Antibiotic Classification and Modes of Action
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Upon completion of this module you will be able to:

- Explain why susceptibility testing is done
- Define the terms, bacteriostatic and bactericidal
- Describe the functional antibiotic classification scheme and list the 5 main groups
- Name at least one antibiotic in each class
- Describe the structure of a Gram-positive and negative cell
- Explain the modes of action for the antibiotics in each of the five functional antibiotic classes
- List examples of natural resistance in each of the five functional antibiotic classes
- Explain why it is not necessary to perform susceptibility testing for certain organism / antibiotic combinations
Microbiologists work with antibiotics every day.

Antimicrobial Susceptibility Testing (AST) is one of the primary functions of the Microbiology Lab.

But, how much do Microbiologists really know about antibiotics?

Let’s review some basic information and see how it can be applied daily.
What is an Antibiotic?

**Antibiotic** is a chemical substance produced by a *microorganism* that inhibits the growth of or kills other microorganisms.

**Antimicrobial agent** is a chemical substance derived from a *biological source* or produced by *chemical synthesis* that kills or inhibits the growth of microorganisms.

The noun “antibiotic” was first used in 1942 by Dr. Selman A. Waksman, soil microbiologist. Dr. Waksman and his colleagues discovered several actinomycetes derived antibiotics.

The two terms are usually used synonymously and that practice will continue throughout this presentation.

The word “antibiotic” will be used to describe:
- a chemical substance derivable from a microorganism or produced by chemical synthesis that kills or inhibits microorganisms and cures infections.
Sources of Antibacterial Agents

- **Natural** - mainly fungal sources
- **Semi-synthetic** - chemically-altered natural compound
- **Synthetic** - chemically designed in the lab

- The original antibiotics were derived from fungal sources. These can be referred to as “natural” antibiotics
  - Organisms develop resistance faster to the natural antimicrobials because they have been pre-exposed to these compounds in nature. Natural antibiotics are often more toxic than synthetic antibiotics.
  - Benzylpenicillin and Gentamicin are natural antibiotics
- Semi-synthetic drugs were developed to decrease toxicity and increase effectiveness
  - Ampicillin and Amikacin are semi-synthetic antibiotics
- Synthetic drugs have an advantage that the bacteria are not exposed to the compounds until they are released. They are also designed to have even greater effectiveness and less toxicity.
  - Moxifloxacin and Norfloxacin are synthetic antibiotics
- There is an inverse relationship between toxicity and effectiveness as you move from natural to synthetic antibiotics
Role of Antibiotics

What is the role of antibiotics?
• To inhibit multiplication

Antibiotics have a bacteriostatic effect.

At which drug concentration is the bacterial population inhibited?
• Minimal Inhibitory Concentration = MIC

Bacteriostatic = inhibits bacterial growth
Quantitative Measure

- MIC = lowest concentration of antibiotic that inhibits growth (measured visually)

- Interpretation of quantitative susceptibility tests is based on:
  - relationship of the MIC to the achievable concentration of antibiotic in body fluids with the dosage given
  - For treatment purposes, the dosage of antibiotic given should yield a peak body fluid concentration 3-5 times higher than the MIC
    or
  - MIC x 4 = dosage to obtain peak achievable concentration
Role of Antibiotics

What is the role of antibiotics?
• To destroy the bacterial population
Antibiotics have a bactericidal effect.

At which drug concentration is the bacterial population killed?
• Minimal Bactericidal Concentration = MBC

Bactericidal = kills bacteria
Quantitative Measure

- MBC = lowest concentration of antibiotic that kills bacteria
There is a much closer relationship between the MIC and MBC values for bactericidal drugs than for bacteriostatic drugs.
How Do Antibiotics Work?

Mechanisms of Action
Antibiotics operate by inhibiting crucial life sustaining processes in the organism: the synthesis of cell wall material, the synthesis of DNA, RNA, ribosomes, and proteins.

Target
The target of the antibiotic should be selective to minimize toxicity...but all antibiotics are toxic to some degree!

The targets of antibiotics should be selective to minimize toxicity.

- Selective Toxicity
  - Harm the bacteria, not the host
Why Do Susceptibility Testing?

- Help patients today
  - To provide guidance to the physician in selection of antimicrobial therapy with the expectation of optimizing outcome

- Help patients tomorrow
  - Build an antibiogram to guide physicians in selecting empiric therapy for future patients with the expectation of optimizing outcome

- Help patients next decade
  - Drive new drug research
  - Monitor the evolution of resistance

We do this testing:
- To provide a guide for therapy
- Allows selection of the most appropriate agent
  - Least expensive
  - Narrowest spectrum
  - Most effective
- To monitor the evolution of resistance

Antibiotic resistance has an impact on individual health and public health.
- Types of resistance seen and frequency
- Which drugs you can expect to be successful
- Emerging and new resistance seen in the community
There are many possible reasons antimicrobials may fail.

Selection of the appropriate antibiotic depends on:
- knowledge of organism’s natural resistance
- pharmacological properties of the antibiotic toxicity, binding, distribution, absorption achievable levels in blood, urine
- previous experience with same species
- nature of patients underlying pathology
- patient’s immune status

Susceptibility testing focuses primarily on the interaction of antimicrobial agents, the organisms and their resistance mechanisms.
Interpreting Susceptibility Results

- MICs are not physical measurements
- There are many factors that play a role in determining clinical outcome
- Susceptibility in vitro does not uniformly predict clinical success in vivo
- Resistance will often, but not always, correlate with treatment failure

Susceptibility tests are essentially artificial measurements.

- in vitro response
- approximate range of effective inhibitory action
- possible error equivalent to one tube dilution

The only true measure of bacterial response to an antibiotic is the clinical response of the patient.

- outcome or in vivo response

One of the real values of AST is to predict resistance

S = success likely, but no guarantee
R = correlates well with treatment failure

Is Resistance Testing a better name for what we do than Susceptibility Testing?
What is the Ideal Antibacterial?

- Selective target – target unique
- Bactericidal – kills
- Narrow spectrum – does not kill normal flora
- High therapeutic index – ratio of toxic level to therapeutic level
- Few adverse reactions – toxicity, allergy
- Various routes of administration – IV, IM, oral
- Good absorption
- Good distribution to site of infection
- Emergence of resistance is slow
Antibiotic Classification and Modes of Action

In the AES Knowledge Base, phenotypes are organized by drug class. The AES decision process attempts to identify a phenotype for each drug class tested. In order to understand and use the software effectively, it is important to have a solid working knowledge of antibiotic classification.

In addition, each drug class typically has a unique mode of action. Bacteria in turn, direct their defenses against these specific modes of action. Understanding why antibiotics fail begins with the classification of antibiotics and their modes of action.
Antibiotic Classification

Grouped by Structure and Function

Five functional groups cover most antibiotics
1. Inhibitors of cell wall synthesis
2. Inhibitors of protein synthesis
3. Inhibitors of membrane function
4. Anti-metabolites
5. Inhibitors of nucleic acid synthesis

Antibiotics are usually classified based on their structure and/or function.

