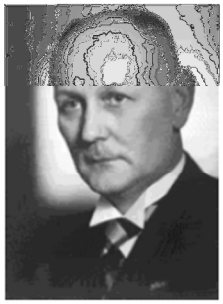
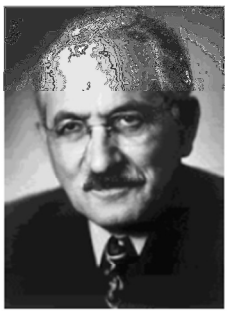
ANTIMICROBIALS

Antibiotic is a chemical substance produced by micro-organisms that is capable of inhibiting the growth of or kill other micro-organisms e.g. Benzylpenicillin and Gentamicin. An **antimicrobial substance** is substances produced either by a microorganism or produced synthetically or semi-synthetically that kills or inhibits the growth of other microorganisms to cure infections e.g. Ampicillin and amikacin are semisynthetic antibiotics while Moxifloxacin and Norfloxacin are synthetic antibiotics. The term antibiotic was first used in 1942 by Dr. Selman A. Waksman, a soil microbiologist, who discovered many actinomycetes derived antibiotics with their colleagues.

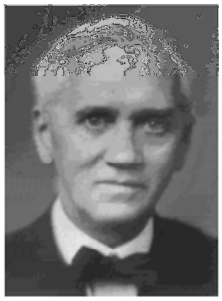
Nobel Laureate involved in antibiotic research:



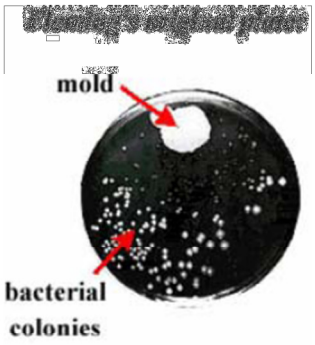
Gerhard Domagk
Nobel Prize: 1939
Prontocil



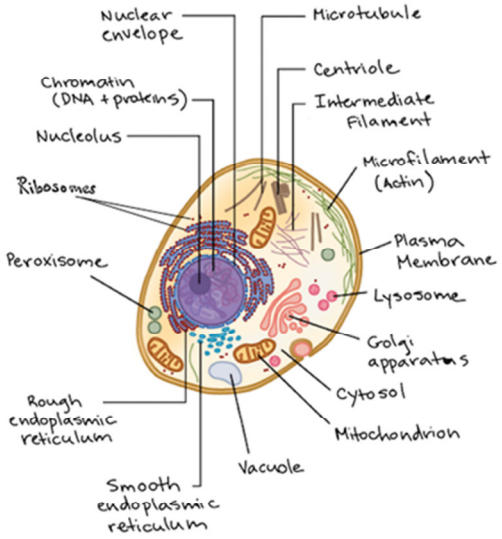
Selman Waksman
Nobel Prize: 1952
Streptomycin



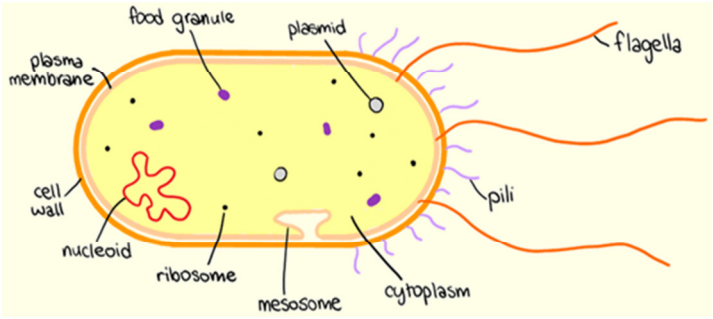
Alexander Fleming
Nobel Prize: 1945
Penicillin



Comparison of eukaryotic and prokaryotic cells:



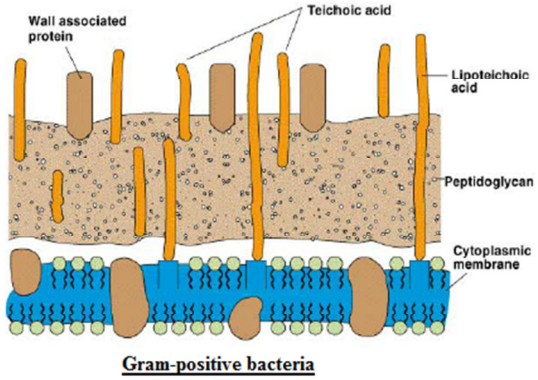
Eukaryotic cell



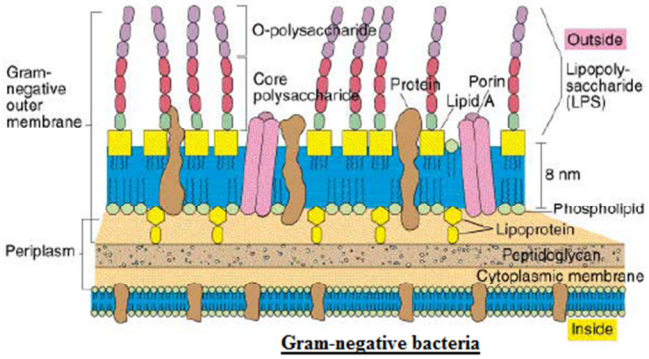
Prokaryotic cell

There are two types of bacteria on the basis of staining developed by Danish Bacteriologist **Hans Christian Gram in 1884**. Therefore, staining techniques named after him as Gram staining and bacteria are either called as **Gram-positive**, if stains purple in Gram staining, or **Gram-negative**, if pink under Gram staining. Gram staining is based on the ability of bacterial cell wall to retain

crystal violet dye during solvent treatment. The cell walls of Gram-positive bacteria have a high peptidoglycan and lower lipid content than Gram-negative bacteria.



Gram-positive bacteria



Gram-negative bacteria

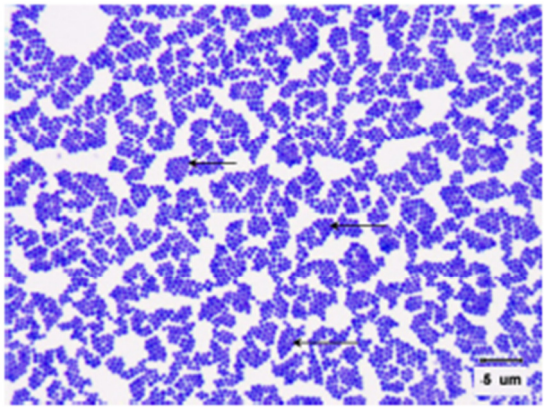
Gram-positive bacteria

Streptococcus

Staphylococcus aureus

Bacillus anthracis

Clostridium botulinum



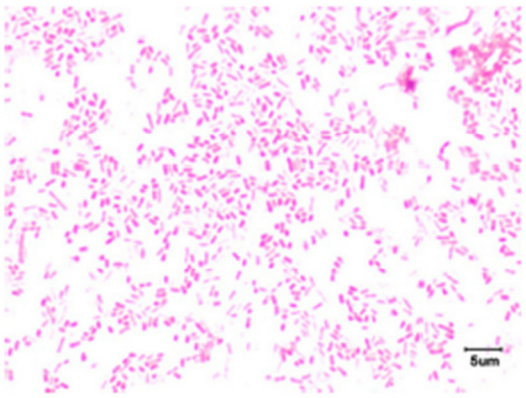
Gram-negative bacteria

Klebsiella pneumoniae

Shigella

Yersinia pestis

Salmonella typhimurium, S. enteriditis



Antibiotics have selective toxicity toward the bacterium rather than the host. Selectivity of the antibiotics varies. It has been observed that higher the selectivity lower the toxicity. Disinfectants are not selective to the bacteria, they lack selectivity.

Therapeutic index: The ratio that compares the blood concentration of antibiotics at which it becomes toxic to the host and the blood concentration of antibiotics at which it is effective. The higher the therapeutic index (TI), the better the antibiotics are.

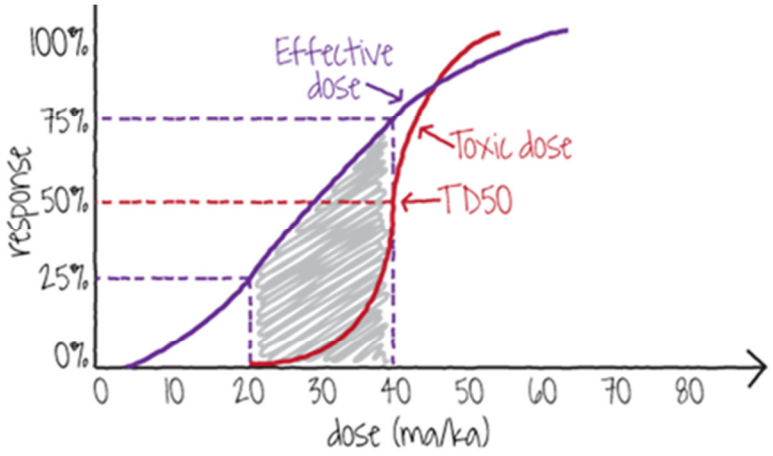
$$TI = \frac{Toxic\ dose}{Dose\ for\ therapeutic\ response} = \frac{TD50}{ED50}$$

For example, if the **TD50** is 200 and the **ED50** is 20 mg, the **therapeutic index (TI)** would be 10. Therefore,

