An Introduction to Medical Microbiology

Microbiology is a subject which deals with living organisms that are individually too small to be seen with the naked eye. It considers the microscopic forms of life and deals about their reproduction, physiology, and participation in the process of nature, helpful and harmful relationship with other living things, and significance in science and industry.

All ecosystems contain microorganisms. Billions of them populate the healthy human body and some of them may be recorded as participants in bodily functions. One of the classical example that the bacteria play a role in the degradation of intestinal contents.

Microbes in Our Lives

1. Living things too small to be seen with the unaided eye are called microorganisms.

2. Microorganisms are important in maintaining Earth's ecological balance.

3. Some microorganisms live in humans and other animals and are needed to maintain good health.

4. Some microorganisms are used to produce foods and chemicals.

5. Some microorganisms cause disease.

Comparing Prokaryotic and Eukaryotic Cells: An Overview

The world of living things is classified in the three **domains** bacteria, archaea, and eucarya. In this system, each domain is subdivided into **kingdoms**. Pathogenic microorganisms are found in the domains **bacteria** and **eucarya**.

Prokaryotes and eukaryotes both contain nucleic acids, proteins, lipids, and carbohydrates. They use the same kinds of chemical reactions to metabolize

food, build proteins, and store energy. It is primarily the structure of cell walls and membranes, and the absence of organelles (specialized cellular structures that have specific functions), that distinguish prokaryotes from eukaryotes.

The chief distinguishing characteristics of **prokaryotes** (from the Greek words meaning prenucleus) are as follows:

1. Typically their DNA is not enclosed within a membrane and is usually a singular, circularly arranged chromosome. Gemma obscuriglobus has a double membrane around its nucleus. (Some bacteria, such as Vibrio cholerae, have two chromosomes, and some bacteria have a linearly arranged chromosome.)

2. Their DNA is not associated with histones (special chromosomal proteins found in eukaryotes); other proteins are associated with the DNA.

3. They generally lack organelles. Advances in microscopy reveal a few membrane-enclosed organelles (for example, some inclusions). However, prokaryotes lack other membraneenclosed organelles such as nuclei, mitochondria, and chloroplasts.

4. Their cell walls almost always contain the complex polysaccharide peptidoglycan.

5. They usually divide by binary fission, where DNA is copied, and the cell splits into two cells. This involves fewer structures and processes than eukaryotic cell division.

Eukaryotes (from the Greek words meaning true nucleus) have the following distinguishing characteristics:

1. Their DNA is found in the cell's nucleus, which is separated from the cytoplasm by a nuclear membrane, and the DNA is found in multiple chromosomes.

Table 1.2 Characteristics of Prokaryotic (Eubacteria) and Eukaryotic (Fungi, Protozoans) Microorganisms

Characteristic	Prokaryotes (bacteria)	Eukaryotes (fungi, protozoans)
Nuclear structure	Circular DNA molecule not covered with proteins	Complex of DNA and basic proteins
Localization of nuclear structure	Dense tangle of DNA in cytoplasm; no nuclear membrane; nucleoid or nuclear equivalent	In nucleus surrounded by nuclear membrane
DNA	Nucleoid and plasmids	In nucleus and in mitochondria
Cytoplasm	No mitochondria and no endoplasmic reticulum, 70S ribosomes	Mitochondria and endoplasmic reticulum, 80S ribosomes
Cell wall	Usually rigid wall with murein layer; exception: mycoplasmas	Present only in fungi: glucans, mannans, chitin, chitosan, cellulose
Reproduction	Asexual, by binary transverse fission	In most cases sexual, possibly asexual

Some Branches of Microbiology

Bacteriology: Small single-celled prokaryotic organisms.

Mycology: The fungi, a group of eukaryotes that includes both microscopic eukaryotes (molds and yeasts) and larger organisms (mushrooms, puffballs)

Protozoology: The protozoa—animal-like and mostly single-celled eukaryotes**.**

Virology: Viruses—minute, noncellular particles that parasitize cells.

Parasitology: Parasitism and parasitic organisms—traditionally including pathogenic protozoa, helminth worms, and certain insects.

Microbial Taxonomy

'Systematics' is the term used to define the study of the diversity of life and their relationships.

'taxonomy' tends to be restricted to the theory and practice of classifying organisms.

Classification attempts to group organisms according to their similarity.

TAXON:- A group or category of related organisms.

There are two key characteristics of taxa are:

1-Members of **lower level** taxa (e.g. Species) are **more similar** to each other than are members of **higher level** taxa (eg. Kingdom or domain).

2-Member of specific taxa are more similar to each other than any are to members of different specific taxa found at the same **hierarchical** متسلسل level (eg. Humans are more similar to apes, i.e., comparison between species, than either is similar to, for example, *Escherichia coli*). Thus once you know that two individuals are member of the same **taxon**, you can inter certain similarities between the two organisms.

