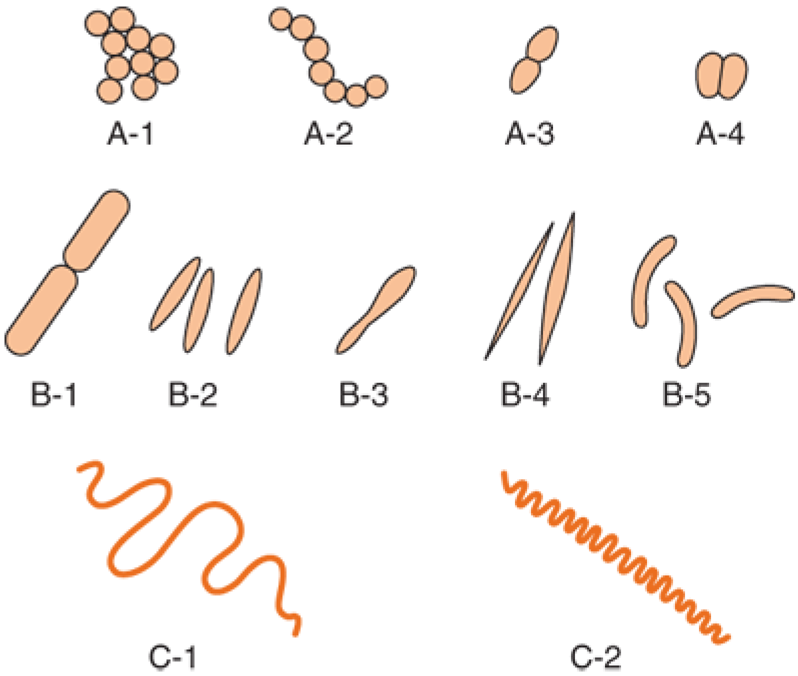
BASIC BACTERIOLOGY

Bacteria are prokaryotes, lacking well-defined nuclei and membrane-bound organelles, and with chromosomes composed of a single closed DNA circle. They come in many shapes and sizes, from minute spheres, cylinders and spiral threads, to flagellated rods, and filamentous chains. They are found practically everywhere on Earth and live in some of the most unusual and seemingly inhospitable places. Evidence shows that bacteria were in existence as long as 3.5 billion years ago, making them one of the oldest living organisms on the Earth.

1- Shape & size of bacteria.

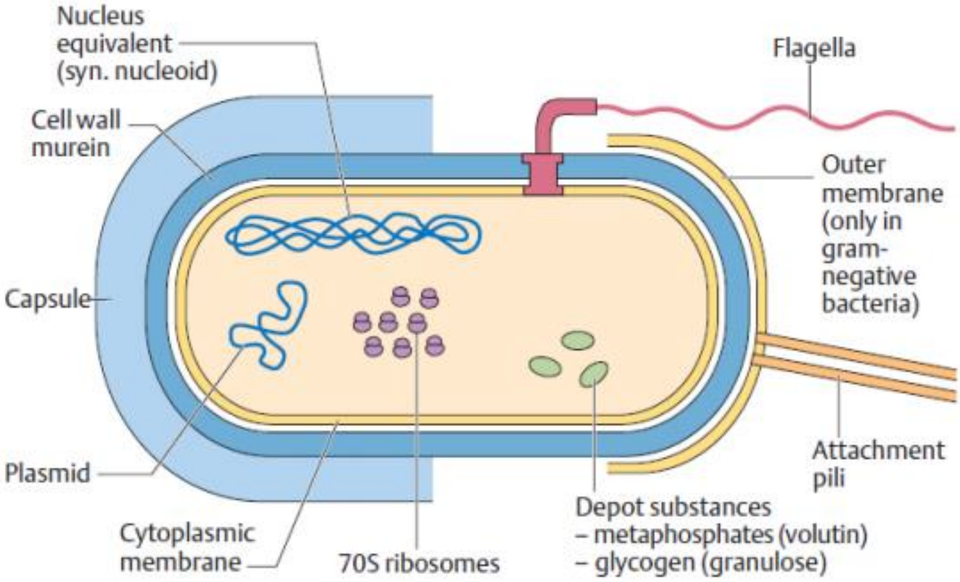
Bacteria are classified by shape into three basic groups: **A-** cocci, **B-** bacilli, and **C-** spirochetes (See Fig below). Some bacteria are variable in shape and are said to be **pleomorphic** (many-shaped). The shape of a bacterium is determined by its rigid cell wall. The microscopic appearance of a bacterium is one of the most important criteria used in its identification. Bacteria range in size from about 0.2 to 5 µm. The smallest bacteria (Mycoplasma) are about the same size as the largest viruses (poxviruses) and are the smallest organisms capable of existing outside a host. The longest bacteria rods are the size of some yeasts and human red blood cells (7 µm).