   - ß-Lactams - Beta-lactam ring
   - Aminoglycosides - vary only by side chains attached to basic structure

2. Function - how the drug works, its mode of action.
   - 5 functional groups
   - These are all components or functions necessary for bacterial growth
   - Targets for antibiotics

In these discussions, we will primarily use the functional classification, but will point out where structural similarities also exist.
1. Inhibitors of Cell Wall Synthesis

- Beta-lactams
  - Penicillins
  - Cephalosporins
  - Monobactams
  - Carbapenems

Glycopeptides
Fosfomycins

Inhibitors of Cell Wall Synthesis

Beta-lactams
- There are about 50 different Beta (ß)-lactams currently on the market
- They are all bactericidal
- They are non-toxic (i.e., they can be administered at high doses)
- They are relatively inexpensive
- Beta-lactams are organic acids and most are soluble in water
1. Inhibitors of Cell Wall Synthesis

**Beta-lactams**

- Similar in structure, as well as, function
- All have a common structural ß-lactam ring
- Antibiotics vary by side chains attached

- All beta-lactams are subject to inactivation by bacterial-produced enzymes called beta-lactamases

Some more common Beta-lactamase enzymes include:

- Penicillinases
- Cephalosporinases
- ESBL’s
- Cephamicinases
- Carbapenemases
Inhibitors of Cell Wall Synthesis

Beta-lactams - Penicillins

Spectrum of Action

1. Natural penicillins
   - **Penicillin G**: Active against Gram-positive organisms that do not produce beta-lactamases, *Neisseria* and some anaerobes

2. Penicillinase-resistant penicillins
   - **Penicillin M**: Active against penicillinase-producing *Staphylococci*

3. Extended-spectrum penicillins
   - **Aminopenicillins**: Slightly less active than Penicillin G against *Pneumococci*, *Streptococci* and *Meningococci*, but active against many strains of *Salmonella*, *Shigella*, and *P. mirabilis*, *H. influenzae*
   - **Carboxypenicillins**: More stable than aminopenicillins to hydrolysis by the β-lactamases of most *Enterobacteriaceae* and *Pseudomonas aeruginosa*
   - **Ureidopenicillins**: Greater activity than carboxypenicillins against Gram-positives, enterics and *P. aeruginosa*

4. Co-Drugs (Beta-lactam + beta-lactamase inhibitor)
   - **β-lactamase inhibitors (BLI) combinations**: Additional activity against beta-lactamase producing organisms, including *Staphylococcus spp.*, some enterics, *H. influenzae* and *Bacteroides spp*

5. Amidinopenicillins
   - **Mecillinam**: Restricted use to urinary infection with *E. coli*. Active against penicillinase and low-level cephalosporinase.
1. Inhibitors of Cell Wall Synthesis

**Beta-lactamase Inhibitors (BLI)**

*Aim: to block β-lactamases*

These enzymatic inhibitors have weak or poor antibacterial activity alone, but a strong affinity for β-lactamases: Clavulanic Acid

- Sulbactam
- Tazobactam

**Combination β-lactams - β-lactamase inhibitor:**

- Amoxicillin + Clavulanic Acid
- Ampicillin + Sulbactam
- Ticarcillin + Clavulanic Acid
- Piperacillin + Tazobactam

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**Beta-lactamase inhibitors** (BLI) have a beta(β)-lactam ring, but have weak or poor antibacterial activity.

- They have a very high affinity for β-lactamases
- They act as a trap, and are hydrolyzed in preference to the β-lactam drug. The drug is left intact to act on the bacteria (cell wall).
- Should be called penicillinase inhibitors, because they are active against:
  - Staph penicillinase
  - Penicillinase of *K. pneumoniae*
  - ESBL (to a greater or lesser degree) - if the penicillinase is being overproduced, the inhibitor effect may be diluted (Inoculum Effect)

**In the case of penicillinase production, where:**

- Amoxicillin → R
- Amoxicillin + Clavulanic acid → usually S

- Inhibitors are active against all penicillinase (PASE and ESBL) but never on cephalosporinase

**NEW ISSUE** - BLI can act as inducers and actually stimulate enzyme (beta-lactamase) production. It is possible to see the following:

- *Pseudo monas*  
  - Ticarcillin = S  
  - Ticarcillin/Clavulanic = R

- *Enterobacteriaceae*  
  - Piperacillin = S  
  - Piperacillin/Tazobactam = R
1. Inhibitors of Cell Wall Synthesis

**Beta-lactams - Cephems**

Spectrum of Action

- **1st generation cephalosporins (C1G):** Narrow spectrum; good Gram-positive activity and relatively modest Gram-negative activity. Inactivated by Gram-neg beta-lactamases (Derived from *Cephalosporium acremonium*).

- **2nd generation cephalosporins (C2G):** Better Gram-negative coverage (more beta-lactamase stability), but less *Staphylococcal* activity.

- **Cephamycins:** Remain susceptible in presence of Extended Spectrum ß-lactamase (ESBL) because they are not a substrate for the enzyme. Can be used as an indicator for ESBL (Derived from *Streptomyces lactamdurans*).

- **3rd generation cephalosporins (C3G):** Wider spectrum of action when compared to C1G and C2G. Less active than narrow spectrum agents against Gram-positive cocci, but much more active against the *Enterobacteriaceae* and *Pseudomonas aeruginosa* (better beta-lactamase stability).

- **4th generation cephalosporins (C4G):** Broadest spectrum of action. Active against high level cephalosporinases of *Enterobacteriaceae* and *Pseudomonas aeruginosa* (not usually used with ESBL producing organisms).

None have activity to MRSA or *Enterococcus spp.*
1. Inhibitors of Cell Wall Synthesis

New Anti- MRSA Cephalosporins

Ceftobiprole
Ceftaroline

A next generation cephalosporins?

Inhibitors of Cell Wall Synthesis

Beta-lactams - Cephems - Ceftobiprole

Spectrum of Action

- **Next generation cephalosporin:** Broad spectrum; active against the common Gram-negative bacteria. Some Gram-positive activity (Drug Resistant *S. pneumoniae*). Notable for activity against MRSA, unlike any other beta-lactam antibiotic. Bactericidal.

- Not yet FDA approved
- Has been added to CLSI® M100-S18, 2008 listing of antibiotics
- No CLSI breakpoints
- Once FDA approved, can no longer say if Staph is resistant to Oxacillin, report all beta-lactams as resistant.
1. Inhibitors of Cell Wall Synthesis

### Beta-lactams: Monobactams

#### Spectrum of Action

- **Aztreonam**: Gram-negatives (*Enterobacteriaceae* and *Pseudomonas*). Not hydrolyzed by most commonly occurring plasmid and chromosomally mediated β-lactamases, and does not induce the production of these enzymes.

### Beta-lactams: Penems

#### Spectrum of Action

- **Carbapenems**: β-lactams with a broad spectrum of action. Gram-positives, except MRSA. Gram-negatives, *Ps. aeruginosa* (except Ertapenem) and anaerobes. Very efficient against high level cephalosporinase and ESBL. Wide diffusion in the body, especially in the cerebrospinal fluid.

