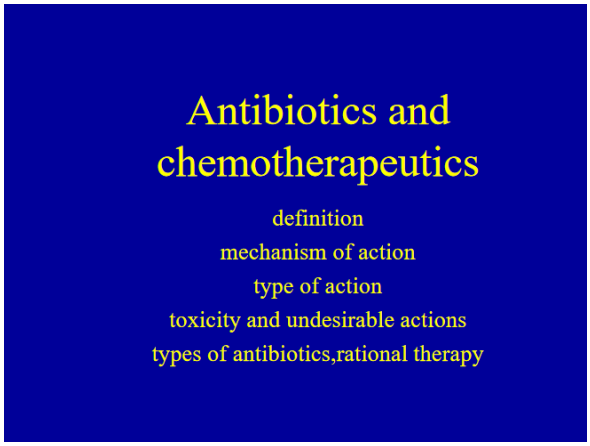
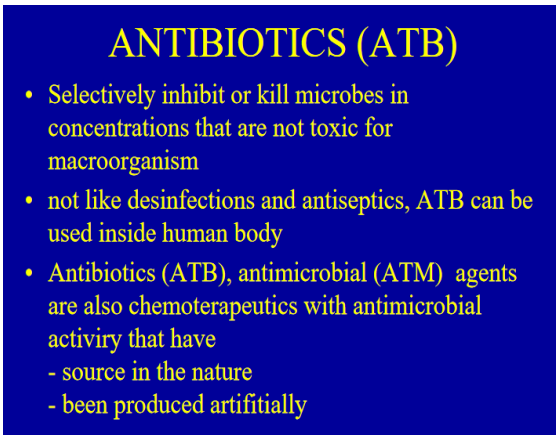
GENERAL MICROBIOLOGY LECTURE 7 and 8

ANTIBIOTICS





ANTIBIOTICS AND CHEMOTHERAPEUTIC AGENTS

The term antibiotic strictly refers to substances that are of biological origin whereas the term chemotherapeutic agent refers to a synthetic chemical. The distinction between these terms has been blurred because many of our newer "antibiotics" are actually chemically modified biological products or even chemically synthesized biological products. The generic terms to refer to either antibiotics or chemotherapeutic agents are antimicrobic or antimicrobial agent. However, the term antibiotic is often used to refer to all types of antimicrobial agents.

MAJOR PRINCIPLES AND DEFINITIONS

SELECTIVITY

Clinically effective antimicrobial agents all exhibit selective toxicity toward the bacterium rather than the host. It is this characteristic that distinguishes antibiotics from disinfectants. The basis for selectivity will vary depending on the particular antibiotic. When selectivity is high the antibiotics are normally not toxic. However, even highly selective antibiotics can have side effects.

THERAPEUTIC INDEX

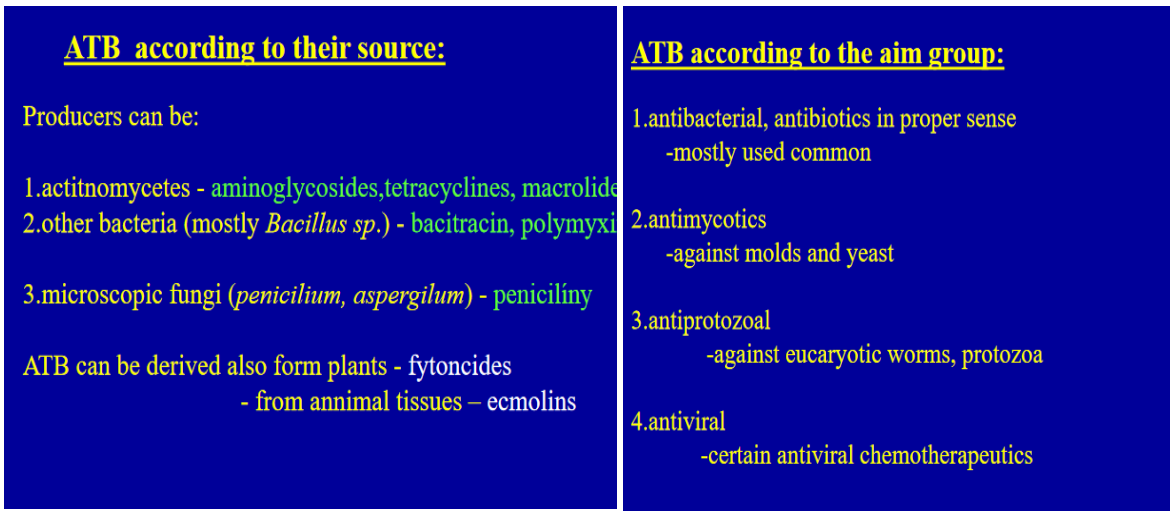
The therapeutic index is defined as the ratio of the dose toxic to the host to the effective therapeutic dose. The higher the therapeutic index the better the antibiotic.

