

## Chapter 10 Antibiotics and chemotherapeutic agents

### I. Definition of an antibiotic

- A. The ideal antimicrobial agent should be nontoxic to the host (selective toxicity), non-allergenic, soluble in body fluids, able to be maintained at therapeutic levels, have a low probability of eliciting resistance, long shelf life, and low cost.

Antibiotic--originally used to denote a chemical substance produced by one microorganism that kills or inhibits the growth of other microbes, the term now applies to both naturally produced substances and those synthesized in the laboratory. Most are produced by either fungi (e.g., penicillin, cephalosporins), *Bacillus* species (e.g., polymyxin, bacitracin), or *Streptomyces* species (streptomycin, tetracycline, erythromycin, kanamycin, neomycin, nystatin). Broad-spectrum antibiotics are those that act on both gram-positive and gram-negative bacteria.

Chemotherapeutic agent (drug)-any chemical (natural or synthetic) that is used in medicine. Ideally, it should attack microorganisms selectively and not harm human cells.

### B. Types and examples of antibiotics

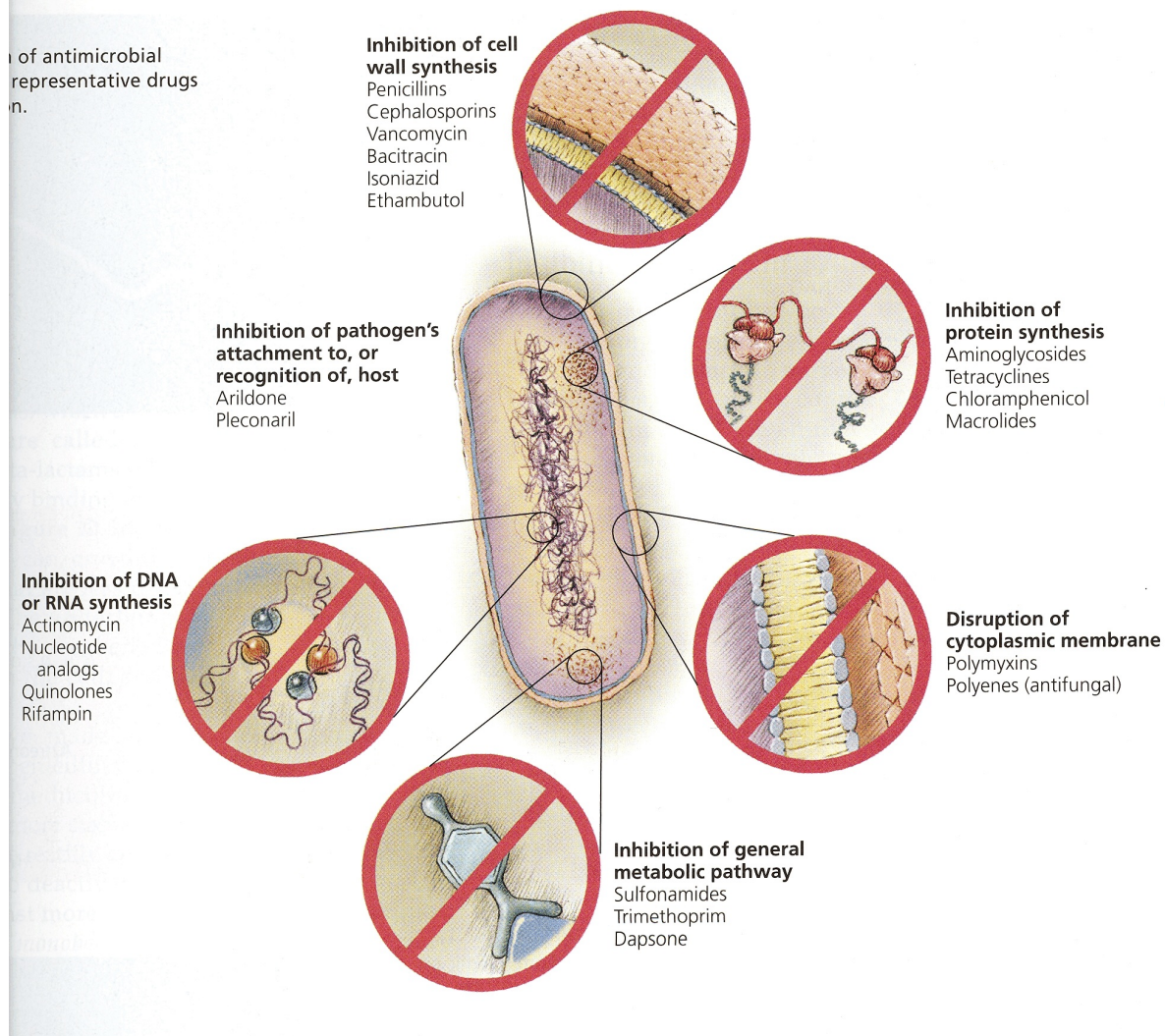
#### 1. Sources

- a. Natural drug-one made by microorganisms.
- b. Synthetic drugs that is made in the laboratory. Semisynthetic drug-one synthesized partly in the laboratory and partly by microorganisms.

#### c. Spectrum of activity

##### **Antibiotics may either be broad or narrow spectrum**

Each antimicrobial drug has a range of microorganisms that it affects. This range is the spectrum of activity of the antimicrobial drug. The spectrum can be narrow (affecting a small number of microorganisms) or broad (affecting a large range of microorganisms). Penicillin has a narrow spectrum of activity and affects only gram-positive, and a few of the gram-negative, bacteria. Tetracycline has a broad spectrum of activity and affects gram-negative and gram-positive bacteria, and rickettsias.



## II. Antimicrobial agents Modes of action

A. Microbes are killed through various means:

### 1. Inhibition of nucleic acid synthesis (DNA/RNA)

a. Narrow spectrum of activity (DNA or RNA synthesis)