BINOMIAL NOMENCLATURE

- Organisms are named using binomial nomenclature (viruses are exceptions)

- Binomial nomenclature employs the names of the two level taxa, genus and species, to name a specie. Binomial nomenclature includes:

i. Genus comes before species (e.g., *Escherichia coli*)

ii. Genus name is always capitalized (e.g., *Escherichia*)

iii. Species name is never capitalized (e.g., *coli*)

iv. Both names are always either ***italicized*** or **<u>underlined</u>** (e.g *Escherichia coli*)

v. The genus name may be used alone, but not the species name (i.e saying or writing "*Escherichia* " alone is legitimate while saying or writing " coli" is not)

Domain (Bacteria, Archaea, Eukarya): Bacteria

Kingdom: Bacteria

Phylum/Division: Proteobacteria

Class: Zymobacteria

Order: Enterobacterales

Family: Enterobacteriaceae

Genus: *Escherichia*

Species: *coli*

Scientific name: *Escherichia coli*

Bacteria

Bacteria are single-celled prokaryotic microorganisms, and their DNA is not contained within a **separate nucleus** as in **eukaryotic** cells. They are approximately 0.1–10.0 μm in size and exist in various shapes, including **spheres** (cocci), **curves**, **spirals** and **rods** (bacilli).

Bacterial Classification

Bacterial classification depends on the following characteristics.

1. Morphology and arrangement
2. Staining
3. Cultural characteristics
4. Biochemical reactions
5. Antigenic structure
6. Base composition of bacterial DNA.

Morphology and staining of bacteria are the commonly used characteristics to classify bacteria.

Morphology of bacteria

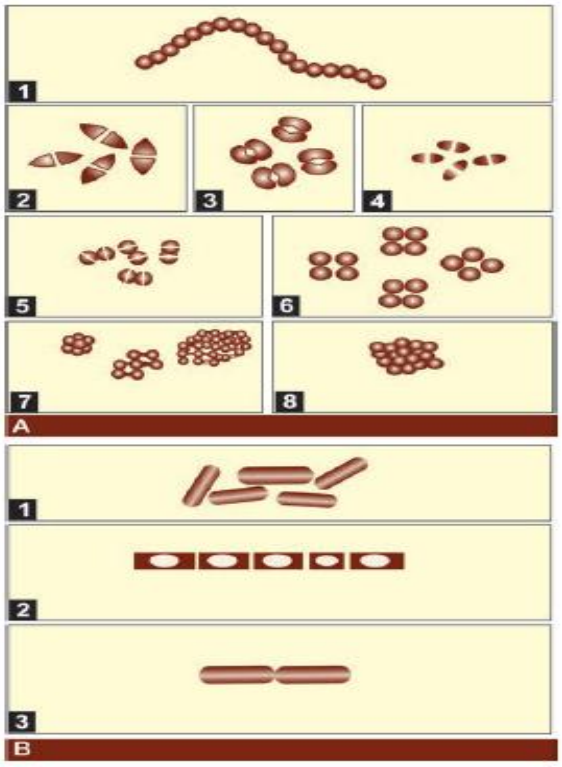
When bacteria are visualized under light microscope, the following morphology are seen.

1. Cocci (singular coccus): Round or oval bacteria measuring about 0.5-1.0μmb in diameter.They are found insingle, pairs, chains or clusters.

2. Bacilli (singular bacillus): Stick-like bacteria with rounded, tapered مستدق الطرف, square or swollen ends; with a size measuring 1-10μm in length by 0.3-1.0μm in width.

3. Coccobacilli (singular coccobacillus): Short rods.

4. Spiral: Spiral shaped bacteria with regular or irregular distance between twisting. Eg. Spirilla and spirochaetes.



Depends to the arrangement the bacteria can be classified into:

- Single cell (coccus كروي, bacillus عصوي and curved or spiral shape منحني او حلزوني).
- Diplococci كرويات ثنائية (2,3,4,5)
- Streptococci كرويات بشكل المسبحة(1)
- Staphylococci(8) كرويات بشكل عناقيد العنب
- Streptobacilli (2, 3 in bacilli shape) عصويات بشكل المسبحة
- Tetrads (6) كرويات تترتب بهية الرباعيات(اربع خلايا)
- Cubic (7) كرويات تترتب بهيئة مكعبات

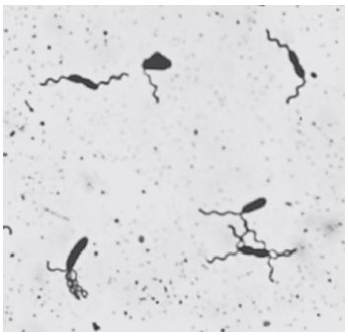
2. Staining of bacteria

Bacterial staining is the process of coloring of colorless bacterial structural components using stains (dyes).

Staining reactions are made possible because of the physical phenomena of capillary osmosis, solubility, adsorption, and absorption of stains or dyes by cells of microorganisms.

Types of microbiological stains

- Basic stains
- Acidic stains
- Neutral stains.