CATEGORIES OF ANTIBIOTICS

Antibiotics are categorized as bactericidal if they kill the susceptible bacteria or bacteriostatic if they reversibly inhibit the growth of bacteria. In general the use of bactericidal antibiotics is preferred but many factors may dictate the use of a bacteriostatic antibiotic. When a bacteriostatic antibiotic is used the duration of therapy must be sufficient to allow cellular and humoral defense mechanisms to eradicate the bacteria. If possible, bactericidal antibiotics should be used to treat infections of the endocardium or the meninges. Host defenses are relatively ineffective at these sites and the dangers imposed by such infections require prompt eradication of the organisms.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The basic quantitative measures of the *in vitro* activity of antibiotics are the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The MIC is the lowest concentration of the antibiotic that results in inhibition of visible growth (*i.e.* colonies on a plate or turbidity in broth culture) under standard conditions. The MBC is the lowest concentration of the antibiotic that kills 99.9% of the original inoculum in a given time. Figure 1 illustrates how to determine the MIC of an antibiotic.



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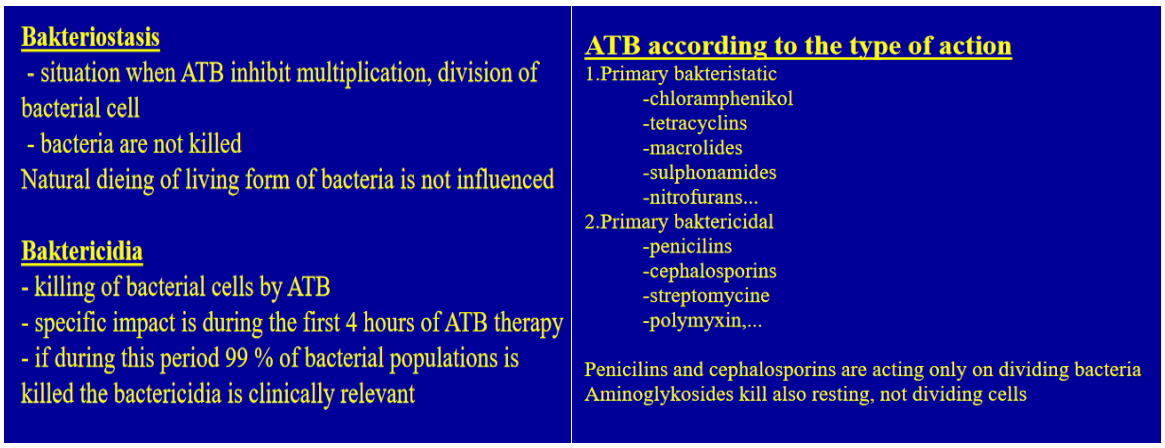

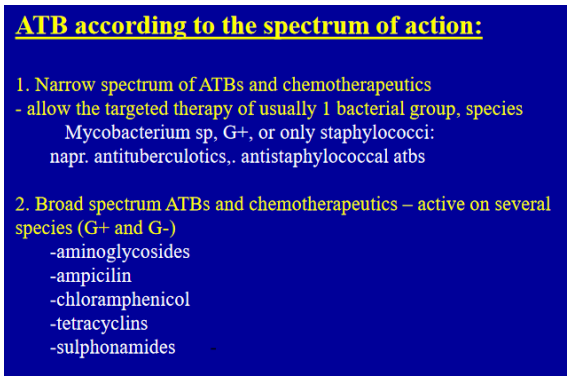

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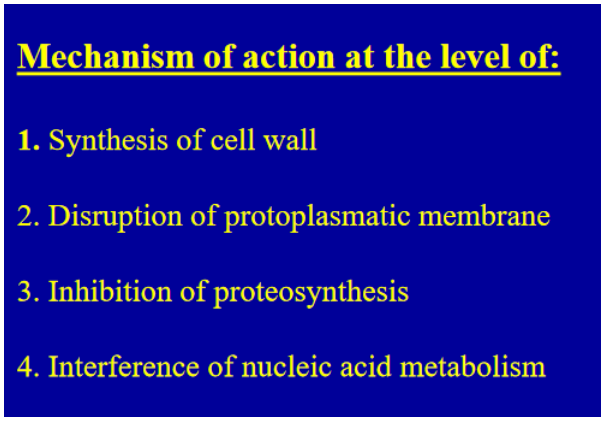