  - Doripenem (new) - Broad spectrum. Particularly good for *Pseudomonas* and other non-fermenters.

- **Penem**: Oral. Primarily for respiratory tract infections. Poor activity with *Serratia, Pseudomonas, Stenotrophomonas.* Faropenem not yet FDA approved.

### Beta-lactams: Carbapenems

- Imipenem
- Meropenem
- Ertapenem
- Doripenem
- Faropenem
Before we can understand the antibiotic modes of action, we need to review the structure and physiology of the bacterial cell.
It is important to understand the growth cycle and physiology of bacteria to appreciate how it influences antimicrobial action.

Bacteria are all around us. Given good growing conditions, a bacterium will divide.

A new cell wall forms at the center and the "bug" splits into two daughter cells, both of which carry the same genetic information as the parent.

If the environment is perfect, the two daughter cells can split into four within 20 minutes.

1, 2, 4, 8, 16, 32, 64...

Typical bacterial growth cycle includes:

- Lag phase
- Exponential (log) phase
- Stationary phase
- Death phase
Typical Structure of a Bacterial Cell (from inside to outside)

DNA  bacterial genetic material

Ribosomes  (protein-making factories), energy-generating systems, digestive system, and everything else are located in the cytoplasm.

Cytoplasmic Membrane or Inner Membrane
  a. Consists of phospholipids and other membrane proteins
  b. Semi-permeable
  c. Regulates pH, osmotic pressure and availability of essential nutrients

Bacterial Cell Wall or Peptidoglycan
  a. Cross-linked mesh that gives a cell its shape, strength and osmotic stability, a protective suit of armour
  b. Porous up to 100,000 Da

The outer layer of  lipopolysaccharide (LPS) and phospholipid material helps protect bacteria from bacteriophages, pH, enzymes, phagocytosis.
  • To multiply, the bacteria must be able to synthesize peptidoglycan, proteins and DNA
  • The cell wall, the ribosomes and DNA are all potential antibiotic targets
Gram-Positive Cell Structure

- The Gram-positive cell wall is **thick** and consists of 90% **peptidoglycan**

- **Teichoic acids** link various layers of peptidoglycan together. Teichoic acids also regulate the autolysin activity in this complex equilibrium.

- The **cytoplasmic membrane** (which defines the intracellular space) consist of:
  - a **lipid bilayer**
  - **intrinsic proteins** which are hydrophobic (mostly enzymes involved in respiration and transmembrane transport)
  - **extrinsic proteins** which are hydrophilic
  - **Penicillin-Binding Proteins (PBPs)**: periplasmic space proteins involved in peptidoglycan synthesis (glycosyltransferase, transpeptidase and carboxypeptidase activities)
Gram-Positive Cell Structure

The Gram-positive Envelope

- Polysaccharide
- Teichoic acid
- Peptidoglycan
- Protein
- Cytoplasmic membrane
- Phospholipid
Gram-Negative Cell Structure

- The **outer membrane** is made up of:
  - phospholipids
  - endotoxin or **lipopolysaccharide (LPS)** - plays an important role in the antibiotic entry into the cell
  - proteins including the **porins** (complexes of three proteins) form aqueous channels that provide a route across the outer membrane for all the water-soluble compounds needed by the bacterium
- The **periplasmic space** contains:
  - peptidoglycan – 5-20% of cell wall
  - various enzymes (in particular, **ß-lactamases**)
- The **cytoplasmic membrane** (which defines the intracellular space) consists of:
  - a **lipid bilayer**
  - intrinsic proteins which are hydrophobic (mostly enzymes involved in respiration and transmembrane transport)
  - extrinsic proteins which are hydrophilic
  - **Penicillin-Binding Proteins (PBPs)** - periplasmic space proteins involved in peptidoglycan synthesis (glycosyltransferase, transpeptidase and carboxypeptidase activities)
Gram-Negative Cell Wall Structure

The Gram-negative Envelope

- O-antigens
- Porin trimer
- Lipopoly saccharide
- Brown's lipoprotein
- Peptido glycans
- Protein

Outer membrane
Periplasmic space
Cytoplasmic membrane
Phospholipid
Peptidoglycan

- is a very high molecular weight polymer composed of many identical subunits
- is a 3-D polymeric macromolecule
- determines cell shape and prevents osmotic lysis, porous up to 100,000 daltons
- contains N-acetylglucosamine (NAG), N-acetylmuramic acid (NAMA), and several different amino acids
- involves 2 types of covalent chemical bonds:
  1. B-1, 4 glycosidic bond between hexose sugars
  2. Peptide bond between amino acids
- In Gram-positive bacteria, peptidoglycan accounts for as much as 90% of the cell wall (approximately 40 layers), with the rest consisting of the teichoic acids.
- Gram-negative bacteria have a thin peptidoglycan layer (accounting for only 5-20% of the cell wall). However, in addition to the cytoplasmic membrane, they have a second phospholipid bilayer external to the peptidoglycan called the outer membrane.
1. Inhibitors of Cell Wall Synthesis

Mode of Action of Beta-lactams

Humans have no cell wall (no peptidoglycan), so this is a good selective target for the antibiotic.
1. Inhibitors of Cell Wall Synthesis

Entry of β-lactams in Gram-Positive Bacteria

β-lactam

Peptido-glycan

Cytoplasmic membrane

PBP

Protein

Beta-lactams are mostly water soluble.

Differences in cell wall composition (more or less lipids) between different bacterial species partially account for their differential susceptibility to β-lactams.

The target of the β-lactam antibiotics for all bacteria is the PBP (Penicillin Binding Protein) in the cytoplasmic membrane.

**Gram-Positives:**

In Gram-positive bacteria, there is no barrier to the entry of β-lactams antibiotics.

The peptidoglycan layers allow the diffusion of small molecules.

However, β-lactams cross the membranes with great difficulty due to high lipid content but, since their target PBP’s are found on the outer surface of the cytoplasmic membrane, they only have to cross the cell wall.

**Natural Resistance**

*Enterococcus* - PBP’s are different from other Gram-positives (also higher lipid content in cell wall), which causes a low level resistance to penicillins and resistance to C1G.
Gram-Negatives:

1. Outer membrane entry through the:

**Porins**: Hydrophilic β-lactams tend to gain access into the periplasmic space using these watery funnels (i.e., *E. coli* organism has about 100,000 of these porins). Porins are transmembrane proteins. They complex together to form water-filled channels through which low molecular weight (<600 daltons) hydrophilic substances readily diffuse. Such diffusion is passive and the concentration in the periplasmic space can reach the level that prevails in the external environment as long as there are no mechanisms to pump the drug back out or which inactivate it.

**Phospholipids**: This mode of entry is less common, but it seems to play a significant part in the case of certain β-lactams. Lipid bilayers support the diffusion of lipophilic compounds (certain β-lactams are lipophilic). However, if the organism’s LPS (endotoxin) is branched (e.g., as in the case of *Pseudomonas aeruginosa*), such diffusion is blocked. This is called impermeability.

