General Outline of Lecture Order
1. Antibiotics that Inhibit Cell Wall Biosynthesis
   beta lactam antibiotics
   glycopeptide antibiotics
2. Antiribosomal Agents
   aminoglycosides, tetracyclines, macrolides
3. Sulfonamides, Antifolates & Macrolides (agents that interfere with nucleic acid biosynthesis)
4. Antituberculosis Agents
5. Antifungal Agents
6. Antiparasitic Agents

I use many structures to explain mechanism of action, activity and to help demonstrate the concepts that are important. You will not be expected to memorize the structures of the antibiotics and you will not see structures on the exam. However, you will be expected to understand certain general structural features that we specifically discuss in class and how the character of the structural features discussed effect things such as distribution, bioavailability, antibiotic activity and other factors that are attributed to these physical characteristics and clinical utility.

See table 3.3 (page 30) for a brief summary of the commonly used antimicrobial agents.

**Initial Reading Assignments:**
- Chapter 3 of text pages 18 - 30
- Chapter 5 of text pages 52 - 61
I. The Bacterial Cell Wall and Sites of Action

Antimicrobial Agents that inhibit cell-wall synthesis are and have been very effective therapeutic agents. One primary reason is that the bacterial cell wall has no mammalian counterpart. Thus the enzymes (over 30 known) and metabolic reactions used by bacteria to synthesize cell wall and cell wall peptidoglycan precursors can be targeted quite specifically by drug therapy. In addition, inhibitors of cell-wall biosynthesis do not have to gain intracellular access to the bacteria; they only need access to their targets in or near the cell wall/peptidoglycan.

A) General features of the bacterial cell-wall and cell-membrane(s)

B) The Gram-positive cell wall.
Gram-positive bacteria have no outer membrane, thus antibiotics that work by inhibiting cell wall peptidoglycan biosynthesis or enzymes involved in cell wall peptidoglycan biosynthesis have unimpeded access to these structures.

C) The Gram-negative cell wall and outer membrane. (SEE Figure 3.2 of text)
Gram-negative bacteria have an outer membrane (a lipid bilayer) that covers the peptidoglycan layer of the cell wall. This impedes the ability of some antibiotics to reach the peptidoglycan and/or enzymes involved in peptidoglycan synthesis. However, a number of antibiotics do penetrate the outer membrane by passing through porin channels. Porin channels (porins) are special proteins that provide access of hydrophilic molecules to the cytoplasmic membrane and the peptidoglycan layer in the periplasmic space.

The peptidoglycan layer is thicker in Gram-positive organisms than in Gram-negative organisms. Penicillin binding proteins (PBPs) are transmembrane or surface proteins in the cytoplasmic membrane and normally function in the synthetic processes of building the peptidoglycan cell wall.

(SEE FIGURES 3.2, 3.3, 3.4 FOR CELL WALL/MEMBRANE STRUCTURES)
Site of Action for Cell Wall Synthesis Inhibitors

A. Glycopeptides (Vancomycin)
B. Beta Lactams (Penicillins/Cephalosporins etc.)
C. Bacitracin
D. Cycloserine
E. Fosfomycin

Membrane

Cytoplasm

Cell Surface

Mature Peptidoglycan

Transpeptidase

Transglycosylase (B)

Immature Peptidoglycan

Lipid II

Lipid I

UDP-GlcNAc (UDP-NAG)

D-alanine

D-alanine

NAG-NAM
II. General Aspects of the Beta-Lactam Antibiotics

A) What are the beta-lactam antibiotics?

The beta-lactams are structurally similar to the D-alanyl-D-alanine terminal dipeptide of cell wall peptidoglycan precursors, thus beta-lactams bind with varying degrees of affinity to proteins that bind D-alanyl-D-alanine. These proteins (transpeptidases) use the D-alanyl-D-alanine in cell wall synthesis and are also called penicillin-binding proteins (PBPs). Each beta-lactam antibiotic contains a highly strained, reactive, 4-membered ring as a part of the pharmacophore.