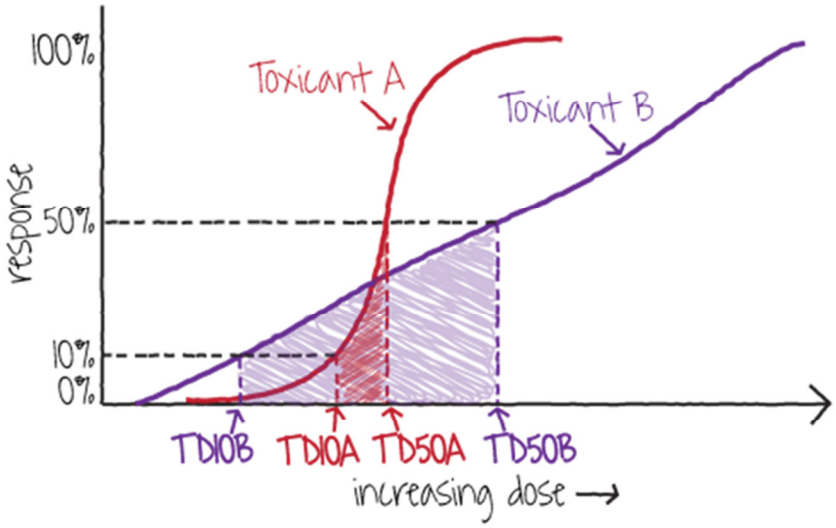
$$TI = \frac{TD50}{ED50} = \frac{200}{20} = 10$$

Margin of Safety (MOS): It is the range of a dose of antibiotics that is above an ineffective level and below the toxic level. The margin of safety of a drug is a concept that tells us how safely we can use a drug for therapeutic purposes without risking too many adverse effects at the same time. The **Margin of Safety (MOS)** is usually calculated as the ratio of the toxic dose to 1% of the population (TD01) to the dose that is 99% effective to the population (ED99).

$$MOS = \frac{TD01}{ED99}$$



The graph shows the relationship between effective dose response and toxic dose response. The shaded area represents the doses at which the substance produces an effective dose response while the toxic dose response remains below the TD50. The slope of a curve shows how dose increases result in responses to the effective or toxic dose.



In above graph, it has been observed that a small increase in dose causes a large increase in response for some substances, as is seen in Toxicant A's steep slope. For other substances, a much larger increase in dose is required to cause the same increase in response, as indicated in Toxicant B's shallow slope.

Minimal inhibitory concentration (MIC): It is the lowest concentration of antibiotic (µg/ml) at which growth of a given strain of bacterial population gets inhibited. It is considered the 'gold

standard' for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. Various concentrations of the antimicrobial substance are inoculated with cultured bacteria, and the results are measured using agar dilution or broth dilution (macro or micro) to determine at what level the MIC endpoint is established. Susceptibility testing is typically conducted using organisms that contribute to an infectious process warranting antimicrobial chemotherapy. A commonly used cocktail of bacteria is known as the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species) which are mainly considered as the leading cause of nosocomial (hospital-related) infections and are known to be resistant to various antimicrobial products. Following the initial dose, >99% reduction of the bacterial counts within 4hr at peak concentration more than three times the MIC.

MIC × 4 = dosage to obtain peak achievable concentration

However, for the re-growing bacteria, MIC might be four to eight times higher.

Minimal bactericidal concentration: It is the lowest concentration of antibiotic (µg/ml) required to kill a bacterium over a fixed, somewhat extended period, such as 18 hrs or 24 hrs, under a specific set of conditions. It is identified by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by a pre-determined reduction such as >99.9%. The MBC is complementary to the MIC; whereas the MIC test demonstrates the lowest level of antimicrobial agent that greatly inhibits growth, the MBC demonstrates the lowest level of antimicrobial agent resulting in microbial death. In other words, if a MIC shows inhibition, plating the bacteria onto agar might still result in organism proliferation because the antimicrobial did not cause death. *Antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC. The Clinical and Laboratory Standards Institute (CLSI) has established protocols and standards for establishing MIC and MBC in products. A common methodology utilized for MIC is CLSI M07-A9, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically.*

Some important facts:

- Quantal dose-response graphs can be characterized by the median effective dose (ED_{50})
- The **median effective dose** is the dose at which 50% of individuals exhibit the specified quantal effect
- The **median toxic dose** is the dose required to produce a defined toxic effect in 50% of subjects
- The **median lethal dose** is the dose required to kill 50% of subjects
- The **therapeutic index** is the ratio of the TD50 to the ED50 , a parameter which reflects the selectivity of a drug to elicit a desired effect rather than toxicity.
- The **therapeutic window** is the range between the minimum toxic dose and the minimum therapeutic dose, or the range of doses over which the drug is effective for most of the population and the toxicity is acceptable.

Category of antibiotics: There are two types of antibiotics viz bactericidal and bacteriostatic. Bactericidal antibiotics are preferred. Bacteriostatic antibiotics are used when the duration of therapy must be sufficient to allow cellular and humoral defense mechanisms to eradicate the bacteria. Serious infections are usually treated with bactericidal antibiotics for prompt eradication of the organisms.

Antibiotic susceptibility testing: It is also known as sensitivity testing, drug resistance testing, culture and sensitivity (C & S) or antimicrobial susceptibility. It is formerly known as bacterial and fungi susceptibility testing. Susceptibility testing is typically conducted using organisms that contribute to an infectious process warranting antimicrobial chemotherapy. A commonly used cocktail of bacteria is known as the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species) which are considered the leading cause of nosocomial (hospital-related) infections and are known to be resistant to antimicrobial products. Susceptibility testing is done by measuring **minimum inhibitory concentration (MIC)** and **minimum bactericidal concentration (MBC)**.

Minimum inhibitory concentration (MIC): It is defined as the lowest concentration of an antimicrobial agent that is bacteriostatic (prevents the visible growth of bacteria). It is used to evaluate the antimicrobial efficacy of various compounds by measuring the effect of decreasing concentration of antibiotics/antiseptic over a defined period in terms of inhibition of microbial population growth.

Minimum bactericidal concentration (MBC): It is the lowest concentration of an antibacterial agent required to kill a bacterium over a fixed, somewhat extended period, such as 18 hours or 24 hours, under a specific set of conditions. The MBC is identified by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by a pre-determined reduction such as ≥99.9%.

Combination therapy: Sometimes a combination of antibiotics is used to treat infections. A combination therapy is required to a) To prevent the emergence of resistant strain, b) to treat emergency cases during the period when an etiological diagnosis is still in progress, and c) to take advantage of antibiotic synergism.

Synergism: An effect of a combination is greater than the sum of the effects of the individual antibiotics.

Antagonism: One interferes with the effects of another antibiotic.

Representative sources of antibiotics	
Micro-organisms	**Antibiotics**
Gram-positive bacilli:	
Bacillus subtilits	Bacitracin
Bacillus polymyxa	Polymyxin
Actinomycetes:	
Streptomyces nodosus	Amphotericin B
Streptomyces venezuelae	Chloramphenicol
Streptomyces aureofaciens	Chlortetracycline and tetracycline
Streptomyces erythraeus	Erythromycin

Streptomyces fradiae	Neomycin
Streptomyces griseus	Streptomycin
Micromonospora purpureae	Gentamycin
Fungi:	
Penicillium notatum	Prnicillin
Penicillium griseofulvum	Griseofulvin
Cephalosporium spp	Cephalothin

Selection of appropriate antibiotic:

1. Knowledge of organism's natural resistance.
2. Pharmacological properties of the antibiotic toxicity. Binding, distribution, absorption, and achievable level in blood and urine,
3. Previous experience with same species.
4. Nature of patient's underlying pathology.
5. Patient's immune status.

Properties of ideal antibiotics:

1. An antibiotic must have selective target and having unique target.
2. They must have bactericidal property and kills the pathogenic bacteria..
3. They must have narrow spectrum – does not kill normal flora.
4. They must have high therapeutic index i.e. ratio of toxic level to therapeutic level and low toxicity to the host.
5. They must have least side effects or adverse effects i.e. toxicity, allergy etc.
6. They must have ability to be administered by various routes such as IV, IM, Oral.
7. They must have good absorption in the host.
8. They must have property of good distribution to the site of infection.
9. They must have slow emergence of resistance.

Antibiotics failure mechanism:

1. **Host factor:** Immune system, underlying diseases, barrier status, foreign bodies.
2. **Site of infection:** Central nervous system (CNS), intramuscular, pulmonary, surgical intervention etc.
3. **Antibiotic properties:** Pharmacokinetics (PK) and pharmacodynamics (PD), metabolism and elimination, tissue penetration, adverse event profile etc.
4. **Pathogen:** Species, virulence factors, resistance mechanisms etc.

Classification of antibiotics: Antibiotics are classified on the basis of chemical structure, origin, range of activity (spectrum of activity), mode of action, effects of their activity, and route of administration.