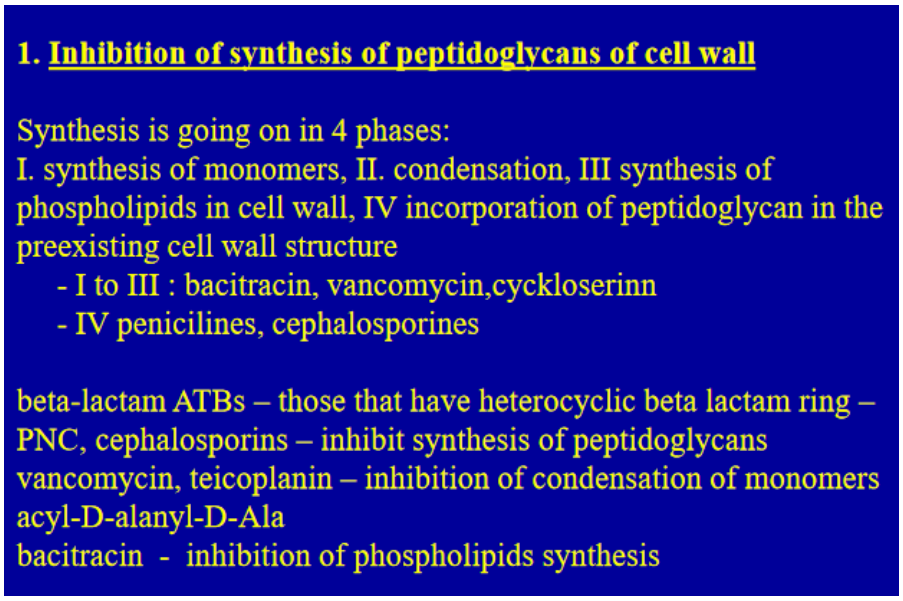

- The peptidoglycan layer is important for cell wall structural integrity, being the outermost and primary component of the wall.
- β-Lactam antibiotics are a broad class of antibiotics that includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.
- β-Lactam antibiotics are bacteriocidal and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls.
- Glycopeptide antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin.
- Glycopeptide antibiotics inhibit the synthesis of cell walls in susceptible microbes by inhibiting peptidoglycan synthesis.

Key Terms

- **beta-lactam antibiotic**: A broad class of antibiotics that inhibit cell wall synthesis, consisting of all antibiotic agents that contains a β-lactam nucleus in their molecular structures. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.
- **Glycopeptide antibiotic**: Glycopeptide antibiotics are composed of glycosylated cyclic or polycyclic nonribosomal peptides. Significant glycopeptide antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin. This class of drugs inhibit the synthesis of cell walls in susceptible microbes by inhibiting peptidoglycan synthesis.
- **peptidoglycan**: A polymer of glycan and peptides found in bacterial cell walls.

Two types of antimicrobial drugs work by inhibiting or **interfering with cell wall synthesis** of the target bacteria. Antibiotics commonly target bacterial cell wall formation (of which peptidoglycan is an important component) because animal cells do not have cell walls. The peptidoglycan layer is important for cell wall structural integrity, being the outermost and primary component of the wall.

The first class of antimicrobial drugs that interfere with cell wall synthesis are the β-Lactam antibiotics (beta-lactam antibiotics), consisting of all antibiotic agents that contains a β-lactam nucleus in their molecular structures. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems. β-Lactam antibiotics are bacteriocidal and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The final step in the synthesis of the peptidoglycan is facilitated by penicillin-binding proteins (PBPs). PBPs vary in their affinity for binding penicillin or other β-lactam antibiotics.

Bacteria often develop resistance to β-lactam antibiotics by synthesizing a β-lactamase, an enzyme that attacks the β-lactam ring. To overcome this resistance, β-lactam antibiotics are often given with β-lactamase inhibitors such as clavulanic acid.

