

### ANTIBIOTICS

<p style="text-align: center;"><b>Antibiotics and chemotherapeutics</b></p> <p style="text-align: center;">definition mechanism of action type of action toxicity and undesirable actions types of antibiotics, rational therapy</p>	<p style="text-align: center;"><b>ANTIBIOTICS (ATB)</b></p> <ul style="list-style-type: none"><li>• Selectively inhibit or kill microbes in concentrations that are not toxic for macroorganism</li><li>• not like disinfections and antiseptics, ATB can be used inside human body</li><li>• Antibiotics (ATB), antimicrobial (ATM) agents are also chemotherapeutics with antimicrobial activity that have<ul style="list-style-type: none"><li>- source in the nature</li><li>- been produced artificially</li></ul></li></ul>
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### **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 antimicrobial 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.

<u>ATB according to their source:</u>	<u>ATB according to the aim group:</u>
<p>Producers can be:</p> <ol style="list-style-type: none"><li>1. actinomycetes - aminoglycosides, tetracyclines, macrolides</li><li>2. other bacteria (mostly <i>Bacillus sp.</i>) - bacitracin, polymyxin</li><li>3. microscopic fungi (<i>penicilium, aspergillum</i>) - penicillin</li></ol> <p>ATB can be derived also from plants - phytoncides - from animal tissues – ecmolins</p>	<ol style="list-style-type: none"><li>1. antibacterial, antibiotics in proper sense - mostly used common</li><li>2. antimycotics - against molds and yeast</li><li>3. antiprotozoal - against eucaryotic worms, protozoa</li><li>4. antiviral - certain antiviral chemotherapeutics</li></ol>

## SELECTIVITY

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<u>Bakteriostasis</u>	<u>ATB according to the type of action</u>
<ul style="list-style-type: none"><li>- situation when ATB inhibit multiplication, division of bacterial cell</li><li>- bacteria are not killed</li></ul> <p>Natural dieing of living form of bacteria is not influenced</p>	<ol style="list-style-type: none"><li>1. Primary bakteriostatic<ul style="list-style-type: none"><li>- chloramphenicol</li><li>- tetracyclins</li><li>- macrolides</li><li>- sulphonamides</li><li>- nitrofurans...</li></ul></li><li>2. Primary baktericidal<ul style="list-style-type: none"><li>- penicilins</li><li>- cephalosporins</li><li>- streptomycine</li><li>- polymyxin,...</li></ul></li></ol>
<p><u>Baktericidia</u></p> <ul style="list-style-type: none"><li>- killing of bacterial cells by ATB</li><li>- specific impact is during the first 4 hours of ATB therapy</li><li>- if during this period 99 % of bacterial populations is killed the baktericidia is clinically relevant</li></ul>	<p>Penicilins and cephalosporins are acting only on dividing bacteria Aminoglykosides kill also resting, not dividing cells</p>

### ATB according to the spectrum of action:

1. Narrow spectrum of ATBs and chemotherapeutics  
- allow the targeted therapy of usually 1 bacterial group, species  
Mycobacterium sp, G+, or only staphylococci:  
napr. antituberculosics, antistaphylococcal atbs
2. Broad spectrum ATBs and chemotherapeutics – active on several species (G+ and G-)
  - aminoglycosides
  - ampicilin
  - chloramphenicol
  - tetracyclins
  - sulphonamides

## CATEGORIES OF ANTIBIOTICS

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### Mechanism of action at the level of:

1. Synthesis of cell wall
2. Disruption of protoplasmatic membrane
3. Inhibition of proteosynthesis
4. Interference of nucleic acid metabolism

### 1. Inhibition of synthesis of peptidoglycans of cell wall

Synthesis is going on in 4 phases:

I. synthesis of monomers, II. condensation, III synthesis of phospholipids in cell wall, IV incorporation of peptidoglycan in the preexisting cell wall structure

