Antibiotics

4th microbiology

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First lecture

Definition of some terms

A chemotherapeutic agent or drug is the use of any chemical agent in the treatment of disease.

An antibiotic agent is usually considered to be a chemical substance made by a microorganism that can **inhibit** the growth or **kill** microorganisms.

An antimicrobic or antimicrobial agent is a chemical substance similar to an antibiotic, but may be synthetic.

Antibiotic (**Greek anti, against, and bios, life**) refers to microbial products that **kill susceptible microorganisms or inhibit their growth.** Drugs such as the **sulfonamides are sometimes called antibiotics** although they are **synthetic chemotherapeutic agents**.

The Development of Chemotherapy

The German physician Paul Ehrlich was fascinated with **dyes** that specifically bind to microbial cells. He reasoned that one of the dyes could be a chemical that would selectively destroy pathogens without harming human cells. By 1904 Ehrlich found that the **dye trypan red** was active against the **trypanosome** that causes African sleeping sickness and could be used therapeutically.

Domagk received the 1939 Nobel Prize in Physiology or Medicine for his discovery of sulfonamides, or sulfa drugs.

Penicillin, the **first true antibiotic** because it is a **natural microbial product**, was first discovered in 1896 by a twenty-one-year old French medical student named Ernest Duchesne. His work was **forgotten** until Alexander Fleming accidentally **rediscovered penicillin in 1928**. After returning from a weekend vacation, Fleming noticed that a Petri plate of staphylococci also had mold growing on it and there were no bacterial colonies surrounding it.

Although the exact result are still unclear, it has been suggested that a ***Penicillium notatum*** spore had contaminated the Petri dish before it had been inoculated with the staphylococci. The mold apparently grew before the bacteria and produced penicillin. The bacteria nearest the fungus were lysed. Fleming correctly deduced that the mold produced a diffusible substance, which he called penicillin. Unfortunately, Fleming could not demonstrate that penicillin remained active *in vivo* long enough to destroy pathogens and he dropped the research.

In 1939 Howard Florey and Ernst Chain, professors at Oxford University, obtained the Penicillium culture from Fleming and set about purifying the antibiotic. Norman Heatley, a biochemist, was enlisted to help. He devised the original assay, culture, and purification techniques needed to produce crude penicillin for further experimentation. When purified penicillin was injected into mice infected with streptococci or staphylococci, almost all the mice survived. Florey and Chain's success was reported in 1940, and subsequent human trials were equally successful.

Fleming, Florey, and Chain received the Nobel Prize in 1945 for the discovery and production of penicillin. The discovery of penicillin stimulated the search for other antibiotics.

Selman Waksman, while at Rutgers University, announced in 1944 that he and his associates had found a new antibiotic, streptomycin, produced by

Streptomyces griseus . This discovery arose from the careful screening of about 10,000 strains of soil bacteria and fungi. it was the first drug to successfully treat tuberculosis. Waksman received the Nobel Prize in 1952, and his success led to a worldwide search for other antibiotic-producing soil microorganisms. Chloramphenicol, neomycin, oxytetracycline, and tetracycline were isolated from other Streptomyces species by 1953 .

General Characteristics of Antimicrobial Drugs:

A successful chemotherapeutic agent has **selective toxicity**: it **kills** or **inhibits** the microbial pathogen while damaging the host as little as possible. The degree of selective toxicity may be expressed in terms of

(1) **Therapeutic dose**: the drug level required for clinical treatment of a particular infection, and

(2) **Toxic dose**: the drug level at which the agent becomes too toxic for the host.

The therapeutic value is the ratio of the toxic dose to the therapeutic dose.

A drug that disrupts a microbial structure or function not found in host cells often has greater selective toxicity and a higher therapeutic value. For example, penicillin inhibits bacterial cell wall peptidoglycan synthesis but has little effect on host cells because they lack cell walls; therefore penicillin's therapeutic value is high. A drug may have a low therapeutic value because it inhibits the same process in host cells or damages the host in other ways. The undesirable effects on the host, or side effects, are of many kinds and may involve almost any organ system. Because side effects can be severe, chemotherapeutic agents must be administered with great care.

Classification of Antibacterial Drugs :

Since Fleming's discovery of penicillin, many antibiotics have been discovered. However, most antibiotics fall into a limited number of classes. Part of the challenge in developing new antibiotics is to find bacterial structures or processes not already targeted. These novel targets have the best chance of beating antimicrobial resistance, at least for a while. Here we present some of the more common antibiotics according to their functional

Classification of antibiotic according to their function (mode of action):

1-Inhibitors of Cell Wall Synthesis.

2- Cell membrane disruption

3- Protein Synthesis Inhibitors.

4- Nucleic Acid Synthesis Inhibition.

5- Metabolic Antagonists.

Classification of antibiotic depending on Manufacture methods :

1. Natural Antibiotics : produced by fermentation (Some bacteria and fungi naturally produce many of the commonly active antibiotics)

2. Semi-Synthetic: Some antibiotics are semisynthetic-natural antibiotics that have been structurally modified by the addition of chemical groups to make them less susceptible to stomach acids and inactivation by pathogens (e.g., ampicillin and methicillin).

3. Synthetic : several important chemotherapeutic agents, such as sulfonamides, trimethoprim, ciprofloxacin, isoniazid, and dapsone, are synthetic manufactured by chemical procedures (**independent of microbial activity**).

Classification of antibiotic depending on their range of effectiveness.

1-Broad-spectrum

2- Narrow-spectrum Antibiotics

Many are narrow-spectrum drugs; that is, they are effective only against a limited variety of pathogens (table 9.1). Others are broad-spectrum drugs that attack many different kinds of bacteria. Many semisynthetic drugs have a **broader spectrum of antibiotic activity than does their parent molecule.** This is particularly true of the semisynthetic penicillin's (e.g., ampicillin, amoxicillin) as compared to the naturally produced penicillin G and penicillin V.

Classified based on the general microbial group they act against:

1- Antibacterial
2- Antifungal
3- Antiprotozoan
4- Antiviral ----anti cancer

A few agents can be used against more than one group; for example, sulfonamides are active against bacteria and some protozoa.

How do antibiotics work? Bactericidal and Bacteriostatic Antibiotics:

Different types of antibiotic, they all work in one of two ways:

A bactericidal antibiotic (penicillin, for instance) kills the bacteria; these drugs usually interfere with either the formation of the bacterium's cell wall or its cell contents. Cidal agent kills the target pathogen, **it may be static at low levels.** The effect of an agent also varies with the target species: **an agent may be cidal for one species and static for another.**

A bacteriostatic stops bacteria from multiplying. If the static agents **is removed**, the microorganisms will recover and grow again. Because static agents do not directly destroy the pathogen, **elimination of the infection depends on the host's own immunity mechanisms**. **A static agent may not be effective if the host is immunosuppressed.**

Factors Influencing Antimicrobial

Drug Effectiveness

It is important to recognize that determine the effectiveness of drug therapy is not a simple matter. Drugs can be administered in several different ways, and **they do not always spread rapidly throughout the body** or **immediately kill all invading pathogens**. A **complex array of factors influences the effectiveness of antimicrobial drugs.**

First: the **drug must actually be able to reach the site of infection**. Understanding the factors that control **drug activity, stability, and metabolism *in vivo*** are essential in drug formulation. For example, the mode of administration plays an important role. A drug such as **penicillin G is not suitable for oral administration because it is**

relatively unstable in stomach acid. Some antibiotics for example, **gentamicin and other aminoglycosides-are not well absorbed from the intestinal tract and must be injected intramuscularly or given intravenously**. Other antibiotics (**neomycin, bacitracin) are so toxic that they can only be applied topically to skin lesions**. Non oral routes of administration are called parenteral routes. **Even when an agent is administered properly, it may be excluded from the site of infection**. For example, **blood clots, necrotic tissue, or biofilms** can protect bacteria from a drug, either because body fluids containing the agent may not easily reach the pathogens or because the agent is absorbed by materials surrounding them.

Second: the pathogen must be susceptible to the drug. **Bacteria in biofilms or abscesses may be replicating very slowly and are therefore resistant to chemotherapy** because many agents affect pathogens only if they are **actively growing and dividing.**

A pathogen, even though growing, may simply not be susceptible to a particular agent. To control resistance, **drug cocktails can be used to treat some infections**. A notable example of this is the use of clavulanic acid (to inactivate penicillinase) combined with ampicillin to treat penicillin-resistant bacteria.

Third: the chemotherapeutic agent must reach levels in the body that exceed the pathogen's MIC value if it is going to be effective. The concentration reached will

1- depend on the amount of drug administered.
2- route of administration
3- and speed of uptake.
4- the rate at which the drug is cleared or eliminated from the body. It makes sense that a drug will remain at high concentrations longer if it is absorbed over an extended period and excreted slowly.

Finally: chemotherapy has been less effective and much more complex by the spread of drug-resistance genes and prevention of drug access by **biofilm components.**

Overcoming Drug Resistance

It is important that physicians be able to treat infectious disease in their patients. The problem is that drugs are being overused, misused. Put simply, the more drugs are used, the more likely it is that bacteria will become resistant to them.

Several strategies can be employed to discourage the emergence of drug resistance. [1]The drug can be given in a **high enough concentration to destroy susceptible microbes** and most spontaneous mutants that might arise during treatment. [2]Sometimes two or even three different drugs can be administered simultaneously with the hope that each drug will prevent the emergence of resistance to the other. This approach is used in treating tuberculosis, HIV, and malaria, for example. When treating tuberculosis (TB), several drugs are administered simultaneously (e.g., isoniazid [INH] plus rifampin, ethambutol, and pyrazinamide). These drugs are administered for 6 to 9 months as a way of decreasing the possibility that the bacterium develops drug resistance. If a patient fails to take prescribed antibiotics as directed (e.g., **does not complete the course of treatment**), resistant mutants survive and increase because of their competitive advantage over nonresistant strains.

[3]Other strategies used to prevent drug resistance include the **strict** control on use of chemotherapeutic drugs, **particularly broad-spectrum drugs**, which should be used only when absolutely necessary.

If possible, **the pathogen should be identified**, **drug sensitivity tests performed**, and the **proper narrow-spectrum drug employed**. Patient compliance is just as important because completing a full course of antimicrobial therapy often prevents full mutation to resistant phenotypes.

Despite efforts to control the emergence and spread of drug resistance, the situation continues to worsen. Thus an urgent need **exists for new antibiotics that microorganisms have never encountered.**

Pharmaceutical and biotechnology companies collect and analyze samples from around the world in a search for completely new antimicrobial agents.

The effectiveness of a chemotherapeutic agent against a pathogen:

It can be obtained from the minimal inhibitory concentration (MIC).

The MIC: is the **lowest** concentration of a drug that **prevents growth** of a particular pathogen.

The minimal lethal concentration (MLC) is the **lowest** drug concentration that **kills the pathogen**.

A cidal drug generally kills pathogens at levels only two to four times the MIC, whereas a static agent kills at much higher concentrations .

How to use antibiotics:

Antibiotics are usually taken by **mouth (orally) as tablet ,capsule, syrup**; however, they can also be administered **by injection** or applied **directly to the affected part of the body(ointments ,**drop **, ,)** . Most antibiotics start having an effect on an infection within a few hours. It is important to complete the whole course of medication to prevent the infection from coming back.

Stopping taking the medication before the end of the course means that there is a higher chance the bacteria will become resistant to future treatments. This is because the ones that survive have had some exposure to the antibiotic and may consequently have built up a resistance to it. Even if an individual feels better, they still need to complete the course of treatment.

Some antibiotics should not be consumed with certain foods and drinks. Others should be taken on an empty stomach ,these would normally be taken about an hour before meals, or 2 hours after. It is important that patients follow the instructions correctly for the medication to be effective. People taking metronidazole should not consume alcohol.

Dairy products should not be consumed when taking tetracycline's, as they might affect the absorption of the medication.

Perfect antimicrobic must be :

1- **Soluble in body fluids**: agent must dissolve in body fluids to be transported in the body and reach the infectious organisms. After a drug is absorbed into the bloodstream it rapidly circulates through the body and the drug moves from the bloodstream into the body's tissues.

[1] **Drugs that dissolve in water** (water-soluble drugs), tend to stay within the blood and the fluid that surrounds cells. [2] **Drugs that dissolve in fat** (fat-soluble drugs), tend to concentrate in fatty tissues. [3] **Other drugs concentrate mainly in only one small part of the body .**

Drugs penetrate different tissues at different speeds, depending on the drug's ability to cross membranes. For example, the antibiotic rifampin, a highly fat-soluble drug, rapidly enters the brain, but the antibiotic penicillin, a water-soluble drug, does not. In general, fat-soluble drugs can cross cell membranes more quickly than water-soluble drugs can.

Some drugs leave the bloodstream very slowly because they bind tightly to proteins circulating in the blood. Others quickly leave the bloodstream and enter other tissues because they are less tightly bound to blood proteins. Some or virtually all molecules of a drug in the blood may be bound to blood proteins. The protein-bound part is generally inactive. As unbound drug is distributed to tissues and its level in the bloodstream decreases, blood proteins gradually release the drug bound to them. Thus, the bound drug in the bloodstream may act as a reservoir for the drug.

Some drugs accumulate in certain tissues which can also act as reservoirs of extra drug. These tissues slowly release the drug into the bloodstream, keeping blood levels of the drug from decreasing rapidly and thereby prolonging the effect of the drug. Some drugs, such as those that accumulate in fatty tissues, leave the tissues so slowly that they circulate in the bloodstream for days after a person has stopped taking the drug.

Distribution of a drug may also vary from person to person. For instance, fat people may store large amounts of fat-soluble drugs, whereas very thin people may store relatively little. Older people, even when thin, may store large amounts of fat-soluble drugs because the proportion of body fat increases with age.

2- **Selectively toxic**: agents must be more toxic to microorganisms than to host cells.

3- **Toxicity not easily altered** : agent should maintain a standard toxicity and not be made more or less toxic by interactions with foods and other drugs or abnormal conditions such as diabetes and kidney disease in the host.

4- **Non allergenic**: agent should not cause an allergic reaction in the host.

5- **Reasonable half life {stability}**: (conserved at a constant therapeutic concentration) is the time it takes for a substance (drug) to lose half of its pharmacologic activity. Typically, this refers to the body's cleansing through the **function of kidneys and liver** in addition to **excretion functions to eliminate a substance from the body.** In a medical context, half-life may also describe the time it takes for the blood plasma concentration of a substance to halve (plasma half-life) its steady-state.

6- **Has a long shelf life**: many drug products may have extended shelf lives beyond their expiration date. However, it is difficult for any one consumer or health care provider to know which product could have an extended shelf life. The ability for a drug to have an extended shelf life would be dependent upon the actual **drug ingredients, presence of preservatives, temperature variations, light, humidity, and other storage conditions**. Once a drug is repackaged into another container, as often happens in the pharmacy, the shelf-life might decline.

7- Unlikely to produce resistance.

8- reasonably priced.

Main Types of Side Effects Associated with Antimicrobial Treatment

1)Toxicity to organs.

2)Allergy – actual drug or breakdown products .

3) Suppress or Disruption of Normal Microflora Can Lead to super infection.

A list of the most common side effects of antibiotics:

Diarrhea, Feeling sick, Fungal infections of the mouth, digestive tract, and vagina.

A list of rare side effects of antibiotics:

Formation of kidney stones (when taking sulphonamides)

Abnormal blood clotting (when taking some cephalosporins)

Sensitivity to sunlight (when taking tetracyclines)

Blood disorders (when taking trimethoprim)

Deafness (when taking erythromycin and the aminoglycosides)

Some patients, especially older adults, may experience inflamed bowels (a type of colitis), which can lead to severe bloody diarrhea. Clindamycin, an antibiotic used for the most serious infections, commonly has this side effect. Penicillins, cephalosporins, and erythromycin can also produce this side effect, but it is much rarer.

Antibiotics should be used with extreme caution for the following individuals:

Anyone with reduced liver or kidney function.

Anyone who is pregnant.

Anyone who is breastfeeding.

Individuals taking an antibiotic, should not take other medicines or herbal therapies without speaking with a doctor first. medicines might also interact with antibiotics.

Spread of drug resistant pathogens

The spread of drug-resistant pathogens is one of the most serious threats to public health in the twenty-first century. There are two types of resistance: **inherent** and **acquired.**

Inherent resistance is that of the cell wall-less mycoplasma's resistance to penicillin, which interferes with peptidoglycan synthesis. Similarly, many Gram-negative bacteria are unaffected by penicillin G because it cannot penetrate the bacterial outer membrane.

Acquired resistance occurs when there is a change in the genome of a bacterium that converts it from one that is sensitive to an antibiotic to one that is now resistant. Resistance in bacteria may be acquired by **a mutation** and passed vertically by selection to daughter cells. More commonly, resistance is acquired by horizontal transfer of resistance genes between strains and species. Exchange of genes is possible by **transformation, transduction or conjugation.**

Mechanisms of Resistance

1) **Alteration of Targets**: which reduces the affinity for antimicrobials either by mutation or by target modification, or quantitative drug target alteration by overproduction of the target (usually affects ribosomes).

2) **Alteration of Membrane Permeability**: Change in the receptor that binds the drug.

3) **Inactivation of antibiotics**: either by hydrolysis or by modification (Enzymes – β-lactamase).

4) **Prevention of accumulation of antimicrobials:** either by [1] **decreasing uptake** or [2] **increasing efflux** of the antimicrobial from the cell via a collection of membrane-associated **pumping proteins**, that pump out drug.

5) **Alteration of Metabolic Pathway** : Development of alternate pathway.

Limiting Resistance

1) Constant exposure to high levels of antibiotic.

2) Use of multiple antibiotics.

3) Restricted use of antibiotics.

Selection of antibiotics

Factors to take into consideration:

1) The identity of the infecting organism.

2) Drug sensitivity of the infecting organism

3) Host factors (site of the infection, status of host defenses).

Empiric therapy prior to completion of lab tests:

1- It may be necessary to begin treatment in patients with serious infections before the lab results.
2- Take samples for culture prior to initiation of treatment.

Host factors

1) Host defenses (immune system).

2) Site of infection .To be effective an antibiotic must be present in the site of infection in a concentration greater than MIC (endocarditis, meningitis, abscesses)

3) Age (infants and elderly highly vulnerable to drug toxicity).

4) Pregnancy and lactation

5) Previous allergic reactions

6) Genetic factors (ie hemolysis in patients with G-6PD deficiency if given sulfonamides). Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme deficiency in humans, affecting 400 million people worldwide. G6PD deficiency confers partial protection against malaria , which probably accounts for the persistence and high frequency of the responsible genes G6PD deficiency can present as neonatal hyperbilirubinemia. In addition, persons with this disorder can experience episodes of brisk hemolysis after ingesting fava beans or being exposed to certain infections or drugs. Infants with prolonged neonatal jaundice as a result of G6PD deficiency should receive phototherapy with a bili light. (newborn jaundice)

Necessary : patients should be instructed to take their medication for the entire prescribed course even though symptoms may subside before the full course has been completed.

Antibiotic combinations :

The result may be additive, synergetic or antagonistic.

1- **Additive response :**one in which the antimicrobial effect of the combination is **equal to the sum** of the effects of the two drugs alone.
2- **synergetic interaction** :one in which the effect of the combination is **greater than** the sum of the effects of the individual agents.
3- Antagonistic response : in certain cases the combination of two antibiotics may be **less effective** than one of the agents by itself . for example, the simultaneous use of **penicillin** and **tetracycline** is often less effective than when either drug is used alone. By stopping the growth of the bacteria, the **bacteriostatic** drug tetracycline interferes with the action of penicillin **bactericidal** , which requires bacterial growth.

Disadvantages of antibiotic combinations

1) Increased risk of toxic and allergic reactions.
2) Possible antagonism of antimicrobial effects.
3) Increased risk of suprainfection.
4) Selection of drug resistant bacteria.
5) Increased cost.

Classification of antibiotic according to their function

Inhibitors of Cell Wall Synthesis

The most selective antibiotics are those that interfere with bacterial cell wall synthesis. Drugs such as penicillins, cephalosporins, vancomycin, and bacitracin have a high therapeutic index because **they target structures and functions not found in eukaryotic cells. the table show some antibiotic and its properties :**

Table 9.1 Properties of Some Common Antibacterial Drugs

Antibiotic Group	Primary Effect	Mechanism of Action	Members	Spectrum	Common Side Effects
Cell Wall Synthesis Inhibition					
Penicillins	Cidal	Inhibit transpeptidation enzymes involved in cross-linking the polysaccharide chains of peptidoglycan Activate cell wall lytic enzymes	Penicillin G, penicillin V, methicillin Ampicillin, carbenicillin	Narrow (Gram-positive) Broad (Gram-positive, some Gram-negative)	Allergic reactions (diarrhea, anemia, hives, nausea, renal toxicity)
Cephalosporins	Cidal	Same as above	Cephalothin, cefoxitin, cefaperazone, ceftriaxone	Broad (Gram-positive, some Gram-negative)	Allergic reactions, thrombophlebitis, renal injury
Vancomycin	Cidal	Prevents transpeptidation of peptidoglycan subunits by binding to D-Ala-D-Ala amino acids at the end of peptide side chains. Thus it has a different binding site than that of the penicillins.	Vancomycin	Narrow (Gram-positive)	Ototoxic (tinnitus and deafness), nephrotoxic, allergic reactions

BACTERIAL CELL WALL STRUCTURE

Gram-Positive Cell Walls

Gram-positive bacteria normally have cell walls that are thick and primarily of peptidoglycan. Peptidoglycan in gram-positive bacteria often contains a peptide inter bridge. In addition, gram-positive cell walls usually contain large amounts of teichoic acids , polymers of glycerol or ribitol joined by phosphate groups (figure). Amino acids such as D -alanine or sugars such as glucose are attached to the glycerol

and ribitol groups. The teichoic acids are covalently connected to the peptidoglycan itself are called Wall Teichoic acid are polymer of ribitol found in some G+ bacteria or bound to plasma membrane lipids are polymer of glycerol they are called lipoteichoic acids (found in all G+) . Has small periplasmic space .

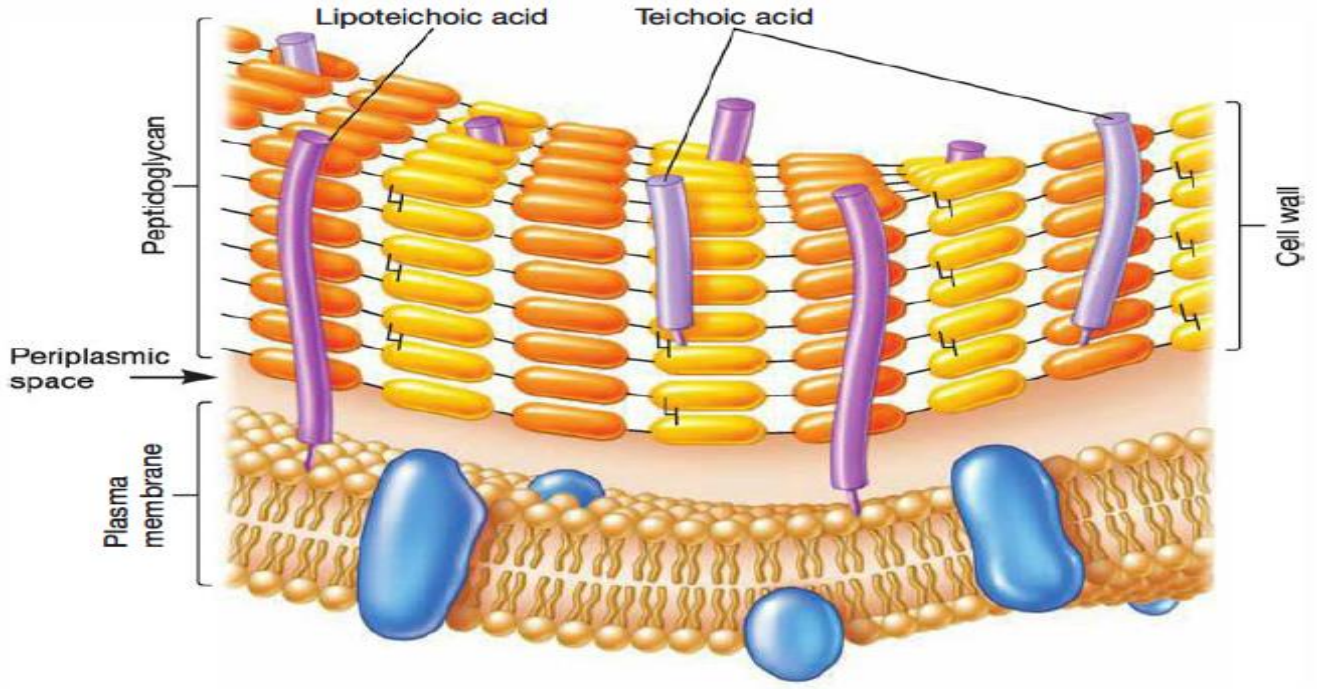


Figure 3.23 Typical Gram-Positive Cell Wall. This component of the cell envelope lies just outside the plasma membrane.

