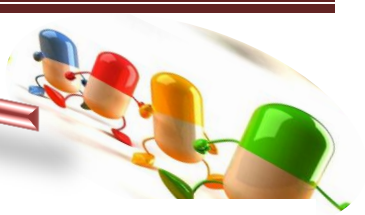
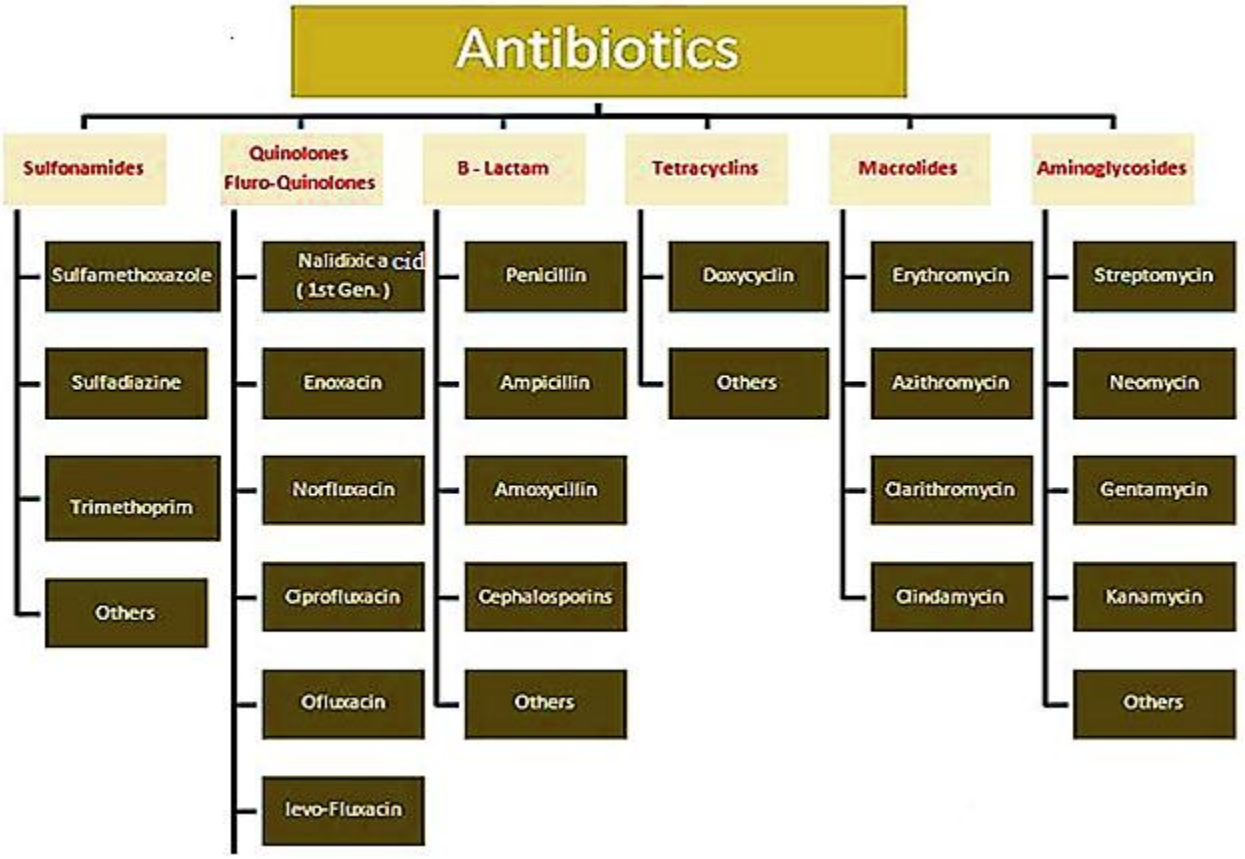


Classification of Antibiotics



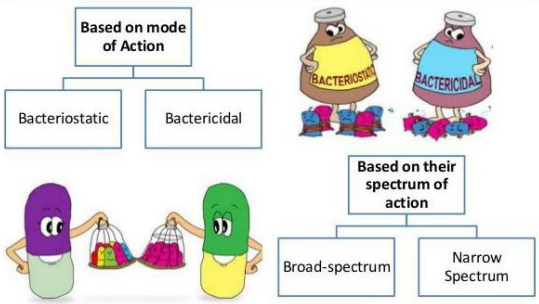
Antibiotic is a chemical substance produced by a *microorganism* that inhibits the growth or kills other microorganisms.