Source: Warren Levinson: Review of Medical Microbiology and Immunology, 14th Edition, www.accessmedicine.com

2- Structure of bacteria.

The structure of a typical bacterium is illustrated in Figure below



A- <u>Nucleus:</u>

In bacteria, the nucleus generally is called a nucleoid or nuclear body.

1. The bacterial nucleus is not surrounded by a nuclear membrane, nor does it contain a mitotic apparatus.

2. Composition. The nucleus consists of polyamine and magnesium ions bound to negatively charged, circular, supercoiled, double-stranded DNA; small amounts of RNA; RNA polymerase; and other proteins.

The DNA of most bacteria is contained in a single circular molecule, called the bacterial chromosome. The chromosome, along with several proteins and RNA molecules, forms an irregularly shaped structure called the nucleoid. This sits in the cytoplasm of the bacterial cell.

In addition to the chromosome, bacteria often contain plasmids – small circular DNA molecules. Bacteria can pick up new plasmids from other bacterial cells (during conjugation) or from the environment. They can also readily lose them – for instance, when a bacterium divides in two, one of the daughter cells might miss out on getting a plasmid. Every plasmid has its own ‘origin of replication’ – a stretch of DNA that ensures it gets replicated (copied) by the host bacterium. For this reason, plasmids can copy themselves independently of the bacterial chromosome, so there can be many copies of a plasmid – even hundreds – within one bacterial cell.

B- Cytoplasm:

The cytoplasm or protoplasm of bacterial cells is where the functions for cell growth, metabolism, and replication are carried out. It is a gel-like matrix composed of water, enzymes, nutrients, wastes, and gases and contains cell structures such as ribosomes, a chromosome, and plasmids. The cytoplasm contains a large number of solute low- and high-molecular weight substances, RNA and approximately 20 000 ribosomes per cell. Bacteria have 70S ribosomes comprising 30S and 50S subunits. Bacterial ribosomes function as the organelles for protein synthesis. The cytoplasm is also frequently used to store reserve substances (glycogen depots, polymerized metaphosphates, lipids). The cytoplasm when seen in the electron microscope has two distinct areas:

(1) An amorphous matrix that contains ribosomes, nutrient granules, metabolites, and plasmids. (2) An inner, nucleoid region composed of DNA.

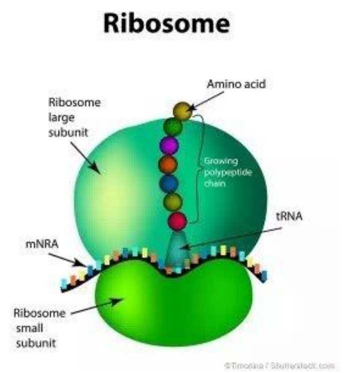
C- Ribosomes:

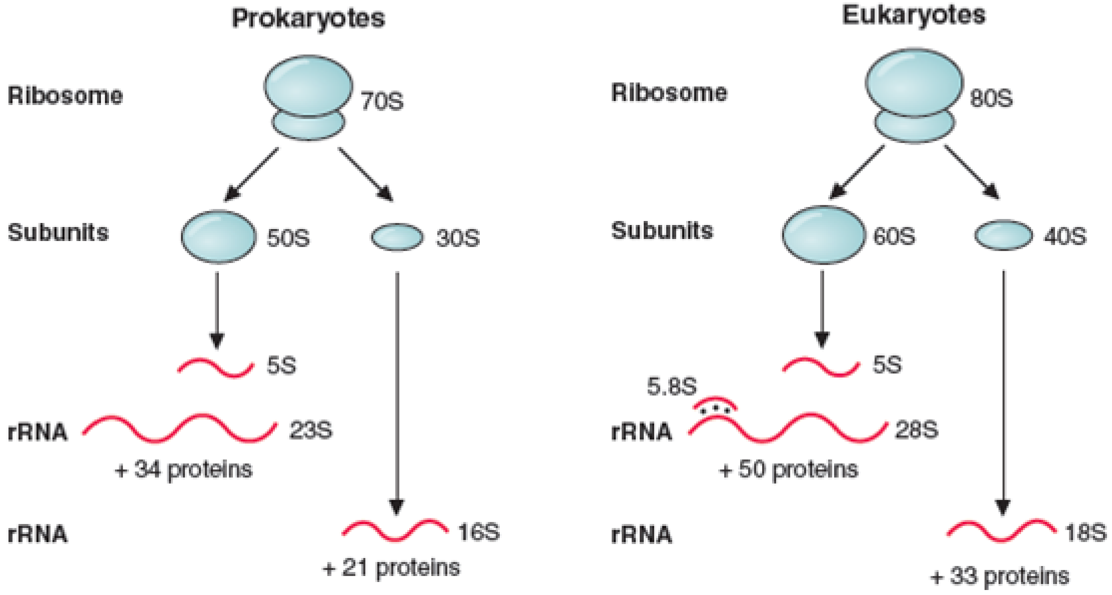
Bacterial ribosomes contain proteins and RNAs that differ from those of their eukaryotic counterparts. Bacterial ribosomes have a sedimentation coefficient of 70S and are composed of 30S and 50S subunits. The genes coding for it are referred to as 16S, and 23S and 5S RNA genes, respectively. **Many antibiotics target ribosomes**, inhibiting protein biosynthesis. Some antibiotics selectively target the 70S ribosomes (e.g., erythromycin), but not 80S ribosomes.