The second class of antimicrobial drugs that interfere with cell wall synthesis are the glycopeptide antibiotics, which are composed of glycosylated cyclic or polycyclic nonribosomal peptides. Significant glycopeptide antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin. This class of drugs inhibit the synthesis of cell walls in susceptible microbes by inhibiting peptidoglycan synthesis. They bind to the amino acids within the cell wall preventing the addition of new units to the peptidoglycan.

SOURCE: https://courses.lumenlearning.com/boundless-microbiology/chapter/functions-of-antimicrobial-drugs/

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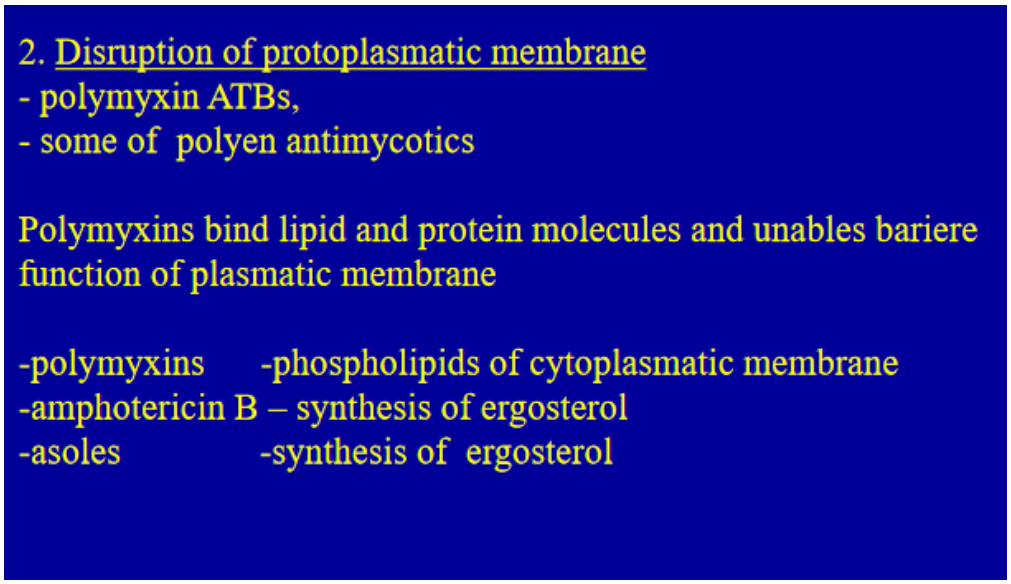

Key Points

- The plasma membrane or cell membrane is a biological membrane that separates the interior of all cells from the outside environment.
- Plasma membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity, and cell signaling. They serve as the attachment surface for several extracellular structures, including the cell wall, glycocalyx, and intracellular cytoskeleton.
- Disrupting the plasma membrane causes rapid depolarization, resulting in a loss of membrane potential leading to inhibition of protein, DNA and RNA synthesis, which results in bacterial cell death.

Key Terms

- **plasma membrane**: The semipermeable membrane that surrounds the cytoplasm of a cell.
- **cell wall**: A thick, fairly rigid layer formed around individual cells of bacteria, Archaea, fungi, plants, and algae, the cell wall is external to the cell membrane and helps the cell maintain its shape and avoid damage.
- **plasma cell**: a form of lymphocyte that produces antibodies when reacted with a specific antigen; a plasmacyte

There are several types of antimicrobial drugs **that function by disrupting or injuring the plasma membrane.** One example is daptomycin, a lipopeptide which has a distinct mechanism of action, disrupting multiple aspects of bacterial cell membrane function. It appears to bind to the membrane causes rapid depolarization, resulting in a loss of membrane potential leading to inhibition of protein, DNA and RNA synthesis, which results in bacterial cell death. Another example is polymyxins antibiotics which have a general structure consisting of a cyclic peptide with a long hydrophobic tail. They disrupt the structure of the bacterial cell membrane by interacting with its phospholipids.