[A.] Classification of antibiotics on the basis of chemical structure:

- ❖ Carbohydrate containing Antibiotics:
- ❖ Pure saccharides antibiotics: examples; Streptozotocin

- ❖ Aminoglycosides: examples; Streptomycin
- ❖ N (a glycosylamine)/O (O-glycoside)/S (a thioglycoside)/C (C-glycoside) glycosides: eg. Chromomycin
- ❖ Other: eg; Lincomycin
- ❖ Macrocyclic lactone antibiotics: eg. Erythromycin
- ❖ Quinolones antibiotics; eg. Fluroquinolone
- ❖ N-containing heterocyclic antibiotics: eg. Beta-lactum
- ❖ O-containing heterocyclic antibiotics: eg. Cycloserine
- ❖ Alicyclic antibiotics: eg. Cycloheximide
- ❖ Aromatic antibiotics (Nitrobenzene): eg. Chloramphenicol
- ❖ Aliphatic amine antibiotics: eg. Spermidine
- ❖ Peptide antibiotics: eg. Polymyxin, Bacitracin, Gramicidin

[B.] Types of antibiotics on the basis of origin;

(a.) Microbial origin:

i. Bacterial origin: *Bacillus polymyxa*: Polymyxin, *Chromobacter violaceum*: Bacitracin, *Micromonospora spp*: Gentamycin

ii. Fungal origin: *Penicillium notatum*: Penicillin**,** *Cephalosporin spp*: Cephalosporin**.**

iii. Actinomycetes origin: *Streptomyces griseus*: Streptomycin, *S. venezuelae*: Chloramphenicol, *S. erythreus*: Erythromycin, *S. mediterranae*: Rifampicin.

(b.) Semi-synthetic origin: Amoxycillin, Ampicillin, Doxycycline, Tigecycline, Sulfonamide etc.

(c.) Synthetic origin: Chloramphenicol (It was extracted from *Streptomyces venezuelae* but now produced synthetically), 4-quinolones, Sulfonamide.

[C.] Classification of antibiotics on the basis of range of activity (spectrum of activity):

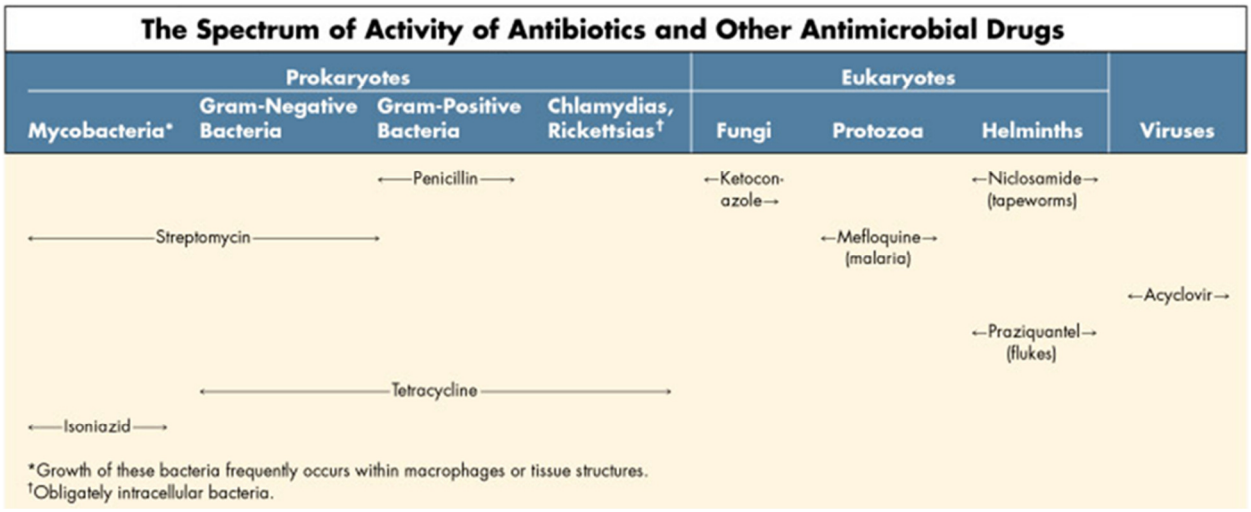
(a.) Narrow spectrum: Active towards relatively fewer micro-organisms e.g. Macrolides, Polymyxin

(b.) Moderate spectrum: Active towards Gram-positive bacteria as well as some systemic and UTI causing Gram-negative bacteria e.g. Aminoglycosides, Sulfonamide.

(c.) Narrow-broad spectrum: Active against Gram-positive and Gram-negative bacteria e.g. Beta-lactam.

(d.) Broad spectrum: Active against Gram-positive and Gram-negative bacteria except *Pseudomonas* and Mycobacteria e.g. Chloramphenicol, Tetracycline.

(e.) **Anti-mycobacterial antibiotics:** Effective against Mycobacteria (Tuberculosis causing bacteria), inhibits the synthesis of mycolic acid e.g. Ethambutol, Rifampicin, Isoniazid, Pyrazinamide.



No antibiotic is effective against all microbes.

[D.] Classification of antibiotics on the basis of mode of action:

(a.) **Inhibitors of cell wall synthesis/peptidoglycan inhibitors:** This class of antibiotics inhibit cell wall synthesis by inhibiting enzymes of peptidoglycan synthesis e.g. Beta-lactam, Penicillin, Bacitracin, Cycloserine, Phosphomycin, Cephalosporin, Vancomycin.

(b.) **Protein synthesis inhibitors:** Inhibits the protein synthesis by inhibiting 30s or 50s ribosomal subunit and thus its assembly or premature disintegration e.g. 30s inhibitors: Aminoglycosides (Gentamycin), Tetracycline; 50s inhibitors: Macrolides, Chloramphenicol, Clindamycin, Linezolid, Streptogramins.

(c.) **Nucleic acid synthesis inhibitors:** Inhibits the enzymes that are required for nucleic acid synthesis e.g. Quinolones, Ciprofloxacin, Nalidixic acid, Metronidazole, Nitrofurantoin.

(d.) **Folic acid synthesis inhibitors (Folate antagonist):** Inhibits the use of folate for the synthesis of DNA and thus kill the bacteria e.g. Sulfonamide, Trimethoprim.

(e.) **Cytoplasmic membrane inhibitor:** Inhibits or disrupts the plasma membrane of bacteria e.g. Polymyxin, Colistin.

[E.] Classification of antibiotics on the basis of effects of their activity:

(a.) **Bactericidal:** Kills bacteria e.g. Aminoglycosides, Penicillin, Cephalosporin.

(b.) **Bacteriostatic:** Inhibits the growth of bacteria e.g. Sulfonamide, Tetracycline, Chloramphenicol, Trimethoprim, Macrolides, Lincosamide.

[F.] Classification of antibiotics on the basis of route of administration:

(a.) **Oral antibiotics:** Such as acid stable antibiotics e.g. Penicillin V.

(b.) **Parental route:** Intravenous administration e.g. Penicillin G.

Classification of antibiotics (according to *British National Formulary* 2012)	
Antibiotic class	**Drug names**
Penicillins	
• Benzylpenicillin	Benzylpenicillin
• Penicillinase-resistant penicillin	Flucloxacillin
• Broad-spectrum penicillins	Amoxicillin, ampicillin, co-amoxiclav
• Antipseudomonas penicillins	Piperacillin, piperacillin plus tazobactam, ticaricillin
Cephalosporins	
• First generation	Cefalexin, cefradine, cefadroxil
• Second generation	Cefuroxime, cefaclor, cefixime
• Third generation	Cefotaxime, ceftazidime, ceftriaxone
Carbapenems and other β-lactams	Ertapenem, imipenem, meropenem, aztreonam
Tetracyclines	Tetracycline, demeclocycline, doxycycline, lymecycline, minocylcine, oxytetracycline
Aminoglycosides	Gentamicin, amikacin, tobramycin, neomycin
Macrolides	Erythromycin, azithromycin, clarithromycin
Clindamycin	Clindamycin
Sulphonamides and trimethoprim	Co-trimoxazole, trimethoprim
Metronidazole	Metronidazole
Quinolones	Nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, moxifloxacin
Glycopeptides	Vancomycin, teicoplanin, telavancin, oritavancin, dalbavancin
TB drugs	Ethambutol, rifampicin, streptomycin
Others	Chloramphenicol, daptomycin, sodium fusidate, linezolid, nitrofurantoin

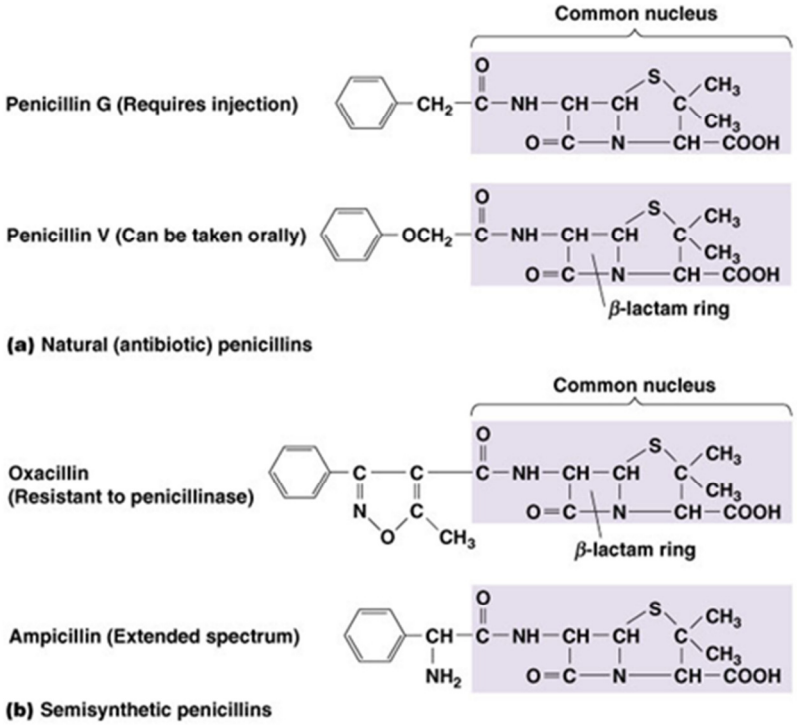
Major structural type of antibiotics:

[A.] Beta-lactam antibiotic: Penicillin

It was first discovered and reported in 1929 by Alexander Fleming which was produced naturally by a fungus named as *Penicillium notatum* (Penicillin G; first antibiotic). A close relative *Penicilliun chrysogenum* is the preferred choice of source of penicillin G. It has a narrow

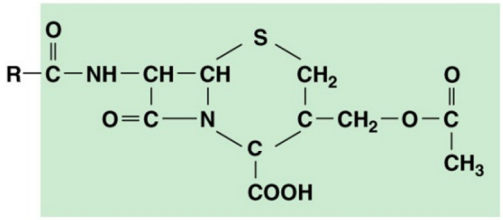
spectrum; only Gram positive bacteria (streptococci) and some Gram-negative bacteria such as *Treponema pallidum* causative agent for syphilis, and meningococci are sensitive to it They are beta-lactam compounds containing a nucleus of 6-animopenicillanic acid (lactam plus thiazolidine) ring and other ring side chains. It was later found that the naturally occurring penicillin can be modified by removing the acyl group to leave 6-aminopenicillanic acid and then adding acyl groups that confer new properties. These modern semi-synthetic penicillins such as

ampicillin, carbenicillin, amoxicillin and oxacillin have various specific properties such as: resistance to stomach acids so that they can be taken orally, a degree of resistance to penicillinase (β-lactamase, a penicillin-destroying enzyme produced by some bacteria) extended range of activity against some Gram-negative bacteria.

