

Lecture: 36: Antibiotics: cell envelope

Faculty: Dr. Alvin Fox, Phone: 733 3288, E-mail: alvin.fox@uscmed.sc.edu

Office: C-19, Building 2

Suggested reading: Murray, 6th Edition, Chapter 8, 20

KEY WORDS

Sterilization/disinfection/antiseptis	β lactam
Antibiotic	Penicillins
Selective toxicity	Cephalosporins/cephamycins
Bactericidal	Monobactam
Bacteriostatic	Clavulinic acid
Minimal inhibitory concentration (MIC)	Penicillinase/ β lactamase
Susceptibility testing	Polymyxin
Penicillin binding proteins (PBP)	Daptomycin
Autolysins	Isoniazid
Cycloserine	Ethionamide
Bacitracin	Ethambutol
Vancomycin	Resistance

Sterilization refers to killing (or removal) of all bacteria in a non-selective fashion. For example, autoclaving involves heating liquids (e.g. media) or solids to 121°C under steam pressure. The materials must be heat resistant. Ethylene oxide has historically been used in hospitals for equipment that can't be heated but there are toxicity concerns; gradually being replaced (e.g. using e.g. using free radicals generated from H₂O₂). Membrane filters have pores that trap bacteria, but allow drugs and small chemicals to pass through; thus pre-sterilized filters can be used to sterilize delicate solutions; similarly air can be treated in rooms with HEPA filters. UV light is used for decreasing bacterial levels on surfaces such as in operating rooms; however it is not fully effective. Gamma radiation is as effective as autoclaving but its major use is in the food industry.

Disinfectants (e.g. phenol-based or quaternary ammonium compounds) can be useful at killing many bacteria on certain instruments, but can't be used for internal consumption or on skin. Antiseptics (e.g. iodine or 70% alcohol) are used topically (e.g. on skin surfaces) to reduce bacterial load.

In contrast antibiotics are agents that are "selectively" toxic for bacteria (either killing [bactericidal] or inhibiting their growth [bacteriostatic]) with minimal harm or side-effects for the patient. By definition these compounds must act on structures found in bacteria, but not in the host. Antibiotics work most efficiently in conjunction with an active immune system to kill

infecting bacteria in the host. After isolation of pure colonies the susceptibility of bacterial isolates can be tested to a variety of antibiotics. The minimal inhibitory concentration (MIC) refers to the lowest concentration of an antibiotic that stops visible growth. The zone of inhibition around a disk impregnated with antibiotic (Kirby-Bauer) is another measure of antibiotic activity.

One major class of antibiotics inhibits the synthesis of peptidoglycan. Once cell wall synthesis (involving penicillin binding proteins) is inhibited enzymatic autolysis of the cell wall can occur. Without the restraining influence of the cell wall the high osmotic pressure inside the cell bursts the inner and/or outer membranes of bacteria destroying them. Thus these antibiotics are generally bactericidal. Several mechanisms are involved in inhibition of peptidoglycan synthesis:

(1) The terminal two amino acids of a peptide side chain of peptidoglycan are unusual amino acids (D-alanine [D-ala] as opposed to its isomer L-alanine [ala]). The antibiotic cycloserine is an analog of D-ala and interferes with enzymatic conversion of L-ala to D-ala and with synthesis of D-ala-D-ala in the cytoplasm. Thus synthesis of peptidoglycan does not occur. Cycloserine is mainly used in treatment of tuberculosis.

(2) The peptidoglycan subunit (containing one side-chain and an attached peptide to be used in cross-bridge formation) is attached to phosphorylated undecaprenol through a second phosphate group. The subunit-diphosphate-undecaprenol complex is passed across the cytoplasmic membrane. After the nascent peptidoglycan monomer leaves the carrier on reaching the cell wall the terminal phosphate on undecaprenol is removed. Bacitracin inhibits the dephosphorylation reaction and in the absence of "free" carrier no more peptidoglycan subunit can be passed across the membrane and cell wall biosynthesis stops.