- I to III : bacitracin, vancomycin, cycloserin
- IV penicilines, cephalosporines

beta-lactam ATBs – those that have heterocyclic beta lactam ring – PNC, cephalosporins – inhibit synthesis of peptidoglycans  
vancomycin, teicoplanin – inhibition of condensation of monomers acyl-D-alanyl-D-Ala  
bacitracin - inhibition of phospholipids synthesis

- The peptidoglycan layer is important for cell wall structural integrity, being the outermost and primary component of the wall.
- $\beta$ -Lactam antibiotics are a broad class of antibiotics that includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.
- $\beta$ -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  $\beta$ -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  $\beta$ -Lactam antibiotics (beta-lactam antibiotics), consisting of all antibiotic agents that contains a  $\beta$ -lactam nucleus in their molecular structures. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.  $\beta$ -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  $\beta$ -lactam antibiotics.

Bacteria often develop resistance to  $\beta$ -lactam antibiotics by synthesizing a  $\beta$ -lactamase, an enzyme that attacks the  $\beta$ -lactam ring. To overcome this resistance,  $\beta$ -lactam antibiotics are often given with  $\beta$ -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/>

## 2. Disruption of protoplasmatic membrane

- polymyxin ATBs,
- some of polyen antimycotics

Polymyxins bind lipid and protein molecules and unables bariere function of plasmatic membrane

- polymyxins      -phospholipids of cytoplasmatic membrane
- amphotericin B – synthesis of ergosterol
- asoles            -synthesis of ergosterol

### 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.

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

### 3. Inhibition of proteosynthesis

- tetracyclins, macrolides, aminoglycosides,...
- inhibition of the binding of aminoacyl-tRNA on receptors of ribosomes
  - lack of aminoacids
  - inhibition of polysomes formation from free ribosomes and mRNA

- chloramphenicol
- tetracyclins
- macrolides
- clindamycin
- aminoglycosides

## **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  $\beta$ -lactam antibiotics such as the penicillins. The  $\beta$ -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*.

## 4. Inhibition of nucleic acid metabolism

### Nucleic acids:

- chinolons            -DNK-gyrase
- rifampicin        -RNK-polymerase
- nitroimidazoles -

### Synthesis of folic acid :

- sulfonamids - synthesis of folic acid
- trimetoprim - reductase of dihydrofolic acid

## 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:

### Antibacterial chemotherapeutics

sulphonamids                      -synthesis of folic acid

sulphonamids                      -//-

+diaminopyrimidins

nitrofurans                      -inhibition of glycid metabolism

chinolons                      -synthesis of nucleic acids

nitroimidazoles –synthesis of nucleic acids anaerob bacteria

## 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 dihydropteridic 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**

**Antitubercotics:** bakteriostatic  
toxic  
only for TBC  
rifampicin  
INH, ETM, PYR, PAS

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 recommended concentrations of ATBs are applied

### Toxic effect

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

### Undesirable effects

Allergy – usually after sensitisation caused by very small dose and given in not natural way

-frequent in PNC

-look for them in history

-polymorphic exanthema, eosinophilia, edema, conjunctivitis, photodermatoses, anaphylactic shock...

-dangerous forms 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

nephrotoxic - amphotericin B

hepatotoxic - rifampicin, ketoconazole

neurotoxic - nitrofurantoin, gentamicin, isoniazid, streptomycin

## ANTIMICROBIAL DRUG RESISTANCE

### Resistance – of bacteria to the effect of ATB of chemotherapeutics

-natural – microbes are out of spectrum of ATB (bacteria without cell wall to PNC)

-primary – not sensitivity of a part of bacterial population, that is normally in spectrum of ATB efficiency and without any influence of preliminary therapy with that ATB

-secondary – not sensitivity of the strain belonging to the spectrum of ATB, that arises after exposition to the that ATB

-mutational – related to previous therapy, mutation – resistance – multiplication of resistant bacteria

- transmissible resistance – mediated by plasmids

- more frequent in G-

- transmission of genetic information

**Transduction** – by bacteriophage to another bacterium

- cross resistance – not sensitivity to several ATB

- bidirectional (relative ATB)

- unidirectional (Gent.-Amikacin),

## 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>