1. Antibiotics known as (amino glycosides, streptomycin, neomycin, netilmicin, tobramycin, gentamicin, amikacin, etc.) bind to the 30S subunit of bacterial ribosomes and block the attachment of the 50S subunit to the initiation complex

**Remember** that eukaryotic ribosomes subunits differ from prokaryotic ribosomes subunits in that they are made up of a 60s and 40s subunit to make an 80s ribosome.

2. trimethoprim, imidazoles and quinolones, rifamycin interfere with bacterial DNA  
Novobiocin is a narrow-spectrum antibiotic that may be bacteriostatic or bactericidal at higher concentrations. It is active mostly against gram-positive bacteria but also against a few gram-negative bacteria. There is a synergistic effect with tetracyclines. Many species of bacteria can develop resistance to novobiocin.

The mechanism of action of novobiocin is the inhibition of deoxyribonucleic acid (DNA) synthesis. The inhibition of nucleic acid synthesis (topoisomerase II inhibitors) was accompanied by an approximately threefold accumulation of all eight nucleoside triphosphates. Dosage: Novobiocin (2% or 5%) in methylcellulose were applied twice daily

2. **Inhibition of Cell wall formation** (*Bacitracin, vancomycin, cephalosporins, and penicillin*), Sulfa drugs-bacteriostatic; active upon vegetative cells resulting in cell lysis due to effects of osmotic pressures.

- a. Mode of action

1. Beta-lactam:

Interference of cross-linkage of NAM subunits of bacterial cell wall subjecting microbes to environmental osmotic pressures.

- a. Beta-lactams ring found in *penicillin* and *cephalosporins* inhibit peptidoglycan formation by irreversibly binding to the enzymes that cross-link NAM subunits.

2. Vancomycin interferes with alanine-alanine cross-link bridges between NAM subunits in many Gram-positive bacteria

3. Bacitracin blocks the secretion of NAG and NAM from cytoplasm.

3. **Inhibition of Protein synthesis**

- a. **Macrolides** (*erythromycin, azithromycin, clarithromycin, dirithromycin, troleandomycin, etc.*) bind reversibly to the 50S subunit. They can inhibit elongation of the protein by the peptidyltransferase, the enzyme that forms peptide bonds between the amino acids.