B) Mechanism of action of the beta-lactam antibiotics

The beta-lactam antibiotics bind to proteins in the cell wall. These proteins have been named penicillin-binding proteins (PBPs) and their natural functions involve binding to D-alanyl-D-alanine where they exert their transamidase or carboxypeptidase activity in cell wall synthesis. A bacteria typically has 3 to 6 different PBPs and each PBP may have 1) different affinities for the different beta-lactams, 2) different modes of action or roles in cell wall biosynthesis. Thus a particular beta-lactam may bind and inhibit one or more PBPs and the effect of this binding depends on the role of the PBP. Additionally, the beta lactams activate autolytic enzymes in the cell wall, which result in lesions causing cell death.

C) Beta-lactamases

Beta-lactamases are enzymes that inactivate beta-lactams through a similar mechanism, rendering the antibiotic inactive. Two approaches have been taken to overcome this type of resistance. 1) Develop beta-lactams that do not bind to the beta-lactamases and thus are effective against bacteria with this type of resistance. 2) Develop inhibitors of beta-lactamases that can be given concurrently with beta-lactamase sensitive beta-lactams antibiotics.
III. Penicillins

A) The original penicillins and how small changes in structure effect activity.

Penicillin V and Penicillin G

The simple difference in R-group renders Pen-V more acid stable so it can be taken orally. Benzathine or procaine salts (hydrophobic organic salts) of penicillin afford a hydrophobic product that has low water solubility. Thus, IM injection of these salt forms results in slow dissolution affording a slow, steady release of antibiotic into the body over a number days (duration depends on salt form used)

Penicillin is susceptible to beta-lactamase inactivation and is most useful against Gram-positive bacteria. Therefore new analogs of penicillin were developed to overcome the beta-lactamase resistance problem and to broaden the spectrum of activity toward Gram-negative bacteria.

B) Penicillinase sensitive, broad spectrum, penicillins

Polar groups (amine/carboxyl) on the R group of the penicillins increases the ability of these agents to enter Gram-negative bacteria through porin transport. Thus they have a broader spectrum of activity than the original penicillins.

The amine substituent on the amino penicillins (ampicillin and amoxicillin) affords increased acid stability. Therefore they have greater bioavailability than penicillin G when taken orally since less is inactivated in the gut before adsorption. The carboxyl and ureido penicillins shown below are given parenterally.
The penicillin derivatives above have a broader spectrum of activity to the gram-negative peptidoglycan layer through increased porin transport. However, these agents are still generally susceptible to inactivation by beta-lactamases. These agents can be given in conjunction with a beta-lactamase inhibitor to counteract beta-lactamase inactivation.

To the right are shown two inhibitors of beta-lactamase, Sulbactam and Clavulanate. Tazobactam is also a more recent beta-lactamase inhibitor (not shown). These compounds bind beta-lactamase in a manner similar to the beta-lactam antibiotics but permanently inhibit the enzyme so that the co-administered antibiotic will not be inactivated.

For example amoxicillin and the beta lactamase inhibitor clavulanate are marketed as “Augmentin”. Ticarcillin and clavulanic acid are combined to provide an injectable called “Timentin”. Piperacillin and tazobactam are a combination with a broad spectrum of antibacterial activity encompassing most Gram-positive and Gram-negative aerobic bacteria and anaerobic bacteria, including many pathogens producing beta-lactamases. Evidence from clinical trials in adults has shown that Piperacillin/Tazobactam, administered in an 8:1 ratio, is an effective treatment for patients with lower respiratory tract, intra-abdominal, urinary tract, gynecological and skin/soft tissue infections.

C) The penicillinase (beta-lactamase) resistant penicillins

The penicillinase resistant penicillins have variable activity against strains of bacteria that produce penicillinases (beta-lactamases). The increased bulkiness (size) of the side chain (R group) of these compounds makes them resistant to either binding or inactivation by beta-lactamases. However, they still bind and inhibit PBPs thus they are effective antibiotics.

