VI. Antimicrobial chemotherapy

NOTE: I expect you to understand the **principles** of antimicrobial therapy and the **general characteristics** of the major **antibiotics listed below**.

A. Based on **selective toxicity - toxic to bacteria, not bad for us**
   1. Selective toxicity based on **differences in physiology** between bacteria and us
   2. Measured as **therapeutic index = toxic dose/therapeutic dose (high is good)**
   3. Also consider **allergenicity**

B. Bactericidal vs. bacteriostatic
   1. **bactericidal - kills bacteria (irreversible)**
   2. **bacteriostatic - stops growth (reversible)**
   3. measured in lab as:
      a. **Minimum Bactericidal Concentration (MBC)** - lowest dose for complete killing
      b. **Minimum Inhibitory Concentration (MIC)** - lowest dose for stasis

C. **Pharmacologic absorption and distribution in body**
   1. oral vs. i.v.
   2. penetration to relevant site (e.g., blood brain barrier or inside of host cells)
   3. rate of excretion
   4. rate of metabolism

E. **Broad spectrum vs. narrow spectrum**
   1. broad good for unknown bacterial agent with serious effects
   2. narrow good for known bacterial agent

F. Existing **antibiotic resistance** among the population

G. **combination therapies**
   1. synergistic
   2. additive
   3. antagonistic

H. Other practical considerations for compliancy
   1. **cost**
   2. **frequency and length of administration**
   3. for pediatrics - taste!

I. Summary of key antimicrobials
   1. **peptidoglycan**
      a. **β-lactams inhibit transpeptidation** (similar in structure to D-Ala-D-Ala)
         i. **penicillins** (methicillin, amoxicillin)
         ii. **cephalosporins** (in third generation)
         iii. **carbapenems**
      b. **monobactams** inhibit transpeptidation
      c. **vancomycin** inhibits transpeptidation (recognizes D-Ala-D-Ala, note difference with β-lactams)
      d. **bacitracin** transport of the subunits across membrane
   2. Outer membrane - polymyxins (similar to cationic detergents)

3. **Protein synthesis - act at ribosome**
   a. **aminoglycosides: gentamicin, kanamycin, neomycin, streptomycin**
   b. **aminocyclitols: spectinomycin**
   c. **tetracycline**
   d. **chloramphenicol**
   e. **macrolides: erythromycin**, azithromycin
4. **DNA synthesis**
   a. **quinolones** inhibit gyrase: **nalidixic acid, ciprofloxacin**
   b. **metronidazole** incorporated into DNA after metabolism in anaerobes
5. **RNA synthesis - rifampin** (rifampicin, rifamycin) and its derivatives
6. **metabolic inhibitors - folic acid inhibitors**
   a. **sulfonamides** - PABA analogs block dihydropteroate synthetase
   b. **trimethoprim** blocks dihydrofolate reductase
7. **Inhibitors of antibiotic resistance** function
   a. **clavulanic acid** inhibits β-lactamase
   b. used in conjunction with amoxicillin (**Augmentin**)

VII. Antibiotic resistance

A. Very serious problem since some bacteria are already resistant to all usable antibiotics
B. Proper and rational use of antimicrobials can help with the problem.
   1. indiscriminate prescription and use
   2. agricultural use