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 Minimal Inhibitory Concentration (MIC) refers to the lowest concentration of an antibiotic that stops visible growth. More simply, the zone of inhibition around a disk impregnated with antibiotic.

-The effect of antimicrobial activity on microorganism:

- **Bactericidal:** killing the bacteria.
- **Bacteriostatic :** inhibit the bacteria growth .

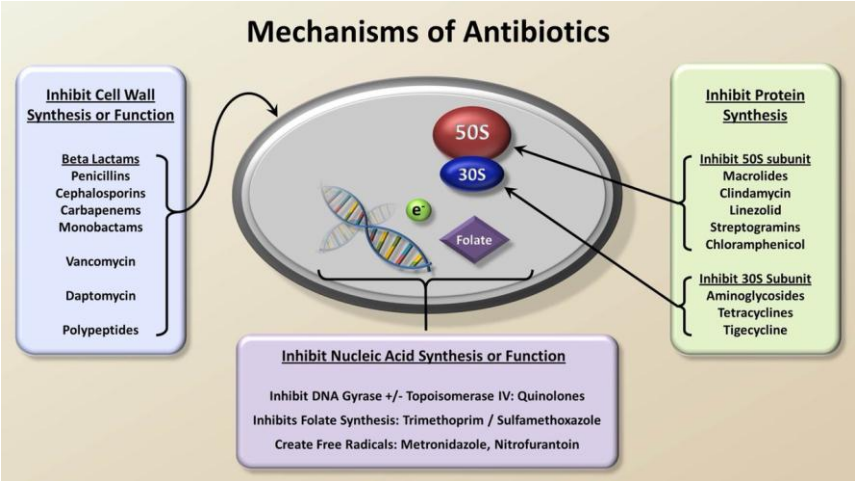


-Selective Toxicity

Antimicrobial agent exhibits selective toxicity, which means that the drug is harmful to a pathogen without being harmful to the host. Often, selective toxicity is relative rather than absolute; **this implies that a drug in a concentration tolerated by the host may damage an infecting microorganism. Selective toxicity may be a function of a specific receptor required for drug attachment, or it may depend on the inhibition of biochemical events essential to the pathogen but not to the host**.

❖ **The mechanisms of action of antimicrobial drugs can be discussed under four headings:**

1. Inhibition of cell wall synthesis
2. Inhibition of cell membrane function
3. Inhibition of protein synthesis (i.e, inhibition of translation and transcription of genetic material)
4. Inhibition of nucleic acid synthesis



1. Inhibition of cell wall synthesis

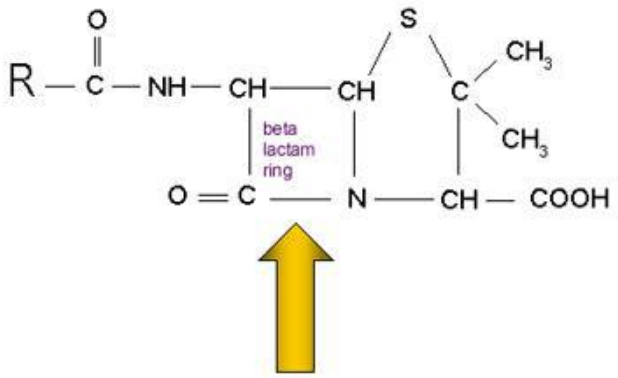
One major class of antibiotics inhibits the synthesis of **peptidoglycan**. Once cell wall synthesis is inhibited, enzymatic autolysis of the cell wall can occur. Without the restraining influence of the cell wall the high osmotic pressure inside the cell bursts

the inner and/or outer membranes of bacteria. All **β-lactam** drugs are inhibitors of bacterial cell wall synthesis and therefore active against growing bacteria.