2. Peptidoglycan entry

3. Periplasmic space entry: β-lactams may encounter β-lactamases

4. Cytoplasmic membrane PBP - bound

**Natural Resistance** - Many Gram-negative organisms are naturally resistant to penicillin G and oxacillin because the drug is prevented from entering the cell by the LPS which blocks the porins.
1. Inhibitors of Cell Wall Synthesis

Interactions between β-lactams and PBPs

- PBPs are enzymes involved in peptidoglycan synthesis (glycosyltransferases, transpeptidases and carboxypeptidases)
- There are many kinds of PBP’s - some essential, some not
- They are numbered by molecular weight
- Inhibition of these enzymes by β-lactams inhibits peptidoglycan synthesis and therefore stops cell growth (bacteriostatic activity)
- β-lactams form stable complexes with PBPs
  - Bind irreversibly to PBP by a covalent bond
- β-lactams also have bactericidal activity. Although peptidoglycan synthesis is stopped, the autolysins remain active. Autolysin activity is progressively potentiated. The peptidoglycan network begins to become disorganized and teichoic acid (which normally regulates autolysin activity by natural inhibition) tends to leak out.
- Without a peptidoglycan layer, the bacterium bursts and eventually all cells die

**NOTE:** Ceftobiprole and Ceftaroline (the next generation cephalosporins) have the ability to inactivate PBP2a which is primarily responsible for oxacillin resistance in *Staphylococci.*
The efficacy of the antibiotic hangs on 2 parameters:

- The rapidity of the drug entrance
- The rate of enzymatic hydrolysis

Periplasmic Space Entry

- The periplasmic space represents a “mine field” for β-lactams due to the presence of β-lactamase enzymes (defense enzymes)
- β-lactamases are found in all bacteria, although in variable amounts and with varying levels of activity (they can even be found in wild-type *E. coli* although at such a low concentration that their effect is insignificant).
- β-lactamases hydrolyze the β-lactam ring. The rate of this hydrolysis depends on the rate on entry of the drug and the level of β-lactamase activity.
- In many cases, the rate of entry is sufficient to guarantee a consistently high enough concentration of the drug to interact with the PBP’s, even if a certain proportion is constantly being enzymatically hydrolyzed.
1. Inhibitors of Cell Wall Synthesis

Beta-lactams
  - Penicillins
  - Cephalosporins
  - Monobactams
  - Carbapenems

Glycopeptides
  - Vancomycin and Teicoplanin

Fosfomycins

Inhibitors of Cell Wall Synthesis

Glycopeptides

- The term ‘Glycopeptide’ included two main compounds with very similar structures and modes of action:
  - Vancomycin and Teicoplanin
- Teicoplanin not FDA approved in the U.S.
- Both are of high molecular weight (1500-2000 daltons)
- Glycopeptides have a complex chemical structure
- Inhibit cell wall synthesis at a site different than the beta-lactams
- All are bactericidal
- All used for Gram-positive infections. (No Gram-negative activity)
- Pharmaceutical research and development has been very active in this area recently resulting in new antimicrobials and classification
1. Inhibitors of Cell Wall Synthesis

**Glycopeptides**

Glycopeptide:
Vancomycin

Lipoglycopeptide:
Dalbavancin
Oritavancin
Telavancin
Teicoplanin

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**Inhibitors of Cell Wall Synthesis**

**Glycopeptides: Glycopeptide**

Spectrum of Action

- **Vancomycin**: MRSA (Methicillin Resistant *Staph. aureus*), *C. difficile*, *Streptococci* including *Strep pneumoniae*. Alternative to Penicillin G in serious infections. Good diffusion in all tissues (except CSF). High toxic effects: ears and kidneys.

**Glycopeptides: Lipoglycopeptide**

Spectrum of Action

- **Dalbavancin**: (Vicuron) 2nd generation lipoglycopeptide. Bactericidal for MRSA and MRSE. 1/week IV dosing – long acting. Not yet FDA approved.
- **Oritavancin**: (Targanta) 2nd generation glycopeptide. Actually a lipoglycopeptide. IV – once per day dosing. For skin and skin structure infections. Activity similar to Vancomycin – better for *Staphylococcus* and *Enterococcus*. Not yet FDA approved. Bactericidal
- **Teicoplanin**: Not FDA approved in the US. Widespread use in Europe.
1. Inhibitors of Cell Wall Synthesis

Mode of Action of Glycopeptides

Vancomycin
1. Inhibitors of Cell Wall Synthesis

Entry of Glycopeptides in Gram-Positive Bacteria

In Gram-Positives:
The drugs enter without any problem because peptidoglycan does not act as a barrier for the diffusion of these molecules.
1. Inhibitors of Cell Wall Synthesis

Entry of Glycopeptides in Gram-Negative Bacteria

In Gram-Negatives:

Glycopeptides are of high molecular weight (1500-2000 daltons), stopping them from passing through the porins of gram-negative bacteria (i.e., glycopeptides have no activity against Gram-negatives).

Gram-negatives are naturally resistant.

Use this property in Microbiology in several ways:

Check Gram reaction - growth around Vancomycin disk would indicate a Gram-negative organism (resistant to Vancomycin).

KVC disks/media - for anaerobe ID’s.
1. Inhibitors of Cell Wall Synthesis

Mode of Action of Glycopeptides

• Glycopeptides inhibit the final cell wall stage of the peptidoglycan synthesis process.

• The ‘pocket-shaped’ glycopeptide binds the **D-ala-D-ala** terminal of the basic sub-unit theoretically waiting to be incorporated into the growing peptidoglycan.

• Because it is so bulky, the glycopeptide inhibits the action of the glycosyltransferases and transpeptidases (which act as a kind of “cement”) - blocks pentaglycine from joining molecules, thereby blocking peptidoglycan growth.

• Glycopeptides are bactericidal, but slow-acting.
1. Inhibitors of Cell Wall Synthesis

Beta-lactams
- Penicillins
- Cephalosporins
- Monobactams
- Carbapenems

Glycopeptides
- Fosfomycins

Inhibitors of Cell Wall Synthesis

Fosfomycins

Spectrum of Action

**Fosfomycin:** Acts to inhibit cell wall synthesis at a stage earlier than the penicillins or cephalosporins. FDA approved 1996. While it is a broad spectrum agent, CLSI® only provides breakpoints for urinary tract infections due to *E.coli* and *E.faecalis*.

Reference Method:

Preferred reference method is agar dilution. Broth dilution should not be performed.