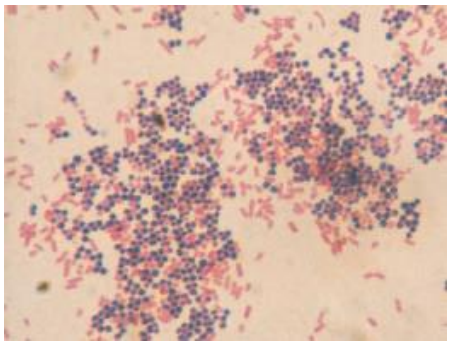


Flagellar stain Jawetz, P.39

Bacterial staining methods include:

- Simple stain.. (ex. Methylene blue stain)
- Differential stain. (ex. Gram stain, Acid fast stain)

- Special stain. (Spore stain, Flagellar stain)

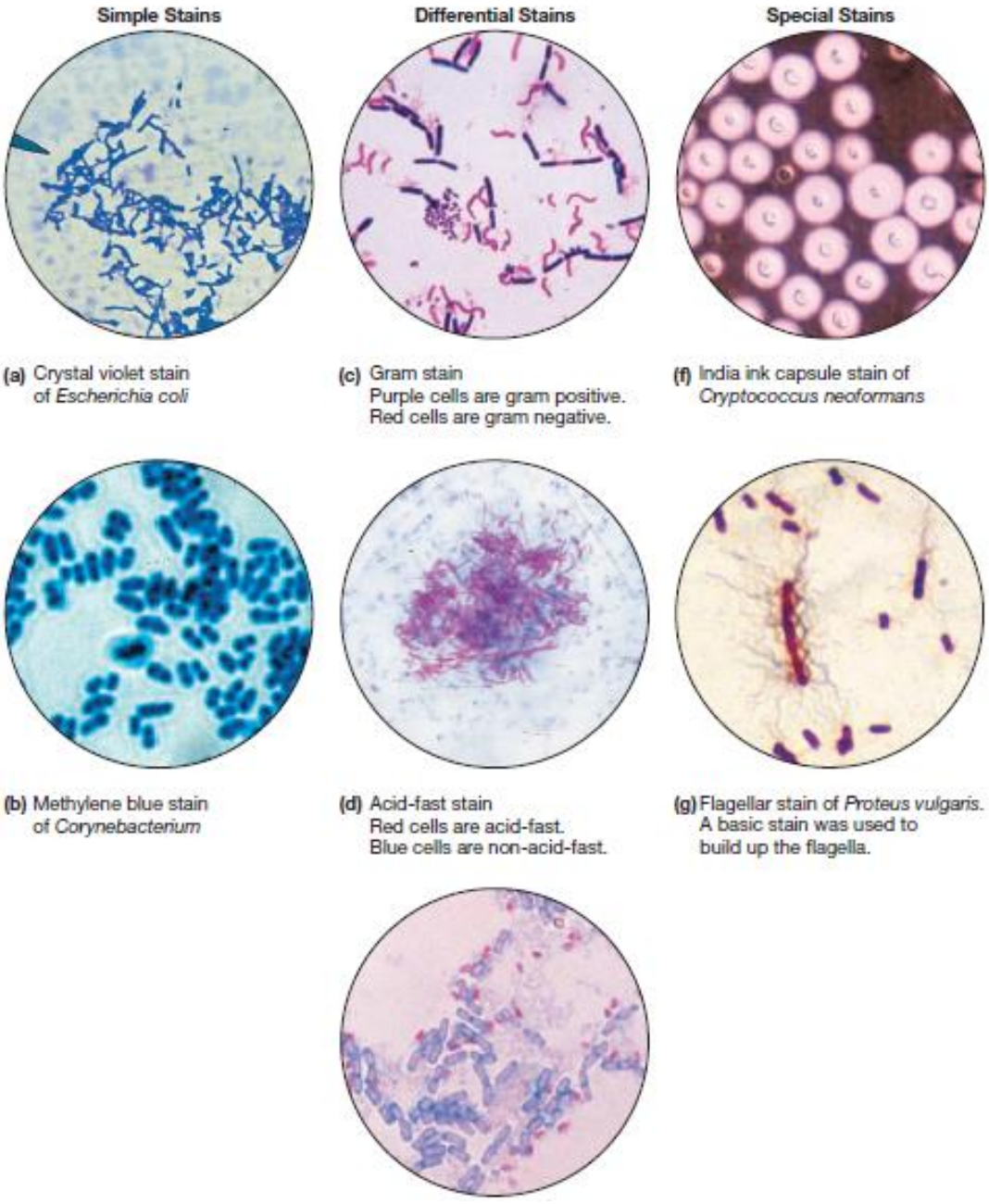


Gram-stain Prescott, P. 28

According to the Gram-stain technique the bacterial cells classified into:

Gram-positive bacteria (purpule color), and Gram-negative bacteria (Pink- color).

Some bacterial cell cannot be stained with Gram- sain according to the difference of structure in the bacterial cell wall, thus it must be stained with other techniques such as Acid- Fast stain.



Bacterial staining Methods

"Prescott, microbiology, p27

Bacterial Cell Structure.... (part one)

The prokaryotic cell is simpler than the eukaryotic cell at every level, with one exception: The cell envelope is more complex.

The general property of the bacterial cell includes....