Gram-Negative Cell Walls

The (figure) shows that gram-negative cell walls are much more complex than gram-positive walls. The thin peptidoglycan layer next to the plasma membrane and bounded on either side by the periplasmic space usually constitutes only 5 to 10 % of the wall weight. In *E. coli*, it is about 2 nm thick and contains only one or two sheets of peptidoglycan.

The periplasmic space of gram-negative bacteria is also extremely different from that of gram-positive bacteria. It ranges in width from **1 nm to as great as 71 nm**. Some recent studies indicate that it may constitute about **20 to 40% of the total cell volume**. When cell walls are disrupted carefully or removed without disturbing the underlying plasma membrane, periplasmic enzymes and other proteins are released and may be easily studied. for example, **hydrolytic enzymes** and **transport proteins**. Some periplasmic proteins are involved in energy conservation. Other periplasmic proteins are involved in peptidoglycan

synthesis and the modification of toxic compounds that could harm the cell. The outer membrane lies outside the thin peptidoglycan layer and is linked to the cell in two ways (figure). **The first is by Braun's lipoprotein, the most abundant protein in the outer membrane**. This small lipoprotein is covalently joined to the underlying peptidoglycan and is embedded in the outer membrane by its hydrophobic end.

The second linking mechanism involves the many adhesion sites joining the outer membrane and the plasma membrane. The two membranes appear to be in direct contact at these sites. Adhesion sites may be regions of direct contact or possibly true membrane fusions.

Possibly the most unusual constituents of the outer membrane are its **lipopolysaccharides (LPSs)** . These large, complex molecules contain both lipid and carbohydrate, and **consist of three parts**: **(1) lipid A (2) the core polysaccharide, and (3) the O side chain.**

The lipid A region contains two glucosamine sugar derivatives, each with three fatty acids and phosphate or pyrophosphate attached. The fatty acids of lipid A are embedded in the outer membrane, while the remainder of the LPS molecule projects from the surface. The core polysaccharide is joined to lipid A. The O side chain or O antigen is a polysaccharide chain extending outward from the core. It has several peculiar sugars and varies in composition between bacterial strains.

The outer membrane is **more permeable than the plasma membrane** and permits the passage of small molecules such as glucose and other monosaccharides. This is due to the presence of **porin proteins** . **Most porin proteins cluster together to form a <u>trimer</u> in the outer membrane**. Each porin protein extents the outer membrane and is more or less tube-shaped; **its narrow channel allows passage of molecules smaller than about 600 to 700 daltons**. specific channel proteins allow other molecules to move through the outer membrane. The permeability varies with the species, for example, *Pseudomonas aeruginosa* has an external membrane less than 100 times the permeability of the outer membrane of *E. coli* (for this *Pseudomonas aeruginosa* more resistant to antibiotics) depending on the type of **genes responsible** for encoding transported proteins .

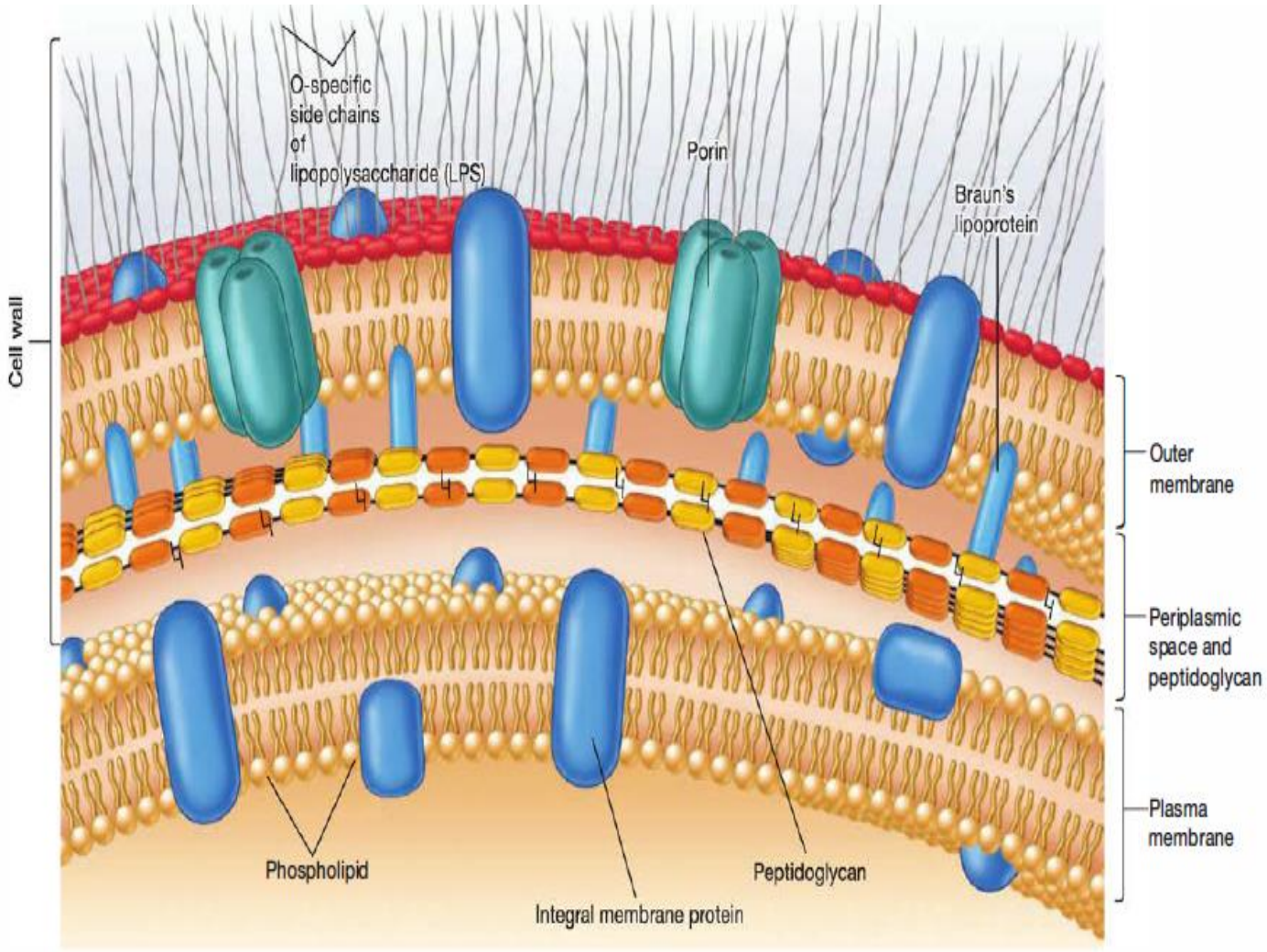


Figure 3.25 Typical Gram-Negative Cell Wall. Notice that these bacteria are bounded by two membranes, the plasma membrane and the outer membrane of the cell wall.

Peptidoglycan Structure

Peptidoglycan is an enormous, mesh like polymer composed of many identical subunits. The polymer contains two sugar derivatives, N -acetylglucosamine and N -acetylmuramic acid, and several different amino acids. **Three of these amino acids are not found in proteins:**

D -glutamic acid, D -alanine, and meso –diaminopimelic acid.(Most proteins are composed of amino acids **L-type** while in the brain and the bacteria are of **type D,** which **prevents them from attacking by most Peptidases** which recognize only the

L-isomers of amino acid residues and also give different and distinctive antigenic structure of the bacteria from eukaryotic).

The peptidoglycan subunit present in most gram-negative and many gram-positive bacteria is shown in figure . The **backbone** of this polymer is composed of alternating N -acetylglucosamine and N -acetylmuramic acid residues bind in glycoside bound β 1-4 . A peptide chain of four alternating D - and L -amino acids is connected to the carboxyl group of N-acetylmuramic acid. Many bacteria replace meso - diaminopimelic acid (**the precursor of lysine**) with another diamino acid, usually L - lysine (figure).

To make a strong, mesh like polymer, peptidoglycan chains must be joined by cross-links between the peptides. Often the **carboxyl group** of the terminal D -alanine is connected directly to the **amino group** of diaminopimelic acid, but a peptide inter bridge may be used instead (figure). Most gram-negative cell wall peptidoglycan **lacks the peptide inter bridge** . With or without a peptide inter bridge, cross-linking results in an enormous peptidoglycan sac that is actually one dense, interconnected network . These sacs have been isolated from gram-positive bacteria and are strong enough to keep their shape , yet they are relatively permeable and elastic (figure).

The differences between G+ & G- in possession of the amino acid **Lysine** in **third** position **instead of DAP** and the **possession of cross bridges** consisting of **Penta glycine** , which varies from one type to another and may be consist of a single amino acid, which are most common in Gr + than Gr-. Some of the Gr + have D.DAP in a third location bound with D-alanine, as in Corynebacterium, Clostridium, Lactobacillus , Bacillus and some replace D.DAP by L.DAP.

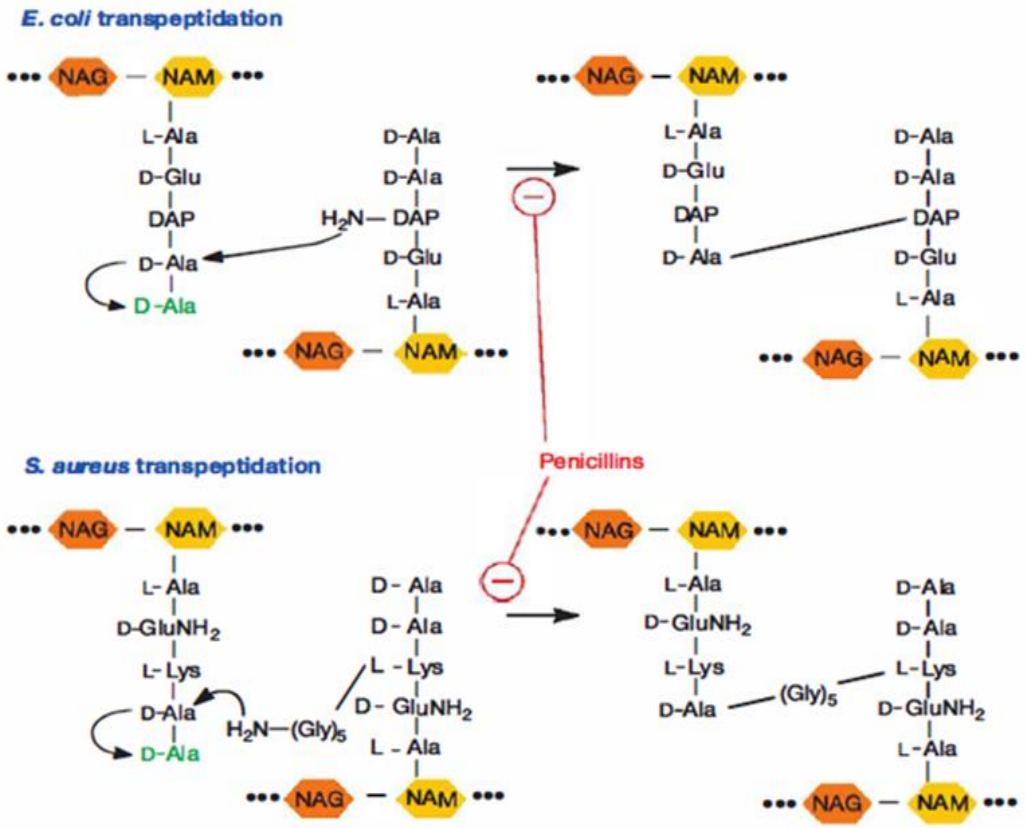


Figure 12.12 Transpeptidation. The transpeptidation reactions in the formation of the peptidoglycans of *E. coli* and *Staphylococcus aureus*.

Biosynthesis of Peptidoglycan

Peptidoglycan is a large, complex molecule consisting of long polysaccharide chains made of alternating N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues. Pentapeptide chains are attached to the NAM groups. The polysaccharide chains are connected through their pentapeptides or by interbridges (figure).

Not surprisingly, such an intricate structure requires an equally intricate biosynthetic process, especially because some reactions occur in the cytoplasm, others in the membrane, and others in the periplasmic space. Peptidoglycan synthesis involves two carriers (figure).

The first, uridine diphosphate (UDP), functions in the cytoplasmic reactions.

1- In the first step of peptidoglycan synthesis, UDP derivatives of NAM and NAG are formed.
2- Amino acids are then added sequentially to UDP-NAM to form the pentapeptide chain.

3- NAM-pentapeptide is then transferred to the second carrier, **bactoprenol** phosphate, which is located at the cytoplasmic side of the plasma membrane. The resulting intermediate is often **called Lipid I.** Bactoprenol is a 55- carbon alcohol and is linked to NAM by a pyrophosphate group .

4- Next, UDP transfers NAG to the bactoprenol- NAM-pentapeptide complex (Lipid I) to generate Lipid II. This creates the peptidoglycan repeat unit. The repeat unit is transferred across the membrane by bactoprenol. If the peptidoglycan unit requires an **interbridge**, it is added while the repeat unit is within the membrane. Bactoprenol stays within the membrane and does not enter the periplasmic space.

5- After releasing the peptidoglycan repeat unit into the periplasmic space, bactoprenol- pyrophosphate is **dephosphorylated** and returns to the cytoplasmic side of the plasma membrane, where it can function in the next round of synthesis.

6- Meanwhile, the peptidoglycan repeat unit is added to the growing end of a peptidoglycan chain. The final step in peptidoglycan synthesis is transpeptidation , which creates the peptide cross-links between the peptidoglycan chains. The enzyme that catalyzes the reaction removes the terminal D-alanine as the cross-link is formed. To grow and divide efficiently, a bacterial cell must add new peptidoglycan to its cell wall in a precise and well-regulated way while maintaining wall shape and integrity in the presence of high osmotic pressure.

7- Because the cell wall peptidoglycan is essentially a single, enormous network, the growing bacterium must be able to degrade it just enough to provide acceptor ends for the incorporation of new peptidoglycan units. It must also reorganize peptidoglycan structure when necessary. This limited peptidoglycan digestion is accomplished by **enzymes known as autolysins** , **some of which attack the polysaccharide chains, while others hydrolyze the peptide cross-links**.

8- Autolysin inhibitors are produced to keep the activity of these enzymes under tight control. Because of the importance of peptidoglycan to bacterial cell wall structure and function, its synthesis is a particularly effective target for antimicrobial agents. Inhibition of any stage of synthesis weakens the cell wall and can lead to lysis.

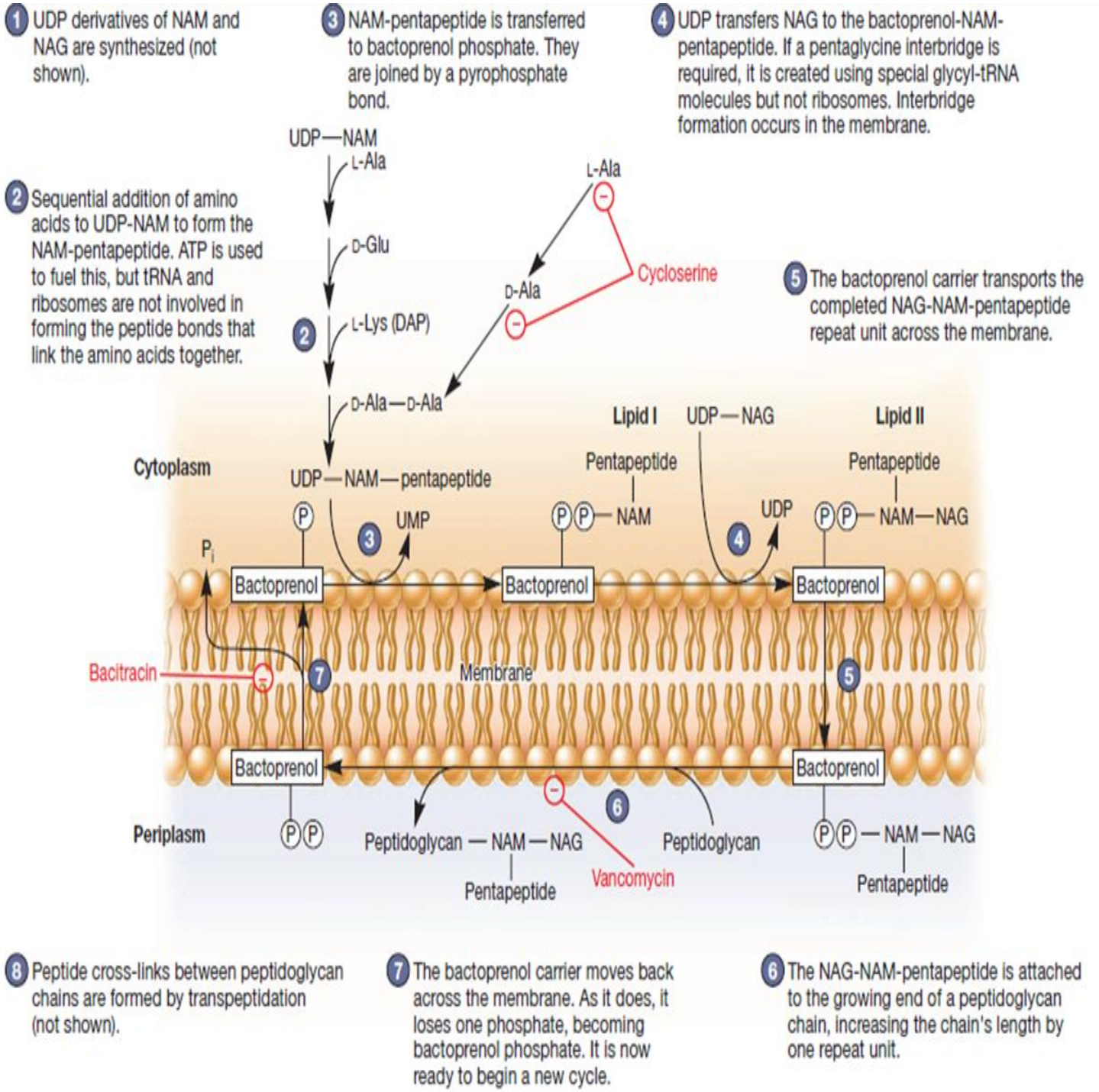


Figure 11.11 Peptidoglycan Synthesis. NAM is *N*-acetylmuramic acid and NAG is *N*-acetylglucosamine. The pentapeptide contains L-lysine in *Staphylococcus aureus* peptidoglycan, and diaminopimelic acid (DAP) in *E. coli*. Inhibition by bacitracin, cycloserine, and vancomycin also is shown. Stage eight is depicted in figure 11.13.

Bacterial cell wall biosynthesis and the steps blocked by antibiotic

1-**Fosfomycin** is bactericidal and inhibits bacterial cell wall biogenesis by inhabit formation glycosidic bound between UDP –NAGA and UDP- NAMA in cytoplasm .

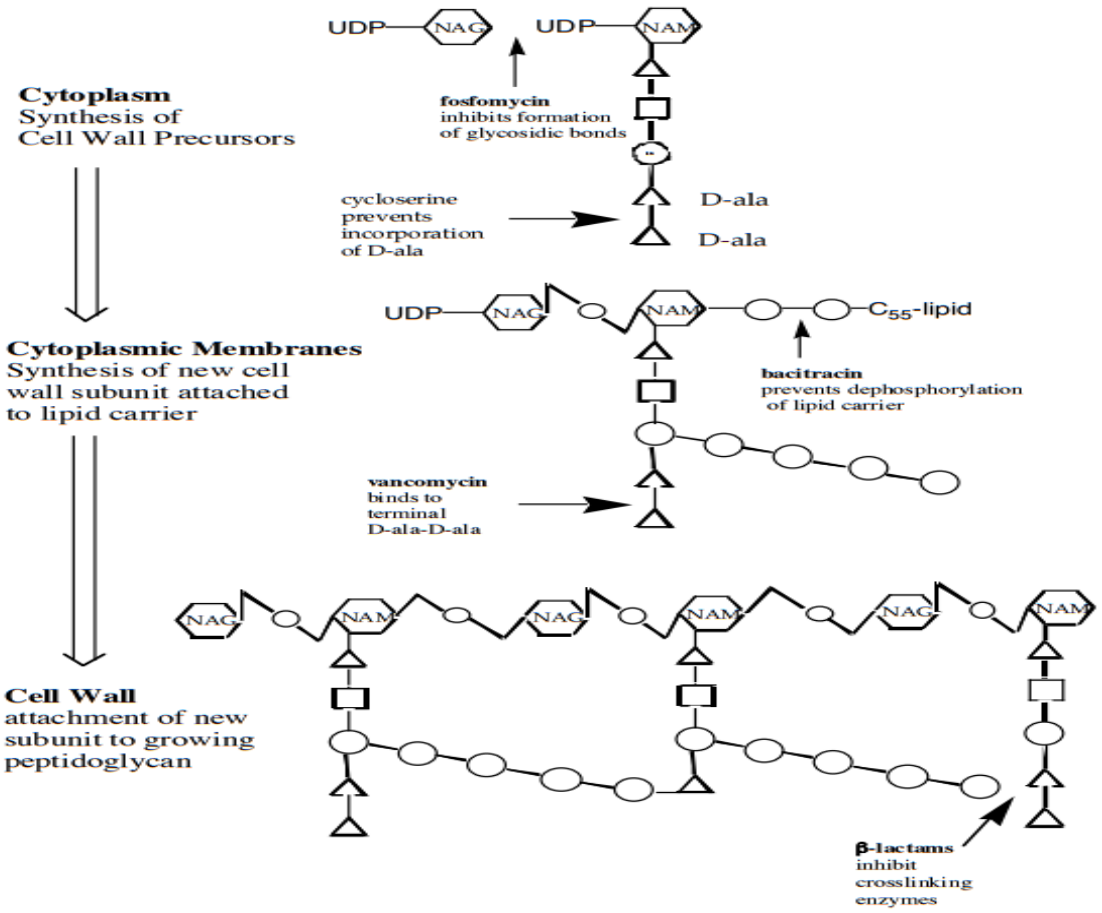
2- The antibiotic **cycloserine** is an analog of D-alanine and interferes with enzymatic conversion of L-alanine to D-alanine in the cytoplasm. Thus, subsequent synthesis of peptidoglycan cannot occur.

3- The peptidoglycan subunit (containing one side-chain and an attached peptide to be used in cross-bridge formation) is passed across the cytoplasmic membrane attached to Bactoprenol diphosphate. After the growing peptidoglycan monomer leaves the carrier on reaching the cell wall, the **Bactoprenol diphosphate is dephosphorylated to its monophosphate form**. **Bacitracin** inhibits the dephosphorylation reaction and in the absence of monophosphorylated carrier peptidoglycan subunit synthesis stops.

4- The final step in peptidoglycan synthesis involves **linking the sugar portion of the peptidoglycan subunit to the glycan backbone of the existing cell wall polymer.** Cross-linking of the peptide portion of the subunit to a peptide in the cell wall then occurs. **During this process D-alanine is enzymatically excised** from the end of a pre-existing peptide side chain allowing it to be cross-linked (by a new peptide bond) to the recently synthesized peptidoglycan subunit. **Vancomycin binds to D-alanine-D-alanine thus inhibits transpeptidation (cross-linking).**

5- **Penicillins also bind to several periplasmic proteins (penicillin-binding proteins, or PBPs)** (PBPs; also known as transpeptidases), which add disaccharide pentapeptides to extend the glycan strands of existing peptidoglycan molecules and cross-link adjacent peptide strands of immature peptidoglycan units, respectively.The beta lactam antibiotics include penicillins (e.g. ampicillin), cephalosporins and monobactams. Thus formation of a complete cell wall is blocked, leading to osmotic lysis. This mechanism is consistent with the observation that penicillins act only on growing bacteria that are synthesizing new peptidoglycan. However, the mechanism of penicillin action is actually more complex.