The prokaryotic is comprised of a 30s (Svedberg) subunit and a 50s (Svedberg) subunit meaning 70s for the entire organelle equal to the molecular weight of 2.7×10^6 Daltons. Prokaryotic ribosomes are about 20 nm (200 Å) in diameter and are made of 35% ribosomal proteins and 65% rRNA.

Type	Size	Large subunit (LSU rRNA)	Small subunit (SSU rRNA)
prokaryotic	70S	50S (5S : 120 nt, 23S : 2906 nt)	30S (16S : 1542 nt)
eukaryotic	80S	60S (5S : 121 nt,[15] 5.8S : 156 nt,[16] 28S : 5070 nt[17])	40S (18S : 1869 nt[18])

(Note: The Svedberg unit (S) offers a measure of a particle's size based on its sedimentation rate under acceleration, i.e. how fast a particle of given size and shape 'settles' to the bottom of a solution. The Svedberg is a measure of time, defined as exactly 10^{-13} seconds (100 fs). For biological molecules, sedimentation rate is typically measured as the rate of travel in a centrifuge tube subjected to high g-force).



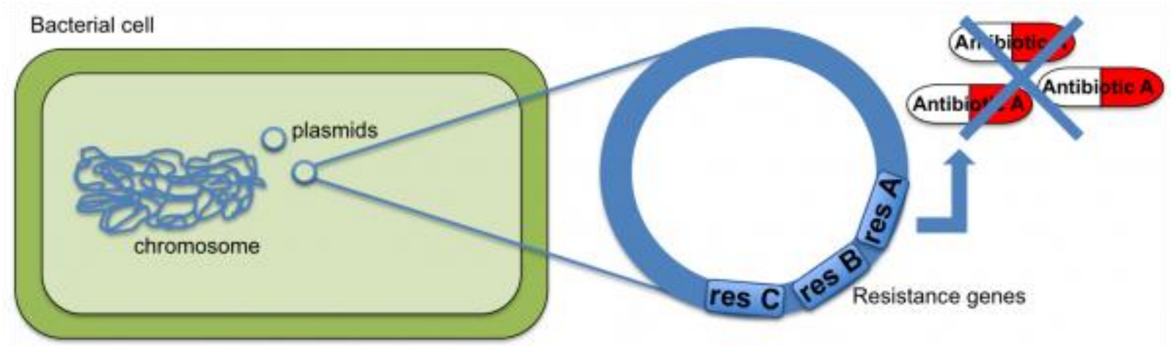


D- <u>Plasmids.</u>

A plasmid is a small, extrachromosomal DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently. They are most commonly found as small circular, double-stranded DNA molecules in bacteria.

1. **Plasmids are small, circular, nonchromosomal, double-stranded DNA molecules that are:**
a. Capable of self-replication. b. Most frequently extra-chromosomal but may become integrated into bacterial DNA.

2. Function: contain genes that confer protective properties such as antibiotic resistance, virulence factors, or their own transmissibility to other bacteria.



E- Cytoplasmic Cell Membrane.