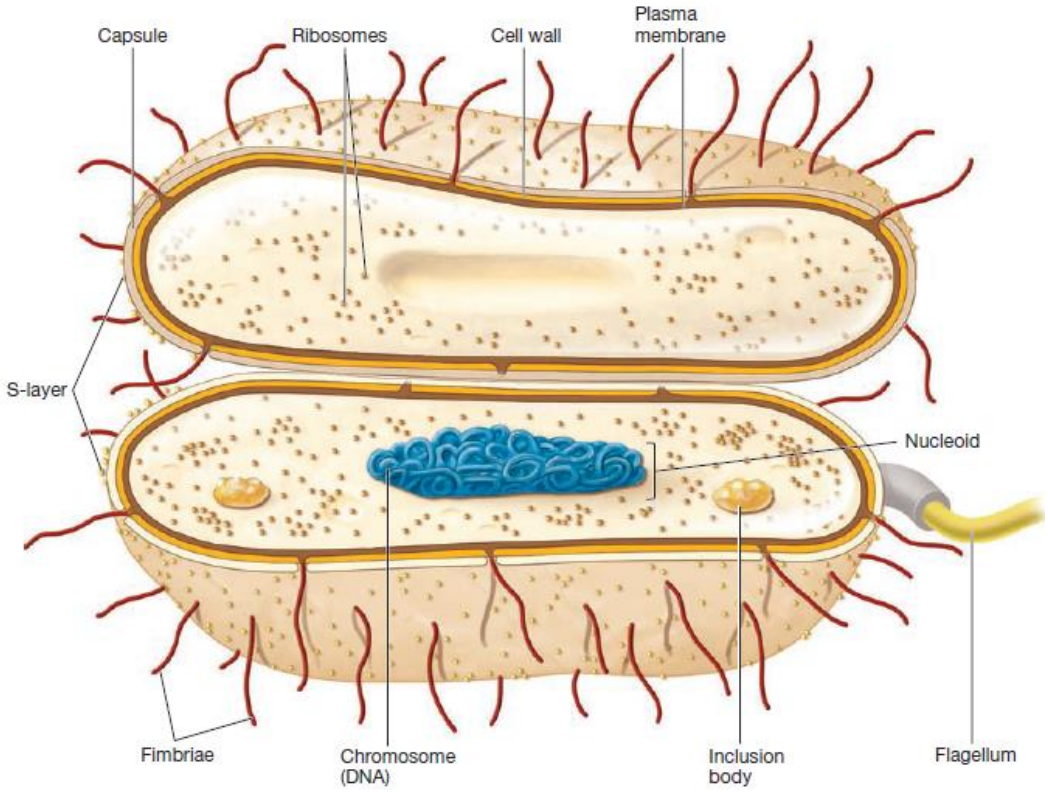
- Typical prokaryotic cell
- Contain both DNA and RNA
- Most grow in artificial media
- Replicate by binary fission
- Almost all contain rigid cell wall
- Sensitive to antimicrobial agent

Bacterial structure is considered at three levels.

1. Cell envelope proper: Cell wall and cell membrane.

2. Cellular element enclosed with in the cell envelope: Mesosomes, ribosomes, nuclear apparatus, polyamines and cytoplasmic granules.

3. Cellular element external to the cell envelope: Flagellum, Pilus and Glycocalyx.



Bacterial Cell Structure

"Prescott, microbiology, p27"

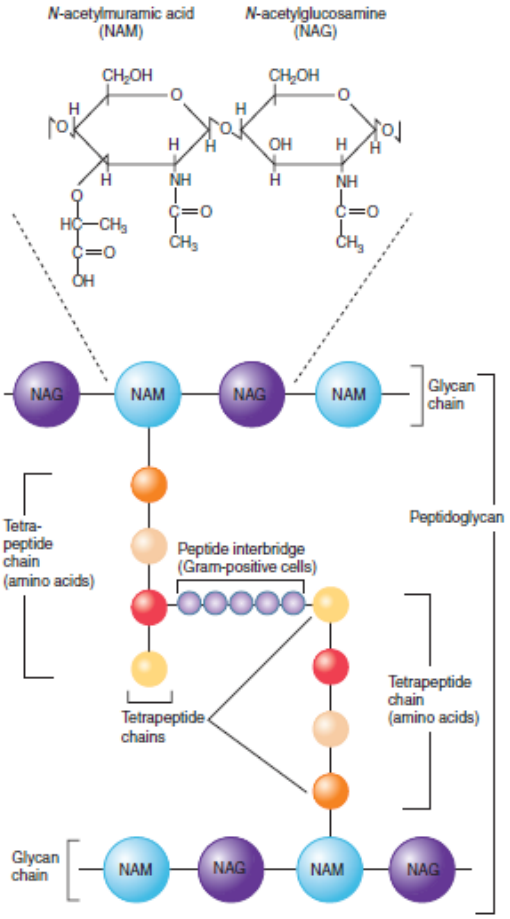
- Willey, J. M., Sherwood, L., Woolverton, C. J., **Prescott**, L. M., & Willey, J. M. (2011). **Prescott's microbiology**. New York: McGraw-Hill.

Cell wall structure

The bacterial cell wall composed of a substance variously referred to as **murein**, **mucopeptide**, or **peptidoglycan** (all, including "cell wall," are synonyms).

Most bacteria are classified as Gram-positive or Gram-negative according to their response to the Gram-staining procedure.