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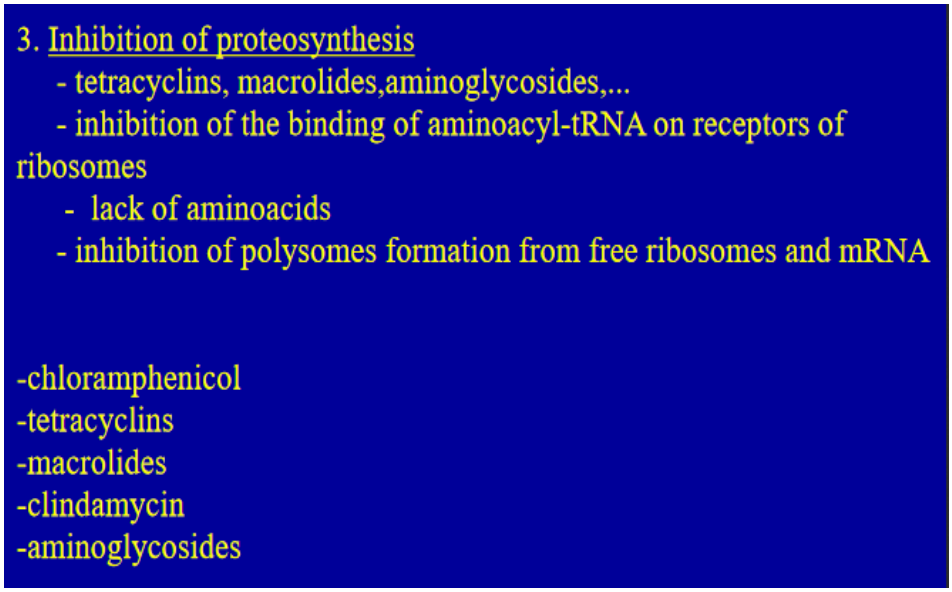


INHIBITORS OF PROTEIN SYNTHESIS

The selectivity of these agents is a result of differences in the prokaryotic 70S ribosome and the 80S eukaryotic ribosome. Since mitochondrial ribosomes are similar to prokaryotic ribosomes, these antimetabolites can have some toxicity. They are mostly bacteriostatic.

ANTIMICROBIALS THAT BIND TO THE 30S RIBOSOMAL SUBUNIT

Aminoglycosides (bactericidal)

Streptomycin, kanamycin, gentamicin, tobramycin, amikacin, netilmicin and neomycin (topical)

a. Mode of action: The aminoglycosides irreversibly bind to the 30S ribosome and freeze the 30S initiation complex (30S-mRNA-tRNA), so that no further initiation can occur. The aminoglycosides also slow down protein synthesis that has already initiated and induce misreading of the mRNA.

b. Spectrum of Activity : Aminoglycosides are active against many gram-negative and some gram-positive bacteria. They are not useful for anaerobic bacteria, since oxygen is required for uptake of the antibiotic, or for intracellular bacteria.

c. Resistance: Resistance to these antibiotics is common

d. Synergy: The aminoglycosides synergize with β-lactam antibiotics such as the penicillins. The β-lactams inhibit cell wall synthesis and thereby increase the permeability of the bacterium to the aminoglycosides.

Tetracyclines (bacteriostatic):

Tetracycline, minocycline and doxycycline

a. Mode of action

The tetracyclines reversibly bind to the 30S ribosome and inhibit binding of aminoacyl-t-RNA to the acceptor site on the 70S ribosome.

b. Spectrum of activity – These are broad spectrum antibiotics and are useful against intracellular bacteria

c. Resistance - Resistance to these antibiotics is common

d. Adverse effects - Destruction of normal intestinal flora often occurs, resulting in increased secondary infections. There can also be staining and impairment of the structure of bone and teeth

Spectinomycin (bacteriostatic)

a. Mode of action - Spectinomycin reversibly interferes with mRNA interaction with the 30S ribosome. It is structurally similar to aminoglycosides but does not cause misreading of mRNA

b. Spectrum of activity - Spectinomycin is used in the treatment of penicillin-resistant *Neisseria gonorrhoeae*

c. Resistance - This is rare in *Neisseria gonorrhoeae*

ANTIMICROBIALS THAT BIND TO THE 50S RIBOSOMAL SUBUNIT

Chloramphenicol, lincomycin, clindamycin (bacteriostatic)

a. Mode of action - These antimicrobials bind to the 50S ribosome and inhibit peptidyl transferase activity.

b. Spectrum of activity - Chloramphenicol - Broad range

Lincomycin and clindamycin - Restricted range

c. Resistance - Resistance to these antibiotics is common

d. Adverse effects - Chloramphenicol is toxic (bone marrow suppression) but it is used in the treatment of bacterial meningitis.