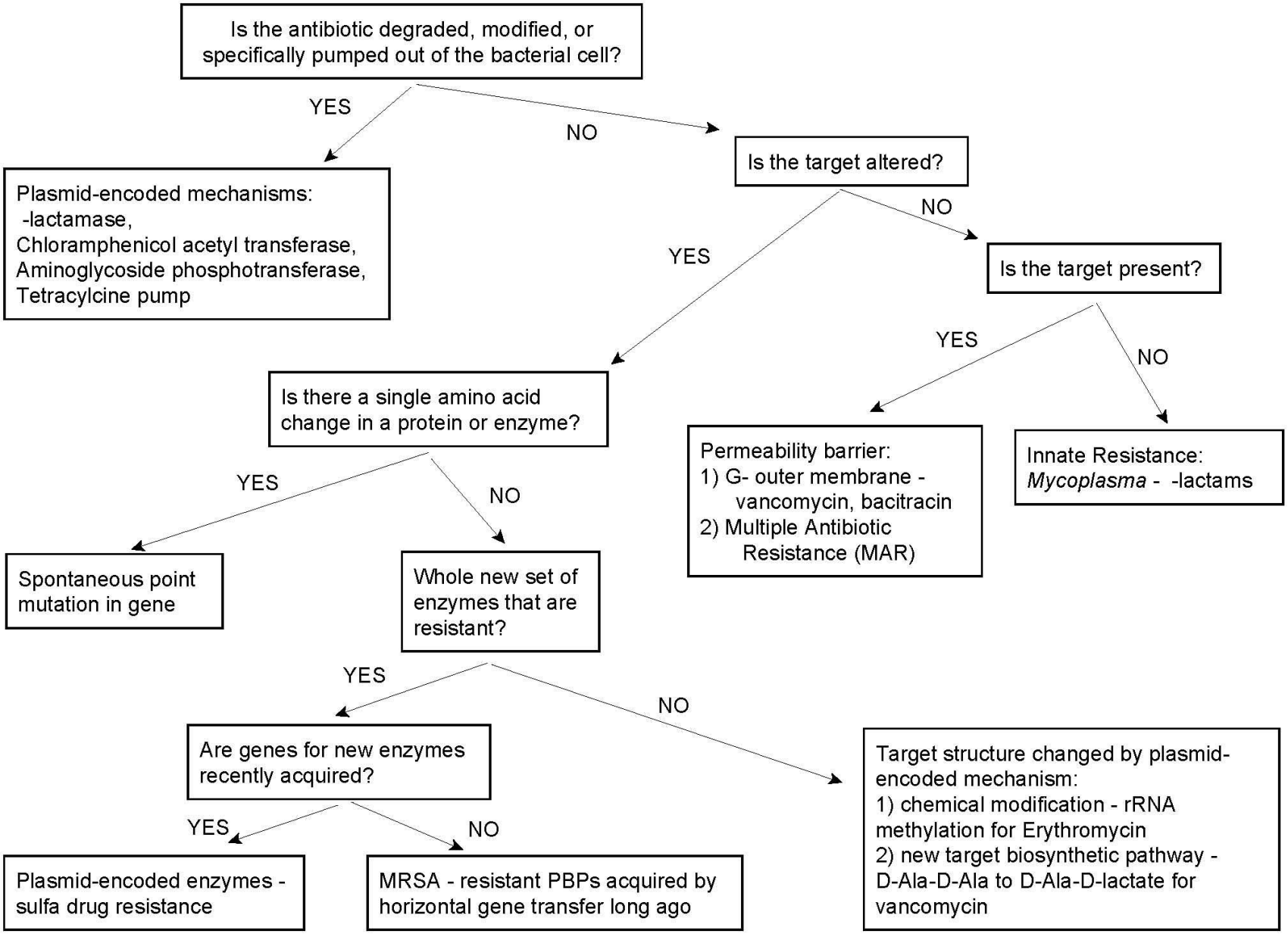
C. General mechanisms of resistance

1. **enzymatically modify or degrade the antibiotic**
   a. most common - **β-lactamase (*bla*)**
   b. chloramphenicol acetyl transferase (*cat*)
   c. aminoglycoside phosphotransferase (*aph*)
2. **alter the target of the antibiotic**
   a. **spontaneous mutation** most common mechanism (retain original function) see *
   b. enzymatic modification
   c. new biosynthetic pathway yielding altered target (vancomycin)
   d. acquire new enzymes
      i. methicillin-resistant *Staphylococcus aureus* - permanent change in genome - penicillin-binding proteins
      ii. plasmid-encoded enzymes - sulfa drugs
3. **change flux of antibiotic**
   a. pump the antibiotic out of the cell (**tetracycline** resistance)
   b. decreased uptake - more specific pores - multiple antibiotic resistance (MAR)
4. **innate resistance**
   - permeability barrier of gram-negatives
   - lack of peptidoglycan in *Mycoplasma*

* from 2.a. (Str - rpsL, Nal - gyrA, Rif - rpoB)

D. Genetics of resistance: plasmid vs. chromosomal

1. If a whole **new enzyme or biosynthetic pathway** is required to degrade the antibiotic or create a resistant pathway, the set of genes is most likely received as a block on an **antibiotic resistance plasmid** (MRSA is an exception)
2. If the **target** has been altered to retain function, but not be inhibited by the antibiotic, a spontaneous **point mutation in the chromosomal gene** could work
3. **vancomycin resistance** is an exception where a plasmid results in the alteration of the target
4. **R plasmids** are the most concern since they can encode **multiple resistances** and they can be **rapidly transferred to diverse bacteria**
5. **spontaneous point mutations** are of less concern because they are less likely to spread among different populations. Instead, the **resistant strain must spread**. (problem in hospital setting)
6. Penicillin resistance of *Streptococcus pneumoniae* is an exception to the usual β-lactamase resistance plasmids - spontaneous mutations accumulating resistance in penicillin-binding proteins


Is the antibiotic degraded, modified, or specifically pumped out of the bacterial cell?
YES
NO
Plasmid-encoded mechanisms:
-lactamase,
Chloramphenicol acetyl transferase,
Aminoglycoside phosphotransferase,
Tetracylcine pump
Is the target altered?
NO
YES
Is the target present?
YES
NO
Is there a single amino acid change in a protein or enzyme?
Permeability barrier:
1) G- outer membrane -
vancomycin, bacitracin
2) Multiple Antibiotic
Resistance (MAR)
Innate Resistance:
Mycoplasma - -lactams
YES
NO
Spontaneous point mutation in gene
Whole new set of enzymes that are resistant?
YES
NO
Are genes for new enzymes recently acquired?
Target structure changed by plasmid-encoded mechanism:
1) chemical modification - rRNA methylation for Erythromycin
2) new target biosynthetic pathway - D-Ala-D-Ala to D-Ala-D-lactate for vancomycin
YES
NO
Plasmid-encoded enzymes - sulfa drug resistance
MRSA - resistant PBPs acquired by horizontal gene transfer long ago

Therapeutic index - toxic dose is toxic for humans - we want a high dose for that. Therapeutic dose is what stops or kills the bacteria - we want a small number there.