Mode of Action:

Inhibits the first step of the peptidoglycan synthesis process
2. Inhibitors of Protein Synthesis

Aminoglycosides  
MLSK  
(Macrolides, Lincosamides, Streptogramins, Ketolides)  
Tetracyclines  
Glycylcyclines  
Phenicols  
Oxazolidinones  
Ansamycins

### Inhibitors of Protein Synthesis

<table>
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<tr>
<th>Category</th>
<th>Examples</th>
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<tbody>
<tr>
<td><strong>Aminoglycosides</strong> - (Bactericidal)</td>
<td>Gentamicin, Tobramycin, Amikacin</td>
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<tr>
<td><strong>MLSK</strong> - (Bacteriostatic)</td>
<td>Erythromycin, Clindamycin, Quinupristin-Dalfopristin (Synercid), Clarithromycin, Azithromycin, Telithromycin</td>
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<tr>
<td><strong>Tetracyclines</strong> - (Bacteriostatic)</td>
<td>Tetracycline, Doxycycline, Minocycline</td>
</tr>
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<td><strong>Glycylcyclines</strong> -</td>
<td>Tigecycline</td>
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<tr>
<td><strong>Phenicols</strong> - (Bacteriostatic)</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td><strong>Oxazolidinones</strong> - (Bactericidal for <em>Streptococci</em>; Bacteriostatic for <em>Enterococcus</em> and <em>Staphylococci</em>)</td>
<td>Linezolid</td>
</tr>
<tr>
<td><strong>Ansamycins</strong> - (Bacteriostatic or Bactericidal depending on organism and concentration)</td>
<td>Rifampin</td>
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2. Inhibitors of Protein Synthesis

- Aminoglycosides
  - MLSK
    (Macrolides, Lincosamides, Streptogramins, Ketolides)
  - Tetracyclines
  - Glycylcyclines
  - Phenicols
  - Oxazolidinones
  - Ansamycins

Aminoglycosides
- Humans do synthesize proteins, but at a much slower rate than bacteria
- Drug can act on bacteria before it does damage to man
- But, since it is a common process, we do tend to see more toxic side effects
- Need to do therapeutic drug monitoring
- All bactericidal
Inhibitors of Protein Synthesis

Aminoglycosides:

- Related in structure and function
- Drugs differ based on location of radical groups attached to the 3 ring basic structure
2. Inhibitors of Protein Synthesis

Aminoglycosides

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<tr>
<th>AMINOGLYCOSIDES</th>
<th>International Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amikacin</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
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<tr>
<td></td>
<td>Tobramycin</td>
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<tr>
<td></td>
<td>Streptomycin</td>
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<tr>
<td></td>
<td>Kanamycin</td>
</tr>
<tr>
<td></td>
<td>Netilmicin</td>
</tr>
</tbody>
</table>

Inhibitors of Protein Synthesis

Aminoglycosides:

Spectrum of Action

- Rapid bactericidal effect
- Broad spectrum of action
  - Gram-negative nosocomial infections and severe systemic infections
  - Gram-positives, except *Streptococcus* and *Enterococcus*. Must combine an aminoglycoside (Gentamicin or Streptomycin) with a penicillin, ampicillin or vancomycin for severe enterococcal infections (Synergy Testing).
- In serious infection, used in association with beta-lactams or fluoroquinolones
- Kanamycin develops resistance quickly
- Hospital use only
- Nephrotoxic and toxic for ears

Drug Dosage Adjustment:

Monitoring aminoglycosides is mandatory. It is important to control the serum level for peak and trough to ensure the bactericidal effect and avoid side effects.
2. Inhibitors of Protein Synthesis

Mode of Action of Aminoglycosides
2. Inhibitors of Protein Synthesis

Protein Synthesis

A quick review of protein synthesis before we begin.
2. Inhibitors of Protein Synthesis

Protein Synthesis

Proteins are made up of at least 20 different amino acids

Thousands of different proteins exist, each with its own function (enzymes, etc.).

Each protein is manufactured in the cell using a code contained in the DNA.

• The code for the 20 essential amino acids consists of at least a 3-base set (triplet) of the 4 bases

• If one considers the possibilities of arranging four things 3 at a time (4X4X4), we get 64 possible code words, or codons (a 3-base sequence on the mRNA that codes for either a specific amino acid or a control sequence).
2. Inhibitors of Protein Synthesis

Protein Synthesis Pathway

- Proteins are manufactured in the cell using a code in the DNA
- DNA is transcribed into RNA by an enzyme called RNA polymerase
- The RNA strand is translated into proteins by the ribosomes
2. Inhibitors of Protein Synthesis

Protein Synthesis Role of Ribosome

- Each ribosome consists of one small sub-unit (30S) and a larger one (50S)
- The ribosome crawls along the messenger-RNA molecule translating the code and assembling the amino acids in the correct order before chemically joining them to create the protein
2. Inhibitors of Protein Synthesis

**Entry of Aminoglycosides**

- **Outer Membrane** entry:
  - Aminoglycosides are positively charged molecules which means they rapidly enter bacteria (negatively charged) since the two charges attract each other.
  - The negative charge of bacteria is due to LPS in the outer membrane and the peptidoglycan (notably the teichoic acid).

- **Cytoplasmic Membrane** entry:
  - The drugs cross the cytoplasmic membrane via respiratory enzymes (involved in aerobic respiration).
  - This is why bacteria without respiratory enzymes (strict anaerobes or facultative anaerobes like streptococci) are **naturally resistant** to aminoglycosides.

**Aminoglycoside Mode of Action**

Target = Ribosome in cytoplasm

1. Outer Membrane entry:
   - Aminoglycosides are positively charged molecules which means they rapidly enter bacteria (negatively charged) since the two charges attract each other.
   - The negative charge of bacteria is due to LPS in the outer membrane and the peptidoglycan (notably the teichoic acid).

2. Cytoplasmic Membrane entry:
   - The drugs cross the cytoplasmic membrane via respiratory enzymes (involved in aerobic respiration).
   - This is why bacteria without respiratory enzymes (strict anaerobes or facultative anaerobes like streptococci) are **naturally resistant** to aminoglycosides.
Aminoglycosides bind to the RNA of the 30S ribosomal sub-unit.

The resulting change in ribosome structure affects all stages of normal protein synthesis.

- Initiation step of translation
- Blocks elongation of peptide bond formation
- Release of incomplete, toxic proteins

Translational errors are frequent and many non-functional or toxic proteins are produced. The incorporation of such abnormal proteins into the cytoplasmic membrane compromises its function.

The bactericidal activity of aminoglycosides ultimately stops protein synthesis and dramatically damages the cytoplasmic membrane.
2. Inhibitors of Protein Synthesis

Aminoglycosides

- **MLSK**
  
  (Macrolides, Lincosamides, Streptogramins, Ketolides)

- Tetracyclines
- Glycylcyclines
- Phenicols
- Oxazolidinones
- Ansamycins

Inhibitors of Protein Synthesis

**MLSK**: (Macrolides, Lincosamides, Streptogramins, Ketolides)

Bacteriostatic

Their spectrum of activity is limited to Gram-positive cocci such as *Streptococci* and *Staphylococci*. These antibiotics are also active against anaerobes.
Inhibitors of Protein Synthesis

MLSK: (Macrolides, Lincosamides, Streptogramins, Ketolides)

Four different classes of antibiotics which are unrelated in terms of structure but which have a similar mode of action and spectrum of activity.
2. Inhibitors of Protein Synthesis

Macrolides, Lincosamides, Streptogramins, Ketolides

MLSK

<table>
<thead>
<tr>
<th>International Common Name</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrolides</td>
<td>Erythromycin</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>Lincomycin, Clindamycin</td>
</tr>
<tr>
<td>Streptogramins</td>
<td>Quinupristin / Dalfopristin Pristinamycin</td>
</tr>
<tr>
<td>Ketolides</td>
<td>Telithromycin</td>
</tr>
</tbody>
</table>

Inhibitors of Protein Synthesis

Spectrum of Action

**Macrolides:** Respiratory infections due to *S. pneumoniae* and *S. pyogenes*, *Mycoplasma, Legionella*, less serious *Staphylococcal* infections.