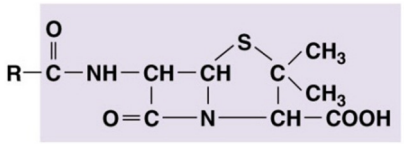


Beta-lactam antibiotic: Cephalosporin

Members of this group of antibiotics are similar to penicillin in their structure and mode of action but have less allergenicity. The first known member of this group of antibiotics was first isolated by Guiseppe Brotzu in 1945 from the fungus *Cephalosporium acremonium* however; the credit had been given to Edward Abraham who had been able to extract the compound.



Cephalosporin nucleus



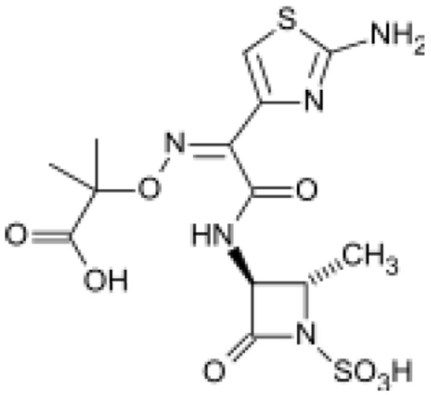
Penicillin nucleus

Cephalosporins contain 7-aminocephalosporanic acid nucleus and side chain containing 3, 6-dihydro-2 H-1,3- thiazane rings. They are used in the treatment of bacterial infections and diseases arising from Penicillinase-producing, Methicillin-susceptible Staphylococci and Streptococci, *Proteus mirabilis,* some *Escherichia coli, Klebsiella pneumonia, Haemophilus influenza, Enterobacter aerogenes* and some *Neisseria*.

They are subdivided into generations (1st-5th) in accordance to their target organism of which later versions are increasingly more effective against Gram-negative pathogens. Cephalosporins have a variety of side chains that enable them get attach to different penicillin-binding proteins (PBPs), to circumvent blood brain barrier, resist breakdown by penicillinase producing bacterial strains and ionize to facilitate entry into Gram-negative bacterial cells.

Beta-lactam antibiotic: Monobactams

The antibiotic was obtained from the bacterium *Chromobacterium violaceum* and first reported by Skyes and co-workers. They are part of beta-lactam compounds but unlike most other beta-lactams, the beta-lactam ring of monobactams stand alone and is not fused to another ring.



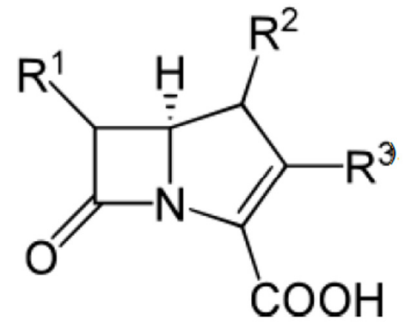
Structure of Monobactam (Aztreonam structure; Bonner & Sykes, 1984)

Aztreonam is the only commercially available monobactam antibiotic, with a narrow spectrum of activity. Aztreonam is active only against aerobic Gram-negative bacteria such as *Neisseria* and *Pseudomonas*; used for treating pneumonia, septicemia and urinary tract infections caused by these groups of bacteria. The monobactams are not effective against Gram-positive bacteria or anaerobes. They are used as injectable and inhalers.

Beta-lactam antibiotic: Carbapenems

This class of antibiotics was discovered out of necessity in 1976. Prior to this time, in the late 1960's, the effectiveness of penicillin was greatly threatened owing to the emergence of beta-lactamase in bacteria which conferred resistance on bacteria against penicillin. This led to the massive search for the potent beta-lactamase inhibitors resulting in finding of olivanic acid produced by a Gram-positive bacterium *Streptomyces clavuligerus* in 1976. However, these acids are chemically unstable and unable to penetrate the bacteria cell wall. Later, two superior beta-lactamase inhibitors clavulanic acid obtained from *Streptomyces clavuligerus* Brown et al.,

1976) and thienamycin obtained from *Streptomyces cottleya* (Kropp et al., 1976) were discovered.



Structure of Carbapenem (Papp-Wallace et. al., 2011)

Thienamycin is considered to be the first "carbapenem" and serves as a standard for every other carbapenem. Later on, a good number of other carbapenems have also been identified. Examples are:

i. Imipenem: broad spectrum, effective against aerobic and anaerobic pathogens, taken orally having minimal side effects;

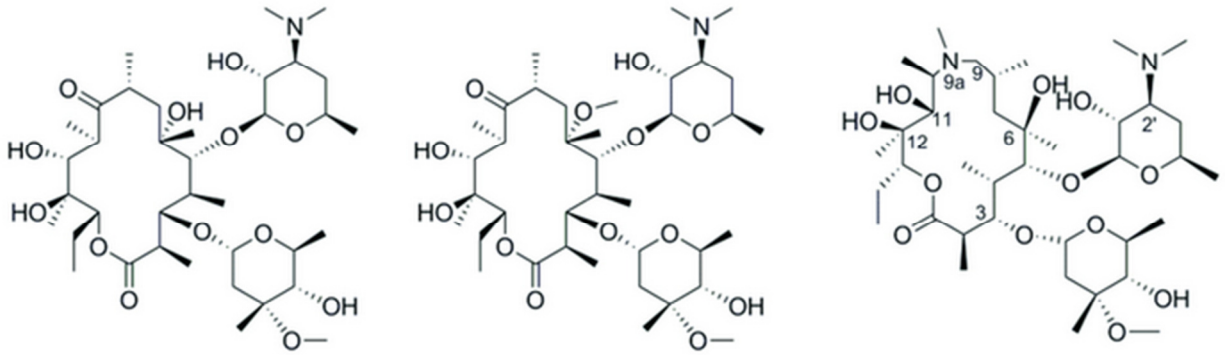
ii. Meropenem: broad spectrum, effective against non-fermentative Gram-negative bacilli; **iii.** Ertapenem – broad spectrum with limited activity against non-fermentative Gram-negative bacilli.

iii. Ertapenem: a broad spectrum with limited activity against non-fermentative Gram-negative bacilli.

They occupy a very important place in fight against bacterial infections, because they are able to resist the hydrolytic action of beta-lactamase enzyme. Among the several hundreds of known beta-lactams, carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria and therefore, they are called as "antibiotics of last resort" administered in gravely ill patients with MDR bacteria.

[B.] Macrolides (Streptomyces-derived antibiotics)

Macrolides are characterized by 14-, 15-, or 16- membered macrocyclic lactose rings with unusual deoxy sugars L-cladinose and D-desosamine attached. The first antibiotic of this class was first discovered and isolated by J. M. McGuire in 1952 as a metabolic product of a soil inhabiting fungus *Saccharopolyspora erythraea (*previous called as (*Streptomyces erythraeus).*

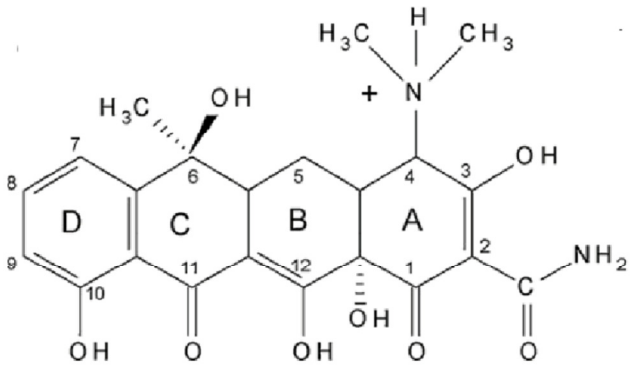


Structure of macrolide antiobiotics

They kill or inhibit micro-organisms by binding to their ribosomal subunit and thus preventing the addition of amino acid to polypeptide chains during protein synthesis. They may cause inflammation. Examples are: erythromycin, azithromycin, telithromycin, clarithromycin, streptomycin, and clindamycin. This class of antibiotics is generally broad spectrum, however, some bacterial species such as *Streptococcus pneumoniae* have resistance against the antibiotics.