Peptidoglycan is a complex polymer consisting, for the purposes of description, of **three parts**: a **backbone**, composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acidconnected by β1→4 linkages; a set of identical tetrapeptide side chains attached to *N*-acetylmuramic acid; and a set of identical peptide cross-bridges.



Components and structure of peptidoglycan.

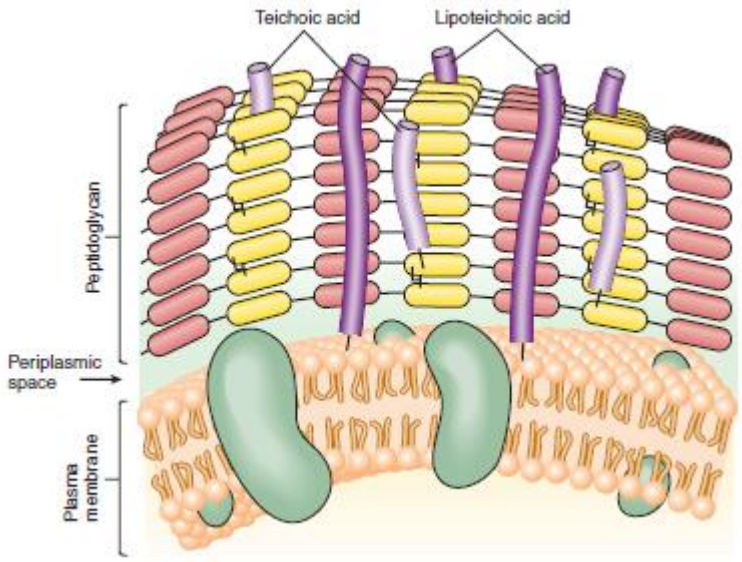
Chemical structure of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM); the ring structures of the two molecules are glucose. Glycan chains are composed of alternating subunits of NAG and NAM joined by covalent bonds. Adjacent glycan chains are cross-linked via their tetrapeptide chains to create peptidoglycan.(Jawetz, p. 25)

- **Brooks**, G. F., Jawetz, E., Melnick, J. L., & Adelberg, E. A. (2013). Jawetz, Melnick & Adelberg's medical microbiology (26th edition.). New York : London: McGraw-Hill Medical.

Diaminopimelic acid is a unique element of bacterial cell walls. It is never found in the cell walls of Archaea or eukaryotes.

Special Components of Gram-Positive Cell Walls

- **Teichoic and teichuronic acids.**
- **Polysaccharides**



Gram-positive cell wall (Envelope), "Jawetz, p.26"

Special Components of Gram-Negative Cell Walls

- **Outer membrane:** The outer membrane is chemically distinct from all other biological membranes. It is a bilayer structure; its inner leaflet resembles in composition that of the cytoplasmic membrane, and its outer leaflet contains a distinctive component, a lipopolysaccharide (LPS).

The outer membrane has special channels, consisting of protein molecules called porins that permit the **passive diffusion** of low-molecular-weight hydrophilic compounds, such as sugars, amino acids, and certain ions.

- **Lipopolysaccharide (LPS)—**The LPS of Gram-negative cell walls consists of a complex glycolipid, called **lipid A**, to which is attached a **polysaccharide** made up of a **core** and a **terminal** series of repeat units.
- **The periplasmic space**—The space between the inner and outer membranes, called the periplasmic space, contains the peptidoglycan layer and a gel-like solution of proteins.
- **The Acid-Fast Cell Wall**

Some bacteria, notably the tubercle bacillus (*Mycobacterium tuberculosis*) and its relatives, have cell walls that contain substantial amounts of waxes,