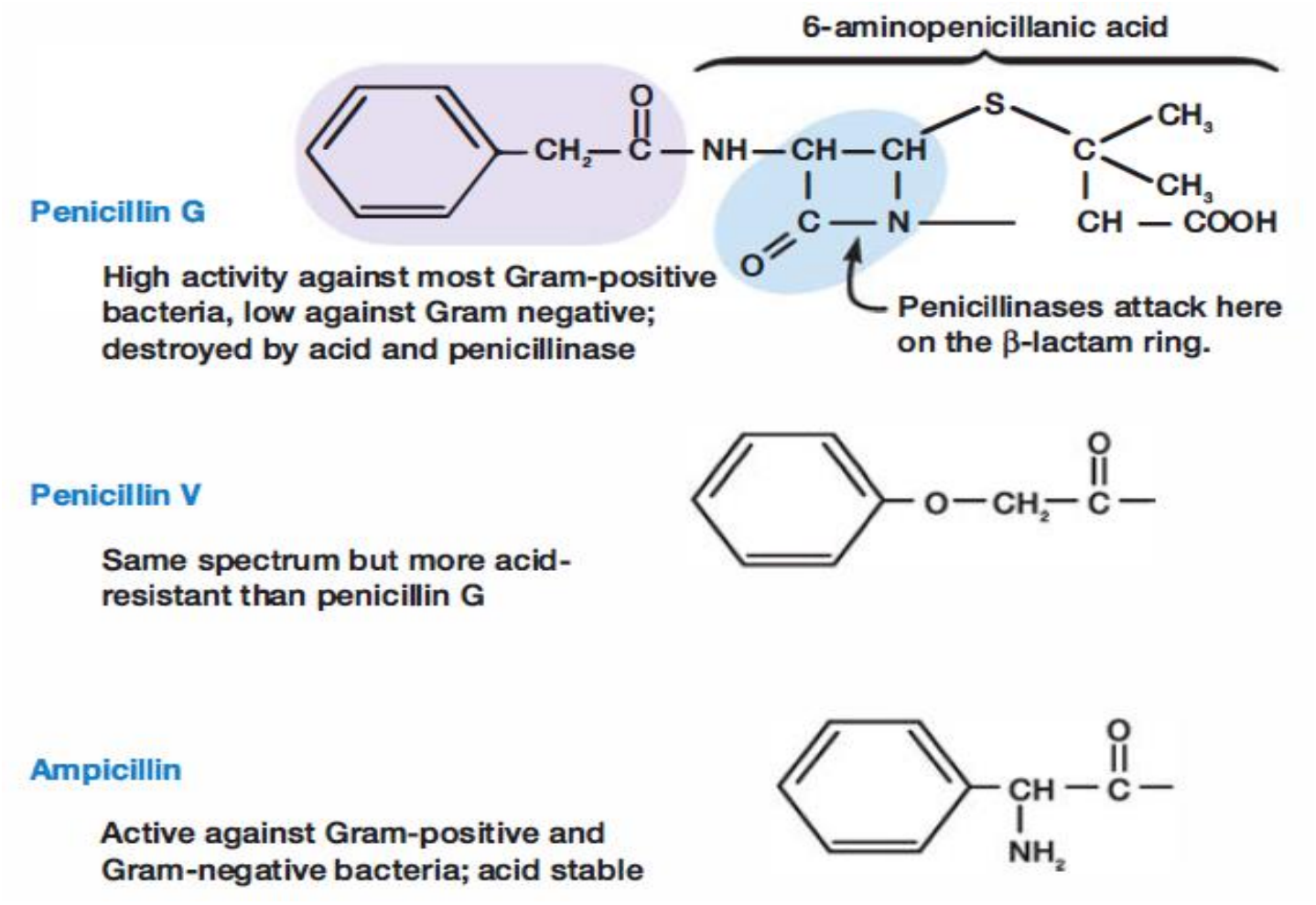
6-penicillins may also destroy bacteria by activating their own **autolytic enzymes**.



Antibacterial Drugs:

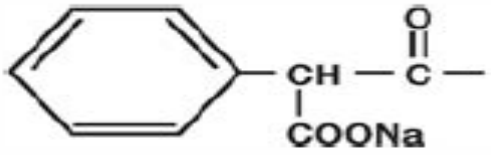
Penicillins

Most penicillins (e.g., penicillin G or benzylpenicillin) are derivatives of **6-aminopenicillanic acid** and differ from one another with respect to the **side chain attached to the amino group** (figure). The most key feature of the molecule is the **β-lactam ring**, which is essential for bioactivity. Many penicillin resistant bacteria produce penicillinases (also called β-lactamases), **enzymes that inactivate the antibiotic by hydrolyzing a bond in the β-lactam ring.**



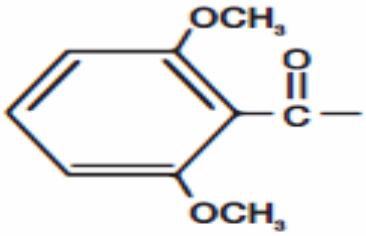
Carbenicillin

Active against Gram-negative bacteria such as *Pseudomonas* and *Proteus;* acid stable; not well absorbed by small intestine



Methicillin

Penicillinase-resistant but less active than penicillin G; acid-labile



Ticarcillin

Similar to carbenicillin but more active against *Pseudomonas*

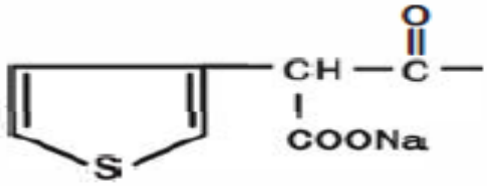


Figure 9.5 Penicillins. The structures and characteristics of representative penicillins. All are derivatives of 6-aminopenicillanic acid; in each case, the shaded portion of penicillin G is replaced by the side chain indicated. The β-lactam ring is also shaded (blue).

Penicillins differ from each other in several ways. The two naturally occurring penicillins, penicillin G and penicillin V, are narrow-spectrum drugs (figure 9.5). Penicillin G is effective against bacteria that cause gonorrhea and some cases of meningitis (the Gram-negative *Neisseria gonorrhea* and *N. meningitis*, respectively), as well as Gram-positive pathogens such as streptococci and staphylococci. However, because of increasing resistance, the Centers for Disease Control and Prevention (CDC) recommends treating gonorrhea with an additional antibiotic.

Penicillin G must be administered by **injection** because it is **destroyed by stomach acid**. Penicillin V is similar to **penicillin G** in spectrum of activity but can be

given orally because it is more resistant to stomach acid. The **semisynthetic penicillins** (as group) have a broader spectrum of activity. **Ampicillin** can be **administered orally** and is effective against Gram-negative bacteria such as Haemophilus (middle-ear infections), Salmonella (gastroenteritis), and Shigella (dysentery). **Carbenicillin** and **ticarcillin** are potent against Pseudomonas and Proteus (wound and respiratory infections).

An increasing number of bacteria have become resistant to natural penicillins and many of the semisynthetic analogues. Physicians sometimes prescribe specific semisynthetic penicillins that are not destroyed by β-lactamases to combat antibiotic-resistant pathogens. These include methicillin (figure 9.5), nafcillin, and oxacillin.

Commonly Encountered Problem when Resistant of Staphylococcal infections (mortality rates are 25% to 63%) **MRSA**: Methicillin Resistant *Staph aureus* and MRSE: Methicillin Resistant Staph epidermis .

Although penicillins are the least toxic of the antibiotics, about 1 to 5% of the adults in the United States develop allergies to them. Occasionally, a person will die of a violent allergic reaction.

Cephalosporins

Cephalosporins are a family of antibiotics originally isolated in 1948 from the fungus Cephalosporium. They contain a β-lactam structure that is very similar to that of the penicillins (figure 9.6). As might be expected from their structural similarities to penicillins, cephalosporins also inhibit the transpeptidation reaction during peptidoglycan synthesis. They are **broad-spectrum** drugs frequently given to patients with penicillin allergies; however, about 10% of patients allergic to penicillin are also allergic to cephalosporins.

Vancomycin

Vancomycin is a **glycopeptide** antibiotic produced by the bacterium ***Streptomyces orientalis***. It is a cup-shaped molecule composed of a peptide linked to a disaccharide. The peptide portion blocks the transpeptidation reaction by binding specifically to the D-alanyl-D-alanine terminal sequence on the pentapeptide portion of peptidoglycan (figure). The antibiotic is bactericidal for the Gram-positive bacteria Staphylococcus and some members of the genera Clostridium (gangrene), Bacillus (food poisoning), Streptococcus ("strep" throat),

and Enterococcus (urinary tract infections). It is given **both orally and intravenously**, and has been particularly important in the treatment of antibiotic-resistant staphylococcal and enterococcal infections. However, vancomycin-resistant strains of Enterococcus **VRE** (mortality rates are 42% to 81%) have become widespread, and cases of resistant *Staphylococcus aureus* have appeared Vancomycin resistance *Staphylococcus aureus* **VRSA**. **Vancomycin resistance poses a serious public health threat**: vancomycin has been considered the "drug of last choice" in cases of antibiotic-resistant *Staph. aureus*.

Teicoplanin

Another **glycopeptide** antibiotic, is produced by ***Actinoplanes teichomyceticus***. It is similar in structure and mechanism of action to vancomycin but has fewer side effects. It is active against staphylococci, enterococci, streptococci, clostridia, Listeria, and many Gram-positive pathogens.

Penicillins side effects

Penicillins are generally very safe drugs with minimum toxicity. Their most **common side effect is** Headaches , diarrhea. Nausea, vomiting, and upset stomach are also common. Vaginal itching and discharge due to either a yeast infection or bacterial vaginosis .Sore mouth and tongue, sometimes with white patches.

In rare cases penicillins can cause immediate or delayed allergic reactions which noticeable as skin rashes, fever, angioedema, and anaphylactic shock. Severe hypersensitivity reactions are more common **after injections than after oral** formulations. **Neurotoxicity** ,very high doses of penicillins, especially in patients with **renal damage, may cause convulsions** .

breast-feeding mothers may pass small amounts of penicillin to their babies. This can result in **allergic reactions**, **diarrhea, fungal infections, and skin rash**.

Interactions - some drugs can interact with penicillin. It is important to check with a doctor before taking multiple medications.

Bleeding problems - some penicillins (carbenicillin, piperacillin, and ticarcillin) can make pre-existing bleeding problems worse.

Oral contraceptives - penicillins can block the effectiveness of oral contraceptives, raising the chances of pregnancy.

Cystic fibrosis - patients with cystic fibrosis are more susceptible to getting fever and skin rash when taking piperacillin.

Kidney disease - patients with kidney disease have an increased risk of side effects.

Phenylketonuria - some stronger, chewable amoxicillin tablets have high levels of aspartame that the body converts to phenylalanine. This is dangerous for anyone with phenylketonuria. (is an inherited disorder that increases the levels of a substance called phenylalanine in the blood).

Gastrointestinal problems - patients with a history of stomach ulcers or other intestinal diseases might be more likely to develop colitis when taking penicillin.

Penicillin allergy

Allergic reactions and interactions

Some patients may develop an allergic reaction to antibiotics , especially penicillins. Side effects might include a rash, swelling of the tongue and face, and difficulty breathing vomiting, light headedness, and low blood pressure. These symptoms typically come on over minutes to hours. Reactions to antibiotics can be very serious, and sometimes fatal, they are called **anaphylactic reactions**. (Anaphylaxis is a serious allergic reaction that is rapid in onset and may cause death).

Allergic reactions to antibiotics can be immediate or delayed hypersensitivity reactions. Anyone who has an allergic reaction to an antibiotic must tell their doctor and/or pharmacist. Around 10 percent of people report an allergy to penicillin but the true number is closer to 1 percent, and only 0.03 percent exhibit life threatening allergic effects.

Alcohol and penicillin Although alcohol does not interact with penicillin, it may alter how effective it is. For this reason, it is not advised that people drink alcohol with penicillin. Alcohol and penicillin also share side effects in some people dizziness and nausea, the side effects could be worsened. It is also worth noting that certain antibiotics do have serious reactions with alcohol, for instance, metronidazole and tinidazole.

Pharmacokinetics

1. All penicillins have short half-lives (0.5-2 h) and must be given 3-4 times per day.

2. Penetration into **cerebral spinal fluid CSF - very low** . Penicillinase-resistant penicillins (methicillin & nafcillin) show very poor penetration. **Also penetrate and crosses the placenta and enters breast milk.**

3. Absorption: Variably absorbed from the gastrointestinal tract. Procaine and benzathine penicillin G intramuscular absorption is delayed and prolonged and results in continuous therapeutic blood levels.
5. Protein Binding: 60%.

6. Metabolism and Excretion: Minimally metabolized by the liver, excreted mainly unchanged by the kidneys.

There are three common ways in which bacteria can develop an resistance to penicillin:

1- **Penicillinase**: sometimes bacteria produce penicillinases (e.g. **beta-lactamase**), enzymes that degrade penicillin. This ability is then transmitted throughout the bacterial group via a plasmid (a small ring of DNA) in a process called conjugation . the bacterial equivalent of sexual reproduction, where new genetic information is shared between individuals.
2- **Altered bacterial structure** : some bacteria slightly change the arrangement of their peptidoglycan wall or the penicillin-binding proteins so that the penicillin can no longer bind to it.
3- **Penicillin removal** : other bacteria develop systems to export penicillin. Bacteria have **efflux pumps (Membrane-associated protein assemblies that transport amphiphilic molecules from cytoplasm to the extracellular environment).** that are used to transport substances out of the cell. Some of these pumps can be repurposed to dispose of penicillin. L-forms??

Protein Synthesis Inhibitors

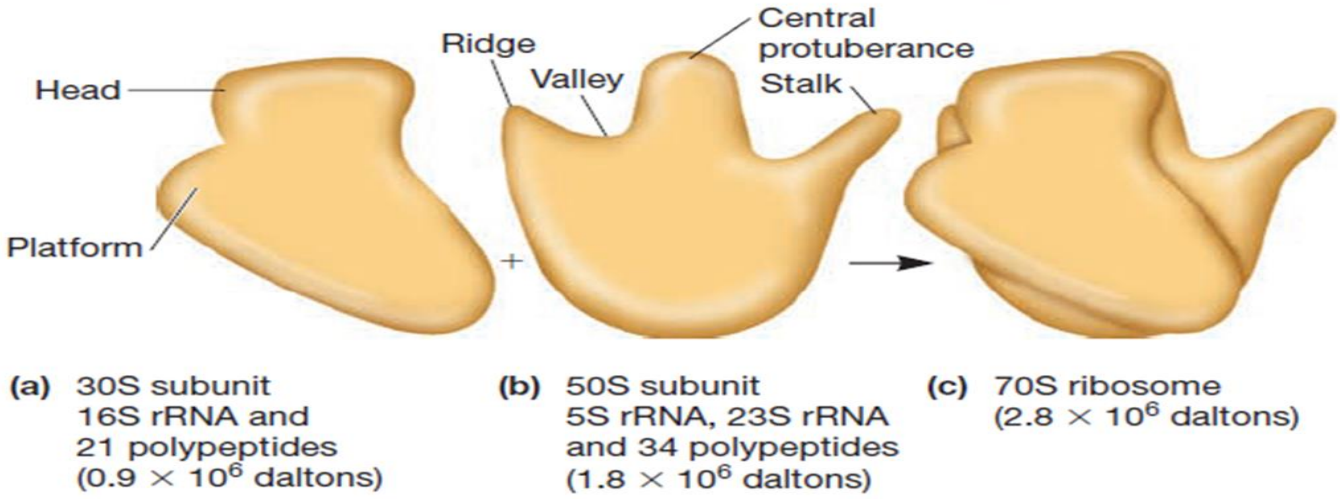
Many antibiotics inhibit protein synthesis by binding with the bacterial ribosome and other components of protein synthesis. Because these drugs distinguish between bacterial and eukaryotic ribosomes, their therapeutic indication is fairly high but not as high as that of cell wall synthesis inhibitors. Several different steps in protein synthesis can be affected by drugs in this group.

Protein Synthesis Inhibition					
Aminoglycosides	Cidal	Bind to small ribosomal subunit (30S) and interfere with protein synthesis by directly inhibiting synthesis and causing misreading of mRNA	Neomycin, kanamycin, gentamicin Streptomycin	Broad (Gram-negative, mycobacteria) Narrow (aerobic Gram-negative)	Ototoxic, renal damage, loss of balance, nausea, allergic reactions
Tetracyclines	Static	Same as aminoglycosides	Oxytetracycline, chlortetracycline	Broad (including rickettsia and chlamydia)	Gastrointestinal upset, teeth discoloration, renal and hepatic injury
Macrolides	Static	Bind to 23S rRNA of large ribosomal subunit (50S) to inhibit peptide chain elongation during protein synthesis	Erythromycin, clindamycin	Broad (aerobic and anaerobic Gram-positive, some Gram-negative)	Gastrointestinal upset, hepatic injury, anemia, allergic reactions
Chloramphenicol	Static	Same as macrolides	Chloramphenicol	Broad (Gram-positive and -negative, rickettsia and chlamydia)	Depressed bone marrow function, allergic reactions

Procaryotic ribosomes are smaller than the ribosomes of eucaryotic cells. Procaryotic ribosomes are called 70S ribosomes (80S in eucaryotes), are constructed of a 50S and a 30S subunit (figure). The S in 70S and similar values stands for Svedberg unit . This is the unit of the sedimentation coefficient, **a measure of the sedimentation velocity in a centrifuge; the faster a particle travels when centrifuged, the greater its Svedberg value or sedimentation coefficient.** The sedimentation coefficient is a function of a particle's molecular **weight, volume,**

and shape. Heavier and more compact particles normally have larger Svedberg numbers and sediment faster.

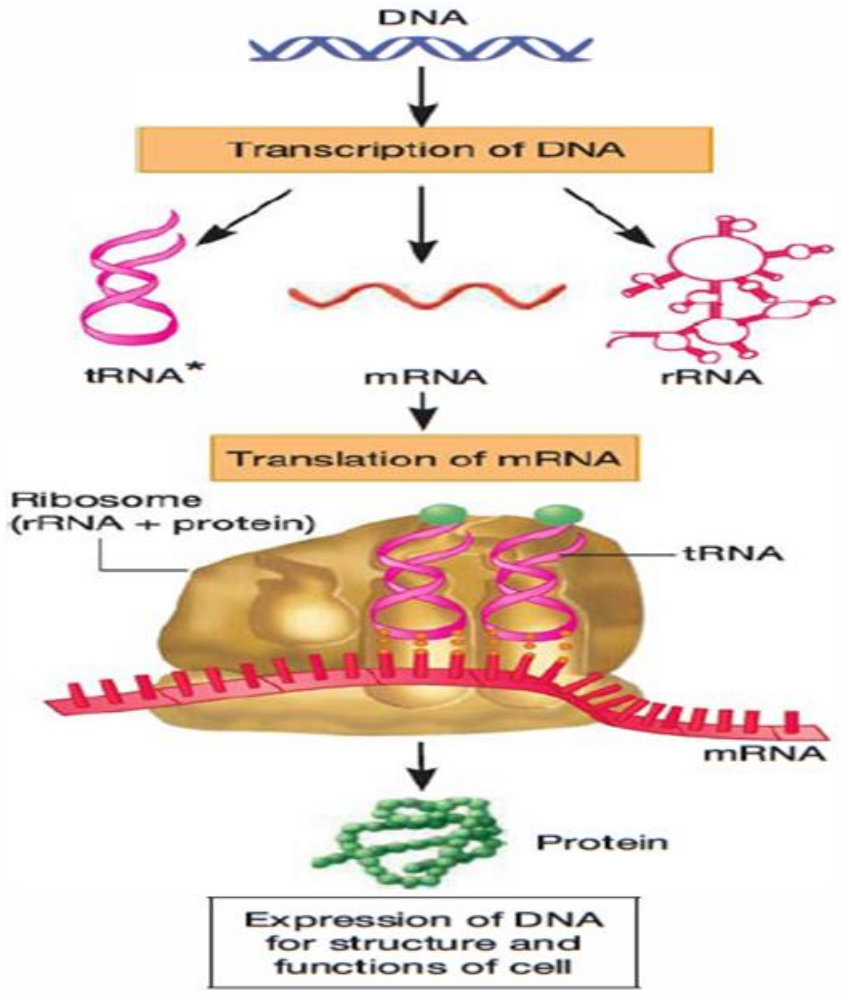
70s ribosomes consists of two subunits small one (30s) compose of (16s rRNA) with 21 (by weight) proteins and the large one (50s) contains proteins (34 by weight) and two molecules of rRNA (23s rRNA, 5s rRNA) is similar to Archaea that contain (23s rRNA, 5s rRNA , and 5.8 s rRNA) and with protein weight 68 is more similar to eukaryotic ribosomes. many antibiotics inhabit Proteins synthesis such as **Gentamycin, Streptomycin bind to 30s, but Chloramphenicol, Erythromycin are bind to 50s subunit** because there is a differences between the ribosomes of prokaryotic and eukaryotic these antibiotics will **only affect bacterial cells without affecting the host cells.**



Transcription and translation

Most of the genes found in bacterial genomes encode proteins. However, DNA does not serve directly as the template for protein synthesis. Rather, the genetic information in the gene is **transcribed** to give rise to **a messenger RNA** (mRNA), which is **translated** into a **protein** . For this to occur, protein-coding genes must contain signals that indicate where transcription should start and stop, and signals in the resulting mRNA that indicate where translation should start and stop. during transcription only one strand of a gene directs mRNA synthesis. This strand is called the template strand, and the complementary DNA strand is known as the **coding strand** because it is the same nucleotide sequence as the mRNA, except in DNA bases. Messenger RNA is synthesized from the 5' to the 3' end in a manner similar to

DNA synthesis. Therefore the polarity of the DNA template strand is 3' to 5 '. In other words, the beginning of the gene is at the 3 ' end of the template strand. The promoter is the binding site for RNA polymerase, the enzyme that synthesizes RNA. and where transcription should begin and then translation to protein .

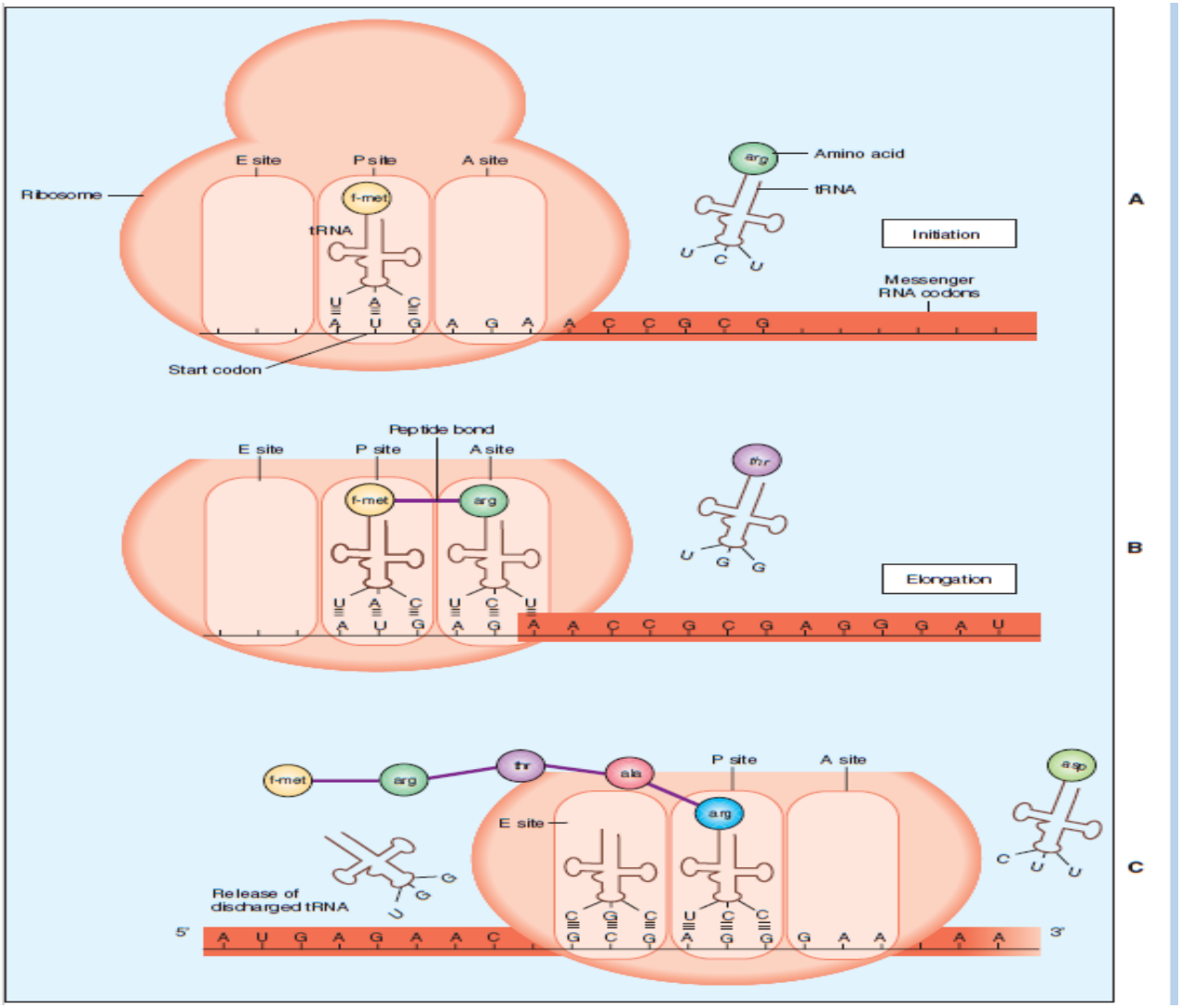


*The sizes of RNA are enlarged to show details.