- b. **Aminoglycosides** (*tetracycline*) blocks bacterial translation by binding reversibly to the 30S subunit and distorting it in such a way that the anticodons of the charged tRNAs cannot align properly with the codons of the mRNA

- c. **Antisense nucleic acids** (*fomiversen*) designed as either single strand RNA or DNA to fit with complementary nucleic acid of pathogen with no effect on human cells.
- 4. **Damaging the plasma membrane** (*Nystatin, amphotericin B, miconazole* and *ketoconazole*)
  - a. Polyenes drugs incorporate into cell membranes causing membrane integrity damage (Pore formation) resulting cellular lysis.
    - 1) Amphotericin B side effects in humans
      - a) binds to cholesterol which is similar to fungal ergosterol
  - b. Azoles (*fluconazole*) and Allylamines (*terbinafine*) antifungal drugs
    - 1) Inhibits the synthesis of ergosterol which is necessary for fungal membrane structure (much like cholesterol is necessary for human membrane structure)
- 5. **Inhibition of general metabolic pathway** (*atovaquone, trimethoprim, amantadine, rimantadine*)
  - a. *Atovaquone* interferes with electron transport system of protozoa and fungi
  - b. Heavy metals such as arsenic, mercury, antimony inactivates enzymes
    - 1) disrupting tubulin polymerization and glucose uptake.
  - c. Sulfonamides and dapsone which act as structural analogs of para-aminobenzoic acid (PABA) inhibit the synthesis of folic acid in many microbes.
    - 1) PABA is precursor for the synthesis of DNA and RNA
    - 2) Sulfonamide compete with PABA molecules for the active site of the enzyme involved in the production dihydrofolic acid.
  - d. Trimethoprim binds to enzyme that converts dihydrofolic acid into tetrahydrofolic acid a precursor for the synthesis of purine and pyrimidine nucleotides
- 6. **Inhibition of pathogen's attachment to or recognition of the host**
  - a. Antiviral agents target host receptors interfering with binding of virus to host cell
  - b. *Amantadine* and *rimantadine* act to neutralize the acid of phagolysosome within a macrophage white blood cell preventing viral uncoating.

### III. Antibiotic Efficacy (How well does the antimicrobial agent work?)

#### A. Diffusion Susceptibility Test

##### 1. Kirby-Bauer test

- a. Petri disk inoculate with standardized amount of pathogen in question
- b. Small uniform disks containing antimicrobial agents arranged on the surface of the Petri disk
- c. After a specific incubation period zone of induction (clearing) measured around each disk
- d. Zone of inhibition compared to medical accepted standard for performance.
  - 1) Results classified as: ***Susceptible, intermediate*** or ***resistant***

#### B. Minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) or minimal lethal concentration (MLC) of an antibiotic

1. the MIC is determined by the smallest concentration of anti-biotic that prevents visible growth

Example: MIC = 2µg/ml (1:100 dilution of 200 µg/ml)

MBC is 20µg/ml (1:10 dilution of 200µg/m.).

The cells that do not show growth in the MIC test are cultured in media free of the anti-microbial. If there is growth, then the antimicrobial is not bactericidal. The MBC can then be calculated.

### IV. Resistance to Antimicrobials

#### A. Microbial susceptibility and resistance

1. **Resistance** to an antibiotic means that a microorganism that was formerly susceptible to the action of that antibiotic is no longer affected by it. Antibiotic resistance can sometimes be transferred among bacteria on extra chromosomal DNA molecules known as plasmids. Resistance may be due to changes in the sensitivity of affected enzymes, changes in the selective permeability of cell walls and membranes, increased production of a competitive substrate, or enzymatic alteration of the drug itself. Unnecessary exposure to antibiotics has brought about a significant increase in antibiotic-resistant microbes.

A variety of mutations can lead to antibiotic resistance. Resistance genes are often on plasmids or transposons that can be transferred between bacteria

#### B. Mechanisms of antibiotic resistance

1. Enzymatic destruction of drug
2. Inducing changes in the cell membrane prevention of penetration of drug into cell
3. Alteration of drug's target site
4. Rapid ejection of the drug; pumping the drug out of the cell
5. Altering the cell's metabolic pathway

#### C. Reasons for antibiotic resistance

1. Misuse of antibiotics selects for resistance mutants. Misuse includes:
  - a. Using outdated, weakened antibiotics
  - b. Using antibiotics for the common cold and other inappropriate conditions
  - c. Use of antibiotics in animal feed
  - d. Failure to complete the prescribed regimen
  - e. Using someone else's leftover prescription