These agents are typically used for beta-lactamase producing Staphylococci.

Some of these agents have increased acid stability affording acceptable oral bioavailability.

The increase hydrophobicity of these agents also increases their serum protein binding and may decrease
D) General metabolic and other characteristics of the penicillins

Penicillins are generally not readily metabolized and are primarily excreted unchanged in the urine.

Allergic responses to the beta-lactams are the most common drug induced allergy in the U.S. Six to eight percent of people in the U.S. will display an allergic response to Penicillin. Most penicillins and some of the other beta-lactams are capable of showing cross sensitivity. Briefly, the mechanism of allergy is a result of the beta-lactam or a reactive metabolic intermediate becoming attached to protein in the body. Once attached to a protein the macromolecular complex can elicit an immune response.

IV. The Cephalosporins

The first cephalosporin (Cephalosporin C) was isolated from the sea near a sewer outlet from a fungus (Cephalosporium sp). While Cephalosporin C was a weak antibiotic and showed little promise, a method was developed for the production of 7-aminocephalosporanic acid that made possible the synthesis of a vast array of cephalosporins with varying properties. Years of development of have produced novel cephalosporin derivatives that are very effective antibiotics.

A) Classification and some general characteristics of the cephalosporins

The cephalosporins are typically divided by “generations” that correspond mainly to an increasing spectrum of activity. Cephalosporins have a mechanism of action similar to the penicillins 1) binding to specific PBPs 2) inhibition of cell wall biosynthesis by blocking transpeptidation of peptidoglycan 3) activation of autolytic enzymes in the cell wall resulting in lesions and cell death. Cephalosporins as a class are inactive against enterococci and MRSA.

While the original cephalosporins were not inactivated by penicillinase, many beta-lactamases (cephalosporinases) degrade the cephalosporins thus organisms producing these enzymes can be resistant to a variety of the cephalosporins.

The cephamycins are typically classified along with the cephalosporins due to similarity in core structure. However, cephamycins are actually obtained as fermentation products from Streptomyces.

B) First Generation Cephalosporins - Representative Agents

First generation cephalosporins are typically very active against gram-positive cocci and have some limited gram-negative activity. While first generation cephalosporins have a relatively broad spectrum of action and are relatively nontoxic they are rarely the drugs of choice for any infection. They do not penetrate the CNS and cannot be used to treat meningitis.

\[
\text{Cephalothin (parenteral)} \quad R \quad X
\]

\[
\text{Cefazolin} \quad N \quad N \quad \text{CH}_2^\text{CH}_3
\]
C) Second Generation Cephalosporins - Representative Agents

Second generation cephalosporins are a “mixed bag” of agents with significant individual differences in activity, toxicity and pharmacokinetics. They are generally active against organisms affected by the first generation agents but, in general, have extended gram-negative activity and are less active against gram-positive bacteria. This reduced activity against gram-positive bacteria seen with many second and third generation cephalosporins can lead to superinfections from staphylococci and enterococci during treatment.

Cefuroxime is available as cefuroxime axetil. This is an ester derivative of cefuroxime (the COOH group above being an ester instead of the acid). The ester is more hydrophobic, improving oral adsorption. However, once absorbed, esterases in the body cleave the ester to give cefuroxime back in the acid form.

D) Third Generation Cephalosporins

The major feature of third generation cephalosporins is their expanded gram-negative coverage and many third generation agents have an increased ability to reach the CNS.
The alpha-iminoacyl group (R group above) found in the third generation cephalosporins is a major structural feature that increases stability to beta-lactamases (just like the big hydrophobic groups did for the penicillins) but, is also quite polar (hydrophilic) which is also effective for increasing gram-negative activity (transport across gram-negative outer membrane).