Figure 13.19 Transcription Yields Three Major Types of RNA Molecules. Messenger RNA (mRNA) molecules arise from transcription of protein-coding genes. They are translated into protein with the aid of the other two major types of RNA: transfer RNA (tRNA) molecules carry amino acids to the ribosome during translation; ribosomal RNA (rRNA) molecules have several functions, including catalyzing peptide bond formation.

The ribosome has three sites for binding tRNAs: (1) the peptidyl or donor site (P site), (2) the aminoacyl or acceptor site (A site), and (3) the exit site (E site). At the beginning of an elongation cycle, the P site is filled with either fMet-tRNA or a tRNA bearing a growing polypeptide chain (peptidyl-tRNA), and the A and E sites are

empty. Messenger RNA is bound to the ribosome in such a way that the proper codon interacts with the P site tRNA (e.g., an AUG codon for fMet-tRNA). The next codon is located within the A site and is ready to accept an aminoacyl-tRNA.

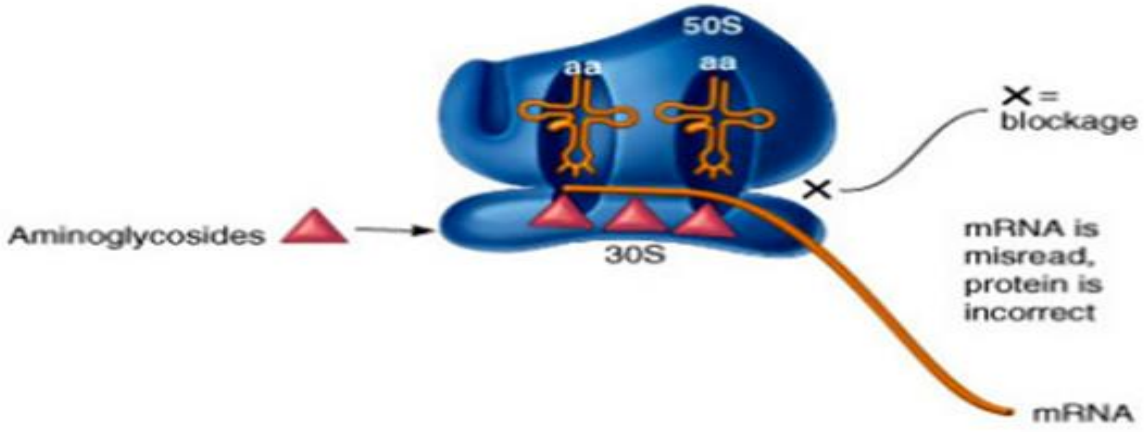


Aminoglycosides

Although considerable variation in structure occurs among several important aminoglycoside antibiotics, all contain a cyclohexane ring and amino sugars (figure 9.8a). **Streptomycin, kanamycin, neomycin, and tobramycin** are synthesized by different species of the bacterial genus **Streptomyces**, whereas **gentamicin** comes from a related genus, ***Micromonospora purpurea***.

Streptomycin's usefulness has decreased greatly due to widespread drug resistance, but it may still be effective when other aminoglycosides should not be used (e.g., due to interactions with other drugs). Gentamicin is used to treat Gram-negative Proteus, Escherichia, Klebsiella, and Serratia infections.

Aminoglycosides bind to the **30S (small) ribosomal subunit** to interfere with protein synthesis(Aminoglycosides interact with the conserved sequences of the 16S rRNA of the 30S subunit near the A site through hydrogen bonds. They cause misreading and premature termination of translation of mRNA).
These antibiotics are **bactericidal** and tend to be most effective **against Gram-negative** pathogens.



It is thought that by binding to bacterial ribosomes, aminoglycosides allow an incorrect amino acid to be brought to the ribosome by transfer RNA (tRNA). This yields a protein with a different amino acid sequence than the normal protein. Any abnormal proteins bound for secretion from the cell are inserted into the plasma membrane The abnormal proteins may be inserted into the cell membrane leading to altered permeability and further stimulation of aminoglycoside transport (figure 9.8b), where they induce changes in metabolic pathways that result in hydroxyl radical formation.

Thus aminoglycosides deliver a two-fold hit: [1]protein synthesis is altered and [2] oxygen radical production increases. It is therefore not surprising that these drugs are **bacteriocidal,** not static.

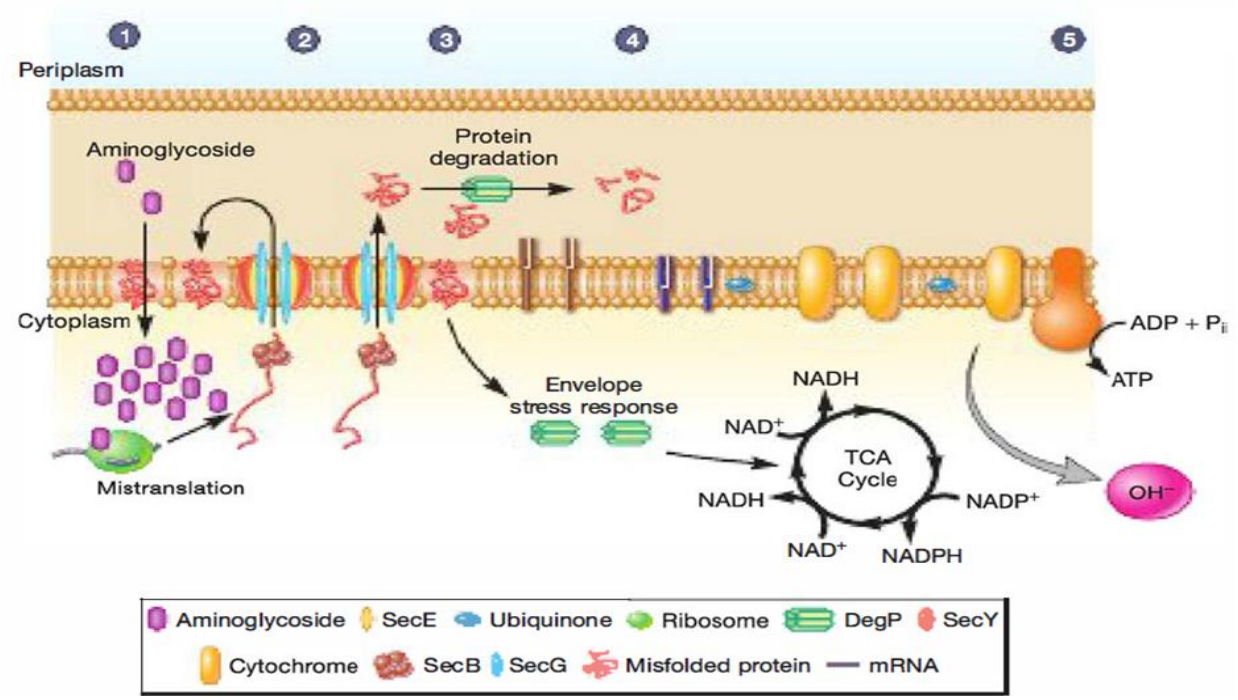


Figure 9.8 Representative Aminoglycoside Antibiotics. (a) Identifying cyclohexane ring and amino sugar.(b) Proposed aminoglycoside killing mechanism. Aminoglycoside (1) enters bacterium, causing mistranslation of bacterial proteins. (2) Mistranslated proteins translocate into periplasm and (3) integrate into the plasma membrane, causing (4) envelope stress that (5) up regulates hydroxyl radical formation.

Side effects

Some side effects are more likely to occur in premature and newborn babies.

upset stomach, throwing up, loss of appetite

Most of the following side effects are not common, but they may be a sign of a serious problem. These side effects may occur even after completed treatment:

loss of hearing, ringing or buzzing in the ears, increased thirst, needing to urinate more or less frequently than usual, skin rash or itchiness, unusual drowsiness, dizziness, or weakness, clumsiness or unsteadiness

Some possible side effects are not common, but they may be a sign of a serious problem :

muscle twitching, seizures, difficulty breathing

Pharmacokinetics

Aminoglycosides are poorly absorbed orally but are well absorbed from the peritoneum, pleural cavity, and joints and from removed skin. Aminoglycosides are usually given intravenous (IV) . Intravenous injection is required to reach levels high enough to treat meningitis.

Aminoglycosides are excreted by kidney filtration and have a serum half-life of 2 to 3 h; the half-life increases exponentially as the glomerular filtration rate(GFR) falls (eg, in renal insufficiency, in the elderly).

Aminoglycoside antibiotics may be given once a day (every 24 hours) or several times a day (for example, every 8 or 12 hours). **Sometimes they are given only every day and a half (every 36 hours) or even less often (once every 2 or more days).**

Resistance

Even when not genetically resistant to antibiotics, bacteria can resist the effects of antibiotics by severely slowing their metabolism, minimizing nutrient uptake and thus antibiotic uptake.

Three general types of enzymes catalyze one of the following modifications of an aminoglycoside molecule (Figure 11-4):

- **Phosphorylation of hydroxyl groups**
- **Adenylylation of hydroxyl groups**
- **Acetylation of amine groups**

Once an aminoglycoside has been modified, its affinity for binding to the 30S ribosomal subunit may be sufficiently reduced or totally lost so protein synthesis is able to continue unchanged .

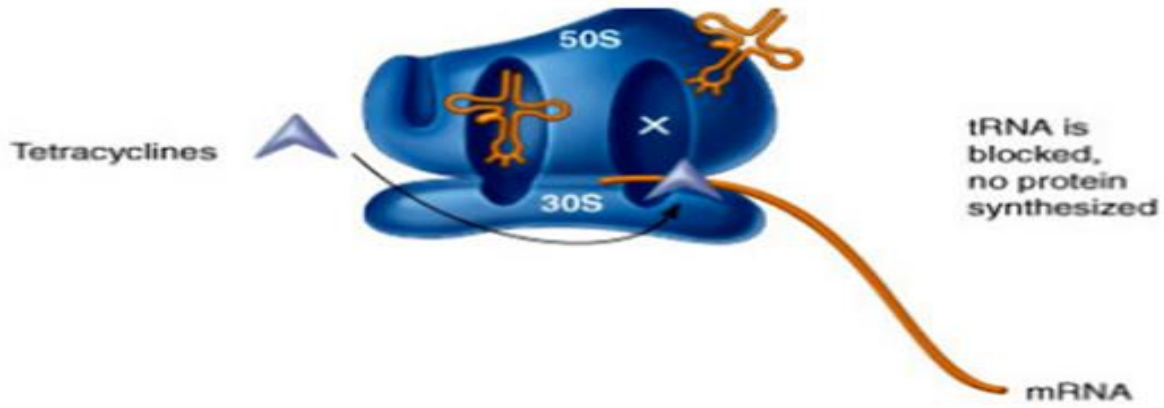
Aminoglycosides enter the gram-negative cell by passing through outer membrane porin channels. Therefore, porin alterations may also contribute to aminoglycoside resistance among these bacteria. Although some mutations that result in altered ribosomal targets have been described, the altered target pathway is thought to be

a rare means for bacteria to achieve resistance to most commonly used aminoglycosides.

Tetracyclines

Oxytetracycline and chlortetracycline are produced naturally by Streptomyces species, whereas other tetracyclines are **semisynthetic**. These antibiotics are similar to the aminoglycosides in that they can combine with the 30S subunit of the ribosome, Periplasm inhibiting protein synthesis. **Their action is only bacteriostatic**, though. Tetracyclines are **broad-spectrum** antibiotics that are active against most bacteria, including the intracellular pathogens rickettsias, chlamydiae, and mycoplasmas.

The tetracyclines interfere with the attachment of the tRNA carrying the amino acids to the ribosome at the 30S portion of the 70S ribosome, preventing the addition of amino acids to the growing polypeptide chain.



They do not interfere with mammalian ribosomes because they do not penetrate very well into intact mammalian cells.

The tetracyclines are a family of antibiotics with a common four-ring structure to which a variety of side chains are attached (figure 9.9).

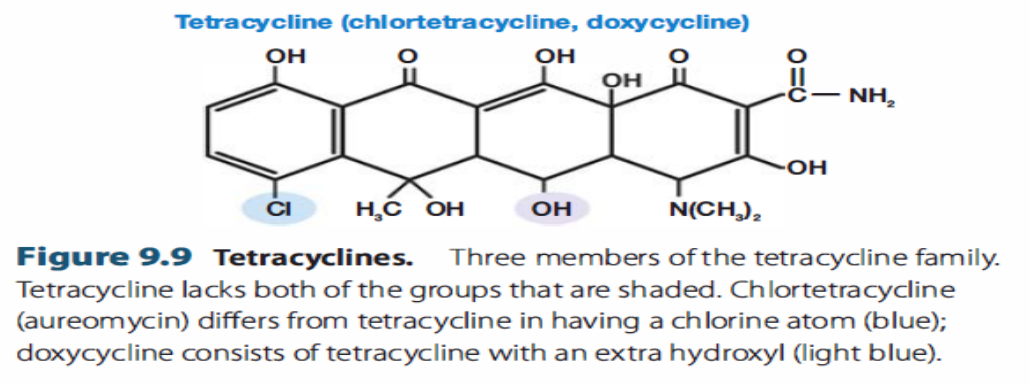


Figure 9.9 Tetracyclines. Three members of the tetracycline family. Tetracycline lacks both of the groups that are shaded. Chlortetracycline (aureomycin) differs from tetracycline in having a chlorine atom (blue); doxycycline consists of tetracycline with an extra hydroxyl (light blue).

Tetracycline is used to treat many different bacterial infections of the skin, intestines, respiratory tract, urinary tract, genitals, lymph nodes, and other body systems. It is often used in treating severe acne, or sexually transmitted diseases such as syphilis, gonorrhea, or chlamydia.

Tetracycline is also used to treat infections you can get from **direct contact with infected animals or contaminated food.**

In some cases, tetracycline is used when penicillin or another antibiotic cannot be used to treat serious infections such as Anthrax, Listeria, Clostridium, Actinomyces, and others.

Pharmacokinetics

Take tetracycline on an empty stomach, at least 1 hour before or 2 hours after a meal.

Do not take this medicine with milk or other dairy products, unless your doctor has told you to. Dairy products can make it harder for your body to absorb the medicine.

Oxytetracycline and tetracycline have half-lives in the range of 6 to 12 hours and are administered two to four times daily. Demeclocycline has a half-life of 16 hours.

Since doxycycline and minocycline are well absorbed and have half-lives of 16 to 18 hours, less frequent and lower doses are possible.

Tetracycline

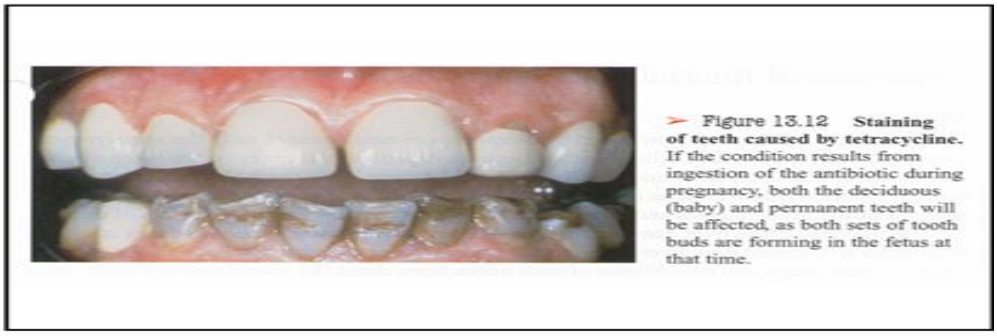
Produced from Streptomyces, Broad spectrum and low cost, Commonly used to treat sexually transmitted diseases. Side effects gastrointestinal disruption, deposition in some tissues .Inhibits proteins synthesis Binds the 30S ribosome and blocks attachment of tRNA.

Tetracycline side effects

Common tetracycline side effects may include:

nausea, vomiting, diarrhea, upset stomach, loss of appetite; white patches or sores inside your mouth or on your lips; swollen tongue, black or "hairy" tongue, trouble swallowing; sores or swelling in your rectal or genital area; or vaginal itching or discharge. Complications with renal disease, Hepatic toxicity usually follows large dose parenteral therapy.

Children can develop permanent brown discoloration of the teeth due to drugdeposition, probably due to its chelating property and the formation of a tetracycline–calcium orthophosphate complex. Therefore, the risk is when the teeth are being calcified . The period of greatest danger is from mid pregnancy to about 4 to 6 months of the postnatal period for the deciduous anterior teeth, and from a few months to 5 years of age for the permanent anterior teeth . However, children up to 8 years old may be susceptible to this complication of tetracycline therapy. Enamel defects and hypoplasia can also occur.



➢ Figure 13.12 **Staining of teeth caused by tetracycline.** If the condition results from ingestion of the antibiotic during pregnancy, both the deciduous (baby) and permanent teeth will be affected, as both sets of tooth buds are forming in the fetus at that time.

Macrolides

The macrolide antibiotics contain a ring structure consisting of 12 to 22 carbons

called a **lactone ring**. The lactone ring is linked to one or more sugars (figure 9.10).

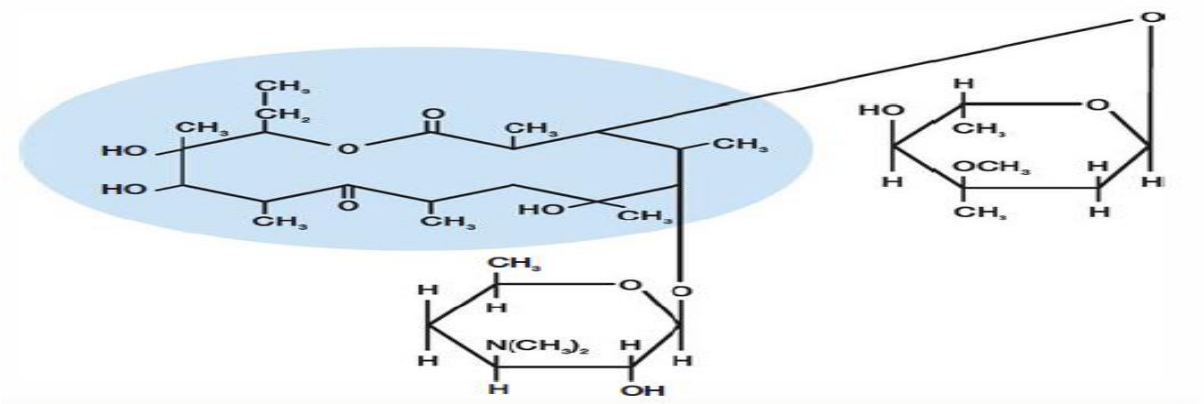


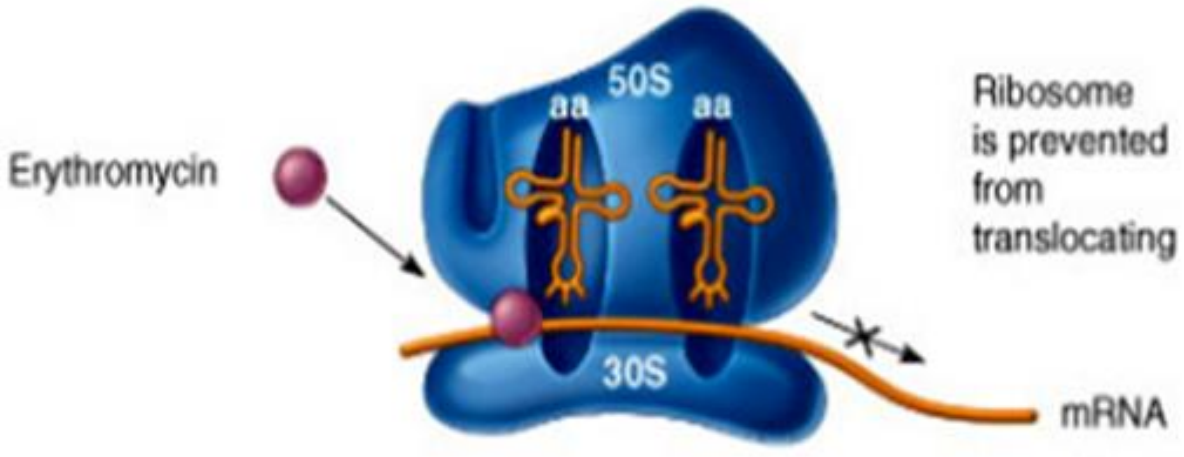
Figure 9.10 Erythromycin, a Macrolide Antibiotic.
Erythromycin is composed of a 14-member lactone ring (shaded), so named because it contains a lactone or cyclic ester group, that is connected to two sugars.

Erythromycin

Produced by Streptomyces • Structure macrolide ring • Commonly used as prophylactic drug prior to surgery • Side effects low toxicity

Erythromycin binds to the 50S ribosomal subunit to inhibit bacterial protein synthesis (**prevents translocation**). Erythromycin is a relatively **broad-spectrum antibiotic** effective against Gram-positive bacteria, mycoplasmas, and a few Gram-negative bacteria, but it is usually only **bacteriostatic**.

It is used with patients who are allergic to penicillin's and in the treatment of whooping cough, diphtheria, diarrhea caused by Campylobacter, and pneumonia from Legionella or Mycoplasma infections.

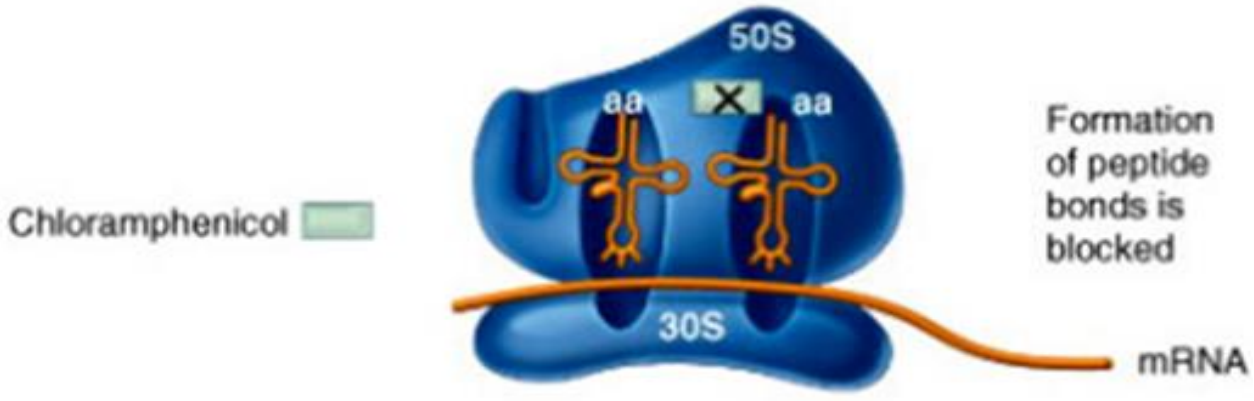


Clindamycin is effective against a variety of bacteria, including staphylococci and anaerobes such as Bacteroides.

Azithromycin (Zithromax), which has bettered erythromycin in use, is particularly effective against many bacteria, including the sexually transmitted *Chlamydia trachomatis.*

Chloramphenicol

Chloramphenicol was first produced from cultures of *Streptomyces venezuelae* **but is now synthesized chemically**. Like erythromycin, this antibiotic binds the **50S ribosomal subunit** to inhibit bacterial protein synthesis (prevents peptide bond formation). It has **a very broad spectrum** of activity but, unfortunately, **is quite toxic**. Consequently this antibiotic is used only in life-threatening situations when no other drug is sufficient. The spectrum includes both aerobes and anaerobes. It can be used topically, orally or parentally. Bioavailability after oral administration is as good as parenteral use and the oral preparation can be used to initiate treatment in emergencies if the injection is not available. Chloramphenicol is not safe in pregnancy and in infant as it may **cause Grey baby syndrome**. This drug can also cause **bone marrow suppression (aplastic anemia)**. Its use as far as possible should be limited to specific indications like typhoid fever, invasive salmonellosis, meningitis, brain abscess and occasionally anaerobic infections.