**Lincosamides:** Gram-positive skin infections and anaerobe infections. Oral administration appropriate for out patient settings.

**Streptogramins: Quinupristin/Dalfopristin (Synercid):** consists of an A and B component (Synergistic).

- **A** - prevents peptide bond formation and changes the ribosome to attract the B component.
- **B** - causes early release of incomplete peptide chains. Used for *E. faecalis* (VRE) and MRSA.

**Ketolides: Telithromycin:** Represents a novel class that has received much attention recently due to their excellent activity against resistant organisms.

Ketolides are semi-synthetic derivatives of erythromycin.

FDA approved for bronchitis, sinusitis, community acquired pneumonia due to *S.pneumoniae* (including Macrolide Resistant strains), *H. influenza*, *M.catarrhalis, S.aureus* (sinusitis), *M.pneumoniae*, and *C.pneumoniae*

Side effects – possible liver damage.
2. Inhibitors of Protein Synthesis

Mode of Action of MLSK

- Telithromycin
- Clindamycin
- Quinupristin-Dalfopristin
- Erythromycin
2. Inhibitors of Protein Synthesis

Entry of MLSK in Gram-Positive Bacteria

The drugs enter a Gram-positive cell without any problem because peptidoglycan does not act as a barrier for the diffusion of these molecules nor does the cytoplasmic membrane.

Target = Ribosome in cytoplasm
• In Gram-negative bacteria there is no entry because MLSK are lipophilic molecules. They cannot cross the outer membrane which is hydrophilic. “Oil and water don’t mix”.

• MLSK are also large molecules that cannot pass through the porins (which are also aqueous channels) – impermeability.

• Most Gram-negatives are naturally resistant to MLSK
2. Inhibitors of Protein Synthesis

Mode of Action - The MLSK Group

- Antibiotics in the MLSK group are structurally distinct but have a similar mode of action by binding the 50S ribosomal subunit.
- During translation, it blocks the initiation step, elongation step or peptide release step of protein synthesis.
- Unfinished or toxic protein is released.
2. Inhibitors of Protein Synthesis

Aminoglycosides

MLSK
(Macrolides, Lincosamides, Streptogramins, Ketolides)

- Tetracyclines
- Glycylcyclines
- Phenics
- Oxazolidinones
- Ansamycins

Inhibitors of Protein Synthesis

Tetracyclines:

- Bacteriostatic
- Chlortetracycline – 1948, the original tetracycline derived from a *Streptomyces spp*

Glycylcyclines:

- New class
- Developed to overcome some of the more common tetracycline resistance mechanisms
- Bacteriostatic
- Broad spectrum
2. Inhibitors of Protein Synthesis

**Tetracyclines**

<table>
<thead>
<tr>
<th>Tetracyclines</th>
<th>International Common Name</th>
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</tbody>
</table>

**Glycylcyclines**

- Tetracycline
- Minocycline
- Doxycycline
- Tigecycline

**Inhibitors of Protein Synthesis**

**Tetracyclines:**
Spectrum of Action: Broad spectrum, but resistance is common which limits its use. Primarily for treatment of genital infections (chlamydiae) and atypicals (Rickettsiae, Mycoplasma). Growth promoter in animal husbandry.

Toxicity: Diffuse well in cells and bones. Not recommended for pregnant women and children (less than 2 years old) because of the toxicity on bones and teeth of the fetus.

Tetracycline = Short acting
Minocycline and Doxycycline = Long acting
Minocycline and Doxycycline are more active than Tetracycline.
   - if Tetra = S, then Mino and Doxy = S
   - if Tetra = R, must test Mino and Doxy (may be S)

**Glycylcyclines: Tigecycline**

Spectrum of Action: Same as the tetracyclines; may have activity against multi-drug resistant organisms.

Derivative of Minocycline.

VT2 - available for Gram Negs.
Must add breakpoints in 2.01 PC software.
Breakpoints are included in 3.01 PC software.
FDA Gram-negative breakpoints = 2/4/8.)
2. Inhibitors of Protein Synthesis

Mode of Action - Tetracycline

<table>
<thead>
<tr>
<th>Mode of Action</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracyclines</td>
<td>Irreversibly binds to the 30S ribosomal sub-unit</td>
</tr>
<tr>
<td></td>
<td>Inhibits elongation step of protein synthesis</td>
</tr>
</tbody>
</table>

Tetracycline exists as a mixture of two forms - lipophillic and hydrophillic.

- Helps the antibiotic gain entry into the Gram-pos and neg cell
- Once inside the cell it complexes with Mg++ ions, making the molecule bigger and trapping it inside the bacterial cell
- It can then go on to reach it’s target - the 30s ribosome
- There it inhibits the elongation step of protein synthesis

Gram-positives have no natural resistance to the tetracyclines.

Of the Gram-negative organisms only Proteus mirabilis is naturally resistant.
2. Inhibitors of Protein Synthesis

Aminoglycosides
MLSK
(Macrolides, Lincosamides, Streptogramins, Ketolides)

Tetracyclines
Glycylcyclines

Phenicols

Oxazolidinones
Ansamycins

Inhibitors of Protein Synthesis
Phenicols:

- Bacteriostatic
- Broad spectrum
2. Inhibitors of Protein Synthesis

Phenicols

Chloramphenicol

Inhibitors of Protein Synthesis
Phenicols: Chloramphenicol

Spectrum of Action:

Toxicity: High toxicity, causes bone marrow aplasia and other hematological abnormalities.
## 2. Inhibitors of Protein Synthesis

### Chloramphenicol

<table>
<thead>
<tr>
<th>Mode of Action</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binds to the 50S ribosomal sub-unit</td>
<td>Inhibits elongation step of protein synthesis</td>
</tr>
</tbody>
</table>

- Relatively small molecule, easily enters Gram-positive and Gram-negative bacteria
- Target is Ribosome
- Binds to 50S subunit where it inhibits elongation step of protein synthesis
2. Inhibitors of Protein Synthesis

Aminoglycosides

MLSK
(Macrolides, Lincosamides, Streptogramins, Ketolides)

Tetracyclines
Glycylcyclines
Phenicols
➢ Oxazolidinones
Ansamycins

Inhibitors of Protein Synthesis

Oxazolidinones:

• Bacteriostatic
• Narrow spectrum
2. Inhibitors of Protein Synthesis

Oxazolidinones

Linezolid

Inhibitors of Protein Synthesis
Oxazolidinones: Linezolid

• Spectrum of Action: Gram-positive infections. Effective for *E. faecium* VRE, MRSA and multi-drug resistant *S. pneumoniae*.
• Trade Name = Zyvox®
2. Inhibitors of Protein Synthesis