Macrolides (bacteriostatic) - Erythromycin (also azithromycin, clarithromycin)

a. Mode of action - The macrolides inhibit translocation of the peptidyl tRNA from the A to the P site on the ribosome by binding to the 50S ribosomal 23S RNA.

b. Spectrum of activity - Gram-positive bacteria, *Mycoplasma, Legionella*

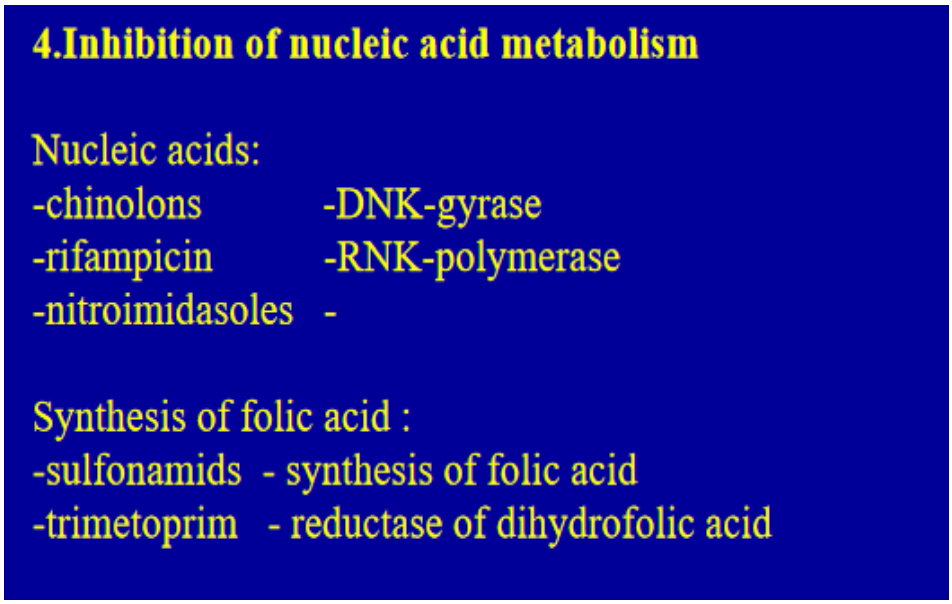
c. Resistance - Resistance to these antibiotics is common. Most gram-negative antibiotics are resistant to macrolides.

ANTIMICROBIALS THAT INTERFERE WITH ELONGATION FACTORS

Fusidic acid (bacteriostatic)

a. Mode of action - Fusidic acid binds to elongation factor G (EF-G) and inhibits release of EF-G from the EF-G/GDP complex.

b. Spectrum of activity - Fusidic acid is only effective against gram-positive bacteria such as *Streptococcus*, *Staphylococcus aureus* and *Corynebacterium minutissimum*.



INHIBITORS OF NUCLEIC ACID SYNTHESIS AND FUNCTION

The selectivity of these agents is a result of differences in prokaryotic and eukaryotic enzymes affected by the antimicrobial agent.

INHIBITORS OF RNA SYNTHESIS AND FUNCTION

Rifampin, rifamycin, rifampicin (bactericidal)

a. Mode of action
These antimicrobials bind to DNA-dependent RNA polymerase and inhibit initiation of RNA synthesis.

b. Spectrum of activity
They are wide spectrum antibiotics but are used most commonly in the treatment of tuberculosis

c. Resistance
Resistance to these antibiotic is common.

d. Combination therapy
Since resistance is common, rifampin is usually used in combination therapy

INHIBITORS OF DNA SYNTHESIS AND FUNCTION

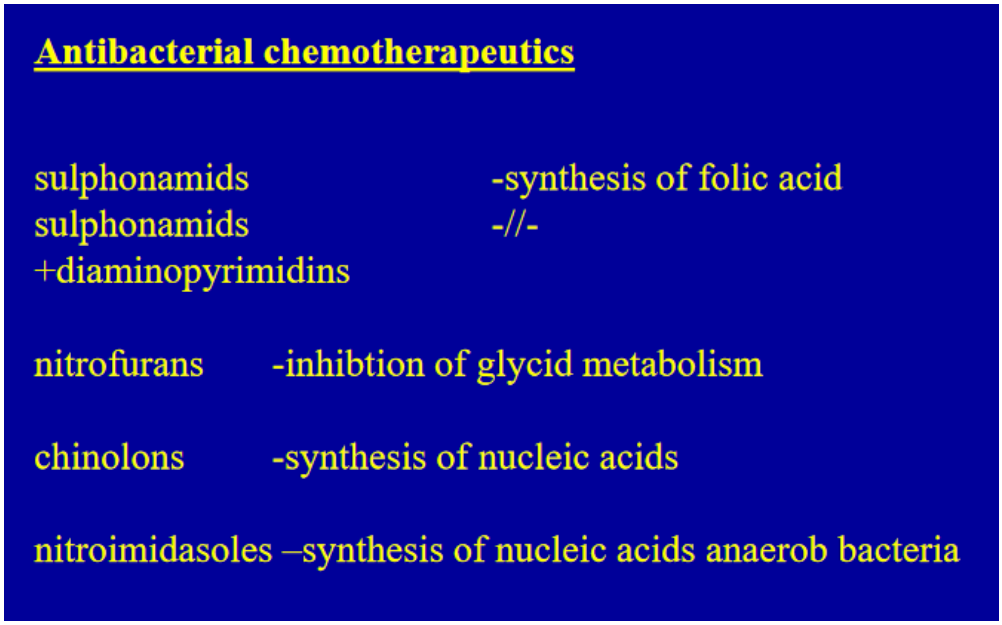
Quinolones - nalidixic acid, ciprofloxacin, oxolinic acid (bactericidal)