Cell membrane disruptors

Cytoplasmic membrane

Membranes are an absolute requirement for all living organisms. The **plasma membrane** encompasses the cytoplasm of both procaryotic and eucaryotic cells. It is the chief point of contact with the cell's environment and thus is responsible for much of its relationship with the outside world. The plasma membranes of procaryotic cells are particularly important because they must **fill an incredible variety of roles**. In addition to retaining the cytoplasm, the plasma membrane also serves as a selectively permeable barrier. **Clearly the plasma membrane is essential to the survival of microorganisms. prokaryotic membranes can differ dramatically in terms of the lipids they contain. Indeed, membrane chemistry can be used to identify particular procaryotic species.**

Fluid Mosaic Model of Membrane Structure

The emerging picture of bacterial plasma membranes is one of a highly organized and **asymmetric system that also is flexible and dynamic** (The most widely accepted model for membrane structure is the fluid mosaic model of Singer and Nicholson, which proposes that membranes are lipid bilayers within which proteins float) **.**

The chemical nature of membrane lipids is critical to their ability to form **bilayers**. Most membrane-associated lipids (e.g., the phospholipids) are structurally asymmetric, with polar and nonpolar ends, and are called **amphipathic** (figure). **The polar ends interact with water and are hydrophilic ; the nonpolar hydrophobic ends are insoluble in water** and tend to associate with one another. In aqueous environments, amphipathic lipids can interact to form a bilayer. The outer surfaces of the bilayer membrane are hydrophilic, whereas hydrophobic ends are buried in the interior away from the surrounding water(because of this feature **any broken in membrane will heal automatically)**.

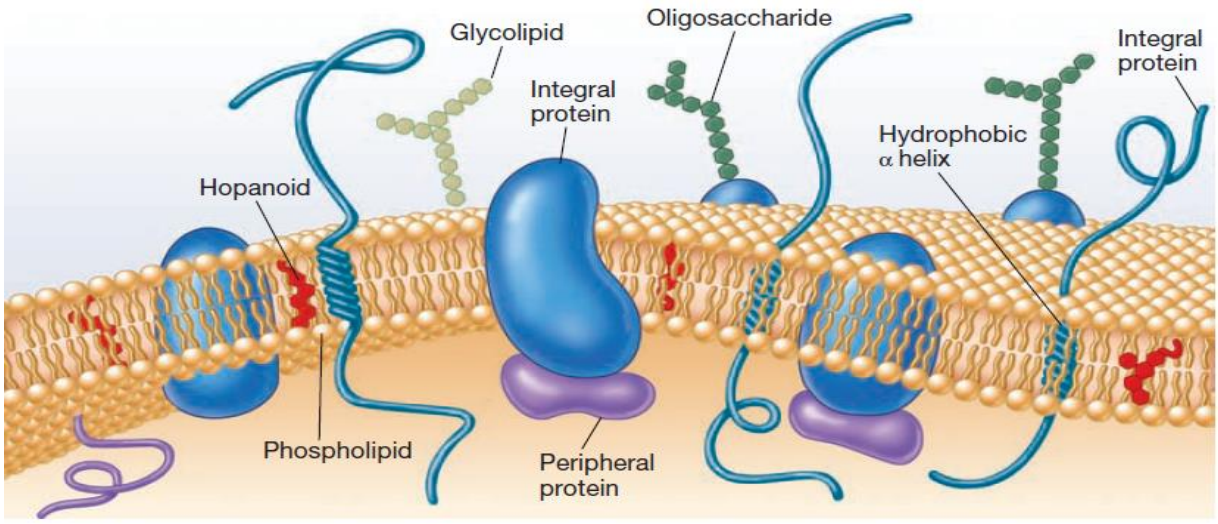


Figure 3.5 The Fluid Mosaic Model of Bacterial Membrane Structure. This diagram shows the integral proteins (blue) floating in a lipid bilayer. Peripheral proteins (purple) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids, and wiggly tails are the hydrophobic fatty acid chains. Other membrane lipids such as hopanoids (red) may be present. For the sake of clarity, phospholipids are shown in proportionately much larger size than in real membranes.

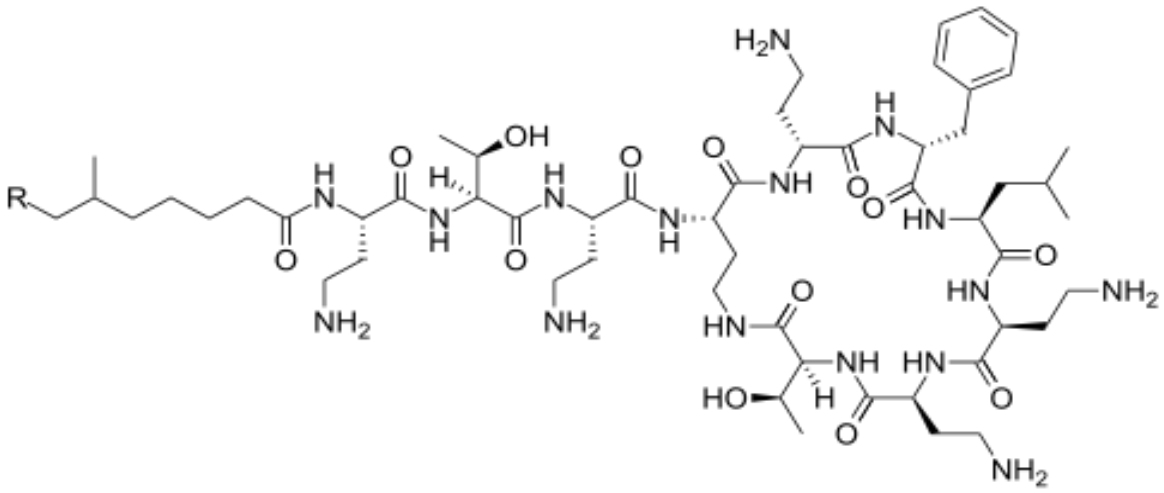
Compounds affected cytoplasmic membrane :

There are some compounds can be associated with the membrane and destroyed it, the Ionophorse is hydrophobic dissolved in lipid that dissolved in membrane lipids and making holes in the membrane, leading to the spread of ions into and out of the membrane , including the antibiotic Valincomycin, Tyrocidin and Polymyxins.

Cell Membrane Disruption					
Polymyxin B	Cidal	Binds to plasma membrane and disrupts its structure and permeability properties	Polymyxin B, polymyxin topical ointment	Narrow—mycobacterial infections, principally leprosy	Can cause severe kidney damage, drowsiness, dizziness

Polymyxin B

Polymyxin B is a **polypeptide bactericidal antibiotic** are a group of cyclic non-ribosomal polypeptide .The polymyxins were discovered in 1947 and introduced to the medical community in the 1950s they are produced by fermentation in different species of *Paenibacillus polymyxa* .Colistin, also called polymyxin E and its parenteral form, are related to polymyxins. Polymyxins can be administered **orally, topically** or parenterally, including intrathecally (in the spinal cord canal) and intraperitoneally. This drug is **toxic to human cells**, since it **can also lyse eukaryotic membranes; this explains its limited clinical use.**



However parenteral administration is primarily used in life threatening infections caused by Gram-negative bacilli or Pseudomonas species that are resistant to other drugs. And multidrug resistant bacteria .

These antibiotics act synergistically with potentiated sulfonamides, tetracyclines and certain other antimicrobials. Polymyxins also limit activity of endotoxins in body fluids and therefore, may be beneficial in therapy for endotoxemia. (namely the prevention of the endotoxin's ability to induce shock through the release of cytokines)

Polymyxin B : **attack the outer membrane of Gram negative bacteria** and the **cytoplasmic membrane of Gram-positive and Gram-negative bacteria**. By binding to the lipid A portion of lipopolysaccharide and also to phospholipids in cell membrane ,**causing loss of membrane integrity, leakage of cytoplasmic**

contents and finally cell death .Since the cell membrane is not exposed in **Gram positive bacteria polymyxin has little activity against** them. (Polymyxins are more effective against Gram-negative than Gram positive bacteria and are effective against all Gram-negative bacteria except Proteus species).

Side effect:

Nephrotoxicity , Neurotoxicity, itching , dermatoses, drug fever, gastrointestinal disturbances and superinfections may develop infrequently during the course of therapy with polymyxins. Leukopenia and granulocytopenia may be possibly associated with the use of colistin. Pain at the site of intramuscular injection or superficial thrombophlebitis may occur.

Resistant:

1- The key initial interaction between the polymyxins and lipopolysaccharides **can be blocked by modification of lipid A .** an increase of the absolute charge of lipid A that lowers the affinity for polymyxins.
2- An efflux pump/potassium system has been found to be associated with the mediation of resistance to cationic antimicrobial peptides in general, including polymyxin B .
3- *K. pneumoniae* increased production of capsule polysaccharide.

Pharmacokinetic

polymyxins accumulate in liver, lung, kidney, heart, and muscles . At 24h, less than 50 % of the doses of polymyxins were bound to tissues; the largest amount was in the skeletal muscle . A plasma protein binding of 55% was reported for colistin .

Metabolic Antagonists

Several valuable drugs act as antimetabolites: they **antagonize, or block**, the functioning of metabolic pathways. The chemical reactions of metabolic pathways are catalyzed by enzymes. Enzymes function by binding a molecule referred to as the enzyme substrate. **Antimetabolites are structurally similar to the substrates of key enzymes and compete with the metabolites for the binding site of these enzymes.** However, once bound to the enzyme, the antimetabolites are different enough to block enzyme activity and further progression of the pathway. By preventing metabolism, they are **broad spectrum but bacteriostatic**; their removal reestablishes the metabolic activity.

Antimetabolites					
Sulfonamides	Static	Inhibit folic acid synthesis by competing with *p*-aminobenzoic acid (PABA)	Silver sulfadiazine, sulfamethoxazole, sulfanilamide, sulfasalazine	Broad spectrum	Nausea, vomiting, and diarrhea; hypersensitivity reactions such as rashes, photosensitivity
Trimethoprim	Static	Blocks folic acid synthesis by inhibiting the enzyme tetrahydrofolate reductase	Trimethoprim (in combination with a sulfamethoxazole)	Broad spectrum	Same as sulfonamides but less frequent
Dapsone	Static	Thought to interfere with folic acid synthesis	Dapsone	Narrow—mycobacterial infections, principally leprosy	Back, leg, or stomach pains; discolored fingernails, lips, or skin; breathing difficulties, fever, loss of appetite, skin rash, fatigue
Isoniazid	Cidal if bacteria are actively growing, static if bacteria are dormant	Exact mechanism is unclear but thought to inhibit lipid synthesis (especially mycolic acid); putative enoyl-reductase inhibitor	Isoniazid	Narrow—mycobacterial infections, principally tuberculosis	Nausea, vomiting, liver damage, seizures, "pins and needles" in extremities (peripheral neuropathy)

Sulfonamides or Sulfa Drugs

Sulfonamides, or sulfa drugs, are structurally related to sulfanilamide, an analogue of p-amino benzoic acid, or PABA (figure 9.11).

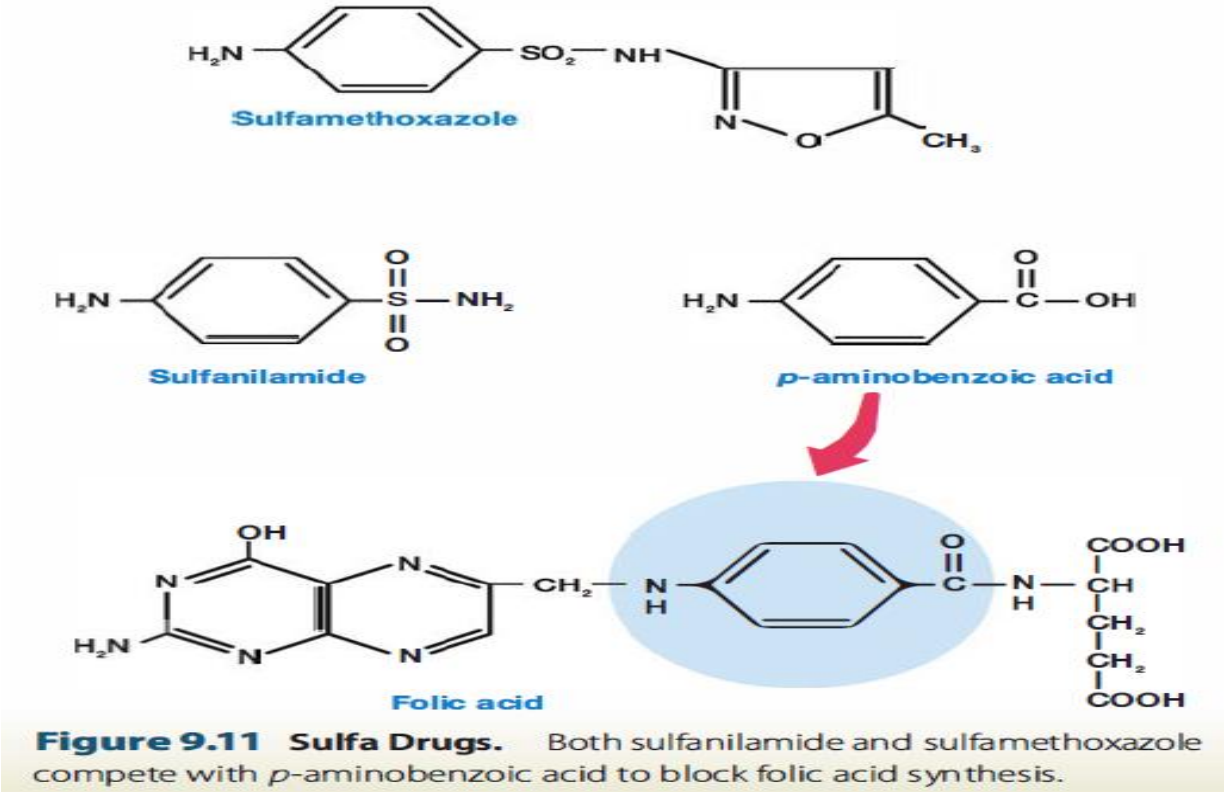


Figure 9.11 Sulfa Drugs. Both sulfanilamide and sulfamethoxazole compete with *p*-aminobenzoic acid to block folic acid synthesis.

PABA is an important component (cofactor) of many enzymes and is needed for folic acid (folate) synthesis. Folic acid is a precursor of **purines and pyrimidines, the bases used in the construction of DNA, RNA, and other important cell constituents (e.g., ATP**). When sulfanilamide or another sulfonamide enters a bacterial cell, it competes with PABA for the active site of an enzyme involved in folic acid synthesis, causing a decline in folate concentration (see figure 10.18).

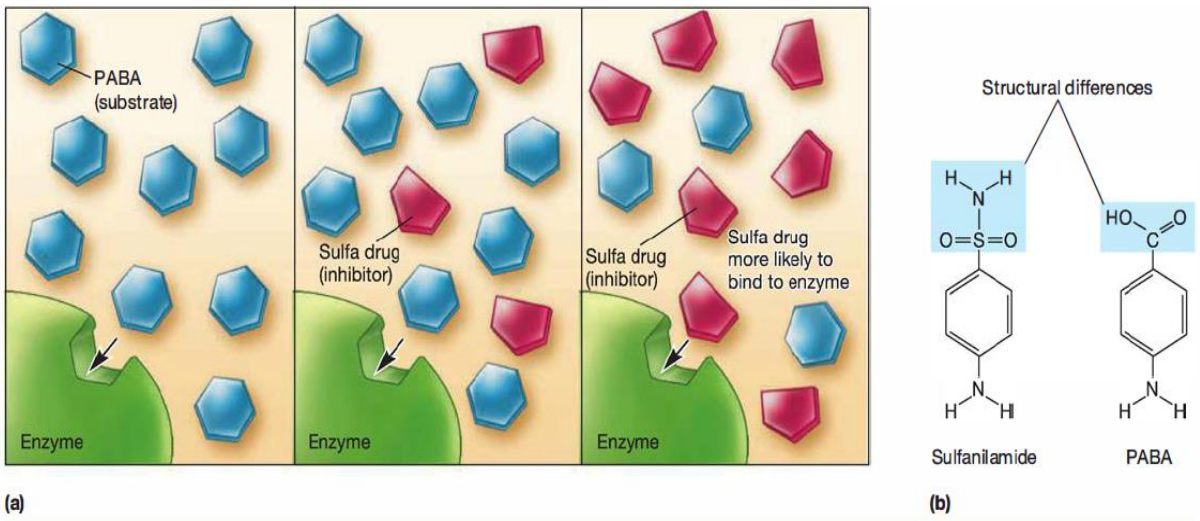


Figure 10.18 **Competitive Inhibition of Enzyme Activity.** (a) A competitive inhibitor is usually similar in shape to the normal substrate of the enzyme and therefore can bind the active site of the enzyme. This prevents the substrate from binding, and the reaction is blocked. (b) Structure of sulfanilamide, a structural analogue of PABA. PABA is the substrate of an enzyme involved in folic acid biosynthesis. When sulfanilamide binds the enzyme, activity of the enzyme is inhibited and synthesis of folic acid is stopped.

The resulting inhibition of purine and pyrimidine synthesis leads to ending of protein synthesis and DNA replication. Sulfonamides are selectively toxic for many bacteria and protozoa because these microbes manufacture their own folate and cannot effectively take up this cofactor, whereas humans do not synthesize folate; instead, we must obtain it in our diet. Sulfonamides thus have a high therapeutic index. However, the increasing resistance of many bacteria to sulfa drugs limits their effectiveness.

Trimethoprim

Trimethoprim is a **synthetic antibiotic** that also interferes with the production of folic acid. It does so by binding to dihydrofolatereductase (DHFR), the enzyme responsible for converting dihydrofolic acid to tetrahydrofolic acid, competing against the dihydrofolic acid substrate (figure 9.12). Trimethoprim is **abroad-spectrum antibiotic** often used to treat respiratory and middle ear infections, urinary tract infections, and traveler's diarrhea.

It is often combined with sulfa drugs to increase effectiveness of treatment by blocking two key steps in the folic acid pathway. The inhibition of two successive steps in a single biochemical pathway means that less of each drug is needed in combination than when used alone. This is termed a synergistic drug interaction.

Nucleic Acid Synthesis Inhibition

The antibacterial drugs that inhibit nucleic acid synthesis function **by inhibiting (1) DNA polymerase and topoisomerases or (2) RNA polymerase, to block replication or transcription, respectively**. **These drugs are not as selectively toxic as other antibiotics because bacteria and eukaryotes do not differ greatly with respect to nucleic acid synthesis.** The most commonly used drugs in this category are the quinolones.

Nucleic Acid Synthesis Inhibition					
Quinolones and Fluoroquinolones	Cidal	Inhibit DNA gyrase and topoisomerase II, thereby blocking DNA replication	Norfloxacin, ciprofloxacin, Levofloxacin	Narrow (Gram-negatives better than Gram-positives) Broad spectrum	Tendonitis, headache, light-headedness, convulsions, allergic reactions
Rifampin	Cidal	Inhibits bacterial DNA-dependent RNA polymerase	R-Cin, rifacilin, rifamycin, rimactane, rimpin, siticox	*Mycobacterium* infections and some Gram-negatives (e.g., *Neisseria meningitidis* and *Haemophilus influenzae* b)	Nausea, vomiting, diarrhea, fatigue, anemia, drowsiness, headache, mouth ulceration, liver damage

Quinolones

The quinolones are synthetic drugs that contain the quinolone ring. They are increasingly used to treat a wide variety of infections.

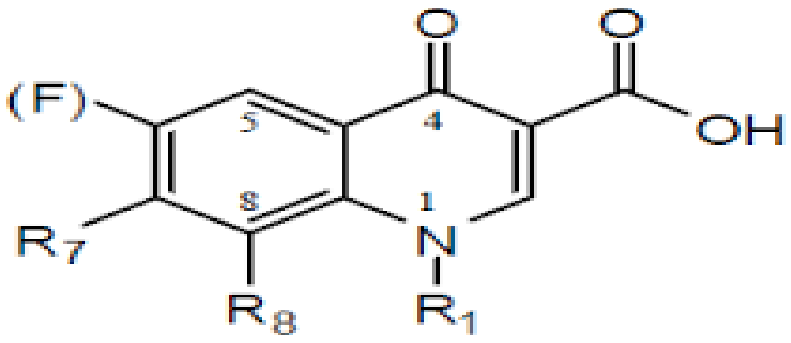


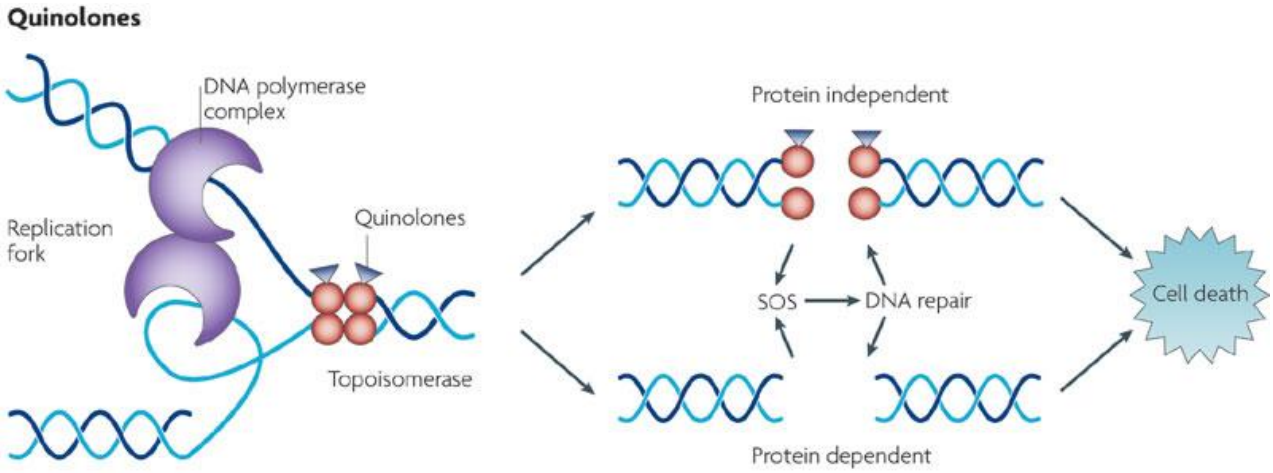
Figure 1: Minimal quinolone structure.

The first quinolone, nalidixic acid, was **synthesized** in 1962. Since that time, generations of fluoroquinolones have been produced. Three of these-ciprofloxacin, norfloxacin, and ofloxacin are currently used in the United States, and more fluoroquinolones are being synthesized and tested.

Quinolones are bacteriocidal drugs, meaning that they kill bacteria. These antibiotic drugs inhibit the bacterial DNA gyrase enzyme which is necessary for DNA replication. Since a copy of DNA must be made each time a cell divides, interfering with replication makes it difficult for bacteria to multiply.

How DNA is packaged is very different in bacteria as opposed to eukaryotes. Bacteria supercoil DNA using DNA gyrase, whereas eukaryotes coil DNA around histone proteins. Because quinolones specifically target (topoisomerase) DNA gyrase, they do not interfere with human DNA.

Fluoroquinolones also inhibit topoisomerase II, another enzyme that untangles DNA during replication. It is not surprising that quinolones are **bactericidal**.



The quinolones are **broad-spectrum antibiotics**. They are highly effective against enteric bacteria such as *E. coli* and *Klebsiella pneumoniae*. They can be used with Haemophilus, Neisseria, *P. aeruginosa*, and other Gram-negative pathogens. The quinolones also are active against Gram-positive bacteria such as *S. aureus, Streptococcus pyogenes*, and *Mycobacterium tuberculosis*. Thus they are used in treating a wide range of infections.

Side Effects of Quinolones

Quinolones typically have few side effects, most commonly including nausea, headache, dizziness, and confusion. Rare but serious adverse events have been reported , phototoxicity, liver enzyme abnormalities, arthropathy (joint problems), as well as cartilage and tendon abnormalities.

Antibiotic Resistance

Resistance to quinolones can develop very quickly, even during a course of treatment. The newer quinolones are rarely used as first-line agents, since overuse and inappropriate use of this class of antibiotic is likely to worsen current problems with antibiotic resistance. A variety of pathogens, including Staphylococcus aureus (pathogenic Staph), Enterococci, and Streptococcus pyogenes (the causative agent of strep throat) now exhibit resistance.

Pharmacokinetics

Food or cations such as calcium, iron, aluminum, magnesium, and zinc may weaken absorption of fluoroquinolones.

Although drug penetration into the central nervous system is minimal, fluoroquinolones distribute to nearly all other body compartments, including the lungs, gallbladder, sputum, bronchi, bones, muscle, genitourinary tissues, lymph, skin, and prostate.

Resistance

Microorganisms develop resistance to fluoroquinolones by altered membrane permeability or through mutations in the DNA-binding region.