Cefepime is listed here but is often considered a fourth generation cephalosporin. The positively charged size chain (at position X above) provides a zwitterionic molecule that more easily penetrates the outer membrane of gram-negative bacteria. In addition, cefepime has excellent penetration to the cerebral spinal fluid.
V. The Monobactams and Carbapenems

A) Monobactams

The monobactams are synthetic beta-lactam derivatives of which aztreonam is the most significant. Aztreonam only binds PBP-3 (common in gram-negative bacteria) and thus has little gram-positive activity. Its spectrum of activity is similar to the cephalosporin ceftazidine. Aztreonam is poorly absorbed orally thus given IM or IV. The monobactams mechanism of action is similar to other beta-lactams but they are resistant to beta-lactamases.

B) Carbapenems

The carbapenems differ from other beta-lactams in that they have a carbocyclic ring adjacent to the four membered beta-lactam ring. However, the mechanism of action is the same. Carbapenems are resistant to most beta-lactamases and are effective against many organisms that are resistant to penicillins or cephalosporins.

Imipenem is given perenterally and rapidly inactivated (hydrolyzed) by renal tubular dipeptidase, also called renal dihydropeptidase-1. Therefore imipenem is co-administered with cilastatin (“primaxin”). Cilastatin inhibits renal tubular dipeptidase and thus imipenem is not as rapidly inactivated.

Meropenem is a newer carbapenem that is inherently stable to renal tubular dipeptidase and therefore Cilastatin does not need to be co-administered.
VI. The Glycopeptide Antibiotics

- **Vancomycin** is currently the only glycopeptide antibiotic used clinically in the U.S. It is active against most Gram-positive bacteria and is most notable for its activity against Staphylococcus, methacillin resistant *Staph. Aureus* (MRSA), enterococci and streptococci.

- **Teicoplanin** is a glycopeptide antibiotic used clinically in Europe.

A) **Mechanism of action for the glycopeptide antibiotics (Bactericidal)**

The glycopeptide antibiotics (vancomycin) inhibit bacterial cell-wall biosynthesis by binding to a C-terminal dipeptide (D-alanyl-D-alanine) on the bacteria’s growing cell-wall peptidoglycan. Since the D-alanyl-D-alanine dipeptide is required for cross-linking of cell-wall peptidoglycan, binding of the glycopeptide to this dipeptide prevents peptidoglycan crosslinking and ultimately leads to cell lysis. This ‘attachment’ of vancomycin to the growing cell wall can also inhibit the transglycosylation step of cell-wall biosynthesis. Thus the binding of glycopeptide antibiotics to cell wall D-alanyl-D-alanine dipeptide inhibits bacterial cell-wall biosynthesis. (This is shown Pictorially below)

B) **Spectrum of Activity**

Vancomycin is active against Gram-positive bacteria but is not active against Gram-negative bacteria. *Gram-positive selective activity is common throughout the glycopeptide class of antibiotics.* This spectrum of activity occurs since the glycopeptide antibiotics must bind to bacterial cell-wall peptidoglycan in order to inhibit bacteria growth. Since Gram-positive bacteria have no outer membrane, cell-wall peptidoglycan is readily accessible by the large glycopeptide structures. However, *Gram-negative bacteria have an outer membrane that is, in general, impermeable to the glycopeptide antibiotics.* Therefore, the glycopeptide antibiotics cannot access peptidoglycan of the growing cell wall in Gram-negative bacteria and are not active against them. Vancomycin does not exhibit cross-resistance to other agents that act by inhibiting cell-wall synthetic pathways since its mechanism of action is different.