**Linezolid**

<table>
<thead>
<tr>
<th>MODE OF ACTION</th>
<th>EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linezolid</td>
<td>Binds to the 50S ribosomal sub-unit</td>
</tr>
<tr>
<td></td>
<td>Inhibits initiation process of protein synthesis</td>
</tr>
</tbody>
</table>

- Relatively small molecule, easily enters Gram-positive bacteria
- Target is Ribosome
- Linezolid disrupts bacterial growth by inhibiting the initiation process in protein synthesis
- This site of inhibition occurs earlier in the initiation process than other protein synthesis inhibitors (e.g., chloramphenicol, clindamycin, aminoglycosides, and macrolides) that interfere with the elongation process
- Because the site of inhibition is unique to linezolid, cross-resistance to other protein synthesis inhibitors has not yet been reported
- Linezolid may also inhibit virulence factor expression and decrease toxin production in Gram-positive pathogens
- It has been demonstrated that linezolid is bacteriostatic against *Enterococci* and *Staphylococci*, and bactericidal for the majority of *Streptococci*
- Gram-negative bacteria appear to be **naturally resistant**
2. Inhibitors of Protein Synthesis

Aminoglycosides
MLSK
(Macrolides, Lincosamides, Streptogramins, Ketolides)

Tetracyclines
Glycylcyclines
Phenicols
Oxazolidinones
▶ Ansamycins

Inhibitors of Protein Synthesis

Ansamycins → Rifamycins → Rifampin (Rifamipicin):

• Bacteriostatic or bactericidal – depending on organism and concentration
• Broad spectrum
• Discovered in 1959
• Natural or semi-synthetic derivative of Amycolatopsis rifamycinica (previously Streptomyces mediterranei)
2. Inhibitors of Protein Synthesis

Ansamycins

Rifampin (Rifamipicin)

Inhibitors of Protein Synthesis

Ansamycins → Rifamycins → Rifampin (Rifamipicin):

Spectrum of Action:
- Primarily Gram-positive organisms and some Gram-negatives
- Used in combinations with other drugs to treat tuberculosis
- Used to treat carriers of \textit{N. meningitidis} (prophylaxis)
- Used in combination with other antibiotics for severe \textit{Staphylococcal} infections (including MRSA)
- Oral or IV administration

Mode of Action:
Forms a stable complex with RNA polymerase and prevents DNA from being transcribed into RNA, thus inhibiting protein synthesis
3. Inhibitors of Membrane Function

NEW CLASS

Lipopeptides

Polymyxins
Cyclic Lipopeptides

Inhibitors of Membrane Function

Lipopeptides: (previously Polypeptides)

Polymyxins have been around since 1940’s and 1950’s.

Polymyxin A,B,C,D,E

- Polymyxin B and E can be used therapeutically
- Polymyxin B – derived from Bacillus polymyxa var. aerosporus
- Polymyxin E – derived from Bacillus polymyxa var. colistinus = Colistin

Colistin exists as two forms:
- Colistin sulfate – intestinal infections, topical, powders, media
- Colistimethate sodium – most active, effective form

- All Bactericidal
3. Inhibitors of Membrane Function

Polymyxins

Polymyxin B

Colistin

Inhibitors of Membrane Function

Lipopeptides: Polymyxins

Polymyxin B

Spectrum of Action:

Narrow spectrum for Gram-negative UTI, blood, CSF and eye infections. Can be used in combination against very resistant *Pseudomonas*, KPC.

- High toxicity – neurotoxic and nephrotoxic
- IV, IM and topical for infected wounds

Colistin (Colistimethate Sulfate)

Spectrum of Action:

Narrow spectrum for Gram-negatives, especially *Pseudomonas aeruginosa* infections in cystic fibrosis patients. It has come into recent use for treating multi-drug resistant *Acinetobacter* infections. Ineffective for *Proteus* and *Burkholderia*.

- High toxicity – neurotoxic and nephrotoxic
- PO, IV and topical
3. Inhibitors of Membrane Function

Mode of Action of Polymyxins

Lipopeptides

- **Polymyxins**
  - Polymyxin B
  - Colistin
- Cyclic Lipopeptides
  - Daptomycin
3. Inhibitors of Membrane Function

Polymyxin Mode of Action

Target = Membrane phospholipids (lipopolysaccharides (LPS) and lipoproteins)

1. Outer and Cytoplasmic Membrane Effect:

   Polymyxins are positively charged molecules (cationic) which are attracted to the negatively charged bacteria.

   The negative charge of bacteria is due to LPS in the outer membrane and the peptidoglycan (notably the teichoic acid).

   The antibiotic binds to the cell membrane, alters its structure and makes it more permeable. This disrupts osmotic balance causing leakage of cellular molecules, inhibition of respiration and increased water uptake leading to cell death.

   The antibiotic acts much like a cationic detergent and effects all membranes similarly. Toxic side effects are common.

   Little or no effect onGram-positives since the cell wall is too thick to permit access to the membrane.

   Gram-positives are naturally resistant.
3. Inhibitors of Membrane Function

➢ Cyclic Lipopeptides

**Daptomycin**

Inhibitors of Membrane Function

Lipopeptides: Cyclic Lipopeptide

**Daptomycin** (Cubicin)

- Cubicin from CUBIST Pharmaceutical got FDA approval on September 12, 2003
- Bactericidal
- Resistance rate appears to be low - requires multiple mutations
- Only ‘Sensitive’ CLSI® breakpoints
3. Inhibitors of Membrane Function

Cyclic Lipopeptides

Daptomycin

FDA approval for skin/skin structure infections

- S. aureus (MSSA and MRSA)
- Beta-hemolytic Streptococci (A,B,C,G)
- E. faecalis (Vanco sensitive)

Inhibitors of Membrane Function

Lipopeptides: Cyclic Lipopeptide

Daptomycin (Cubicin)

Spectrum of Action:

Daptomycin: Daptomycin is bactericidal. Active against Gram-positive bacteria (MRSA, MSSA) including those resistant to methicillin, vancomycin and linezolid.

- 1/day IV dosing
- Requires Ca++ in the media

Mode of Action:

Binds to components (calcium ions) of the cell membrane of susceptible organisms and causes rapid depolarization, inhibiting intracellular synthesis of DNA, RNA, and protein.

Gram-negatives appear to be naturally resistant.
4. Anti-Metabolites

(Folate Pathway Inhibitors)

Sulfonamides
Trimethoprim/Sulfamethoxazole

Anti-Metabolites
• Called folate pathway inhibitors or anti-metabolites

Folic acid is essential for the synthesis of adenine and thymine, two of the four nucleic acids that make up our genes, DNA and chromosomes.

Humans do not synthesize folic acid. Good selective target.