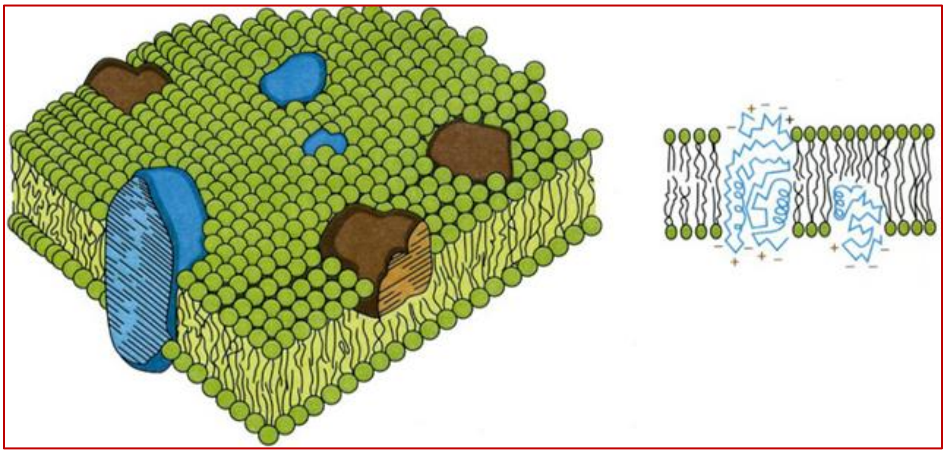
The plasma membrane, also called the cytoplasmic membrane, is the most dynamic structure of a procaryotic cell. Its main function is a s a selective permeability barrier that regulates the passage of substances into and out of the cell. Bacterial membranes are composed of 40 percent phospholipid and 60 percent protein. The composition of a phospholipid bilayer is similar in microscopic appearance to that in eukaryotic cells. They are chemically similar, but eukaryotic membranes contain cholesterol, whereas prokaryotes generally do not. The only prokaryotes that have sterols in their membranes are members of the genus Mycoplasma. The membrane has four important functions:

(1) Selective permeability and active transport of molecules into the cell.

(2) Energy generation by oxidative phosphorylation.

(3) Synthesis of precursors of the cell wall.

(4) Secretion of enzymes and toxins.



F- The Cell Wall.

The cell walls of most bacteria gain their relatively rigid quality from a unique macromolecule called peptidoglycan (PG). The term peptidoglycan is derived from the peptides and the sugars (glycan) that make up the molecule. This compound is composed of a repeating framework of long glycan chains cross-linked by short peptide fragments to provide a strong but flexible support framework (figures below). Peptidoglycan is only one of several materials found in cell walls, and its amount and exact composition vary among the major bacterial groups.

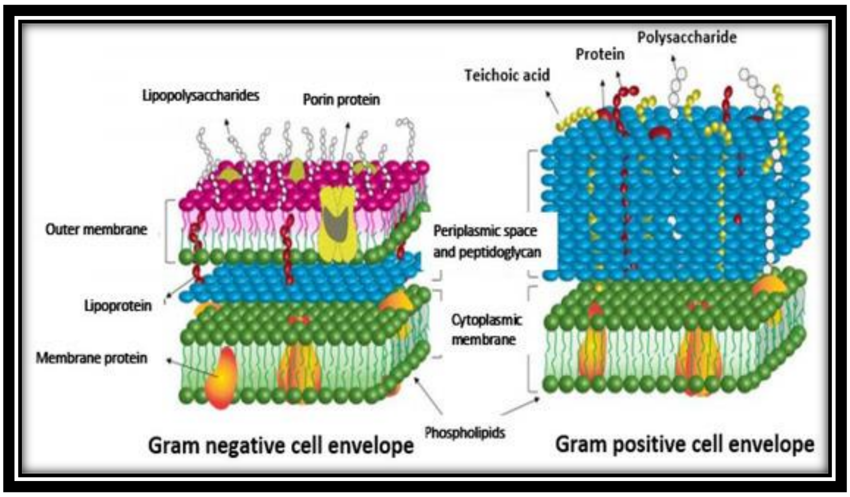
The gram-negative cell wall is more complex in morphology because it contains an outer membrane (OM), has a thinner shell of peptidoglycan, and has an extensive space surrounding the peptidoglycan. The outer membrane is somewhat similar in construction to the cell membrane, except that it contains specialized types of polysaccharides and proteins. As well as, there is lipopolysaccharides (LPS), which are part of the outer membrane of gram-negative cell walls that is responsible for many of the features of disease, such as fever and shock