C) **Distribution, Adsorption, Toxicity**

Vancomycin is a *large molecule and is poorly absorbed from the GI tract.* Therefore, vancomycin is given intravenously for systemic infections such as staphylococcal infections including endocarditis. Oral vancomycin is used in antibiotic-associated colitis and against GI infections. Approximately 90% of vancomycin undergoes urinary excretion and has a half-life of 5-6 hours. Adverse reactions are rare, typically mild, and include impairment of auditory acuity (ototoxicity), renal damage (nephrotoxicity), phlebitis and skin rashes.
Practice Questions:

1. Which of the following is a penicillinase sensitive penicillin that has broader activity against gram-negative bacteria than penicillin G.
   a) Amoxicillin
   b) Penicillin V
   c) Nafcillin
   d) Cefepime

2. Why is imipenem co-administered with cilastatin
   a) Imipenem would be inactivated by beta-lactamases if cilastatin (a beta-lactamase inhibitor) were not co-administered.
   b) Imipenem is unstable in solution so cilastatin is added to decrease hydrolysis of the drug.
   c) Imipenem is degraded by transpeptidases in the bacteria so cilastatin is given to block the bacterial transpeptidases.
   d) Imipenem is hydrolyzed by renal tubular dipeptidase so cilastatin (an inhibitor of renal tubular dipeptidase) is given to block imipenem inactivation.

3. The penicillins, cephalosporins, cabapenems and monobactams all have which of the following in common.
   a) They are all very effective against gram-positive bacteria since the peptidoglycan layer in the cell-wall is exposed and readily accessible.
   b) They all bind penicillin binding protein-1 and inhibit its transpeptidase activity.
   c) They all have a 4-membered beta-lactam ring as a part of their pharmacophore.
   d) None of them penetrate the central nervous system or the cerebral spinal fluid.

4. What do tazobactam, sulbactam, and clavulanate have in common?
   a) Their antibiotic activity is inhibited by beta-lactamases.
   b) They are commonly co-administered with carbapenems to block carbapenemase inactivation.
   c) They are beta-lactamase inhibitors that can be administered with penicillinase sensitive penicillins.
   d) They are typically co-administered with the cephamycins.

5. The glycopeptide antibiotic vancomycin inhibits cell wall synthesis of Gram-positive bacteria via which of the following mechanisms?
   a) Binding PBPs and inhibiting transpeptidation and transglycosylation.
   b) Binding C-terminal D-alanyl-D-alanine dipeptide of cell wall peptidoglycan.
   c) Binding porin channel proteins and physically blocking transport of cell wall precursors.
   d) Binding NAG-NAM saccharides of peptidoglycan thus blocking cell wall biosynthesis.

6. Why is vancomycin typically active against Gram-positive but not Gram-negative bacteria?
   a) Gram-positive bacteria have an outer lipid membrane that is readily accessible to the large vancomycin structure.
   b) Gram-positive bacteria have large porin channels that allow large antibiotics such as vancomycin to enter the cell.
   c) Vancomycin binds and inhibits a number of transpeptidase enzymes in Gram-positive bacteria while it does not bind the transpeptidases of Gram-negative bacteria.
   d) Gram-positive bacteria do not have an outer membrane like Gram-negative bacteria thus vancomycin can access peptidoglycan in Gram-positive but not Gram-negative bacteria.

7. Which of the following statements pertaining to the use of antibiotics to treat mycobacterial tuberculosis infections is incorrect?
   a) Mycobacteria grow and multiply at a very high rate; thus long term therapy is required.
   b) Mycobacteria exist intracellularly; thus the drug must be able to enter cells to effectively rid the body of tuberculosis.
   c) Mycobacteria infections typically contain organisms that are resistant to a single anti-tuberculosis agent; thus multi-drug therapy is used.
   d) Mycobacteria are naturally quite impermeable to many antibiotics due to their lipophilic outer layer.

8. Which of the following is a beta-lactamase resistant penicillin?
   a) Amoxicillin
   b) Penicillin V
   c) Nafcillin
   d) Cefaclor
   e) Resistomycin
9. Which inhibitor of bacterial cell wall synthesis has a mechanism of action that involves binding to the D-alanyl-D-alanine terminal dipeptide of peptidoglycan biosynthetic precursors?

   a) Penicillin  
   b) Isoniazid  
   c) Vancomycin  
   d) Cephalomycin

These practice questions were knowledge based. Exam questions will be more problem solving based.

Answers: 1a 2d 3c 4c 5b 6d 7a 8c 9c