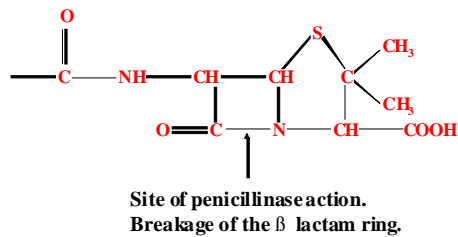
(3) The final step in peptidoglycan synthesis involves linking the sugar portion of the peptidoglycan subunit to the glycan backbone of existing cell wall polymer. Cross-linking of the peptide portion of the subunit to a peptide in the cell wall then occurs. During this process D-ala is enzymatically excised from the end of a pre-existing peptide side-chain allowing it to be cross-linked (by a new peptide bond) to the recently synthesized peptidoglycan subunit. Vancomycin binds to D-ala-D-ala thus sterically inhibits transpeptidation (cross-linking). Replacement of one of the D-ala in the peptide side chain of peptidoglycan leads to resistance.

(4) The beta lactam (β lactam) antibiotics include penicillins (e.g. ampicillin), cephalosporins and monobactams. They bind to and inhibit enzymes (penicillin binding proteins) involved in the transpeptidation (cross-linking) of peptidoglycan. A region of penicillin is an analog of the D-ala-D-ala including the peptide bond. These antibiotics have in common the 4-membered lactam ring. Attached to the lactam, penicillins have an additional 5 membered ring and cephalosporins (and cephamycins, ring oxygen replaces sulfur) a 6-membered ring. Monobactams consist of the lactam ring alone and display antibiotic activity. Carbapenems are more complex in structure.

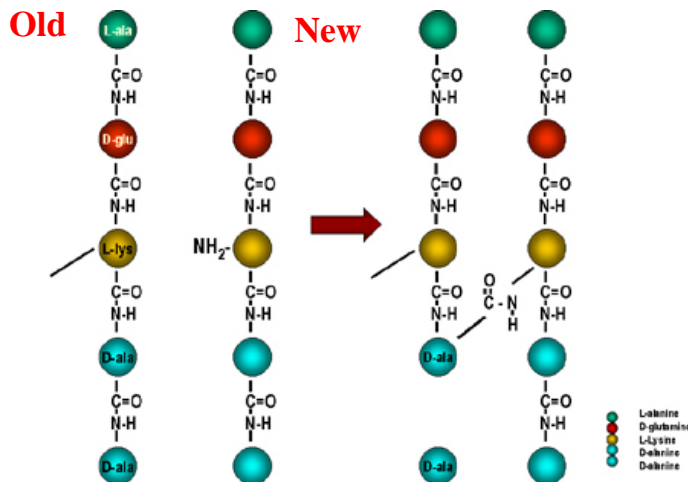
Various chemical side chains have been synthetically linked to the ring structures producing a host of antibiotics with different properties in the host. Many older penicillins display little activity against Gram negative bacteria, since they do not penetrate the outer membrane. Cephalosporins and other newer penicillins are active against Gram negative bacteria, since they can penetrate the outer membrane. Other chemically modified penicillins have lower elimination rates from patients, decreasing frequency of administration.

Penicillins can be destroyed by β lactamase (penicillinase) produced by resistant bacterial strains. Clavulanic acid is also a β lactam, which binds strongly to β lactamases inhibiting their activity but has limited potency as an antibiotic. It is usually used in conjunction with certain penicillins allowing their use against otherwise resistant bacteria. Another form of resistance involves a change in the structure of penicillin binding proteins such that the antibiotic does not bind efficiently. In the case of Gram negative bacteria, penicillins pass across the outer membrane using porins. Resistance may develop from mutation leading to modified porins.

STRUCTURE OF PENICILLIN



Cross-linking of peptidoglycan



Action on membranes

Polymyxin B: Binds to the lipid A portion of lipopolysaccharide and also to phospholipids. However, it binds preferentially to lipid A. This disrupts the outer membrane of Gram negative bacteria. Since the cell membrane is not exposed in Gram positive bacteria polymyxin has little activity. This drug is toxic to human cells, since it can also lyse eukaryotic membranes; this explains its limited clinical use.

Daptomycin: causes depolarization of the cell membrane

Isoniazid, Ethambutol, Ethionamide:

Mainly used for treating tuberculosis!

Isoniazid and ethionamide –block synthesis of mycolic acid

Ethambutol - block synthesis of arabinogalactan