Antifungal Drugs

Treatment of fungal infections generally has been less successful than that of bacterial infections largely because as eukaryotes, fungal cells are much more similar to human cells than are bacterial cells. Many drugs that inhibit or kill fungi are therefore **quite toxic for humans and thus have a low therapeutic index.** In addition, most fungi have a **detoxification system that modifies many antifungal agents, limiting drug effectiveness**. Most antifungals are **fungistatic** .a few drugs are useful in treating many major fungal diseases.

Effective antifungal agents frequently either **remove fungal membrane sterols** or **prevent their synthesis**. Similarly, because fungal **cell walls contain chitin**, the **enzyme chitin synthase is the target for antifungals** such as polyoxin D and nikkomycin.

Three drugs containing imidazole (miconazole, ketoconazole and clotrimazole) are **broad-spectrum** agents available as creams and solutions for the treatment of infections such as athlete's foot, and oral and vaginal candidiasis. They are thought to **disrupt fungal membrane permeability** and **inhibit sterol synthesis**.

Nystatin a polyene antibiotic from **Streptomyces**, is used to control Candida infections of the skin, vagina, or intestinal tract. **It binds to sterols and damages the membrane, leading to fungal membrane leakage**.

Systemic fungal infections are very difficult to control and can be fatal. Three drugs commonly used against systemic mycoses are amphotericin B, 5-flucytosine and fluconazole.

Amphotericin B from *Streptomyces* spp. **binds to the sterols in fungal membranes, disrupting membrane permeability and causing leakage of cell constituents**. It is quite toxic to humans and used only for serious, life-threatening infections.

Subcutaneous mycoses are typically treated with **combinations of drugs** that would be used for superficial and systemic mycoses. The goal of this combination therapy is to provide less toxic drugs over longer time periods, with shorter term exposure to those that have greater toxic side effects. In this way, subcutaneous fungi are

continuously targeted with antifungal drugs, while allowing patients breaks from the side effects of the more toxic drugs .

Antiviral Drugs

- Antiviral drugs interfere with critical stages in the virus life cycle (amantadine, rimantadine, ritonavir) or inhibit the synthesis of virus-specific nucleic acids (zidovudine, adenine arabinoside, acyclovir) .
- Drug combinations (cocktails) appear to be more effective than monotherapies.

Because viruses enter host cells and make use of host cell enzymes and constituents, it was long thought that a drug that blocked virus multiplication would be toxic for the host. However, the discovery of inhibitors of virus-specific enzymes and replication cycle processes has led to the development of antiviral drugs. Some important examples are shown in figure .

Most antiviral drugs disrupt critical stages in a virus's multiplication cycle. Probably the most publicized antiviral agent is Tamiflu (generically, oseltamivir phosphate) . Tamiflu (figure) inhibits the viral molecule neuraminidase, which is essential for release of newly synthesized influenza A virus particles from host cells. Thus Tamiflu received much attention during the 2009-2010 H1N1 influenza pandemic. While Tamiflu is not a cure for neuraminidase-expressing viruses, clinical trials show that patients who take Tamiflu within 48 hours of influenza infection are relieved of flu symptoms 1.3 days faster than patients who do not take Tamiflu. Unfortunately, prophylactic use has resulted in viral resistance to Tamiflu.

It is important to recognize that Tamiflu is not a substitute for yearly flu vaccination.

Amantadine (figure) and rimantadine can also be used to prevent influenza A illness. When given within the first 48 hours of infection, these drugs reduce the incidence of influenza by 50% to 70% in an exposed population. Amantadine blocks the penetration and uncoating of influenza virus particles.

Several drugs are commonly used to treat illnesses caused by viruses with DNA genomes. Adenine arabinoside (vidarabine) disrupts the activity of viral DNA polymerase and several other enzymes involved in DNA and RNA synthesis and function. It is given intravenously or applied as an ointment to treat herpes infections (e.g., cold sores and genital herpes).

A third drug, **acyclovir,** is also used in the treatment of herpes infections. Upon phosphorylation, acyclovir also inhibits viral DNA polymerase. Unfortunately, acyclovir-resistant strains of herpes have developed. Effective acyclovir derivatives are now available.

Valacyclovir is an orally administered prodrug form of acyclovir. Prodrugs are inactive until metabolized. Another kind of drug, foscarnet (figure), also inhibits the virus's DNA polymerase, and is very effective at treating illnesses caused by herpes simplex viruses and cytomegalovirus.

Since the early days of human immunodeficiency virus (HIV) treatment, much effort has been focused on developing new drugs. These drugs target and interfere with critical steps in the viral replicative processes (figure 9.16). There are now four categories of drugs used in combination to manage HIV infection:

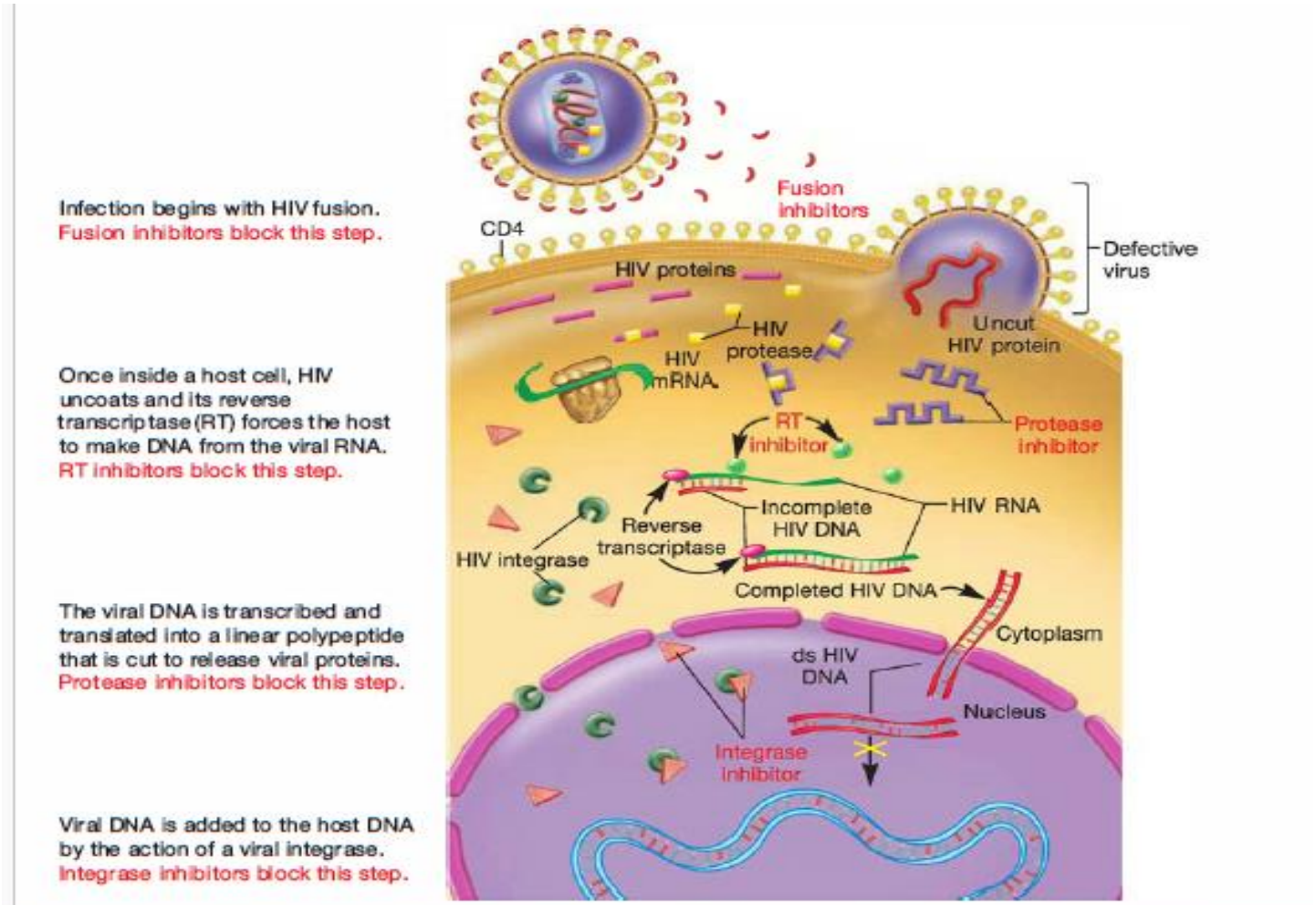
(1) nucleoside reverse transcriptase inhibitors (NRT is) , which are nucleoside analogues that produce faulty viral DNA (e.g., azidothymidine or AZT) ;

(2) non nucleoside reverse transcriptase inhibitors (NNRT is) , which prevent HIV DNA synthesis by selectively binding to and inhibiting the reverse transcriptase enzyme;

(3) protease inhibitors (PIs) , which block the activity of the HIV protease that is needed for the production of all viral proteins; and

(4) fusion inhibitors (FIs) , a relatively new category of drugs that prevent HIV entry into cells (figure 9.16) . Inhibition of reverse transcription, which catalyzes an early step in the multiplication cycle of the virus-the conversion of the virus's RNA genome into double-stranded DNA (figure 9.16), blocks viral DNA synthesis and halts HIV replication. Protease inhibitors are effective because HIV, like many RNA viruses, synthesizes polyproteins that must be cleaved into the individual proteins required for virus replication.

The most successful treatment approach to date in combating HIV/AIDS is to use drug combinations. Most effective is a cocktail of agents given at high dosages to prevent the development of drug resistance. But because HIV can remain dormant in memory T cells, it can survive drug cocktails and reactivate. Thus patients are not completely cured with drug treatment, requiring drug therapy for life. Unfortunately, side effects can be very severe, and treatment is prohibitively expensive for those without medical insurance.



amples of How Anti-HIV Agents Block HIV Replication. Viral infection can be interrupted by correct use of drugs that target spec
e HIV replicative process.

Anti protozoan Drugs

- The mechanisms of action of most drugs used to treat protozoan infection are unknown.
- Some anti protozoan drugs interfere with critical steps in nucleic acid synthesis, protein synthesis, electron transport, or folic acid synthesis.

As with other antimicrobial therapies, antiprotozoan drug development starts by identifying a unique target to which a drug can bind and thus prevent some vital function. However, because protozoa are eukaryotes, **the potential for drug action on host cells and tissues is greater than it is when targeting bacteria**.

Most of the drugs used to treat protozoan infections have significant side effects; even so, the side effects are usually acceptable when weighed against the parasitic problem.

The number of antiprotozoan drugs is relatively small, and the mechanism of action for most of these drugs is not completely understood.

Malaria, caused by any of five species of the genus **Plasmodium**, kills about a million people annually most of them children. Drugs to treat and prevent malaria include **chloroquine** and **qualaquine** to treat malaria . These drugs suppress protozoan reproduction and are effective in eradicating asexual stages of the protozoan's life cycle that occur in red blood cells. Several mechanisms of action have been reported.

Amoebic dysentery is usually treated with **metronidazole.**

Anaerobic organisms, such as the causative agent **Entamoeba**, readily reduce it to the active metabolite within the cytoplasm. A number of **antibiotics that inhibit bacterial protein synthesis are also used to treat protozoan infection.**

These include the aminoglycosides clindamycin and paromomycin. Different aminoglycoside antibiotics bind to different sites on RNAs. RNA binding interferes with the normal expression and function of the RNA, resulting in cell death.

The **antifungal atovaquone** is used to treat **toxoplasmosis,** caused by *Toxoplasma gondii*. Toxoplasmosis is a life threatening infection in immunocompromised individuals and can cause severe birth defects in human fetuses. Atovaquone interferes with eukaryotic electron transport to kill the protozoan parasite. Pyrimethamine and dapsone are also used to treat Toxoplasma .

New approaches for developing effective antimicrobials drugs

Increase drug resistance requires new approaches:

1- Prevent iron scavenging abilities.

One of the first lines of defense against bacterial infection is the withholding of nutrients to prevent bacterial outgrowth in a process termed nutritional immunity. The most significant form of nutritional immunity is the sequestration of nutrient iron . The vast majority of vertebrate iron is intracellular, sequestered within the

iron storage protein ferritin or complexed within the porphyrin ring of heme as a cofactor of hemoglobin or myoglobin. Further, the aerobic environment and neutral pH of serum ensures that extracellular iron is insoluble and hence difficult to access by invading pathogens. This difficulty is enhanced by the serum protein transferrin, which binds iron with an association constant of approximately 10^{36} . Taken together, these factors ensure that the amount of free iron available to invading bacteria is vastly less than what is required to replicate and cause disease.

2- Inhibit genetic controls (riboswitches)

Aptamers are artificial nucleic acids that selectively bind small molecules. it has become clear that nature has already devised its own aptamers that play important regulatory roles. RNA sensors have been discovered in both Gram-positive and Gram-negative bacteria that function as molecular switches in response to direct binding of structurally diverse metabolites. These natural RNA aptamers, called 'riboswitches', are imbedded in the leader sequences of numerous metabolic genes. Riboswitches are able to repress or activate their related genes at both transcriptional and translational levels.

a new class of regulatory RNA that needs the same principles of alternative structure formation to control transcription elongation and translation initiation depending on the metabolic status of the cell. The uniqueness of these RNA systems is that they do not require any intermediary sensory molecules (i.e. protein factors or tRNA) to govern the attenuation process; they behave as sensors of small molecules themselves. Such natural RNA aptamers, also known as riboswitches, appear to control expression of a wide spectrum of metabolic genes in bacteria and possibly in higher organisms as well.

3- Probiotics and prebiotics : Recent advances in understanding the human gut microbiome have demonstrated that specific microbial products, often attributed to these bacteria, have a profound influence on immune system function. The lactobacilli and bifidobacteria have been shown to stimulate immune maturation and minimize inflammation. Other health benefits attributed to the consumption of fermented milks involve minimizing lactose intolerance, lowering serum cholesterol, and possibly protecting against colon cancer. Several lactobacilli have antitumor compounds in their cell walls. While these "good" bacteria cannot numerically overtake entrenched gut microflora, they

have been shown to change the metabolism of other microorganisms in the gut, such that sugars from fruits and vegetables are more thoroughly catabolized.

Prebiotics : Prebiotics are food ingredients that induce the growth or activity of beneficial microorganisms (e.g., bacteria and fungi). The most common example is in the gastrointestinal tract, where prebiotics can alter the composition of organisms in the gut microbiome.

In diet, prebiotics are typically non-digestible fiber compounds that pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous bacteria that colonize the large bowel by acting as substrate for them. They were first identified and named by Marcel Roberfroid in 1995. As a functional food component, prebiotics, like probiotics, are conceptually intermediate between foods and drugs. Prebiotics are also present in mother's milk. These human milk oligosaccharides are thought to enhance the population of bifidobacteria present in the infant gut as well as discourage pathogens that may cause infections in infants. Many brands of infant formula are now supplemented with oligosaccharide prebiotics to mimic this effect.

4- Phage therapy :Bacteriophages are viruses that infect bacteria their name translates as 'bacteria eaters'. Until recently, they received little attention from Western doctors widely available and effective antibiotics were much easier to use. In the former Soviet Union, however, access to cutting-edge antibiotics was severely limited, and some scientists used bacteriophages to treat many infections.

Voluntarily letting bacterial viruses into our body is an unpleasant idea for many of us, even if they kill pathogenic bacteria this in part is why phage therapy has been slow to take off in Western countries. With antibiotic resistance becoming an ever more real issue, though, the US National Institute of Allergy and Infectious Diseases is planning large scale clinical trials of phage-based therapies .

One advantage of bacteriophages over antibiotics is their availability:

thought to be the most abundant organisms on Earth, they are so diverse that no two identical phages have ever been found. This means that the bacterial hosts and phage co-evolve so when bacteria become resistant to a phage the

phage will often evolve to re-infect it. Because of this, phage are described as 'bacteria specific'. Of course, there are difficulties that need to be addressed before bacteriophages can progress beyond the experimental stage.

While phage therapy is unlikely to completely replace antibiotics, scientists can imagine it being used on topical infections as an alternative therapy in cases where antibiotics have proved ineffective.

5- Antivirulence drugs

Traditional antibiotics inhibit the growth of bacteria or kill them outright. A novel class of drugs called **antivirulence drugs will inactivates the specific proteins the bacterium uses to attach to our cells, preventing it from establishing an infection**.(block biofilm formation the first step of infection) .

Because antivirulence drugs 'disarm' rather than kill bacteria, they may not drive development of antibiotic resistance because susceptible organisms can still pass on their genetic material: resistance is not selected for. A study of antivirulence drugs has shown that drug resistant bacterial strains will not come to dominate susceptible ones; this means that the drug can remain effective . An antivirulence drug has recently been found to be effective against MRSA infections in mice .

MRSA is a dangerous strain that causes infections in hospitals, care homes and even in gym locker rooms, so to find a drug that is effective against this bacterium is a positive step forward.

6-Bacteriocins

Bacteriocins are **proteins** produced by bacteria that are **toxic to similar or closely related bacteria**. Essentially, they are narrow-spectrum antibiotics that bacteria produce to eliminate competitors. Bacteriocins that attack pathogens and are produced by bacteria that are harmless to us would make ideal antibiotics.

A number of bacteriocins are now being studied for potential use as antibacterial medication. They are also increasingly used to prevent the growth of dangerous bacteria in food, extending shelf life and delaying food spoilage . One example is nisin, which is approved and used in food production and is known as E234.

(Affected cell wall biosynthesis & cell membrane disruption)

7-Combination therapy Using multiple therapies (4 or more medications at once) to treat a *single* disease . Conditions treated with combination therapy include tuberculosis, leprosy, cancer, malaria, and HIV/AIDS. One major benefit of combination therapies is that they reduce development of drug resistance

8- Nanotechnology

Nanotechnology has been defined by the U. S. National Nanotechnology Initiative (NNI) as "understanding and control of matter at dimensions of roughly 1 to 100 nm where unique phenomena enable novel applications". the nanometer scale (1-100 nanometers, one nanometer being equal to 1 x 10^{-9} of a meter), that is, at the atomic and molecular levels, and the exploitation of novel phenomena and properties of matter at that scale". Several applications of nanotechnology are being developed.

Nanoparticles

nanoparticles make existing antibiotics more effective against heavily ntibiotic-resistant microbes. While scientists continue to search for new antibiotics, light activation is giving new life to existing drugs that lose their effectiveness against antibiotic-resistant microbes.

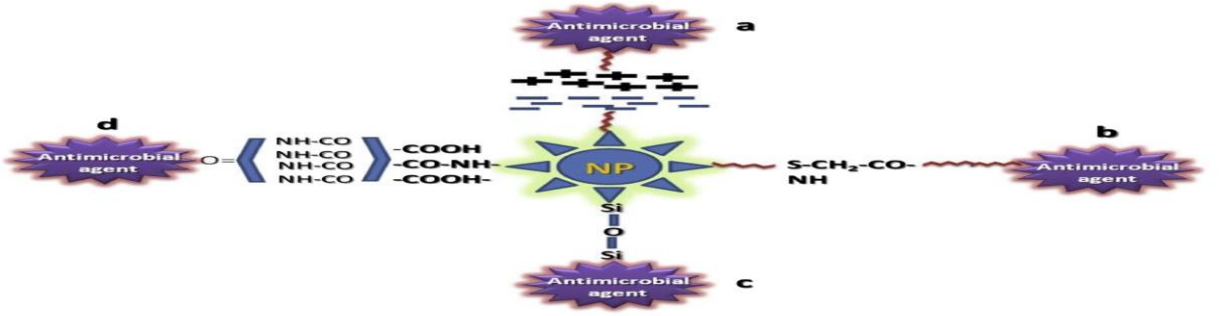
The findings are important because antibiotic-resistant infections will kill about 230 million people by 2050 and cost the global economy $100 trillion, according to *Review on Antimicrobial Resistance*, a report commissioned several years ago by the U.K. prime minister.

Because bacteria mutate frequently and exchange genetic information readily with other bacteria, antibiotic-resistant variants that cannot be treated with conventional medicines could spread rapidly.

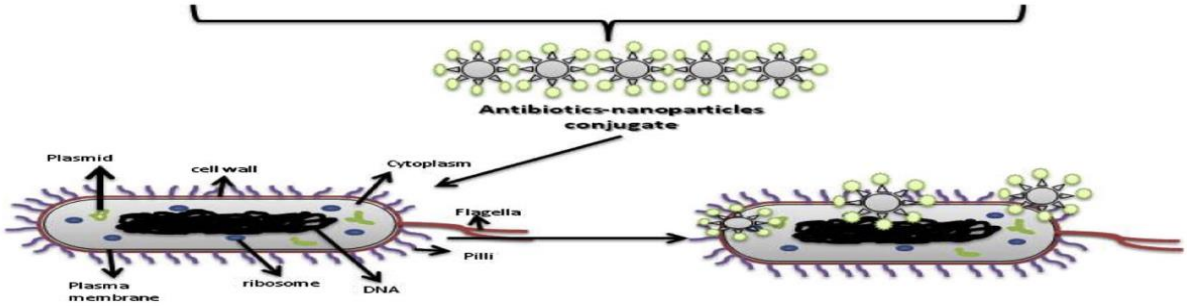
Several characteristics in particular make nanoparticles strong candidates as a traditional antibiotic drug alternative. **Firstly,** they have a high surface area to volume ratio, which increases contact area with target organisms . **Secondly**, they may be synthesized from polymers, lipids, and metals. **Thirdly**, different chemical structures, such as fullerenes and metal oxides, allow for a diverse set of chemical functionalities.

The key to nanoparticle efficacy against antibiotic resistant strains of bacteria lies in their small size. On the nano scale, particles can behave as molecules when interacting with a cell which allows them to easily penetrate the cell membrane and interfere in vital molecular pathways if the chemistry is possible.

Silver nanoparticles improve the activity of amoxicillin, penicillin, and gentamicin in bacteria by altering membrane permeability and improving drug delivery. nanoparticles themselves may have antimicrobial properties enhanced or induced with the addition of organic drugs. **Gold particles**, while not inherently antimicrobial, were discovered to express antimicrobial properties when functionalized with ampicillin.



(a) Electrostatic interaction, **(b)** Bifunctional linkage, **(c)** Silinization **(d)** Lipophilic interaction



- **Forming pit on cell wall**
- **Interference in DNA synthesis**
- **Inhibit respiratory chain enzymes**

Liposomes

Despite the discovery of new antibiotics, treatment of intracellular infections often fails to eradicate the pathogens completely. One major reason is that many antimicrobials are difficult to transport through cell membranes and have low activity inside the cells, thereby imposing negligible inhibitory or bactericidal effects on the intracellular bacteria . In addition, antimicrobial toxicity to healthy tissues poses a significant limitation to their use . Therefore, the delivery of the drug to the bacterial cells is currently a big challenge to the clinicians. This is on top of the problems posed by the emerging Multi-Drug Resistant species. Moreover, the reduced membrane permeability of microorganisms has been cited as a key mechanism of resistance to antibiotics.

Liposomes are composed of small vesicles of a bilayer of phospholipid, encapsulating an aqueous space ranging from about 30 to 10000 nm in diameter . They are composed of one or several lipid membranes enclosing discrete aqueous compartments. The enclosed vesicles can encapsulate water-soluble drugs in the aqueous spaces, and lipid soluble drugs can be incorporated into the membranes. They are used as drug carriers in the cosmetic and pharmaceutical industry. The main routes of liposome administration are parenteral, topical and inhalation, and, in a few occasions, possibly other routes of administration can be used. Majority of current products are administered parenterally.

Antibiotics

4th Microbiology Dr. Sahar AL Saleem

Introduction:

In 1910, Paul Ehrlich a German Physician , developed The first antimicrobial salvarsan for the treatment of syphilis

In 1939, Domagk (German chemist) received Nobel prize in medicine for his discovery of sulfonamides or sulfa drug .

In 1928, Alexander Fleming , British scientist was working with Cultures of *staphylococcus* when he notice that colonies growing near contaminating mold and there were no bacterial colonies surrounding it.

He identified The mold as a species of *Penicillium* and showed that it was producing a bacteria _ killing substances , he called this penicillin.

In 1939, Howard Florey and Ernst chain were successful in their attempt to purify penicillin and injected in to mice infected with streptococci or staphylococci almost all the mice survived .

In 1945, Fleming Florey and chain received the Noble prize for the discovery and production of penicillin which stimulated the search for other antibiotics.

In 1944, Selman Waksman fund anew antibiotic streptomycin by Streptomyces griseous it was the first drug to successfully treat tuberculosis.

This discovery arose from the careful screening of about 10,000 strains of soil bacteria and fungi.

His success led to a worldwide search for other antibiotic producing soil microorganisms , chloramphenicol , neomycin , oxytetracycline.

Development of new generation of drugs :-

In 1960s, scientists discovered that they could alter the chemical structure of drugs such as penicillin G ,and give them new properties , for example ,penicillin G ,which is mostly active against Gram-positive bacteria ,can be altered to produce ampicillin , a drugs that kills additional Gram-negative bacteria species as well .