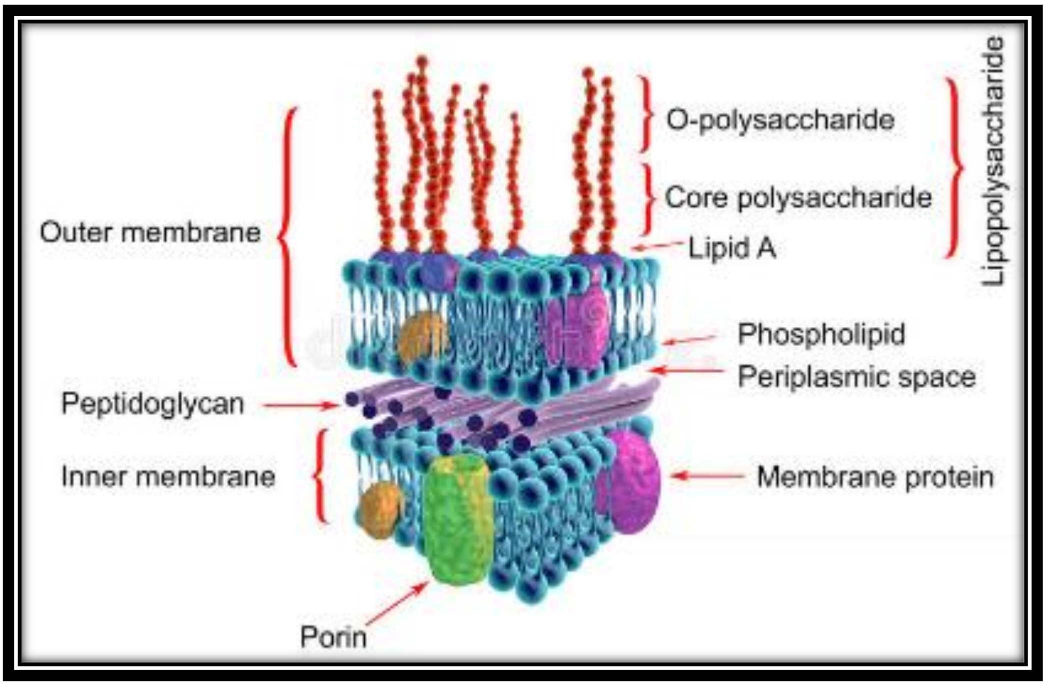
Lipopolysaccharides (LPS), also known as lipoglycans and endotoxins, are large molecules consisting of:

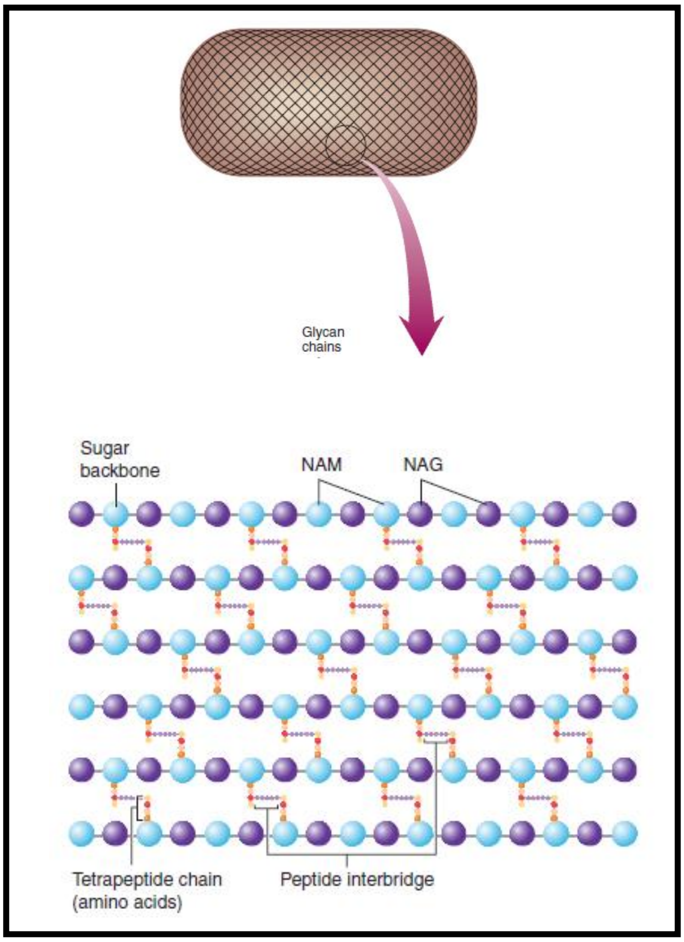
(1) A phospholipid called lipid A, which is responsible for the toxic effects.