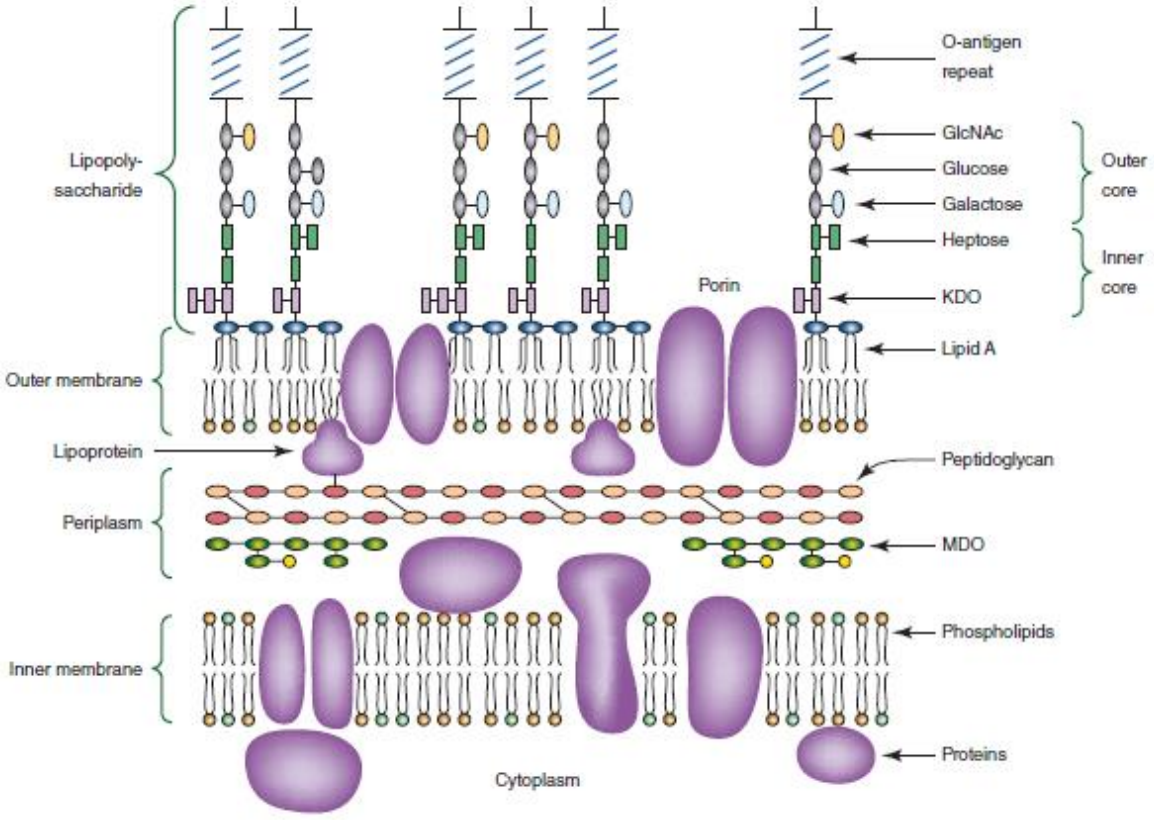
complex branched hydrocarbons (70–90 carbons long) known as **mycolic acids.**

Protoplasts, Spheroplasts, and L Forms

Removal of the bacterial wall may be accomplished by **hydrolysis** with **lysozyme** (as described above) or by **blocking peptidoglycan synthesis** with an **antibiotic** such as penicillin.

In osmotically protective media, such treatments liberate **protoplasts** from Gram-positive cells and **spheroplasts** from Gram-negative cells.

If such cells are able to grow and divide, they are called **L forms**. L forms are difficult to cultivate and usually require a medium that is solidified with agar as well as having the right osmotic strength.



Molecular representation of the envelope of a Gram-negative bacterium.

***Ovals* and *rectangles* represent sugar residues, and circles depict the polar head groups of the glycerophospholipids (phosphatidylethanolamine and phosphatidylglycerol). The core region shown is that of *E. coli* K-12, a strain that does not normally contain an O-antigen repeat unless transformed with an appropriate plasmid. MDO, membrane-derived oligosaccharides. (Jawetz, p. 27)**

Capsule and Glycocalyx

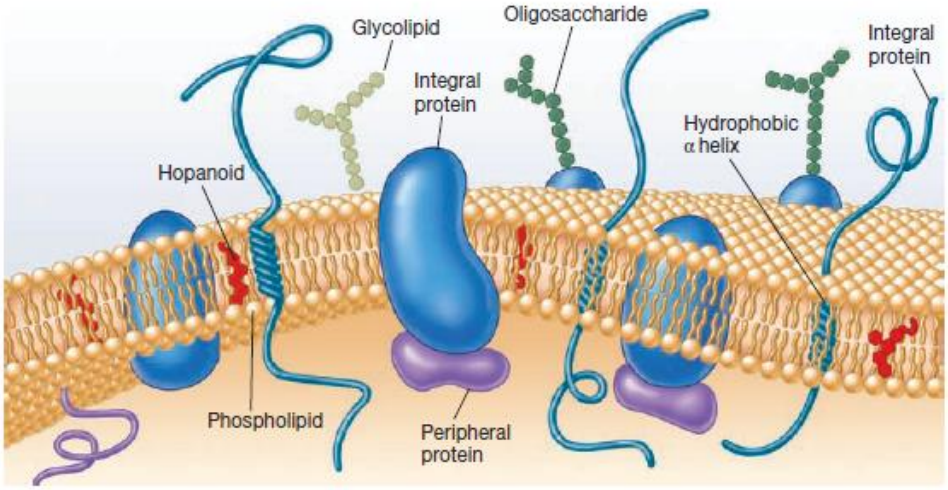
Many bacteria synthesize copious amounts of **extracellular polymers** when growing in their **natural** environments. With few exceptions (the **poly-*d* glutamic acid** capsules of ***Bacillus anthracis***), and the **mixed amino acid** capsule of ***Yersinia pestis***, **the extracellular** material is **polysaccharide**. The terms capsule and slime layer are frequently used to describe polysaccharide layers; the more inclusive شمولي term **glycocalyx** is also used. Glycocalyx is defined as the polysaccharide-containing material lying outside the cell. A **condensed**, **well-defined** layer **closely surrounding** the cell that **excludes particles**, such as India ink, is referred to as a **capsule**. If the glycocalyx is **loosely associated** with the cell and **does not exclude particles**, it is referred to as a **slime layer**.

Cell membrane (also named as cytoplasmic membrane)

- It is a delicate tri-laminar unit membrane.
- It accounts for 30% of the dry weight of bacterial cell.
- It is composed of 60% protein, 20-30% lipids and 10-20%carbohydrate.