MIC/MBC - Because all antibiotics can be measured with an MIC (if they're dead, they won't grow), you usually hear about sensitivity/resistance measured as the MIC, since bacteriostatic antibiotics do not have MBCs.

penetration of antibiotics to relevant site - Some bacteria live within our cells, but some antibiotics don't get inside our cells. These would be ineffective. Some bacteria get to places where some antibiotics have a difficult time getting to (e.g., cerebrospinal fluid across the blood-brain barrier). Therefore, some antibiotics are contraindicated for treating meningitis.

combination therapies - mixing antibiotics is not always beneficial. You will here about cases where mixing antibiotics is essential (e.g., treating tuberculosis). Many antibiotics only work if the bacteria are actively growing. Therefore, if you mix a bacteriostatic antibiotic with a bactericidal antibiotic you could have problems, because the static will stop bacterial growth, but then the cidal will not be effective.

vancomycin - beta-lactams act by mimicking the substrate for transpeptidases and therefore bind to and inhibit the penicillin-binding proteins. Vancomycin is the opposite. It mimics the penicillin-binding proteins and binds to the substrate (D-Ala-D-Ala) and prevents the enzyme from getting to the substrate.

Vancomycin and bacitracin are too big to get through the gram-negative outer membrane porins, so they are ineffective against gram-negatives. Note that if they could get into the periplasm, they would probably be effective.

polymyxins - Because the polymyxins act by binding to membrane components (LPS of gram-negatives) as opposed to completely unique components like peptidoglycan, their specificity (therapeutic index) is not very high.

sulfa drugs and trimpethoprim - remember that we completely lack the target of the sulfa drugs, while we do have the target of trimpethoprim - it is just different enough between bacteria and us that there is a good therapeutic index.

clavulanic acid - Note that this is not really an antibiotic. If you added it to a bacterial culture it would not harm the bacteria. However, if the bacterium expressed beta-lactamase (see next section) and you added clavulanic acid plus a beta-lactam, the bacteria would die because the clavulanic acid would inactivate the resistance mechanism of the bacteria.

Antibiotic resistance - The list of degrading/modifying enzymes is not complete, but should make the point.

Alter the target - spontaneous mutation - I gave the example of DNA gyrase. If a mutation occurs within the *gyrA* gene so that the amino acids recognized by the quinolones is changed, but the gyrase retains its function, then the bacteria will be resistant to quinolones.

Enzymatic modification - A good example is an enzyme that methylates a ribosomal RNA that is targeted by erythromycin.

New biosynthetic pathway - See my figure on the web. Vancomycin binds to D-Ala-D-Ala and prevents penicillin binding proteins from working. With some forms of vancomycin resistance new enzymes are made that change the D-Ala-D-Ala to D-Ala-D-lactate. This modified form still functions in cross linking, but is not bound by vancomycin. This change in the peptidoglycan building block could not have been formed by a single point mutation. There are four or fives new genes involved.

Acquire new enzymes - The methicillin resistant *S. aureus* acquired a block of PBPs that are not affected by methicillin (one of the last effective antibiotics against Staph) a long time ago from an unknown source by genetic exchange. Therefore, a given Staph strain does not change its sensitivity to methicillin. It is either MRSA or not.

For the sulfa drugs and trimpethoprim, some bacteria acquire a new set of folic acid biosynthetic genes on a plasmid.

Decreased uptake - Some gram-negatives can express porins that let less antibiotic in (you can think of them as being tighter).

Genetics of resistance - MRSA is an exception to new enzymes being plasmid-encoded because the “new” resistance PBPs that replaced the “old” sensitive PBPs were acquired a long time ago, and they now reside in the chromosome.

Vancomycin resistance - The target is altered, not by point mutation, but by new enzymes. These enzymes happen to reside on a transposon that resides on a plasmid. You can see why the medical community is worried about vancomycin resistance moving around and ultimately getting into MRSA.

***Streptococcus pneumoniae* beta-lactam resistance** - The major way bacteria become beta-lactam resistant is by plasmid-encoded beta-lactamase. The MRSA became resistant by acquiring new genes by genetic exchange and recombination into the chromosome. You generally do not see point mutations conferring beta-lactam resistance because there are numerous PBPs, and a mutation in one will not confer complete resistance. What is happening with *S. pneumoniae* is that people are not taking their full regimen of beta-lactams for diseases such as otitis media (middle ear infection), hence selective pressure against mutation is not maintained. Therefore, the bacteria are accumulating mutations that will make the beta-lactams useless.