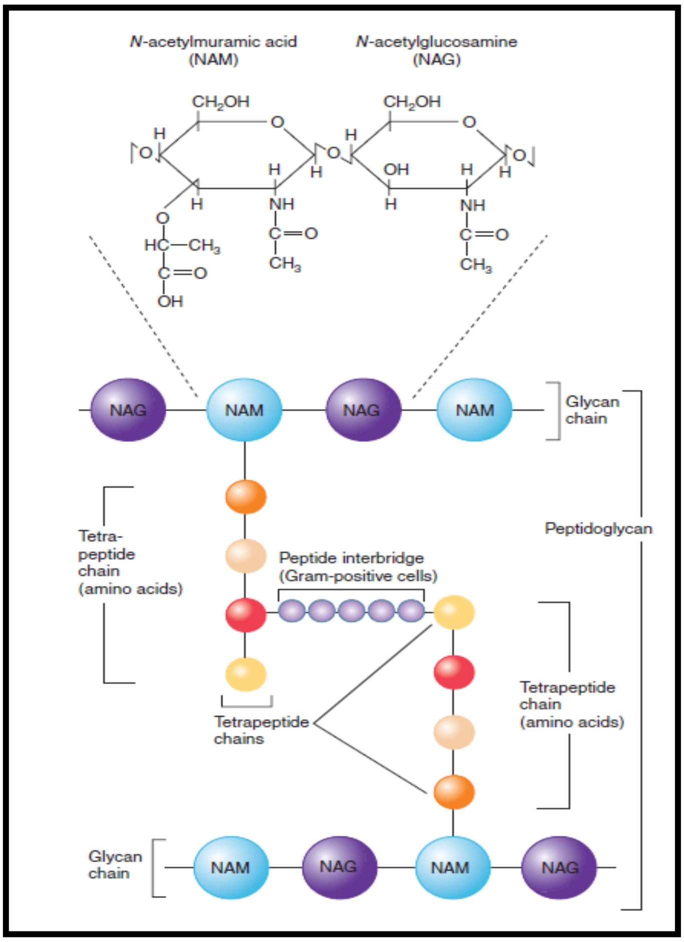
(2) A core polysaccharide of five sugars that is attached to lipid A attached to lipid A by unusual sugars called **keto-deoxyoctulonate**

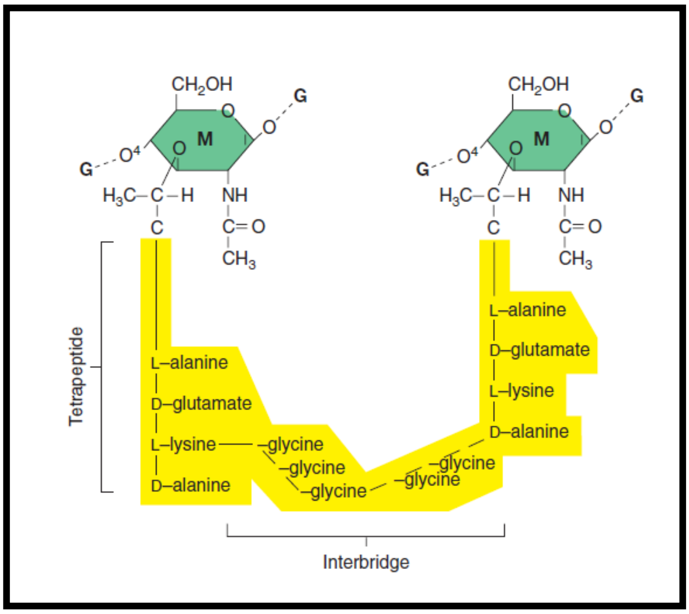
(3) An outer polysaccharide (O antigen) consisting of up to 25 repeating units of three to five sugars.






Glycan chains
Sugar backbone
NAM
NAG
Tetrapeptide chain (amino acids)
Peptide interbridge


N-acetylmuramic acid (NAM)
N-acetylglucosamine (NAG)
NAG
NAM
Glycan chain
Peptidoglycan
Tetra-peptide chain (amino acids)
Peptide interbridge (Gram-positive cells)
Tetrapeptide chains
Tetrapeptide chain (amino acids)


L–alanine
D–glutamate
L–lysine
D–alanine
glycine
Tetrapeptide
Interbridge

ANTIMICROBIAL CHEMOTHERAPY

Antimicrobial choice is related to the mechanism of drug action in one of the following general categories:

A. Inhibits bacterial cell wall biosynthesis.

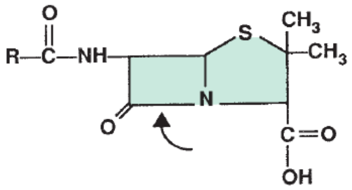
B. Inhibits bacterial protein synthesis..