The Fluid Mosaic Model of Membrane Structure

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. Most membrane-associated lipids are structurally asymmetric, with polar and nonpolar ends, and are called amphipathic. The polar ends interact with water and are hydrophilic; the nonpolar hydrophobic ends are insoluble in water and tend to associate with one another.



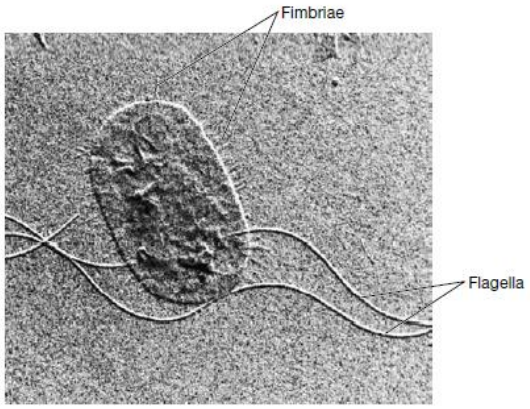
Bacterial Plasma Membrane Structure. This diagram of the fluid mosaic model of bacteria membrane structure shows the integral proteins (blue) floating in a lipid bilayer. Peripheral proteins (purple) are associated loosely with the inner membrane surface. Small spheres represent the hydrophilic ends of membrane phospholipids and wiggly tails, the hydrophobic fatty acid chains.Other membrane lipids such as hopanoids (red) may be present. (Prescott, p. 45)

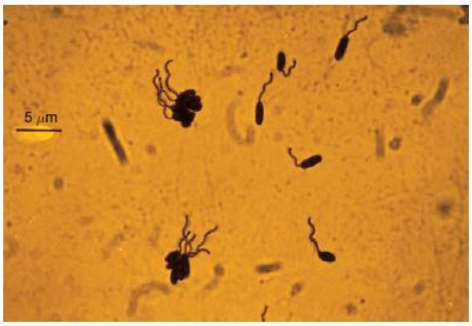
Flagella

Bacterial flagella are thread-like appendages composed entirely of protein. They are threadlike locomotor appendages extending outward from the plasma membrane and cell wall.

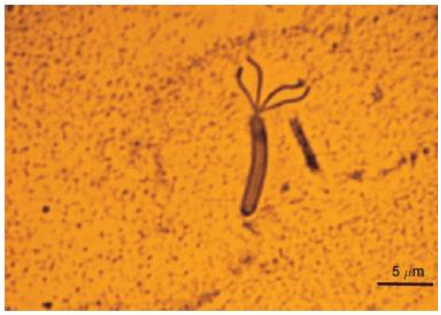
Bacterial species often differ distinctively in their patterns of flagella distribution and these patterns are useful in identifying bacteria. Four types of arrangement are known: **monotrichous** (trichous means hair) (single polar flagellum), it is said to be a **polar** flagellum., **lophotrichous** (lopho means tuft) (multiple polar flagella), **amphitrichous** (amphi means on both sides)(single flagellum found at each of two opposite poles), and **peritrichous (**Flagella are spread fairly evenly over the whole surface) (peri means around) (multiple flagella distributed over the entire cell).

A bacterial flagellum is made up of several protofilaments, each made up of thousands of molecules of a protein subunit called **flagellin**..

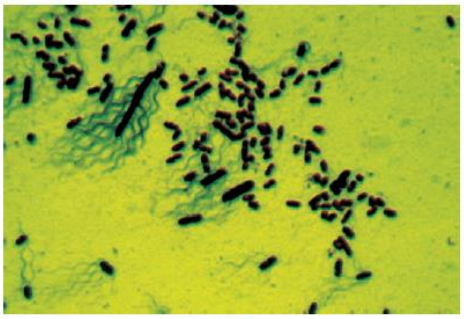




(a) *Pseudomonas*—monotrichous polar flagellation



(b) *Spirillum*—lophotrichous flagellation



(c) *P. vulgaris*—peritrichous flagellation

Flagellar Distribution. Examples of various patterns of flagellation as seen in the light microscope. (a) Monotrichous polar (*Pseudomonas*). (b) Lophotrichous (*Spirillum*). (c) Peritrichous (*Proteus vulgaris*, X600). (Prescott, p.68)

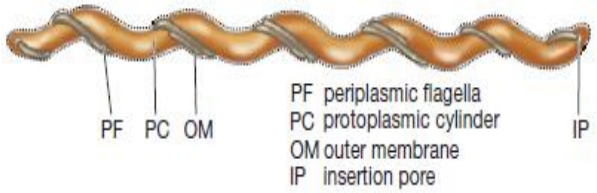
Swarming: An increasing number of bacterial species has been found to exhibit an interesting type of motility called swarming. This motility occurs on moist surfaces and is a type of group behavior in which cells move in unison across the surface.

Swarmming phenomena

Twitching الحركة الوخزية: motility is characterized by short, intermittent, jerky motions of up to several micrometers in length and is normally seen on very moist surfaces.

