Sessions). In broth dilution, tubes with liquid media and increasing concentrations of antibiotic are inoculated with the organism. After 24 hours, the tubes are observed. The first tube (the tube with the lowest concentration of antibiotic) that has no visible growth represents the MIC.

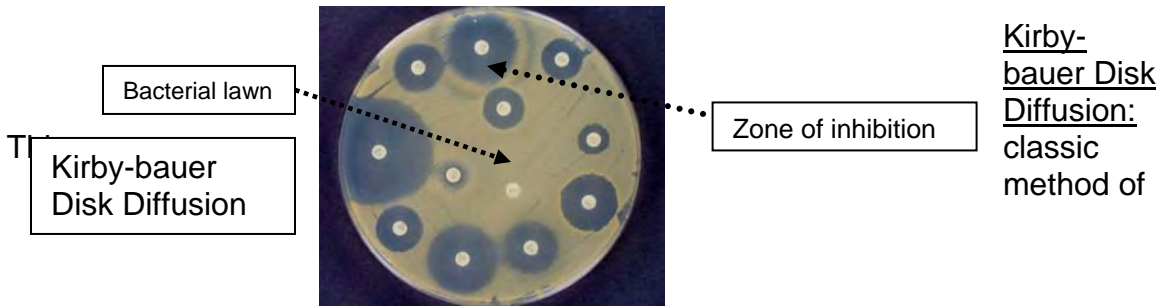


In the figure on the left, the MIC=8 µg/ml. If the organism is *Klebsiella pneumoniae* and the antibiotic is cefepime (a cephalosporin), the organism would be considered susceptible to cefepime (the NCCLS guideline says that and MIC \leq 8 µg/ml is

susceptible.) Though note that the MIC is one dilution away from being 'intermediate'!

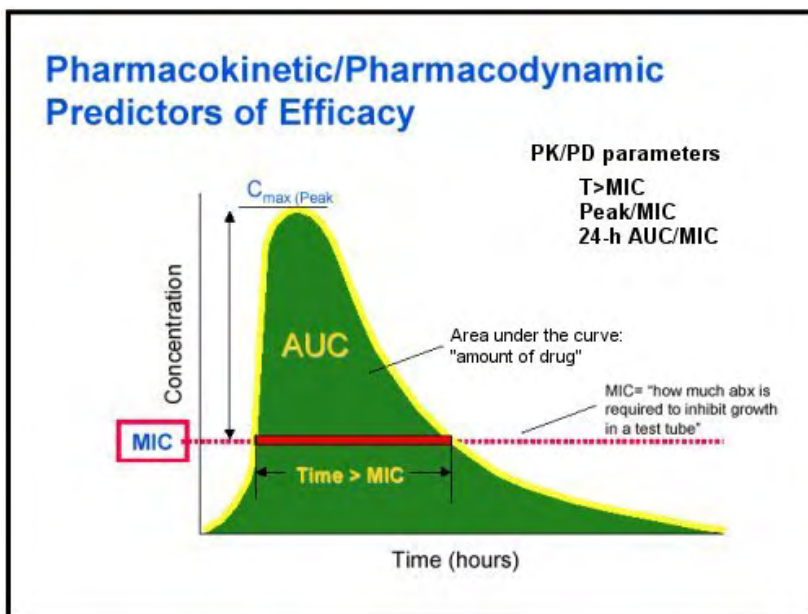
E-test (Epsilometer test): The e-test can also determine the MIC. The e-strip contains antibiotics in a gradient from a low to high concentration. The organism is inoculated onto a plate of solid media, the e-strip is placed on the media, and the plate is incubated for 24 hours. Antibiotic on the e-strip will diffuse into the solid media (also on a gradient). The plate is observed and there will be an elliptical-shaped inhibition of growth. The lowest point on the e-strip the corresponds to an absence of growth equals the MIC. In this example, the MIC appears to be 4 mg/L (though it is difficult to read here).





determining antibiotic susceptibility does not yield an MIC value—it just tells you if the organism is susceptible, intermediate, or resistant. In this method the organism is inoculated onto a plate of solid media. One or more antibiotic disks are placed onto the plate and the antibiotic will diffuse into the media. The plates are incubated for 24 hours and then observed. For each antibiotic disk, the zone of inhibition around it is measured. Depending on the diameter of the zone of inhibition, the organism will be classified as sensitive, intermediate, or resistant (NCCLS guidelines are used). Obviously, if there is no zone of inhibition, the organism is resistant to the antibiotic.