C. Inhibits bacterial nucleic acid synthesis.

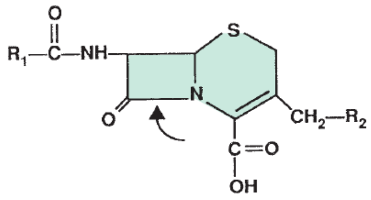
In general, antimicrobial drugs can be either bacteriostatic (inhibit growth) or bactericidal (kill).

A. Inhibitors of Bacterial Cell Wall Biosynthesis

Penicillin is given to patients with an infection caused by bacteria. Some types of bacterial infections that may be treated with penicillin include pneumonia, strep throat, meningitis, syphilis and gonorrhea, according to the National Library of Medicine. Penicillins have a β-lactam ring the integrity of which is required for antibacterial activity.

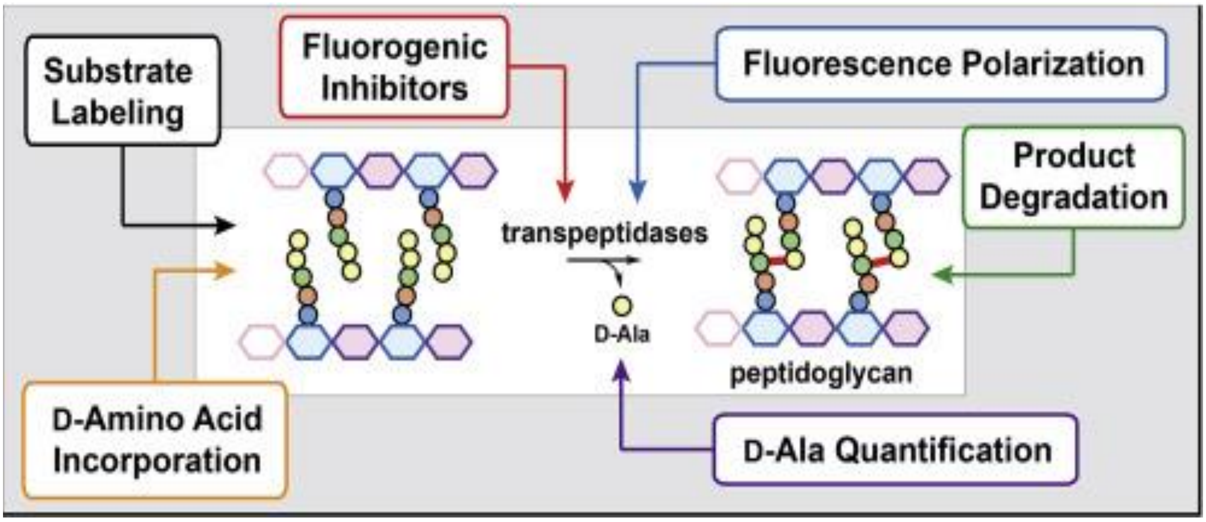


Penicillin nucleus



Cephalosporin nucleus

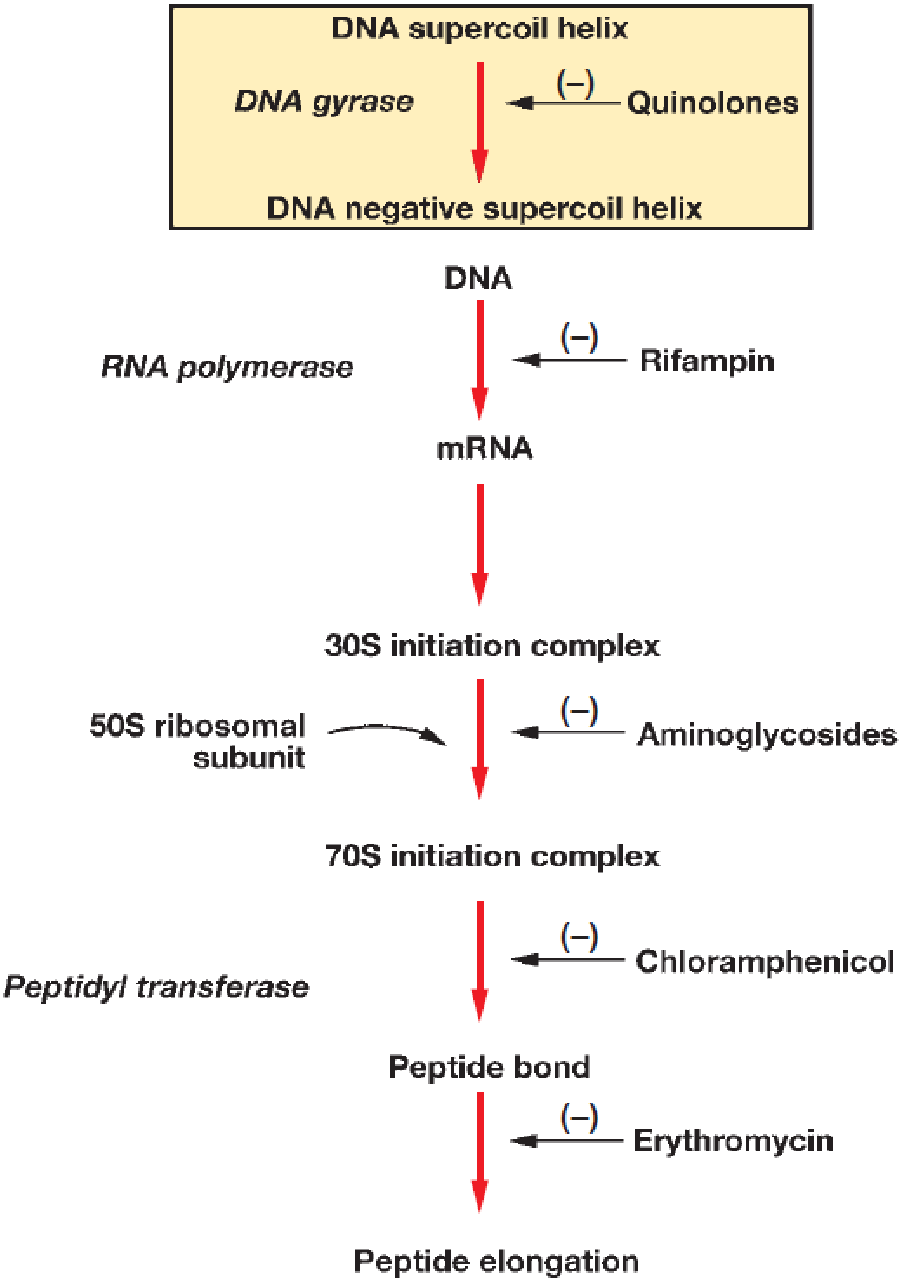
Penicillins inactivate bacterial transpeptidases (or penicillin-binding proteins (PBPs) and prevent the cross-linking of peptidoglycan polymers that is essential for bacterial cell wall integrity. This results in loss of rigidity and a susceptibility to rupture. Penicillins also bind to, and inactivate, penicillin-binding proteins (PBPs) involved in cell wall synthesis.



Penicillins are bactericidal for growing cells. Gram-positive bacteria with thick external cell walls are particularly susceptible. The major cause of resistance is the production of a-lactamases (penicillinases). The genes for β-lactamases can be transmitted during conjugation or as small DNA.

B. Inhibits bacterial protein synthesis.

A protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins. In general, protein synthesis inhibitors work at different stages of prokaryotic mRNA translation into proteins, like initiation, elongation, and termination.



C. inhibition of bacterial nucleic acid synthesis.

The nalidixic **acid**, ciprofloxacin, and norfloxacin, work by inhibiting enzymes that are required for bacterial **DNA synthesis**. So, in contrast to the rifamycins, which **inhibit** transcription of **DNA** into RNA, the quinolones and fluoroquinolones **inhibit DNA** replication.

The antifolate drugs (sulphonamides and dihydrofolate reductase inhibitors) act by blocking the production of thymine. The antifungal agent 5-fluorocytosine interferes with these early stages of DNA synthesis. Through conversion to 5-fluorouracil then to 5-fluorodeoxyuridylic acid (5-F-dUMP).

The anti-human immunodeficiency virus (HIV) drug azidothymidine (AZT). AZT works by selectively inhibiting HIV's reverse transcriptase, the enzyme that the virus uses to make a DNA copy of its RNA. Reverse transcription is necessary for production of HIV's double-stranded DNA, which would be subsequently integrated into the genetic material of the infected cell (where it is called a provirus)