a. Mode of action
These antimicrobials bind to the A subunit of DNA gyrase (topoisomerase) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis.

b. Spectrum of activity -
These antibiotics are active against Gram-positive cocci and are used in urinary tract infections

c. Resistance
This is common for nalidixic acid and is developing for ciprofloxacin

ANTIMETABOLITE ANTIMICROBIALS:



INHIBITORS OF FOLIC ACID SYNTHESIS
The selectivity of these antimicrobials is a consequence of the fact that bacteria cannot use pre-formed folic acid and must synthesize their own folic acid. In contrast, mammalian cells use folic acid obtained from food.

Figure 5 summarizes the pathway of folic acid metabolism and indicates the sites at which antimetabolites act.

Sulfonamides, sulfones (bacteriostatic)

a. Mode of action
These antimicrobials are analogues of para-aminobenzoic acid and competitively inhibit formation of dihydropteric acid.

b. Spectrum of activity
They have a broad range activity against gram-positive and gram-negative bacteria and are used primarily in urinary tract infections and in *Nocardia* infections.

c. Resistance
Resistance to these antibiotics is common

d. Combination therapy
The sulfonamides are used in combination with trimethoprim. This combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

Trimethoprim, methotrexate, pyrimethamine (bacteriostatic)

a. Mode of action
These antimicrobials bind to dihydrofolate reductase and inhibit formation of tetrahydrofolic acid.

b. Spectrum of activity
They have a broad range activity against gram-positive and gram-negative bacteria and are used primarily in urinary tract infections and in *Nocardia* infections.

c. Resistance
Resistance to these antibiotics is common

d. Combination therapy
These antimicrobials are used in combination with the sulfonamides. This combination blocks two distinct steps in folic acid metabolism and prevents the emergence of resistant strains.

ANTI-MYCOBACTERIAL AGENTS

Anti-mycobacterial agents are generally used in combination with other antimicrobials since treatment is prolonged and resistance develops readily to individual agents.

Para-aminosalicylic acid (PSA) (bacteriostatic)

a. Mode of action
This is similar to sulfonamides

b. Spectrum of activity
PSA is specific for *Mycobacterium tuberculosis*

Dapsone (bacteriostatic)

a. Mode of action
Similar to sulfonamides

b. Spectrum of activity
Dapsone is used in treatment of leprosy

Isoniazid (INH) (bacteriostatic)

a. Mode of action
Isoniazid inhibit synthesis of mycolic acids.

b. Spectrum of activity -
INH is used in treatment of tuberculosis

c. Resistance
Resistance has developed

Undesirable effect

arising when normal general doses and recomended concentrations of ATBs are applied

Toxic effect

arising when high doses and elevated plasmatic concentration are reached, or are cause by higher reactivity of organisation or unfunctional elimination ways

Undesirable effects

Alergy – usually after sensibilisation caused by very small dose and given in not natural way

- -frequent in PNC
- -look for them in history
- -polymorphic exanthema,eosinofilia,edema,conjunctivitis photodermatoses,anaphylactic shock...
- -dangerouse formes in parenteral application of ATB
- -can arise as early or late, whenever during therapy or after it ended
- -reaction could be caused by other molecules (adjuvants, conservations)