#### D. Retarding Drug Resistance

1. using sufficiently high concentrations of a drug for a sufficient time to kill all sensitive cells and inhibit others long enough for the body's defenses to destroy them
2. using antimicrobials in combination, promoting synergism, the interplay between drugs that results in efficacy that exceeds the efficacy of either drug alone
3. limiting the use of antimicrobials to necessary cases, avoiding indiscriminate prescribing and uncontrolled use
4. developing new variations of existing drugs

#### D. Nosocomial

1. infections acquired during a hospital stay. They are often extremely resistant to antibiotics and very difficult to treat. A group of gram-negative bacteria are resistant to disinfectants and antiseptics; they are the Pseudomonads (genus *Pseudomonas*). Pseudomonads may even thrive and grow in the presence of disinfectants and antiseptics. The resistance of these bacteria is thought to be due to the presence of porins in the membrane. Porins are proteins that form channels, which permit small molecules to flow into and out of the cell. Porins may allow some microbes to excrete the antibiotic prior to affect. Organisms, such as *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and Metabolic Resistant: *Staphylococcus aureus*, produce an enzyme known to break the beta-lactam side chain penicillin.

## Kinds of Antimicrobial Agents and their Primary Modes of Action

1. The table below is a summary of the classes of antibiotics and their properties including their biological source and mode of action.

**Table 1. Classes of antibiotics and their properties**

Chemical class	Examples	Biological source	Spectrum (effective against)	Mode of action
Beta-lactams (penicillins and cephalosporins)	Penicillin G, Cephalothin	Penicillium notatum and Cephalosporium species	Gram-positive bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Semisynthetic penicillin	Ampicillin, Amoxycillin		Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Clavulanic Acid	Clavamox is clavulanic acid plus amoxycillin	Streptomyces clavuligerus	Gram-positive and Gram-negative bacteria	"Suicide" inhibitor of beta-lactamases
Monobactams	Aztreonam	Chromobacter violaceum	Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Carboxypenems	Imipenem	Streptomyces cattleya	Gram-positive and Gram-negative bacteria	Inhibits steps in cell wall (peptidoglycan) synthesis and murein assembly
Aminoglycosides	Streptomycin	Streptomyces griseus	Gram-positive and Gram-negative bacteria	Inhibit translation (protein synthesis)
	Gentamicin	Micromonospora species	Gram-positive and Gram-negative bacteria esp. Pseudomonas	Inhibit translation (protein synthesis)
Glycopeptides	Vancomycin	Streptomyces orientales	Gram-positive bacteria, esp. Staphylococcus aureus	Inhibits steps in murein (peptidoglycan) biosynthesis and assembly

Lincomycins	Clindamycin	Streptomyces lincolnensis	Gram-positive and Gram-negative bacteria esp. anaerobic Bacteroides	Inhibits translation (protein synthesis)
Macrolides	Erythromycin	Streptomyces erythreus	Gram-positive bacteria, Gram-negative bacteria not enterics, Neisseria, Legionella, Mycoplasma	Inhibits translation (protein synthesis)
Polypeptides	Polymyxin	Bacillus polymyxa	Gram-negative bacteria	Damages cytoplasmic membranes
	Bacitracin	Bacillus subtilis	Gram-positive bacteria	Inhibits steps in murein (peptidoglycan) biosynthesis and assembly
Polyenes	Amphotericin	Streptomyces nodosus	Fungi	Inactivate membranes containing sterols
	Nystatin	Streptomyces noursei	Fungi (Candida)	Inactivate membranes containing sterols
Rifamycins	Rifampicin	Streptomyces mediterranei	Gram-positive and Gram-negative bacteria, Mycobacterium tuberculosis	Inhibits transcription (bacterial RNA polymerase)
Tetracyclines	Tetracycline	Streptomyces species	Gram-positive and Gram-negative bacteria, Rickettsias	Inhibit translation (protein synthesis)
Semisynthetic tetracycline	Doxycycline		Gram-positive and Gram-negative bacteria, Rickettsias Ehrlichia, Borrelia	Inhibit translation (protein synthesis)
Chloramphenicol	Chloramphenicol	Streptomyces venezuelae	Gram-positive and Gram-negative bacteria	Inhibits translation (protein synthesis)