As stated above, in order for a pathogen to be sensitive to an antibiotic, the antibiotic concentration at the site of infection must be at or preferably above the MIC. Some antibiotics are **concentration dependent** agents--the higher the concentration of antibiotic above the MIC, the more killing occurs. Other antibiotics are **time-dependent** agents. For these agents, little is gained as the concentration increases above the MIC. For these agents, it is the duration of time that the concentration of antibiotic remains above the MIC that influences the extent of bacterial killing. These concepts are related to antibiotic pharmacodynamics (what the drug does to the body/bacteria) and must be



weighed with the potential ill effects of antibiotics such as toxicity. For instance, aminoglycosides are concentration dependent agents—so the higher the concentration above the MIC, the better killing it achieves. However, aminoglycosides can also cause ototoxicity. Ototoxicity is related to the peak dose, but

also to the total amount of drug exposure (area under the curve or 'AUC'), which

may be even more important. Therefore, one must carefully dose these antibiotics to achieve a favorable risk/benefit profile.

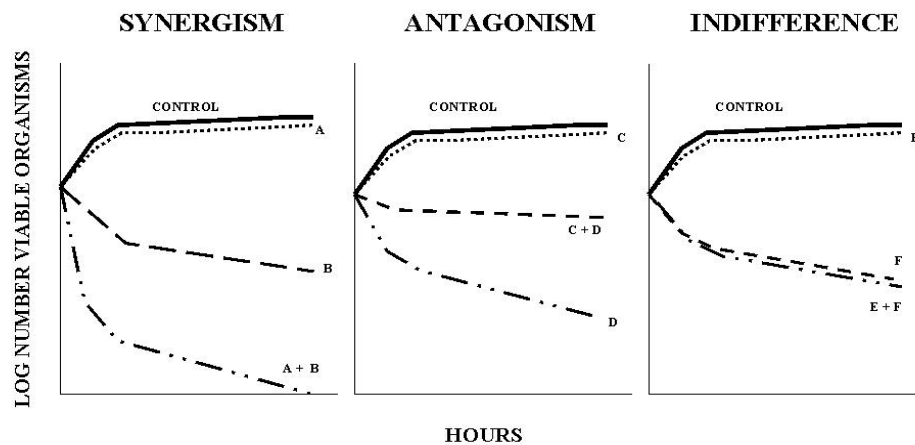
Some pharmacokinetic considerations are addressed below. (Pharmacokinetics are, essentially, what the body does to the drug.)

- 1) Absorption:** if the agent is not administered IV, what % of the drug is absorbed? What other drugs, foods, or medical conditions might affect drug absorption? For instance, absorption of oral quinolones is diminished by the concomitant administration of antacids.
- 2) Volume of Distribution:** this refers to the distribution of a drug throughout the body—a large distribution volume describes a drug widely distributed throughout the body while a small distribution volume refers to a drug with a more limited distribution (*i.e.* only in the bloodstream with little tissue penetration).
- 3) Metabolism:** Drug metabolism, which mainly occurs in the liver, helps facilitate the removal of a drug from the body. Drug-drug reactions may interfere with drug metabolism. For instance, rifampin can increase the metabolism of other drugs such as protease inhibitors (use to treat HIV infection). Administration of these drugs together could result in ineffective treatment for HIV.
- 4) Excretion:** Refers to elimination of a drug from the body which is either renal or non-renal. A patient's ability to excrete an antibiotic must be considered (*e.g.* a person in renal failure would not be able to excrete a renally cleared antibiotic and could end up with toxic levels.)

Using Antibiotics in Combination:

The effect may be:

- 1) Synergistic
- 2) Antagonistic
- 3) Indifferent



Synergism is when the combination of two antibiotics is more lethal than the sum of the 2 antibiotics if given separately (it's a super-additive effect). A classic

example of antibiotic synergism is in the treatment of Enterococcal endocarditis where gentamicin (an aminoglycoside) is used in combination with ampicillin (a penicillin). Used alone, gentamicin ('A' in Panel 1 above) has very little effect because it can't get inside the cell. Ampicillin ('B') does have a bactericidal effect. However, when used in combination, gentamicin can get inside the cell and bacterial killing is enhanced.

Antibiotic resistance will be addressed in more detail later in the course. However, there are four major mechanisms of antibiotic resistance to keep in mind:

- 1) Alteration of target
- 2) Prevention of access to target
- 3) Inactivation of agent
- 4) Failure to convert an inactive precursor agent to its active form

All drugs, including antibiotics, have pharmacodynamic and pharmacokinetic properties. Essentially, pharmacodynamics is what the drug does to the body/organism and pharmacokinetics is what the body does to the drug. These must both be considered when prescribing an antibiotic.