The initial step in drug action consists of binding of the drug to cell receptors **(penicillin-binding proteins [PBPs])** After a β -lactam drug has attached to one or more receptors, the transpeptidation reaction is inhibited, and peptidoglycan synthesis is blocked. The **next step** probably involves removal or inactivation of an inhibitor of autolytic enzymes in the cell wall. This activates the lytic enzyme and results in lysis.Thus, these antibiotics are generally **bactericidal**.

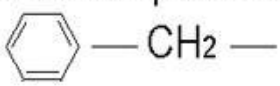
Penicillin

Penicillin is made by the mold *Penicillium chrysogenum*. During fermentation the mold forms 6-aminopenicillanic acid which has a thiazolidine ring and a beta-lactam ring fused together. Many penicillin's display little activity against Gram negative bacteria? since they **do not penetrate the outer membrane**.



Site of penicillinase action (break in β lactam ring)

R = side chain . In natural penicillins this is



Cephalosporins : Are active against some **G ve+** bacteria have the same mode of action as other β -lactam antibiotic (pencillins) but are less susceptible to β -lactamase .

Cephalosporins and other newer penicillins are active against **G ve-** bacteria, since they can penetrate the outer membrane

Penicillin can be destroyed by **beta lactamase** (penicillinase) produced by resistant bacterial strains. **Clavulinic acid**, also has a beta lactam component which binds strongly to beta lactamases inhibiting their activity. It is used in conjunction with certain penicillins allowing their use against otherwise resistant bacteria.

Polymyxin B

Polymyxin B binds to the **lipid A** portion of **lipopolysaccharide** and also to phospholipids. However, it binds preferentially to lipid A. This disrupts the **outer membrane** of Gram negative bacteria. In Gram positive bacteria polymyxin has little activity against them? This drug is toxic to human cells, since it can also lyse eukaryotic membranes; this explains its limited clinical use.

Vancomycin

Vancomycin is a drug of last resort against Gram-positive bacteria. It is a glycopeptide made by an *Acinobacter* species.

It is very hydrophilic and forms hydrogen binds with terminal **D-alanyl-D-alanine** moieties of the **NAM/NAG-subunits** and stops polymerization of the subunits to form long chains, to form finally **peptidoglycan**.

Bacitracin

Bacitracin is a cyclic polypeptide produced by ***Bacillus subtilis*** . Bacitracin inhibits **dephosphorylation** of **C55-isoprenyl pyrophosphate** which transports **peptidoglycan** components bacterial cell walls outside the inner membrane.

2.Protein Synthesis Inhibitors

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

A. *Antimicrobials That Bind To The 30s Ribosomal Subunit*

❖ **Aminoglycosides** (bactericidal)

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 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.

❖ **Tetracyclines** :bacteriostatic

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.

❖ **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*

B. 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.**

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.

b. Spectrum of activity

Gram-positive bacteria, Mycoplasma, Legionella

C.*Antimicrobials That Interfere With Elongation Factors*

Fusidic acid (bacteriostatic)

a. Mode of action

Fusidic acid binds to **protein 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.*

3.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.

A.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. 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 .

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.

- **Mechanisms of Resistance :**

1- 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.

2- Inactivation of the antimicrobial agent

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

3- Altered target site

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

4- Replacement of a sensitive pathway

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

Classification of Bacteria

1- Growth on Media:

One criterion is growth on bacteriologic media. In contrast to viruses and most parasites, many bacterial pathogens can be isolated on solid agar-containing media. The general cultivation of most bacteria requires media rich in metabolic nutrients. These media generally include agar, a carbon source, and an acid hydrolysate or enzymatically degraded source of biologic material (eg, casein). Because of the undefined composition of the latter, these types of media are referred to as **complex**

media. Media can be **nonselective** or **selective**; the latter are used to distinguish among the various bacteria in a clinical sample containing many different organisms.

A. Nonselective Media:

Blood agar and chocolate agar are examples of complex, nonselective media, which support the growth of many different bacteria. These media are intended to cultivate as many species as possible, thus giving rise to numerous types of bacterial colonies.