Other change to penicillin created the drug methicillin ,which is less susceptible to enzymes used by some bacteria to inactivate penicillin.

All of these derivatives retain the core portion of penicillin G ,called 6-aminopenicillanic acid (6-APA) .

Definition of some terms :-

Antibiotic: A compound that is naturally produced by certain molds and bacteria that inhibits the growth of or kills other microorganisms .

Chemotherapeutic agent : Any chemical that used is used to treat a disease .

An antimicrobial drug : A chemical that inhibits the growth of or kills microorganisms. This term similar to an antibiotic but my be synthesis .

Features of Antimicrobial Drugs .

Most modern antibiotics come from microorganisms that normally reside in the soil ,these include species of the bacteria *Streptomyces* and *Bacillus* and the fungi *Penicillum* and *Cephalosporium* ,to produce an antibiotics a carefully selected strains of the appropriate species .

Medically useful antimicrobial drugs has :-

Selective Toxicity ;it kills or inhibit the microbial pathogen while damaging the host as little as possible ,antimicrobial that disrupts a microbial structure or function not found in host cells often has greater selective toxicity and higher therapeutic value ,for example ,Penicillin inhibit bacterial cells wall peptidoglycan synthesis but has little effected on host cells because they lack cell wall .

The toxicity of a given drugs is expressed as the Therapeutic index the drug level required for clinical treatment of particular infection.

Antimicrobial action ; Antimicrobial drugs may either kill microorganisms or inhibit growth their growth those that inhibit growth are called bacteriostatic these drugs depend on the normal host defense , during that kill bacteria are bactericidal these drugs are particularly useful in situations in which the normal host defense can't be relied on to remove or destroy pathogens.

Classification of Antimicrobial Drugs :-

A-Classification of antibiotic depending on their range of effectiveness:

1-Broad-spectrum antibiotics.

2-Narrow-spectrum antibiotics.

Many are narrow-spectrum drugs ,they are effective only against a limited variety of pathogens.

Antimicrobial that affect a wide range of bacteria these are very important in the treatment of acute life threatening disease.

B-Classification of antibiotic depending on Manufacture methods :

1- Natural antibiotics : produced by fermentation (some bacteria and fungi naturally produce many of the commonly active antibiotics)

2- semi-synthetics : some antibiotics are semisynthetic-natural antibiotics that have been structurally modified by the addition of chemical groups to make them less susceptible to stomach acids inactivation by pathogens (e.g., ampicillin and methicillin).

3- synthetic : several important chemotherapeutic agents such as sulfonamides ,trimethoprim, ciprofloxacin are synthetic manufactured by

chemical procedures (independent of microbial activity).

C-Classification of antibiotic to their function (mode of action):

1- inhibitors of cell wall synthesis.
2- cell membrane disruption.
3- protein synthesis inhibitors.
4- Nucleic Acid synthesis inhibition.
5- Metabolic Antagonists.

D-Classified based on the general microbial group they act against:

1- Antibacterial
2- Antifungal
3- Antiprotozoan
4- Antiviral ----- anti cancer

Factors Influencing Antimicrobial:-

1-Tissue Distribution ,Metabolism, and Excretion of the drug:

Antimicrobial differ not only in their action and activity, but also in how they are distributed ,metabolized ,and excreted by the body .For example ; only some drugs are able to cross from the blood into the cerebrospinal fluid .Drugs that are unstable in acid are destroyed by stomach acid when taken orally ,and so these drugs must give intravenous or intramuscular injection .

2- Effect of Combination of Antimicrobial Drugs:

Combination of antimicrobial are sometimes used to treat infections , but care must be taken when selecting the combinations because some drugs will counteract the effects of other .

When the action of one drug enhances the activity of another ,the combination is called **Synergistic** ,Combinations in which the activity of one interferes with the other are called **Antagonistic** , Combination that are neither synergistic nor antagonistic are called **Additive**.

3-Side Effects:

a-Allergic Reactions:

Penicillin or other related drugs usually results in a fever or rash ,but sometimes can cause **Life-threating anaphylactic shock .**

b- Toxic Effects:

Several antimicrobial are toxic at high concentration or cause adverse reactions .For example ,aminoglycosides can damage kidneys .

c-Suppression of Normal Flora:

The normal flora plat an important role in host defense by excluding pathogens ,when the composition of normal flora is altered ,which happens when a person takes an antimicrobial ,pathogens normally unable to compare my multiply to high numbers .

4-Resistance to Antimicrobial .

The mechanisms and equation of Resistance will be discussed later.

Antibiotics

4th Microbiology **Dr. Sahar AL Saleem**

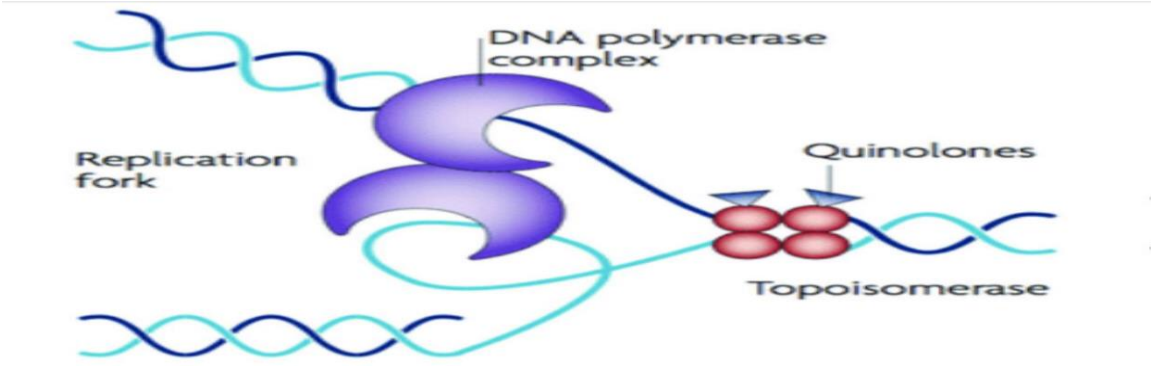
Mechanism action of Antibacterial Drugs:

3- Antibacterial medication that inhibit Nucleic Acid Synthesis.

Enzyme that are required for nucleic acid synthesis are the targets of some groups of antimicrobial drugs. These include the Fluoroquinolones and the Rifamycin.

1-The Fluoroquinolones.

Mechanism of action: The synthetic drugs called the **Fluoroquinolones** inhibit one or more of a group of enzymes called **Topoisomerases** ,which maintain the supercoiling of closed circular DNA within bacterial cell. One type of topoisomerase, called **DNA gyrase** or **TopoisomeraseII**, breaks and rejoins strands to relieve the strain caused by the localized unwinding of DNA during replication and transcription. Consequently, inhibition of this enzyme prevents these essential cell processes (**figure 1**).



(Figure 1) action of Fluoroquinolones

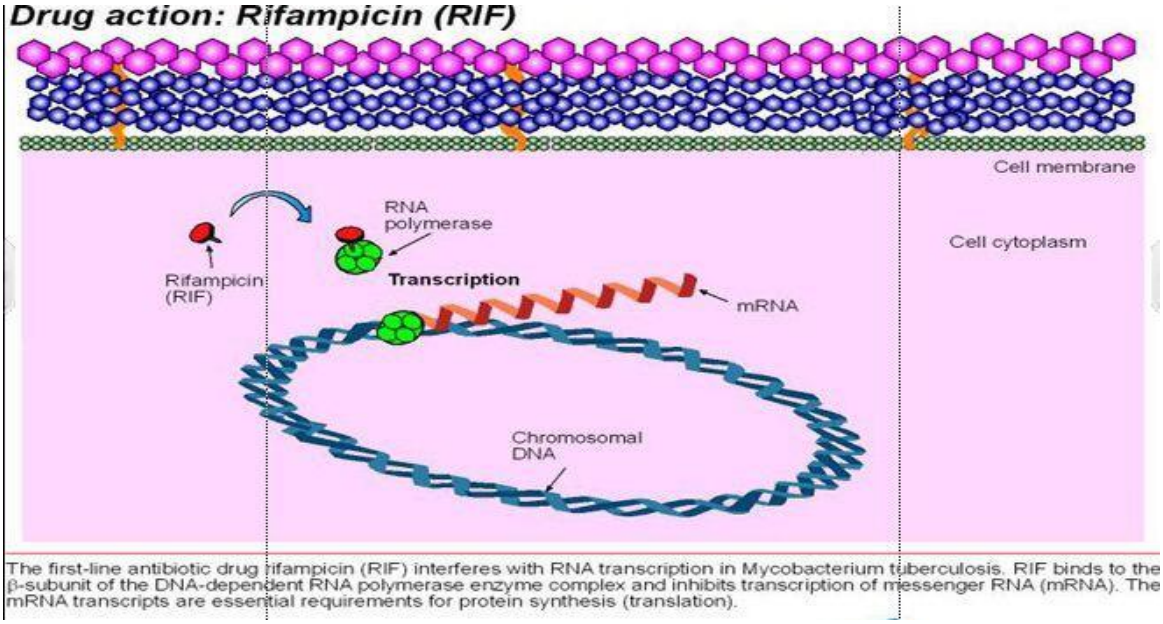
The fluoroquinolones are bactericidal against a wide variety of bacteria, including both Gram-positive and Gram-negative bacteria.

Example of Floroquinolones: include Ciprofloxacin and Ofloxacin.

Resistance: acquired resistance is most commonly due to an alteration in the DNA gyrase target.

2-The Rifamycins

Mechanism of action: The Rifamycins have a unique mechanism of action, selectively inhibiting bacterial DNA-dependent RNA synthesis, by block prokaryotic **RNA polymerase** from initiating transcription, this is due to the high affinity of Rifamycins for the prokaryotic RNA polymerase (**figure 2**).



(Figure 2) action of Rifampcin

The Rifamycins are a group of antibiotics that are synthesized either naturally by the bacterium *Amycolatopsis rifamycinica* or artificially. Rifampin , which is the most widely used Rifamycin, exhibits bactericidal activity against many Gram-positive and Gram-negative bacteria as well as members of the genus Mycobacterium.

Side effects: Rifamycins can cause side effects including reddish-orange pigment appears in urine and tears.

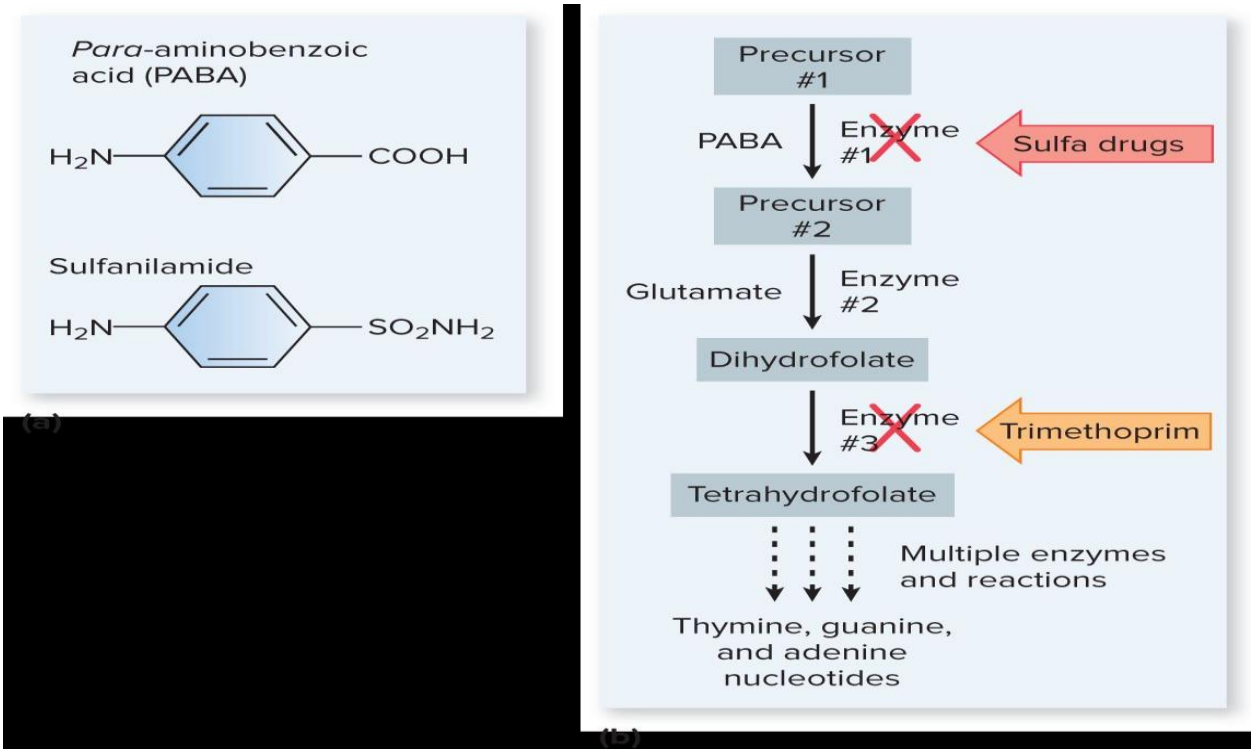
Resistance: Rifamycin develops rapidly and is due to a mutation in the gene that encode RNA polymerase.

4- Antibacterial Medication that inhibit metabolic pathways.

There are few antibacterial medications interfere with metabolic pathways. Among the most useful are the Folate inhibitors-sulfonamide and Trimethoprem. These each inhibit different steps in the pathways that leads initially to the synthesis of folic acid and ultimately to the synthesis of a coenzyme required for nucleotide biosynthesis. Animal cells lack the enzymes in the folic acid is a dietary requirement.

1-The Sulfonamides

Sulfonamide and related compound, collectively referred to as Sulfa Drugs, inhibit the growth of many Gram-positive and Gram-negative bacteria. They are structurally similar to para-aminobenzoic acid (**PABA**), a substrate in the pathway for folic acid biosynthesis. Because of this similarity, the enzyme that normally binds PABA preferentially binds sulfa drugs, resulting in its competitive inhibition. Human cells lack this enzyme, providing the basis for the selective toxicity of the sulfonamides is most often due to the acquisition of a plasmid-encoded enzyme that has a lower affinity for the drug (**figure 3**).



(**Figure 3**) **Inhibitors of the Folate Pathway**

2-Trimethoprim

Trimethoprim inhibits the bacterial enzyme that catalyzes a metabolic step following the one inhibited by sulfonamide. Fortunately, the drug has little effect on the analogous enzyme in human cells.

The combination of trimethoprim and a sulfonamide has a synergistic effect, and they are often used to treat urinary tract infections.

The most common mechanism of resistance is a plasmid-encoded alternative enzyme that has a lower affinity for the drug. Unfortunately, the genes encoding resistance to trimethoprim and sulfonamide are often carried on the same plasmid.

Antibacterial medications that interfere with processes Essential to *Mycobacterium tuberculosis*.

Only a limited range of antimicrobials can be used to treat infections caused by *Mycobacterium tuberculosis* . this is due to several factors , including their slow growth and their waxy cell wall, which is impervious to many drugs.

A group of five medications, called the **first-line drugs**, are preferred because they are the most effective as well as the least toxic. These are generally given in combination to prevent the development of resistant mutants; a small fraction of the infecting population might spontaneously develop resistance to one drug, but the other drug will eliminate those. The second line medications can be used if the first-line drugs are not an option; however, they are either less effective or more toxic.

Antibiotic

Dr. Sahar AL Saleem

4th Microbiology

How to use Antibiotic :

Antibiotics are usually taken by :

1- Mouth (orally)as tablets, capsule , syrup .
2- Injection (intramuscular , intravenous).
3- Directly to the affected part of the body (ointments, drops)

Most antibiotics start having an effect on an infection within a few hours .it is important to complete the whole course of medication to prevent the infection from coming back .

Stopping taking the medication before end of the course means that there is a higher chance the bacteria will become resistant to future treatment . this is because the once that survive have had some exposure to the antibiotic and may consequently have built up a resistant to it. Even if an individual feels better , they still need to complete the course of treatment .

Some antibiotics should not be consumed with certain foods and drinks , others should be taken on an empty stomach , these would normally be taken about an hour before meals , or 2 hours after . it is important that patients follow the instructions correctly for the medication to be effective .

Antibiotics should be used with extreme caution for the following individual s :

1 - Anyone with reduced liver or kidney function .

2 – Pregnant women .

3 – Breast feeding **.**

Anyone taking an antibiotic ,should not take other medicines or herbal therapies without speaking with a doctor first, medicines might also interact with antibiotics .

Perfect antimicrobial must be :

1- **Soluble in body fluids :** agent must dissolve in body fluids to be transported in the body and reach the infectious organisms. After a drug is absorbed into the bloodstream it rapidly circulates through the body and the drug moves from the bloodstream into the body's tissues , according to that there are 3 types of drugs :

1- Drugs that dissolve in water (water-soluble drugs) tend to stay within the blood and the fluid that surrounds cells.
2- Drugs that dissolve in fat (fat-soluble drugs) tend to concentrate in fatty tissues .
3- Other drugs concentrate mainly in only one small part of the body .

During penetrate different tissue at different speeds, depending on the drug's ability to cross membranes ,For example , the antibiotic Rifampin , a highly fat-soluble drugs, rapidly enters the brain, but the antibiotic penicillin ,a water-soluble drugs, can cross cell membrane more quickly than water-soluble drugs .

Some drugs leave the bloodstream very slowly because they bind tightly to proteins circulating in the blood. Other quickly leave the blood stream and enter other tissues because they are less tightly bound to blood proteins. Some or virtually all molecules of a drug in the blood may be bound to blood proteins. The protein-bound part is generally inactive. As unbound drug is distributed to tissues and its level in the bloodstream decreases, blood proteins gradually

release the drug bound to them. Thus, the bound drug in the bloodstream may act as a reservoir for the drug.

Some drugs accumulate in certain tissues which can also act as reservoir drug. These tissues slowly release the drug into the bloodstream, keeping blood levels of the drug from decreasing rapidly and thereby prolonging the effect of the drug. Some drugs , such as those that accumulate in fatty tissues, leave the tissues so slowly that they circulate in the bloodstream for days after a person has stopped taking the drug .

Distribution of a drug may also vary person to person, for instance ,fat people may store large amounts of fat-soluble drugs, whereas very thin people may store relatively little. Older people, even when thin, may store large amounts of fat-soluble drugs because the proportion of body fat increases with age .

2- **Selectively toxic:** agents must be more toxic to microorganisms than to host cells.
3- **Toxicity not easily altered:** agent should maintain a standard toxicity and not be less toxic by interactions with foods and other drugs, or abnormal conditions such as diabetes and kidney diseases in the host.

4- **Non allergic**

5- **Reasonable half life (stability):** is the time it takes for a substance (drug) to lose half of its activity. Typically, this refers to the body's cleaning through the function of kidneys and liver , in addition to excretion function to eliminate a substance from the body .

6- **Unlikely to produce resistance** .

7- **Reasonably priced .**

Mechanisms of action of Antibacterial Drugs:

A number of bacterial processes utilize enzymes or structures that are either different ,absent ,or not commonly found in eukaryotic cells .several microbial processes ,including the synthesis of bacterial cell walls ,proteins ,and nucleic acids ,metabolic pathways ,and the integrity of the cytoplasmic membrane , are the target of most Antimicrobial drugs (figure 1).

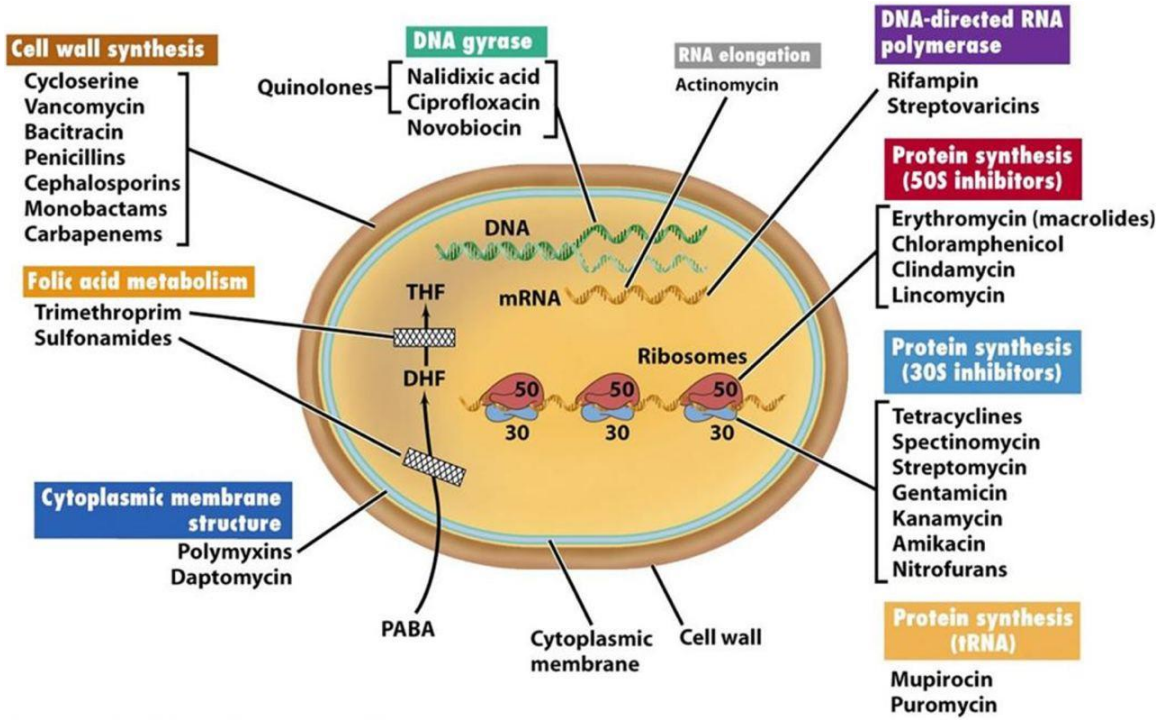


Figure 1: targets of antimicrobial drugs

A group of antibiotics called *B*-lactam drugs will be covered in the greatest detail ,because they serve serves as excellent of some of the important features of antimicrobials.

Antibacterial Medications that inhibit Cell Wall Synthesis .

Bacterial cell wall are unique in that they contain peptidoglycan composed of strand of alternating subunits of *N*- acetylglucos-amine (NAG) and *N*- acetylmuramic acid (NAM) .

These strands, called glycan chains ,are interconnected through peptide bridges between the amino acid side chains of NAM . the intact structure provides the cell the rigidity to maintain integrity (figure 2).

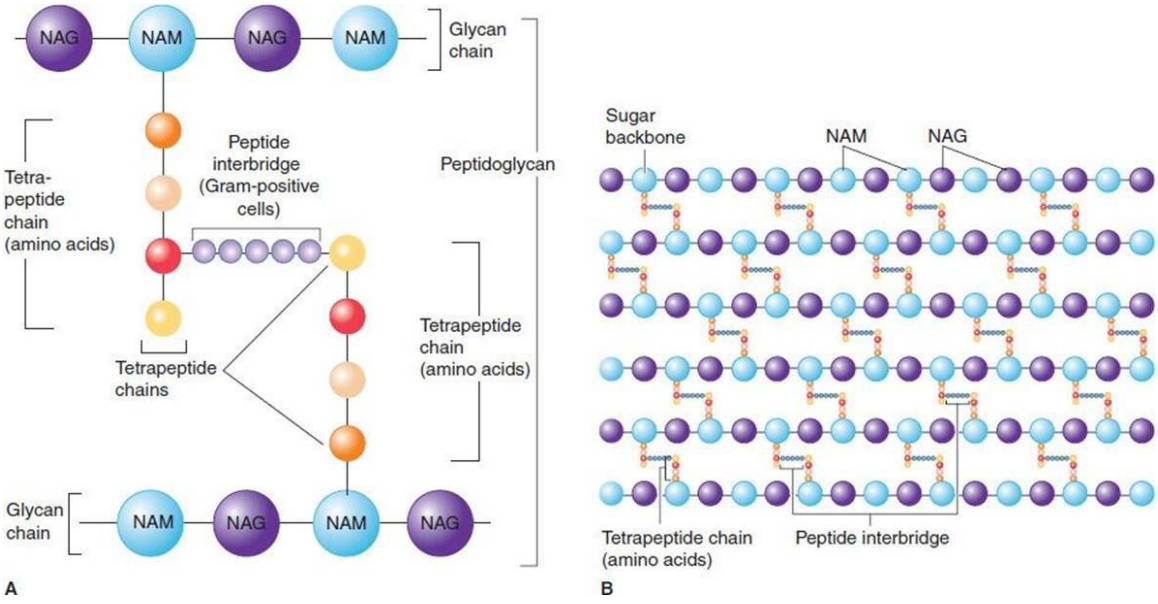


Figure 2: structure of peptidoglycan

Because peptidoglycan is specific to bacteria ,drugs that interfere just with cell wall synthesis do not affect eukaryotic cells ,and they usually have a very high therapeutic index .