Sulfonamides
• Bacteriostatic
• Introduced in 1930’s – first effective systemic antimicrobial agent
• Used for treatment of acute, uncomplicated UTI’s

Trimethoprim/Sulfamethoxazole
• TMP/SXT is bactericidal
• Broad spectrum
• Synergistic action
Anti-Metabolites

Trimethoprim/Sulfamethoxazole:

Spectrum of Action: Prescribed for treatment of certain UTI's, otitis media in children, chronic bronchitis in adults, enteritis and Travelers' Diarrhea.
4. Anti-Metabolites

Mode of Action of Anti-metabolites

[Chemical structures]

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March 2008
4. Anti-Metabolites

Competitive Antagonism

- The drug resembles a microbial substrate and competes with that substrate for the limited microbial enzyme
- The drug ties up the enzyme and blocks a step in metabolism

TMP/SXT Mode of Action
4. Anti-Metabolites

Synthesis of Tetrahydrofolic Acid

Pathway for the Synthesis of Tetrahydrofolic Acid, a cofactor needed for the Synthesis of DNA and RNA

- Sulfonamides such as sulfamethoxazole tie up the enzyme pteridine synthetase while trimethoprim ties up the enzyme dihydrofolic acid reductase. As a result, tetrahydrofolic acid is not produced by the bacterium.

- Sulfonamides, trimethoprim alone and in combination block folic acid essential for the synthesis of adenine and thymidine that make up the DNA, RNA. Therefore, folate pathway inhibitors do not have direct antibiotic activity but the end result is the same, the bacteria is unable to multiply.

- The combination SXT (trimethoprim-sulfamethoxazol) is synergistic and the association provides a bactericidal effect

- Natural Resistance
  
  Enterococcus – low level and poorly expressed
  
  S. pneumoniae
  
  Ps. aeruginosa (impermeability)
5. Inhibitors of Nucleic Acid Synthesis

- Quinolones
- Furanes
5. Inhibitors of Nucleic Acid Synthesis

Quinolones

- Ciprofloxacin
- Levofloxacin
- Gatifloxacin
- Moxifloxacin

Inhibitors of Nucleic Acid Synthesis

Quinolones:
- Humans do synthesize DNA - shared process with bacteria
- Do tend to see some side effects with Quinolones
- Some drugs withdrawn from market quickly
- All are bactericidal
## Inhibitors of Nucleic Acid Synthesis

### Quinolones

<table>
<thead>
<tr>
<th>Quinolones</th>
<th>International Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1st Generation – Narrow Spectrum</strong></td>
<td>Nalidixic Acid</td>
</tr>
<tr>
<td></td>
<td>Cinoxacin</td>
</tr>
<tr>
<td><strong>Fluoroquinolones</strong></td>
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<tr>
<td></td>
<td>Ciprofloxacin</td>
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<td></td>
<td>Enoxacin</td>
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<td>Garenoxacin</td>
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<td>Levofloxacin</td>
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<td>Lomefloxacin</td>
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<td>Norfloxacin</td>
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<td>Sparfloxacin</td>
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<td>Gatifloxacin</td>
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<td></td>
<td>Moxifloxacin</td>
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<tr>
<td></td>
<td>Trovafloxacin</td>
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</tbody>
</table>

**5. Inhibitors of Nucleic Acid Synthesis**

### Quinolones:

- **1st Generation Quinolones**: Only for Gram-negatives, used to treat urinary tract infections because they reach high concentrations in the site of infection.

- **Fluoroquinolones**: Garenoxacin
  
  Gram-negative and Gram-positive coverage including Anaerobes, Atypicals, *S.pneumoniae* and *Pseudomonas*.
  
  In development for VT2 cards, not yet approved.

- **Fluoroquinolones**: Ciprofloxacin, Levofloxacin, Norfloxacin, Ofloxacin
  
  More effective (lower MIC values).
  
  Spectrum extended to cover *Staphylococci*, *Streptococci* and *Pneumococci* (sparfloxacin).
  
  More widespread tissue distribution (they reach the intestine and the lungs).
  
  Ciprofloxacin and Ofloxacin used for systemic infections.

- **Fluoroquinolones**: Sparfloxacin, Gatifloxacin, Moxifloxacin
  
  Trovafloxacin removed from market very quickly after release cardiac arrhythmias, liver destruction, phototoxicity.
  
  Gatifloxacin (Tequin®) removed from market 05/01/06 - diabetes.
5. Inhibitors of Nucleic Acid Synthesis

Mode of Action of Quinolones

[Chemical structures of quinolones]
5. Inhibitors of Nucleic Acid Synthesis

Entry of Quinolones

Quinolone Mode of Action

• Small and hydrophilic, quinolones have no problem crossing the outer membrane.

• They easily diffuse through the peptidoglycan and the cytoplasmic membrane and rapidly reach their target.

• Target = Topoisomerases, e.g., DNA-gyrase

• Rapid bactericidal activity
5. Inhibitors of Nucleic Acid Synthesis

Supercoiling of DNA

- The bacterial chromosome is supercoiled by the enzyme DNA gyrase
- A fully supercoiled chromosome will be about 1 micron in diameter - small enough to fit in the bacteria
- The bacterial chromosome consists of a single circle of DNA
- DNA is double-stranded forming a left-handed double helix
- A typical *E. coli*’s chromosome is 1400 microns long
- The *E. coli*’s bacterial cells are 2-3 microns long
5. Inhibitors of Nucleic Acid Synthesis

Quinolones

- Quinolones inhibit DNA synthesis

- All topoisomerases can relax DNA but only gyrase can also carry out DNA supercoiling (‘supercoiling’ process is necessary to compact the bacterial chromosome which is 1000 times longer than the bacterial cell). Topoisomerases are also involved in DNA replication, transcription and recombination.

- The main quinolone target is the DNA gyrase which is responsible for cutting one of the chromosomal DNA strands at the beginning of the supercoiling process. The nick is only introduced temporarily and later the two ends are joined back together (i.e., repaired).

- The quinolone molecule forms a stable complex with DNA gyrase thereby inhibiting its activity and preventing the repair of DNA cuts

**Natural Resistance**

- Gram Positives – 1\textsuperscript{st} generation quinolones
  - *S.pneumoniae* – decreased activity to Ofloxacin and Ciprofloxacin
  - *Ps. aeruginosa* – decreased activity to Norfloxacin and Ofloxacin
5. Inhibitors of Nucleic Acid Synthesis

Quinolones

- Furanes
5. Inhibitors of Nucleic Acid Synthesis

Furanes

Nitrofurantoin

Nitrofurans: Nitrofurantoin

- Spectrum of Action: Urinary Tract Infections caused by Gram-negative and Gram-positive organisms
- Broad spectrum
- Bactericidal
- Oral

Mode of Action:

The drug works by damaging bacterial DNA. In the bacterial cell, nitrofurantoin is reduced by flavoproteins (nitrofuran reductase). These reduced products are highly active and attack ribosomal proteins, DNA, respiration, pyruvate metabolism and other macromolecules within the cell. It is not known which of the actions of nitrofurantoin is primarily responsible for its bactericidal activity.

Natural Resistance:

Pseudomonas and most Proteus spp. are naturally resistant.
Questions?