B. Selective Media

Because of the diversity of microorganisms that typically reside at some sampling sites (eg, the skin, respiratory tract, intestines, vagina), selective media are used to eliminate (or reduce) the large numbers of irrelevant bacteria in these specimens. The basis for selective media is the incorporation of an inhibitory agent that specifically selects against the growth of irrelevant bacteria.

Examples of such agents are:

• Sodium azide—selects for gram-positive bacteria over gram-negative bacteria

• Bile salts (sodium deoxycholate)—select for gram-negative enteric bacteria and inhibit gram-negative

mucosal and most gram-positive bacteria

• Colistin and nalidixic acid—inhibit the growth of many gram-negative bacteria

Examples of selective media are MacConkey agar (contains bile) that selects for the Enterobacteriaceae and CNA blood agar (contains colistin and nalidixic acid) that selects for Staphylococci and Streptococci.

C. Differential Media:

Upon culture, some bacteria produce characteristic pigments, and others can be differentiated on the basis of their complement of extracellular enzymes; the activity of these enzymes often can be detected as zones of clearing surrounding colonies grown in the presence of insoluble substrates (eg, zones of **hemolysis in agar medium containing red blood cells).**

Many of the members of the Enterobacteriaceae can be differentiated on the basis of their ability to metabolize lactose. For example, whereas pathogenic salmonellae and shigellae do not ferment lactose on a MacConkey plate form white colonies, lactose-fermenting members of the
Enterobacteriaceae (eg, *E coli) form red or pink colonies.*

2- Microscopy

Historically, the Gram stain, together with visualization by light microscopy, has been among the most informative methods for classifying the eubacteria. This staining technique broadly divides bacteria on the basis of fundamental differences in the structure of their cell walls . This typically represents the first step in identifying individual microbial specimens (eg, are they gram negative or gram positive) grown in culture or even directly from patient specimens (eg, urine specimens).

3- Biochemical Tests

Tests such as the **oxidase test, which uses an artificial electron** acceptor, can be used to distinguish organisms on the basis of the presence or absence of a respiratory enzyme, cytochrome C, the lack of which differentiates the Enterobacteriaceae from other gram-negative rods. Similarly, **catalase activity can be** used, for example, to differentiate between the gram-positive cocci; the species staphylococci are catalase positive whereas the species streptococci are catalase negative. If the organism is demonstrated to be catalase positive (*Staphylococcus spp.), the*
species can be subdivided by a coagulase test into *Staphylococcus aureus (coagulase positive) or Staphylococcus epidermitidis* (coagulase negative).

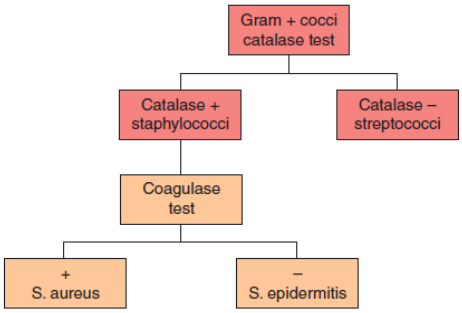


FIGURE Algorithm for differentiating the gram-positive cocci.

4- Immunologic Tests -- Tests—Serotypes, Serogroups :

The designation "sero" simply indicates the use of antibodies that react with specific bacterial cell surface structures such as lipopolysaccharide (LPS), flagella, or capsular antigens.

Numerical Taxonomy :

The Analytical Profile Index (APIs) is a method commonly used to identify a wide range of microorganisms. APIs consist of a number of plastic strips, each of which has about 20 miniature compartments containing biochemical reagents. Almost all cultivatable bacterial groups and more than 550 different species can be identified using the results of these API tests.

FIGURE API™ test demonstrating how bacteria can be differentiated using a series of biochemical tests. Each small compartment contains a dehydrated powder that can be inoculated from a bacterial culture. After incubation, the colorimetric changes can be scored numerically to produce a number that matches to a specific bacterial species and genus. (Courtesy of bioMerieux, Inc.)