A number of medically useful antimicrobial drugs interfere with cell wall synthesis , among these ,the Penicillin and Cephalospo-rin are the most widely used . they are members of large group referred to as *B*-Lactam drugs ,these drugs have chemical structure called *B*-Lactam ring (figure 3).

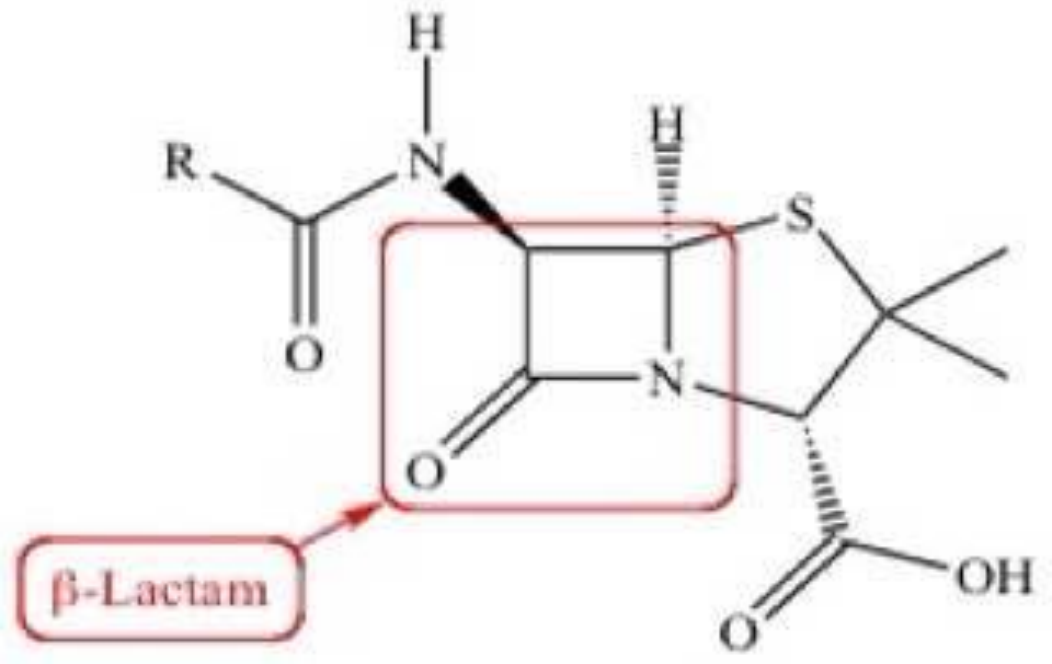


Figure 3 : B-Lactam Ring of Penicillin

Other drugs that target peptidoglycan synthesis include: Vancomycin and Bacitracin .

The *B*-Lactam Drugs:

These drugs inhibit enzymes involved in the final steps of cell wall synthesis. They have been extensively studied because they are such important antimicrobial agents , having very few side effects other than allergic reactions . their properties such as acid stability ,spectrum of activity , and half-life can be modified by chemically altering their structure.

The enzymes inhibited by *B*-lactam drugs mediate the formation of the peptide bridges between adjacent strands of peptidoglycan .they are called **Penicillin Binding Protein(PBPs)**, unfortunately ,the name causes some confusions because it seems to imply that the natural biological function of the enzymes is to bind Penicillin ,when in fact their function is associated with peptidoglycan biosynthesis. In addition , the **PBPs** may bind any of a number of *B*-lactam drugs, not just the penicillin .

The *B*-lactam ring of the penicillin and other similar drugs bears structural similarity to the normal substrate of the **PBPs** . by mimicking that substrate ,the *B*-lactam drugs are bound by **PBPs** and thus competitively inhibit their enzymatic activity . this causes a disruption in cell wall biosynthesis ,leading to a series events that causes the cells to lyse (figure 4).

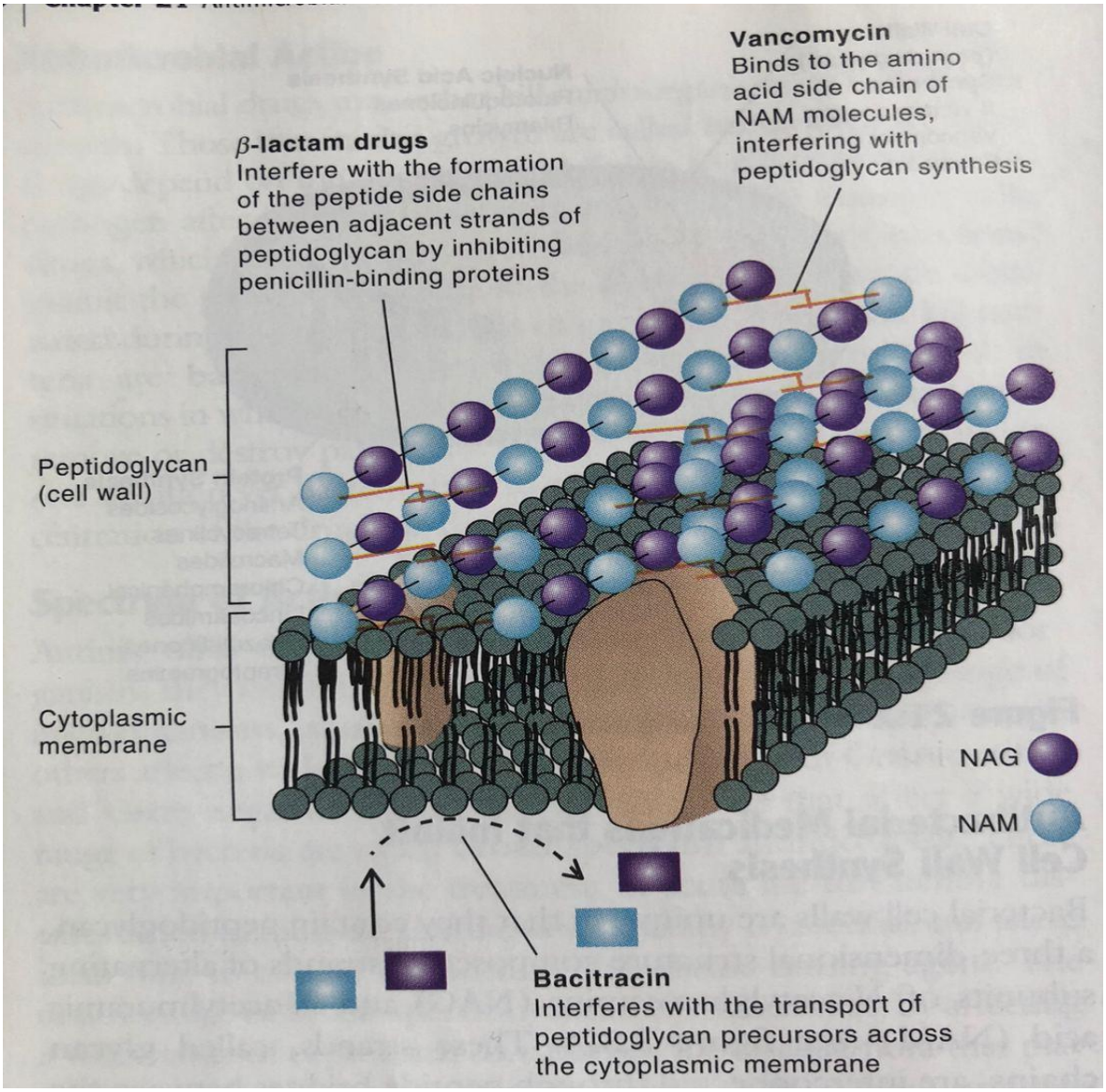


Figure 4 : Antimicrobial Medications that interferes with cell wall Biosynthesis

The different *B*-lactam drugs vary in their spectrum of activity , some are more active against Gram-positive bacteria, whereas others are more active against Gram-negative bacteria .one reason for this difference arise from the architecture of the cell wall .the peptidoglycan layer of Gram-positive bacteria directly contact the outside environment ,making it readily accessible to drugs . in contrast ,the outer membrane of Gram-negative bacteria excludes many antimicrobials ,making many of these organisms innately resistant to many medications, including certain *B*-lactam drugs . Another difference is the affinity of an organism's PBPs for particular *B*-lactam drugs .

The **PBPs** of Gram-positive bacteria differ somewhat from those of Gram-negative bacteria ,and the **PBPs** of obligate anaerobes differ from those of aerobes .

Some bacteria can resist the effects of certain *B*-lactam drugs by synthesizing an enzyme called a *B*-lactamase that breaking the critical *B*-lactam ring, destroying the activity of the antibiotic .

Types of Antimicrobial drugs that inhibit cell wall synthesis:

1- The penicillins

Each member of the family of Penicillin shares a common portion, 6-aminopenicillanic acid (6-APA) only the side chain has been modified in the laboratory to create penicillin derivatives, the family of penicillin can be loosely grouped into several categories :

a-**Natural penicillin**: These are the original penicillin produced naturally by the mold *Penicillium chrysogenum.* Natural penicillin are narrow-spectrum antibiotics effective against gram-positive bacteria and some gram-negative cocci. Natural penicillin include penicillin G and penicillin V. Penicillin V is more stable in acid and therefore better absorbed than penicillin G when taken orally.

b-**Broad-spectrum penicillin:** they are active against penicillin-sensitive Gram-positive bacteria, yet they are also active against Gram-negative organisms. They include ampicillin and amoxicillin.

c-Extended-spectrum penicillin: These have greater activity Pseudomonas spp, Gram-negative bacteria, and they less activity against Gram-positive organisms. Include ticarcillin and piperacillin.

2- The Cephalosporin : they are derived from an antibiotic derived produced by the fungus *Acremonium cephalosporium* , generally included in this family of drugs are closely related group of antibiotics that are made by a genus of filamentous bacteria related to Streptomyces. The chemical structure of the cephalosporins makes them resistant to inactivation by certain B-lactamases. Like the penicillins, the cephalosporins have been chemically modified to produce a family of various related antibiotics, they are grouped as the ,first(Cephalexin), second(Cefaclor) ,third (Cefixime) ,and fourth (Cefepime) generation cephalosporin . The later generation are generally more effective against Gram-negative bacteria and are less susceptible to destruction by B-lactamase .

3-Vancomycin: they bind to the terminal amino acids of the peptide side chain of NAM molecules that are being assembled to form glycan chains. By doing so, it blocks synthesis of peptidoglycan, resulting in weakening of the cell

wall, and cell lyses. Vancomycin is important medication for treating infections caused by Gram-positive bacteria that are resistant to B-lactam drugs .

4-Bacitracin : inhibits cell wall biosynthesis by interfering with the transport of peptidoglycan precursors across the cytoplasmic membrane ,works against Gram-negative bacteria, Its toxicity limits its use to topical applications .

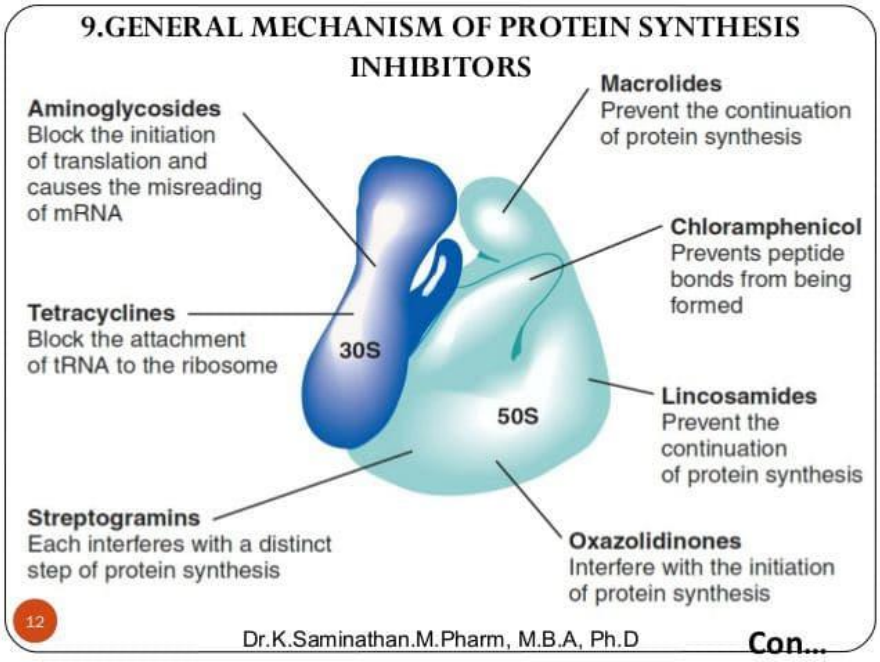
Antibiotics

4th Microbiology Dr. Sahar AL Saleem

Mechanisms action of Antibacterial Drugs:

2- Antibacterial Medications that inhibit Protein Synthesis.

Several types of antibacterial drugs inhibit prokaryotic protein synthesis by binding with the bacterial ribosomes (figure 1) .while all cells synthesize proteins ,these drugs distinguish between bacterial and eukaryotic ribosomes ,the structure of prokaryotic **70S** ribosome (**S** stands for Svedberg unit , this is the unit of the sedimentation coefficient , a measure of the sedimentation velocity in a centrifuge), which composed of a **30S** and a **50S** subunit ,is different enough from the eukaryotic 80S ribosome to make it a suitable target for selective toxicity.

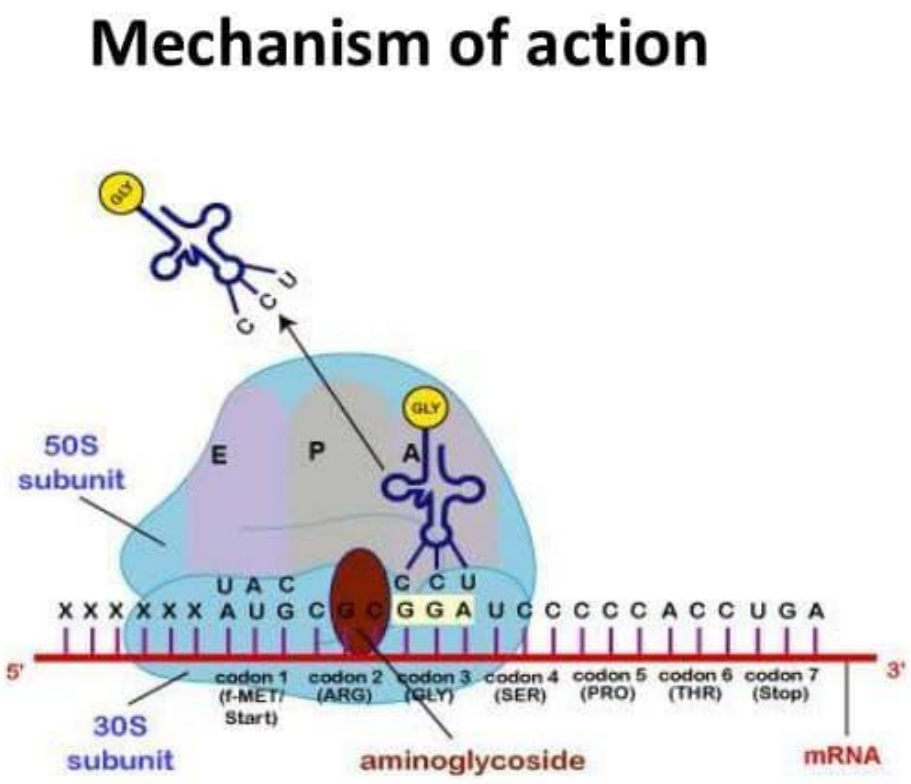


(figure 1) **Antibacterial medication that Inhibit Prokaryotic Protein Synthesis**

The major classes of antibiotics that inhibit protein synthesis are the aminoglycosides, the tetracycline's, and the macrolides others include the lincosamides and chloramphenicol, of these only the aminoglycosides are bactericidal the others are all bacteriostatic.

1-The Aminoglycosides.

Mechanism of action: The aminoglycoside irreversibly bind to the **30S** ribosomal subunit, causing it to distort and malfunction. this blocks the initiation of translation and cause misreading of mRNA by ribosomes , this yield a protein with a different amino acid sequence than the normal protein. Any abnormal proteins may be inserted into cell membrane leading to altered permeability and further simulation of aminoglycoside transport (figure 2).



(figure 2) **Mechanism action of Aminoglycoside**

Aminoglycosides are actively transported into bacterial cells by a process that requires respiratory metabolism. Consequently, they are active against most Gram-negative aerobic and facultative anaerobic bacilli ,but not against Gram – negative anaerobes and most Gram-positive bacteria .

To extend their spectrum of activity , the aminoglycosides are sometimes used in a synergistic combination with a *B*-lactam drugs . The *B*-lactam drug interferes with cell wall synthesis, which , in turn , allows the aminoglycosides to more easily enter cells.

Examples of aminoglycosides : **Streptomycin ,Tobramycin, Kanamycin, Neomycin**, and **Amikacin** . these are synthesis by different species of **Streptomyces** , whereas **Gentamycin** comes from a related genus, *Micromon**ospora purpurea***.

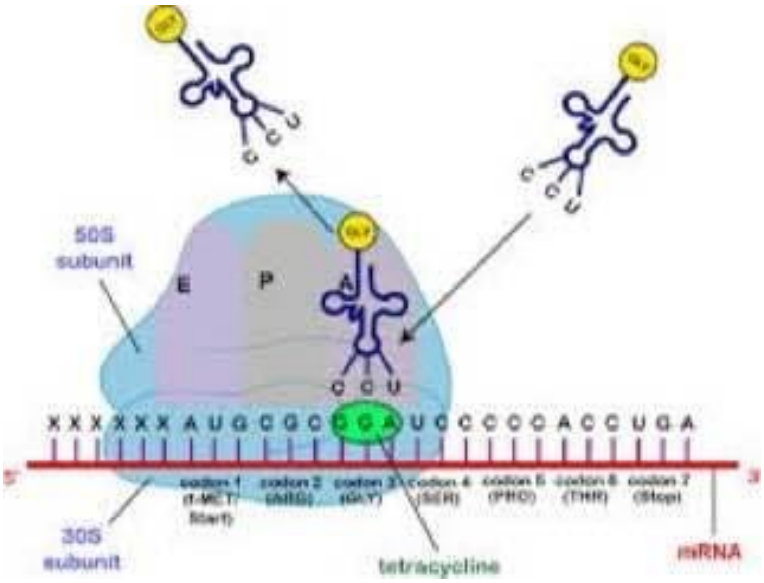
Side Effects : Unfortunately , Aminoglycoside can cause side effects including ,hearing loss and kidney damage ,skin rash or itchiness ,consequently ,they are generally used only when other alternative are not available .

Pharmacokinetics: Aminoglycosides are poorly absorbed orally but are well absorbed from the peritoneum ,pleural cavity, and joints ,they are usually given intravenous (IV).

Resistance: Bacteria can resist the effects of aminoglycosides by several mechanisms ,like enzymatic modification and inactivation of the aminoglycosides mediated by : aminoglycoside **acetyltransferase , phosphtransferase** or **nucleotidyltransferase** its affinity for binding to the **30S** ribosomal subunit may be sufficiently reduced or totally lost so protein synthesis is able to continue unchanged. Aminoglycosides enter the Gram-negative cell by passing through outer membrane porin channel ,therefore porin alteration may also contribute to aminoglycoside resistance among these bacteria .

2- The Tetracyclines.

Mechanism of action: The tetracyclines reversibly bind to the **30S** subunit, blocking the attachment of tRNA to the ribosome and preventing the continuation of protein synthesis (figure 3). These drugs are actively transported into prokaryotic but not animal cells ,they do not interfere with mammalian ribosomes because they don't penetrate very well into intact mammalian cells. They are produced from Streptomyces, broad spectrum, effective against Gram-positive and Gram-negative bacteria.



(figure 3) **Mechanism action of Tetracycline**

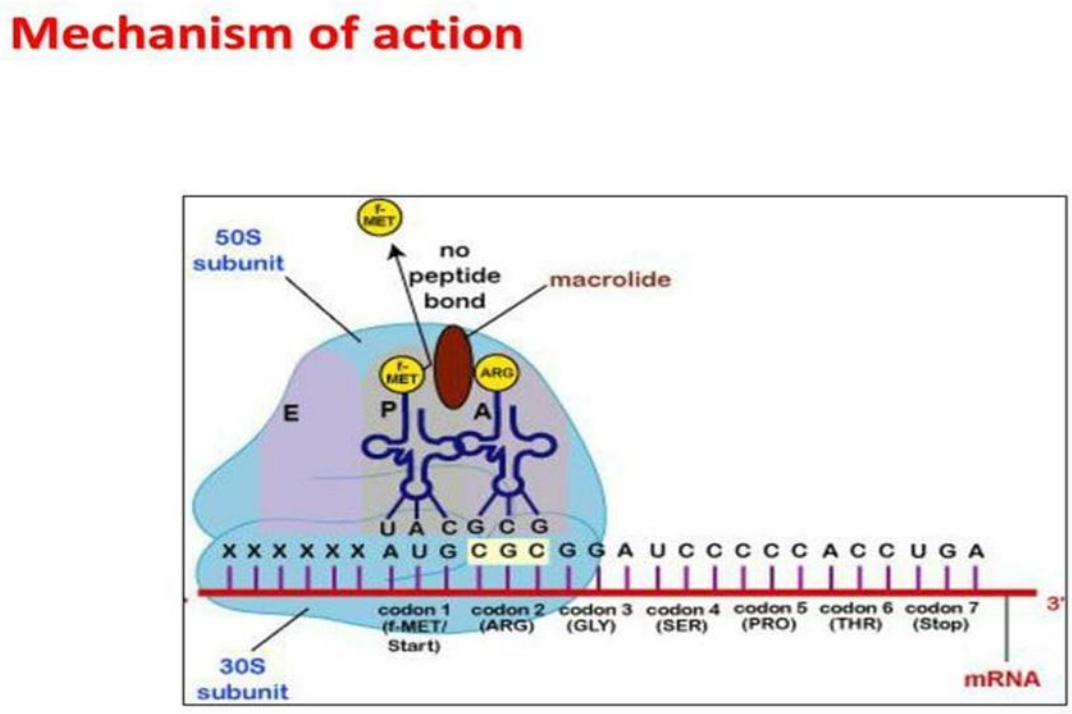
Pharmacokinetics: the Tetracycline used to treat many different bacterial infections of the skin, respiratory tract ,urinary tract , and sexually transmitted diseases such syphilis and gonorrhea.

Side effects: Tetracyclines can cause common side effects include: nausea, vomiting, diarrhea, loss of appetite, sores inside the mouth, swollen tongue, discoloration in teeth when used by young children.

Resistance: resistance to the tetracyclines is widespread among Gram-positive and Gram-negative bacteria and can be the result of pumping the drug out of the cell before it reaches it site of action (efflux), protection of the ribosomal binding site.

3- The Macrolides

Mechanism of action: The macrolides reversibly bind to the **50S** ribosomal subunit and prevent the continuation of protein synthesis(figure 4). They are effective against a variety of bacteria, including many Gram-positive bacteria, Mycoplasma, and few Gram-negative bacteria .



(figure 4) **Mechanism action of Macrolids**

Pharmacokinetics: they are useful in treating respiratory infections ,skin , sexually transmitted disease, they often serve as the drug of choice for patients who are allergic to penicillin.

Example of Macrolides: include **Erythromycin**, **Clarithromycin**, **Azithromycin**.

Resistance: resistance to all macrolides can occur through modification of the ribosomal RNA target. Other mechanisms of resistance include the production of an enzyme that chemically modifies the drug and alterations that result in decreased uptake of the drug.

4- Chloramphenicol

Mechanism of action: Chloramphenicol binds to the **50S** ribosomal subunit, preventing peptide bonds from being formed and, consequently, blocking protein synthesis.

Pharmacokinetics: Although it is active against a wide range of bacteria it is generally used as a last resort for life-threatening infections in order to avoid a lethal side effect.

Side effects: aplastic anemia is characterized by the inability of the body to form white and red blood cells.

5- Lincosamides

Mechanism of action: Lincosamides bind to the **50S** ribosomal subunit and prevent the continuation of protein synthesis, inhibiting a variety of Gram-negative and Gram-positive bacteria.

Example: The most commonly used Lincosamide is **Clindamycin**.

Pharmacokinetics: They are particularly useful for treating infections resulting from intestinal perforation.

Side effects： the most common side effects is diarrhea, colitis.