[C.] Tetracycline

The member of this class of antibiotics was first discovered as chlorotetracycline by Yellapragada Subbarao (also discovered ATP as an energy source of a cell and methotrexate as an anti-cancer drug) and his junior, Benjamin Duggar in 1945 from soil bacterium of the genus Streptomyces. Later, they named the bacterium as *Streptomyces aureofaciens* because of its golden color and antibiotic as aureomycin.



General structure of tetracycline antibiotics

This class of antibiotics have four hydrocarbon rings and they are known by name with the suffix „–cycline". Historically, members of this class of antibiotics are grouped into different generations based on the method of synthesis. Those obtained from biosynthesis are said to be First generation, derivatives of semi-synthesis are Second generation, and those derived from total chemical synthesis are Third generation e.g.

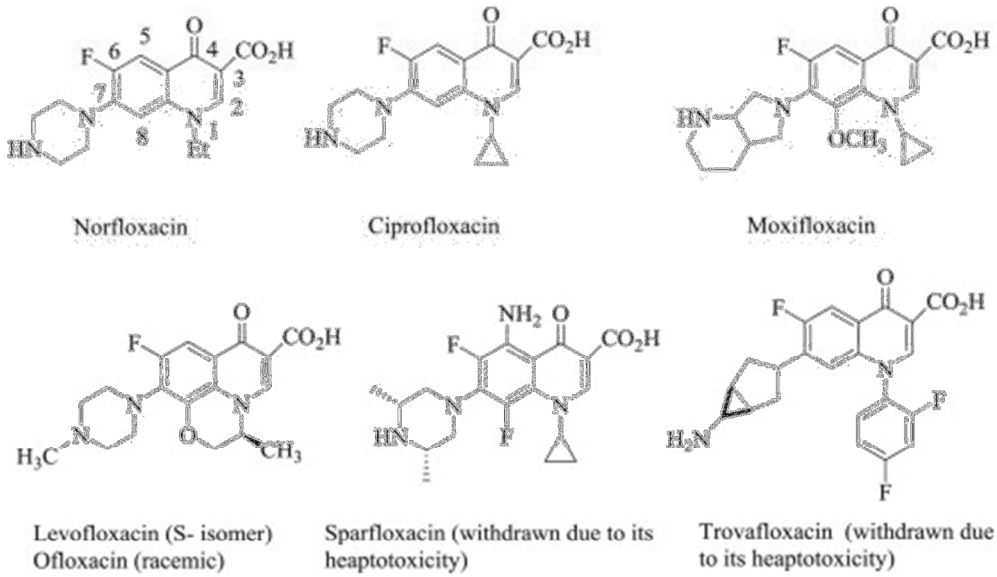
First generation: Tetracycline, Chlortetecycline, Oxytetracycline and Demeclocycline.
Second generation: Doxycycline, Lymecycline, Meclo cycline, Methacycline, Minocycline, and Rolitetracycline.
Third generation: Tigecycline.

Tetracyclines inhibit micro-organisms by binding to their ribosomal subunit and thus preventing the addition of amino acid to polypeptide chains during protein synthesis. It is taken orally two hours before or after meal for better absorption, causes teeth discoloration in patient below 8 years old and used in treating malaria, elephantiasis, amoebic parasites and rickettisia. Now-a-days, numerous bacteria have gained resistant to this class of antibiotics.

[D.] Quinolones

This class of antibiotics was first discovered as nalidixic acid by Scientists involved in search of antimalarial drugs as an impurity during the development of quinine in the early sixties which interfere with DNA replication and transcription in bacteria. Two major groups of compounds have been developed from the basic molecule: quinolones and naphthyridones which include cinoxacin, norfloxacin, ofloxacin, ciproxacin, temafloxacin, sparfloxacin, nalidixic acid, enoxacin etc. They consist of two rings but recent generations of quinolones possess an added ring structure which enables them to extend their spectrum of antimicrobial activity to some bacteria, particularly anaerobic bacteria resistant to quinolone, and members are known by the suffix–oxacin, such as floxacin, ciprofloxacin and levofloxacin.

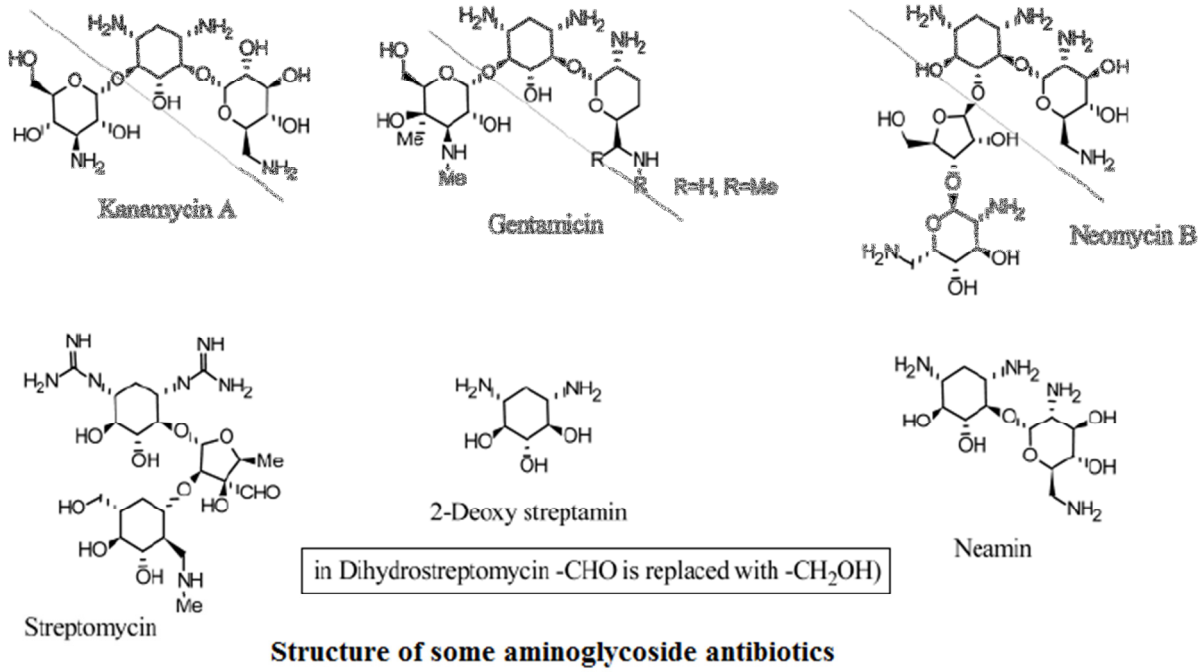
Modifications in the basic structure of quinolones are reported to have improved their bioavailability and increased both their spectrum of activity and potency; enhancing their performance in the treatment of various forms of illnesses such as urinary, systemic and respiratory tract infections.



Structure of some quinolones

[E.] Aminoglycosides (Streptomyces or an actinomycete)-derived antibiotics

Streptomycin was the first antibiotic of this class isolated from soil bacteria (Actinomycetes; a Gram-positive bacteria) *Streptomyces griseus* (state microbe of New Jersey, America because it was discovered in New Jersey soil) in 1943 by American biochemists Selman Waksman, Albert Schatz, and Elizabeth Bugie. It has been greatly used against *Mycobacterium tuberculosis*, the causal agent of tuberculosis among. The aminoglycosides are compounds of usually 3-amino sugars connected by glycosidic bonds.



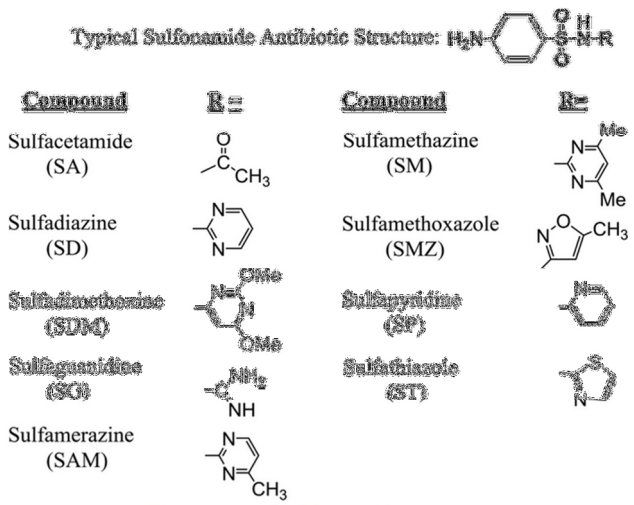
Structure of some aminoglycoside antibiotics

They are broad spectrum antibiotics effective against aerobic Gram-negative bacilli, and certain Gram-positive bacteria. They inhibit micro-organisms by binding to their ribosomal subunit and

thus preventing the addition of amino acid to polypeptide chains during protein synthesis. Streptomycin, a oldest antibiotic of this class, severely used in the treatment of bubonic plague, tularemia and tuberculosis, however, it was found to be highly toxic.

[F.] Sulphonamides or sulfonamides or sulfa drugs

Sulfonamides are generally considered as bacteriostatic rather than bactericidal. They has been reported to become bactericidal if their concentration is sufficiently high.



Structure of sulfonamide

They are said to be first group of antibiotics used in the therapeutic medicine, and are still used in medicinal and veterinary practices. Any *sulfonamide* can be considered as *derived* from a sulfonic acid by replacing a hydroxyl group with an amine group. They inhibit both Gram-positive and Gram-negative bacteria such as *Nocardia, E. coli, Klebsiella, Salmonella, Shigella* and *Enterobacter*, *Chlamydia trachomatis* and some Protozoa, and are widely used in the treatment of various infections including tonsillitis, septicemia, meningococcal, meningitis, bacillary dysentery and some urinary tract infections.