Toxic effects

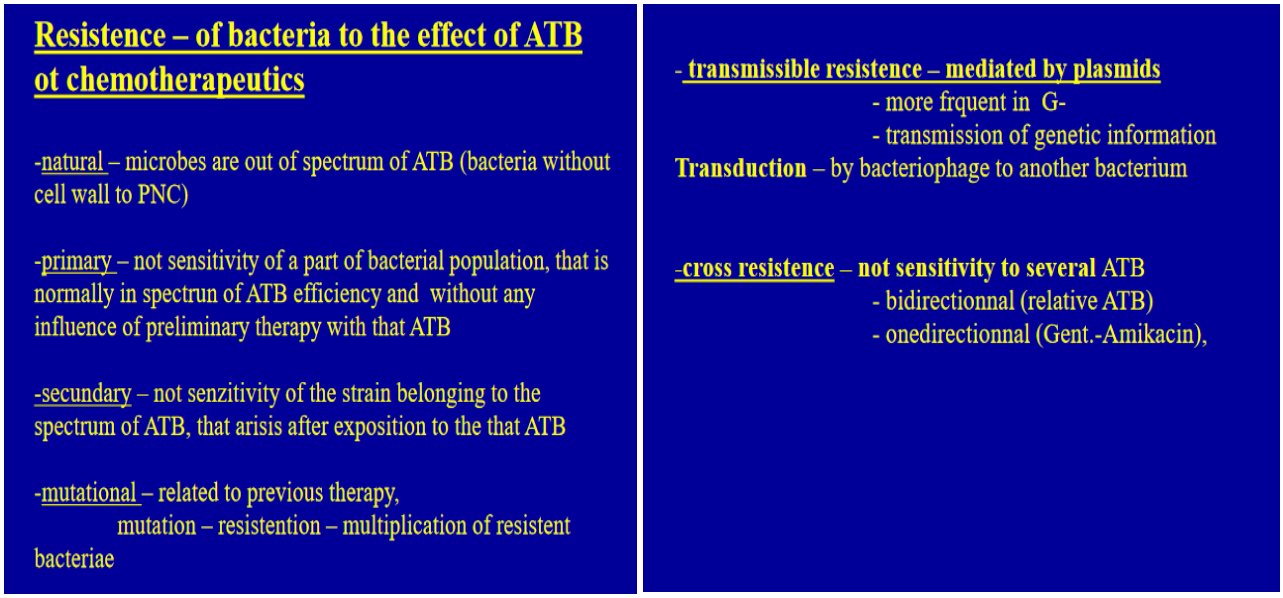
hematotoxic - gancyclovir,chloramphenicol

nefrotoxic - amfotericin B

hepatotoxic - rifampicin, ketokonasol

neurotoxic - nitrofurantoin, gentamycin, izoniazid, streptomycin

ANTIMICROBIAL DRUG RESISTANCE



PRINCIPLES AND DEFINITIONS

Clinical Resistance

Clinical resistance to an antimicrobial agent occurs when the MIC of the drug for a particular strain of bacteria exceeds that which is capable of being achieved with safety *in vivo*. Resistance to an antimicrobial can arise:

- By mutation in the gene that determines sensitivity/resistance to the agent
- By acquisition of extrachromosomal DNA (plasmid) carrying a resistance gene.

Resistance that appears after introduction of an antimicrobial agent into the environment usually results from a selective process, *i.e.* the antibiotic selects for survival of those strains possessing a resistance gene. Resistance can develop in a single step or it can result from the accumulation of multiple mutations.

Cross Resistance

Cross resistance implies that a single mechanism confers resistance to multiple antimicrobial agents while multiple resistance implies that multiple mechanisms are involved. Cross resistance is commonly seen with closely related antimicrobial agents while multiple resistance is seen with unrelated antimicrobial agents.

MECHANISMS OF RESISTANCE

Altered permeability of the antimicrobial agent

Altered permeability may be due to the inability of the antimicrobial agent to enter the bacterial cell or alternatively to the active export of the agent from the cell.

Inactivation of the antimicrobial agent

Resistance is often the result of the production of an enzyme that is capable of inactivating the antimicrobial agent.

Altered target site
Resistance can arise due to alteration of the target site for the antimicrobial agent.

Replacement of a sensitive pathway
Resistance can result from the acquisition of a new enzyme to replace the sensitive one.

SOURCE:

BACTERIOLOGY - CHAPTER SIX ANTIBIOTICS - PROTEIN SYNTHESIS, NUCLEIC ACID SYNTHESIS AND METABOLISM

Available from: http://www.microbiologybook.org/mayer/antibiot.htm