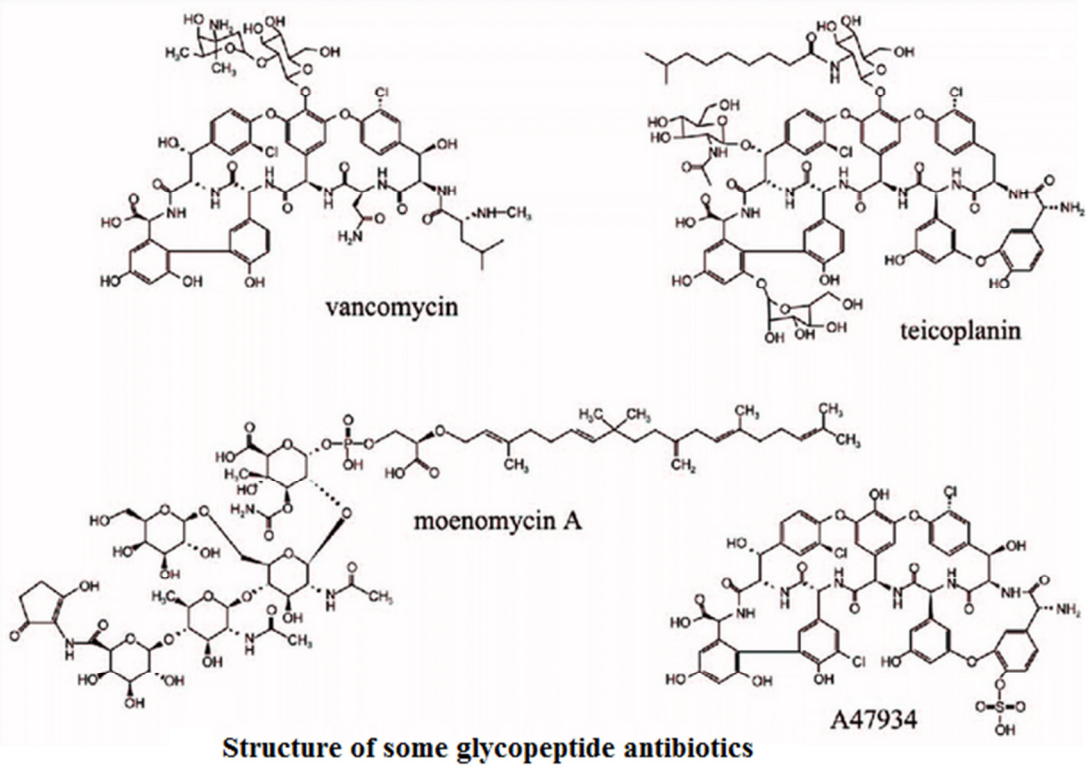
Although, sulfonamides are adjudged good and effective in treating various diseases and infections, they are recommended and administered with caution because of their toxicity and side effects, some of which include urinary tract disorders, hemolytic anemia, porphyria, and hypersensitivity reactions.

[G.] Glycopeptides

Glycopeptide antibiotics (GPAs) were originally obtained as natural products, but now its semi-synthetic derivatives with improved activity and pharmacokinetic properties are also available. Glycopeptides are made of a cyclic peptide of 7 amino acids, to which 2 sugars are bound. Binding of the antibiotic to its target occurs via the formation of 5 hydrogen bonds with the peptidic backbone of the drug. Sometimes, an additional chlorine and/or sugar is/are attached to the backbone of the drug (as is the case in oritavancin) during synthesis that increases their efficiency. Similarly, a lipophilic side chain enhances antibacterial potency and prolongs half-life of glycopeptides. After entering unhindered, it binds to D-ala-D-ala terminal of the basic subunit, inhibits glycosyl transferase and transpeptidase, and blocks pentaglycine from joining molecules thereby blocking peptidoglycan growth.

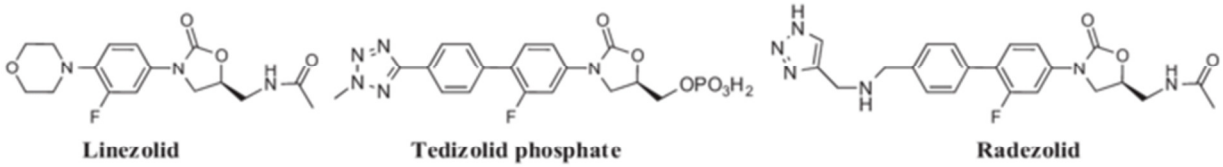
[H.] Oxazolidinones

Oxazolidinones are a group of synthetic antibiotics. Linezolid represents the first member of this group approved for clinical application in the year 2000. They have a broad spectrum of activity against Gram-positive bacteria including methicillin- and vancomycin-resistant staphylococci, vancomycin-resistant enterococci, penicillin-resistant pneumococci and anaerobes. Although the mechanism of action of oxazolidinone is not yet fully understood, they are reported to interfere with protein synthesis by binding to the P site of the ribosomal 50S subunit.



Structure of some glycopeptide antibiotics

Linezolid is used for treatment of respiratory tract and skin infections caused by Gram-positive bacterial pathogens. They easily penetrate and accumulate in the tissue including bone, lung, vegetations (plant-like growth in tissues), haematoma and cerebrospinal fluid. Linezolid administrations are usually safe; side effects such as myelosuppression, resulting to anemia and thrombocytopenia are often encountered in cases when treatment is prolonged. This group of antibiotics includes linezolid, tedizolid, posizolid, radezolid, MRX-1, T145, and AZD5847.



Chemical structures of oxazolidinone antibiotics

Antibiotics mode of action

The mechanisms of antibiotic actions are as follows:

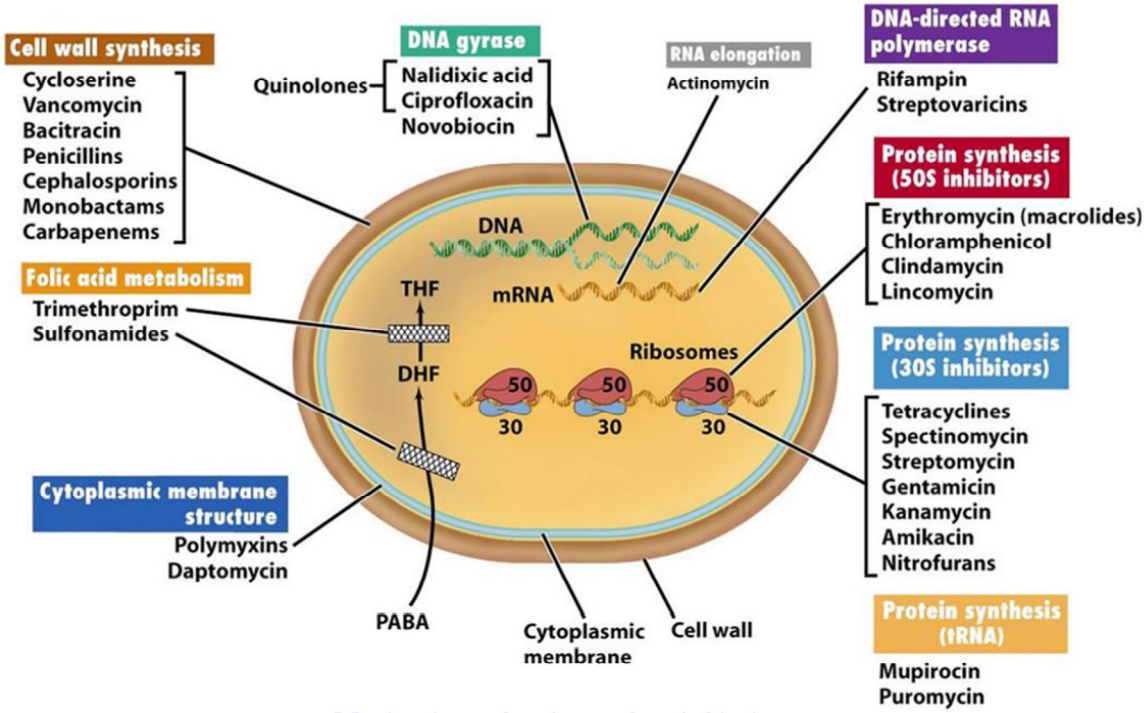
[A.[Inhibition of cell wall synthesis

[B.] Breakdown of cell membrane structure or function

[C.] Inhibition of structure and function of nucleic acids

[D.] Inhibition of protein synthesis

[E.] Blockage of key metabolic pathways

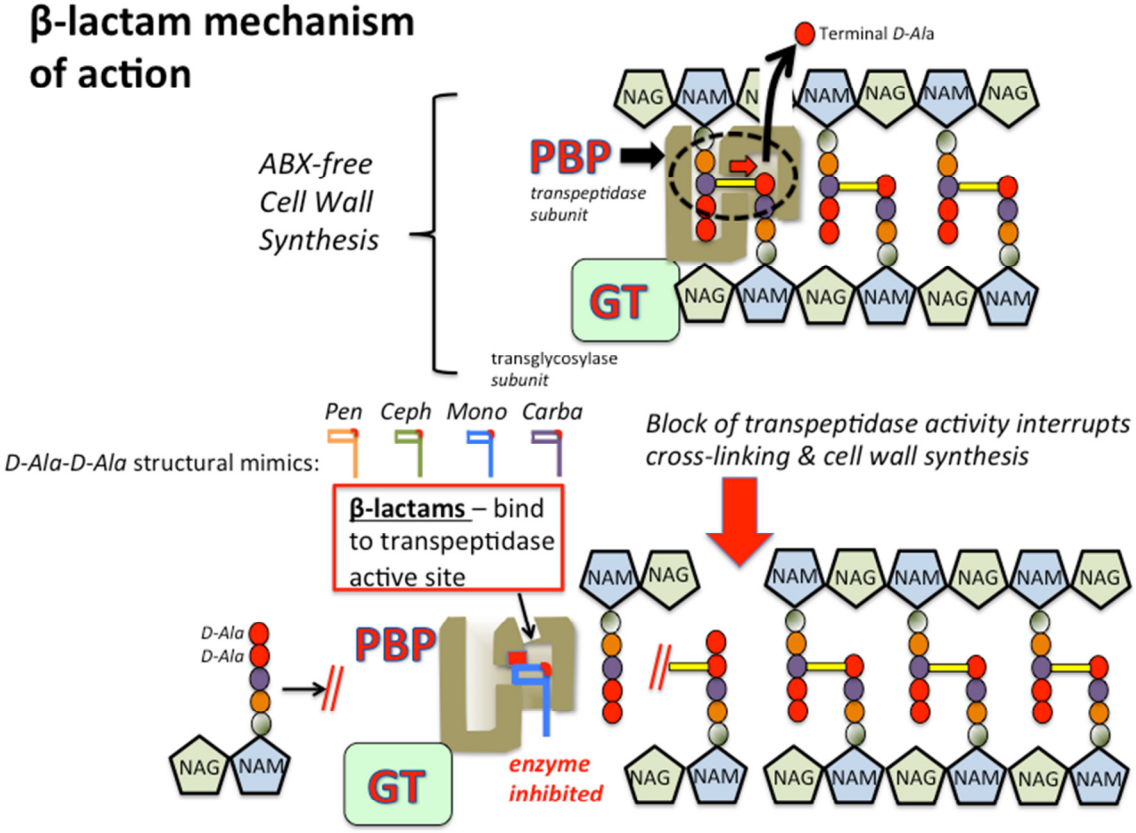


Mechanism of actions of antiobiotics

[A.] ***Inhibition of cell wall synthesis:*** Many antibiotics attack on the different stages of peptidoglycan cell wall synthesis of bacteria which occurs in three stages:

- **The first stage takes place in the cytoplasm**, where the low-molecular-weight precursors UDP-GlcAc and UDP-MurNAc-L-Ala-D-Glu-*meso*-Dap-D-Ala-D-Ala are synthesized. Many antibiotics affect this stage.
  - UTP and N-acetylglucosamine α-1-P are converted to UDP-N-acetylglucosamine, which is subsequently converted by the enzyme phosphoenolpyruvate: UDP-GIcNAc-3-enol-pyruvyltransferase. **Fosfomycins** block this transfer by a direct nucleophilic attack on the enzyme.
  - Three amino acids are added to the muramyl peptide to yield a tripeptide. The dipeptide D-alanyl-D-alanine is synthesized from two molecules of D-alanine by the enzyme D-alanyl-D-alanine synthetase. D-Alanine is produced from L-alanine by an alanine racemase. Cycloserine inhibits both alanine racemase and D-alanyl-D-alanine synthetase, owing to the structural similarity **cycloserine** binds to the enzymes better than the D-alanine.
- **2nd stage is catalyzed by membrane-bound enzymes**. The precursor molecules are transferred sequentially to a carrier in the cytoplasmic membrane. This carrier is a phosphorylated undecaprenyl alcohol. The lipid carrier functions as a point of attachment to the membrane for the precursors and allows for transport of the subunits across the hydrophobic interior of the cytoplasmic membrane to the outside surface. **Bacitracin**, a peptide antibiotic, specifically interacts with the pyrophosphate derivate of the undecaprenyl alcohol, preventing further transfer of the muramylpentapeptide from the precursor nucleotide to the nascent peptidoglycan.

- **The third stage of cell wall synthesis involves polymerization and the attachment of nascent peptidoglycan to the cell wall.** Polymerization occurs by transfer of the new peptidoglycan chain from its carrier in the membrane to the non-reducing *N*-acetylglucosamine of the new saccharide-peptide that is attached to the membrane. The new peptidoglycan is attached to preexisting cell wall peptidoglycan by a transpeptidase reaction D-alanyl-D-alanine terminus of two peptides. Transpeptidase enzyme cleaves the peptide bond between two D-alanyl residues in the pentapeptide and become acylated via the carbonyl group of the penultimate D-alanine residue. This final reaction can be inhibited by β-lactam antibiotics. These antibiotics undergo an acylation reaction with the transpeptidases that cross-link the peptide polymers. **Antibiotics include Penicillins (penams), Cephalosporins (oxacephems and cephamycins), Penems, Thienamycins (carbapenems), and Aztreonam (monobactams).**



Mechanism of action of beta-lactam antibiotics

Fig: Top: *In the absence of drug, transpeptidase enzymes (also known as Penicillin Binding Proteins; PBP) in the cell wall catalyze cross-links between adjacent glycan chains, which involves the removal of a terminal D-alanine residue from one of the peptidoglycan precursors (highlighted by the broken oval). Glycosyltransferases (GT), which exist as either separate subunits, or tightly associated with transpeptidases (e.g. as is the case for PBP-2) create covalent bonds between adjacent sugar molecules NAM & NAG. The net result of covalent bonds between both the peptide and sugar chains creates a rigid cell wall that protects the bacterial cell from osmotic forces that would otherwise result in cell rupture.* ***Bottom:*** *Beta-lactam antibiotics, which include penicillins (Pen), cephalosporins (Ceph), monobactams (Mono) and carbapenems (Carba) bear a structural resemblance to the natural D-Ala-D-Ala substrate for the transpeptidase, and exert their inhibitory*

*effects on cell wall synthesis by tightly **binding to the active site of the transpeptidase (PBP)**. NAG: N-acetylglucosamine; NAM: N-acetylmuramic acid.*

Vancomycin also interefere with the cell wall synthesis:

- Vancomycin interrupts cell wall synthesis by forming a complex with the C-terminal D-alanine residues of peptidoglycan precursors.
- Complex formation at the outer surface of the cytoplasmic membrane prevents the transfer of the precursors from a lipid carrier to the growing peptidoglycan wall by transglycosidases.
- Biochemical reactions in the cell wall catalyzed by transpeptidases and D,D-carboxypeptidases are also inhibited by vancomycin and other glycopeptide antimicrobials.
- Because of its large size and complex structure, vancomycin does not penetrate the outer membrane of gram-negative organisms thus active only on GPBs.

[B.] Breakdown of cell membrane structure and function:

- **Permeabilizes cell membranes for sodium and potassium ions**: Ionophore antibiotics. **Valinomycin** permeabilizes membranes for K^+ of both prokaryotic and eukaryotic cells for potassium and therefore cannot be used for antimicrobial chemotherapy. However, **Monensin** (in cattle) and **salinomycin** (in pigs) are used exclusively in veterinary practice can inhibit bacteria, protozoa (coccidia) and metazoan parasites.
- **Binds to the cytoplasmic membrane and then forms oligomeric pores** viz., permeabilization of liposomes by Lipopeptide antibiotics. **Daptomycin** permeabilizes liposomes only when they contain phosphatidylglycerol (PG) thus active on GPBs, outer membrane of GNBs lacking PG interferes its activity.
- **Binding to LPS to disrupt outer membrane,** Cyclopeptide antibiotics, **polymyxin B and E (colistin)**. Lipopolysccharide (LPS) contains several negative charges interacting with positively charged polymyxins, besides several hydrophobic interactions between the two molecules also disrupt outer membranes. Amino groups in polymyxin B pairs with the phosphates of lipid A in LPS.
- **Quasi-ionophore antibiotics** that include channel-forming agents such as **gramicidin** and the **polyene** antibiotics. The polyene antibiotics, which act by binding to membrane sterols, contain a rigid hydrophobic centre and a flexible hydrophilic section. They interact with fungal cells to produce a membrane-polyene complex that alters the membrane permeability, resulting in internal acidification of the fungus with exchange of K^+ and sugars; loss of phosphate esters, organic acids, nucleotides; and eventual leakage of cell protein.
- **Interfering with the synthesis of lipid membranes. Imidazoles: miconazole, ketoconazole, clotrimazole, and fluconazole.** These compounds inhibit the incorporation of subunits into ergosterol and may also directly damage the fungal cell membrane.

[C.] ***Inhibition of nucleic acid synthesis:*** Many antibiotics interfere with nuclei acid synthesis by blocking replication or stopping transcription.

- **Inhibitors of RNA synthesis:** Bind to DNA-dependent RNA polymerase and inhibit initiation of RNA synthesis e.g. **rifampin, rifamycin, rifampicin** (bactericidal).
- **Inhibitors of DNA synthesis:** Bind to the A subunit of DNA gyrase (topoisomerase) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis e.g. **quinolones, fluoroquinolones, oxolinic acid** (bactericidal).
- **Agents that impair the template function of DNA.** C**hloroquine** and **miracil D (lucanthone)** inhibit plasmodia and schistosomes, respectively by intercalating into the DNA and thereby to inhibit further nucleic acid synthesis. ***Acridine dyes such as proflavine*** *act by intercalation mechanism, but because they have toxicity and carcinogenicity in mammals they are not used as antibacterial agents.*
- **Agents that damage DNA.** Furanes (e.g. nitrofurantoin) reduced by nitrofuran reductase. The product attack ribosomal protein, DNA, pyruvate metabolism, respiration within cell

[D.] ***Inhibition of protein synthesis:*** A number of antibiotics bind either 30S or 50S subunit of ribosomes and disrupts the process of protein synthesis.

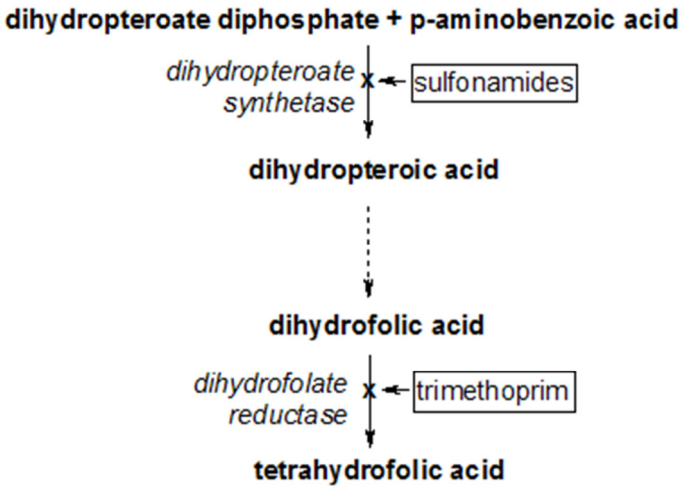
i.) Interfering with initiation of protein synthesis

- **Antimicrobials that bind to the 30s ribosomal subunit**
  - Irreversibly bind to the 30S ribosome and freeze the 30S initiation complex (30S-mRNA-tRNA) e.g. **aminoglycosides** (bactericidal).
  - Reversibly bind to the 30S ribosome and inhibit binding of aminoacyl-t-RNA to the acceptor site on the 70S ribosome e.g. **tetracyclines, streptomycin, spectinomycin, etc** (bacteriostatic).
  - Reversibly interferes with mRNA interaction with the 30S ribosome without causing misreading of mRNA unlike aminoglycosides e.g. **spectinomycin** (bacteriostatic).
- **Antimicrobials that bind to the 50s ribosomal subunit**
  - Bind to the 50S ribosome to inhibit peptidyl transferase activity e.g. **erythromycin, clindamycin, lincomycin, chloramphenicol** (bacteriostatic).
  - Inhibit translocation of the peptidyl tRNA from the A to the P site on the ribosome by binding to the 50S ribosomal 23S RNA e.g. **macrolides** such as **lincosamide** and **streptogramin** (bacteriostatic).

ii.) Affecting peptide elongation: Binds to elongation factor G (EF-G) and inhibits release of EF-G from the EF-G/GDP complex e.g. **fusidic acid** (bacteriostatic).

[E.] ***Blockage of key metabolic pathway (Competitive inhibitors):*** Some antibiotics like sulphonamides and trimethoprim have been shown to mimic a substrate needed for cellular metabolism of bacteria. This deception cause bacterial enzymes to attach themselves to the antibiotic rather than the normal substrate. In particular, analogues of

para-aminobenzoic acid (PABA) competitively inhibiting formation of dihydropteric acid (a pterin which is an immediate precursor of dihydrofolic acid), leading to inhibition of folic acid such as **sulfonamides, sulfones, para-aminosalicylic acid (PSA), depsone** (bacteriostatic).



Trimethoprim, metho-trexate, and **pyrimethamine** bind to dihydrofolate reductase and inhibit the formation of tetrahydrofolic acid thus disrupt the synthesis of folic acid.

Folic acid is vital in the metabolism of nucleic acid and amino acids; for this reason, sulphonamides ultimately disrupt the production of nucleic acids (DNA and RNA) and amino acids.

Inhibits Cell Wall Synthesis			
Penicillins (bactericidal: blocks cross linking via competitive inhibition of the transpeptidase enzyme)			
Class/Mechanism	*\Drugs*	*Indications (**Drug of Choice)*	*Toxicity*
Penicillin	Penicillin G Aqueous penicillin G Procaine penicillin G Benzathine penicillin G Penicillin V	*Strep. pyogenes (Grp.A)*** *Step. agalactiae (Grp.B)*** *C. perfringens(Bacilli)***	Hypersensitivity reaction Hemolytic anemia
Aminopenicillins	Ampicillin Amoxicillin	Above + ↑ Gram-negative: *E. faecalis***, *E. Coli***	Above
Penicillinase-resistant-penicillins	Methicillin,Nafcillin, Oxacillin, Cloxacillin, Dicloxacillin	Above + PCNase-producing *Staph. aureus*	Above + Interstitial nephritis
Antipseudomonal penicillins	Carbenicillin, Zicarcillin, Piperacillin	*Above* + *Pseudomonas aeruginosa***	Above
Cephalosporins (bactericidal: inhibits bacterial cell wall synthesis via competitive inhibition of the transpeptidase enzyme)			
1st generation	Cefazolin Cephalexin	*Staph. aureus*** *Staph. epidermidis*** Some Gram-negatives: *E. Coli, Klebsiella*	Allergic reaction Coombs-positive anemia (3%)
2nd generation	Cefoxitin Cefaclor Cefuroxime	Above + ↑ Gram-negative	Allergic Reaction ETOH Disulfiram reaction
3rd generation	Ceftriaxone	Above +	Allergic Reaction

	Cefotaxime Ceftazidime Cefepime (4th gen)	↑ Gram-negative *Pseudomonas*	ETOH Disulfiram reaction
Other Cell Wall Inhibitors			
Vancomycin (bactericidal: disrupts peptioglycan cross-linkage)	Vancomycin	MRSA** PCN/Ceph allegies** *S. aureus, S. epidermidis*	Red man syndrome Nephrotoxicity Ototoxicity
Beta-lactamase Inhibitors (bactericidal: blocking cross linking)	Clavulanic Acid Sulbactam Tazobactam	*S aureus*** *S epidermis*** *E.Coli*** *Klebsiella***	Hypersensitivity Reaction Hemolytic anemia
Carbapenems	Imipenem (+ cilastatin), Meropenem, Doripenem, Ertapenem	Broadest activity of any antibiotic (except MRSA, Mycoplasma)	
Aztreonam	Aztreonam	Gram-negative rods, Aerobes, Hospital-acquired infections	
Polymyxins	Polymyxin B, Polymyxin E	Topical Gram-negative infections	
Bacitracin	Bacitracin	Topical Gram-positive infections	
Protein Synthesis Inhibition			
Anti-30S ribosomal subunit			
Aminoglycosides (bactericidal: irreversible binding to 30S)	Gentamicin, Neomycin, Amikacin, Tobramycin, Streptomycin	Aerobic Gram-negatives *Enterobacteriaceae* *Pseudomonas*	Nephrotoxicity Ototoxicity
Tetracyclines (bacteriostatic: blocks tRNA)	Tetracycline, Doxycycline, Minocycline, Demeclocycline	*Rickettsia* *Mycoplasma* *Spirochetes* (Lyme's disease)	Hepatotoxicity Tooth discoloration Impaired growth Avoid in children < 12 years of age
Anti-50S ribosomal subunit			
Macrolides (bacteriostatic: reversibly binds 50S)	Erythromycin Azithromycin Clarithromycin	*Streptococcus* *H. influenzae* *Mycoplamsa pneumonia*	Coumadin Interaction (cytochrome P450)
Chloramphenicol (bacteriostatic)	Chloramphenicol	*H influenzae* Bacterial Meningitis, Brain absces	Aplastic Anemia Gray Baby Syndrome
Lincosamide (bacteriostatic: inhibits peptidyl transferase by interfering with amino acyl-tRNA complex)	Clindamycin	*Bacteroides fragilis* *S aureus* *Coagulase-negative Staph & Strep* Excellent Bone Penetration	Pseudomembranous colitis Hypersensitivity Reaction
Linezolid (variable)	Linezolid	Resistant Gram-positives	
Streptogramins	Quinupristin Dalfopristin	VRE GAS and S. aureus skin infections	
DNA Synthesis Inhibitors			
Fluoroquinolones (bactericidal: inhibit DNA gyrase enzyme, inhibiting DNA synthesis)			
1st generation	Nalidixic acid	*Steptococcus, Mycoplasma, Aerobic Gram +*	Phototoxicity Achilles tendon rupture

			Impaired fracture healing
2nd generation	Ciprofloxacin, Norfloxacin, Enoxacin, Ofloxacin, Levofloxacin	As Above +*Pseudomonas*	as above
3rd generation	Gatifloxacin	As above + Gram-positives	as above
4th generation	Moxifloxacin Gemifloxacin	As above + Gram-positives + anaerobes	as above
Other DNA Inhibitors			
Metronidazole (bacteridical: metabolic biproducts disrupt DNA)	Metronidazole (Flagyl)	Anaerobics	Seizures Crebelar dysfunction ETOH disulfram reaction
RNA Synthesis Inhibitors			
Rifampin: bactericidal inhibits inhibits RNA polymerase)	Rifampin	*Staphylococcus* *Mycobacterium* (TB)	Body fluid discoloration Hepatoxicity (with INH)
Mycolic Acids Synthesis Inhibitors			
Isoniazid	Isoniazidz	TB Latent TB	
Folic acid Synthesis Inhibitors			
Trimethoprim/Sulfonamides (bacteriostatic: inhibition with PABA)	Trimethoprim/Sulfamethoxazole (SMX) Sulfisoxazole Sulfadiazine	UTI organisms *Proteus* *Enterobacter*	Thrombocytopenia Avoid in third trimester of pregnancy
Pyrimethamine	Pyrimethamine	Malaria *T. gondii*	

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