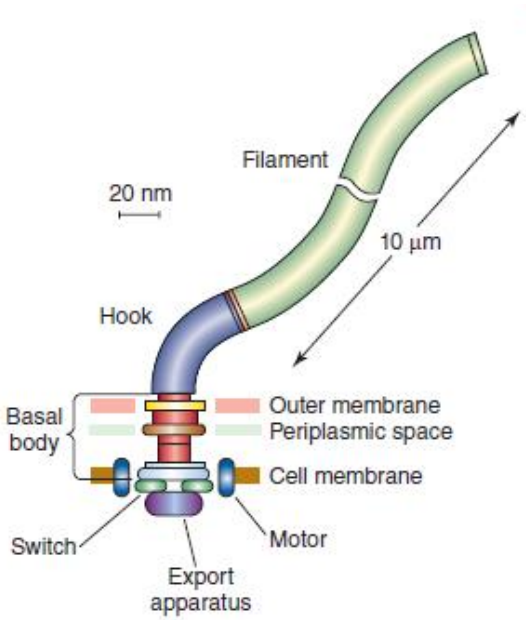
In contrast to the jerky movement of twitching motility, **gliding motility** is smooth.

Spirochete Motility: Spirochetes have flagella that work in a distinctive manner. In many spirochetes, multiple flagella arise from each end of the cell and wind around the cell (. The flagella do not extend outside the cell wall but rather remain in the periplasmic space and are covered by the outer membrane.



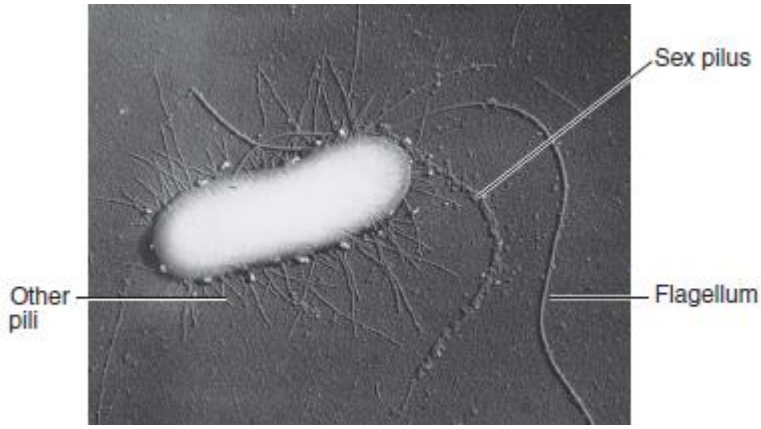
They are highly **antigenic** (H antigens), and some of the immune responses to infection are directed against these proteins.

The flagellum is attached to the bacterial cell body by a complex structure consisting of a **hook** and a **basal body**. Flagella are made stepwise. **First**, the **basal body** is assembled and inserted into the cell envelope. Then **the hook** is added, and **finally**, the **filament** is assembled progressively by the addition of flagellin subunits to its growing tip.



Pili and Fimbriae

Many procaryotes have short, fine, hairlike appendages that are thinner than flagella. These are usually called fimbriae (s., fimbria). Another type of pillus called sex pillus. Fimbriae are responsible for more than attachment. Type IV fimbriae are present at one or both poles of bacterial cells. They can aid in attachment to objects, and also are required for the twitching motility that occurs in some bacteria such as *P. aeruginosa*, *Neisseria gonorrhoeae*, and some strains of *E. coli*.



Pili of different bacteria are antigenically distinct and elicit the formation of antibodies by the host.

Endospores

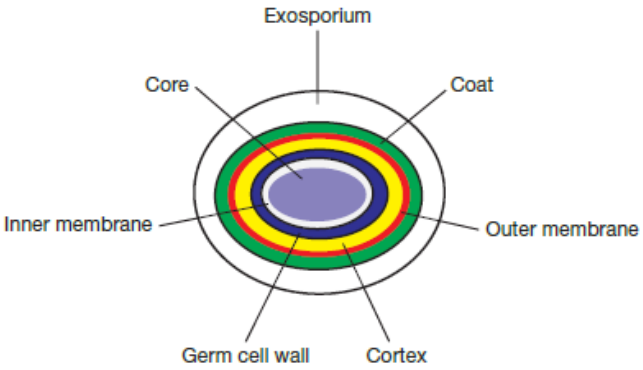
Members of several bacterial genera can form endospores. The two most common are Gram-positive rods: the obligately aerobic genus ***Bacillus*** and the obligately anaerobic genus ***Clostridium***.

The process, **sporulation**, is triggered by near depletion of any of several nutrients (carbon, nitrogen, or phosphorous). Each cell forms a single internal spore that is liberated when the mother cell undergoes autolysis.

The spore is a **resting cell**, **highly resistant to desiccation**, **heat**, and **chemical agents**; when returned to favorable nutritional conditions and activated, the spore **germinates** to produce a **single vegetative cell**. The **location** of an endospore within a cell is species-specific and can be used to determine the identity of a bacterium.

Properties of Endospores

1. **Core**—The core is the spore protoplast. It contains a complete chromosome, all the components of the proteinsynthesizing apparatus, and an energy-generating system based on glycolysis.

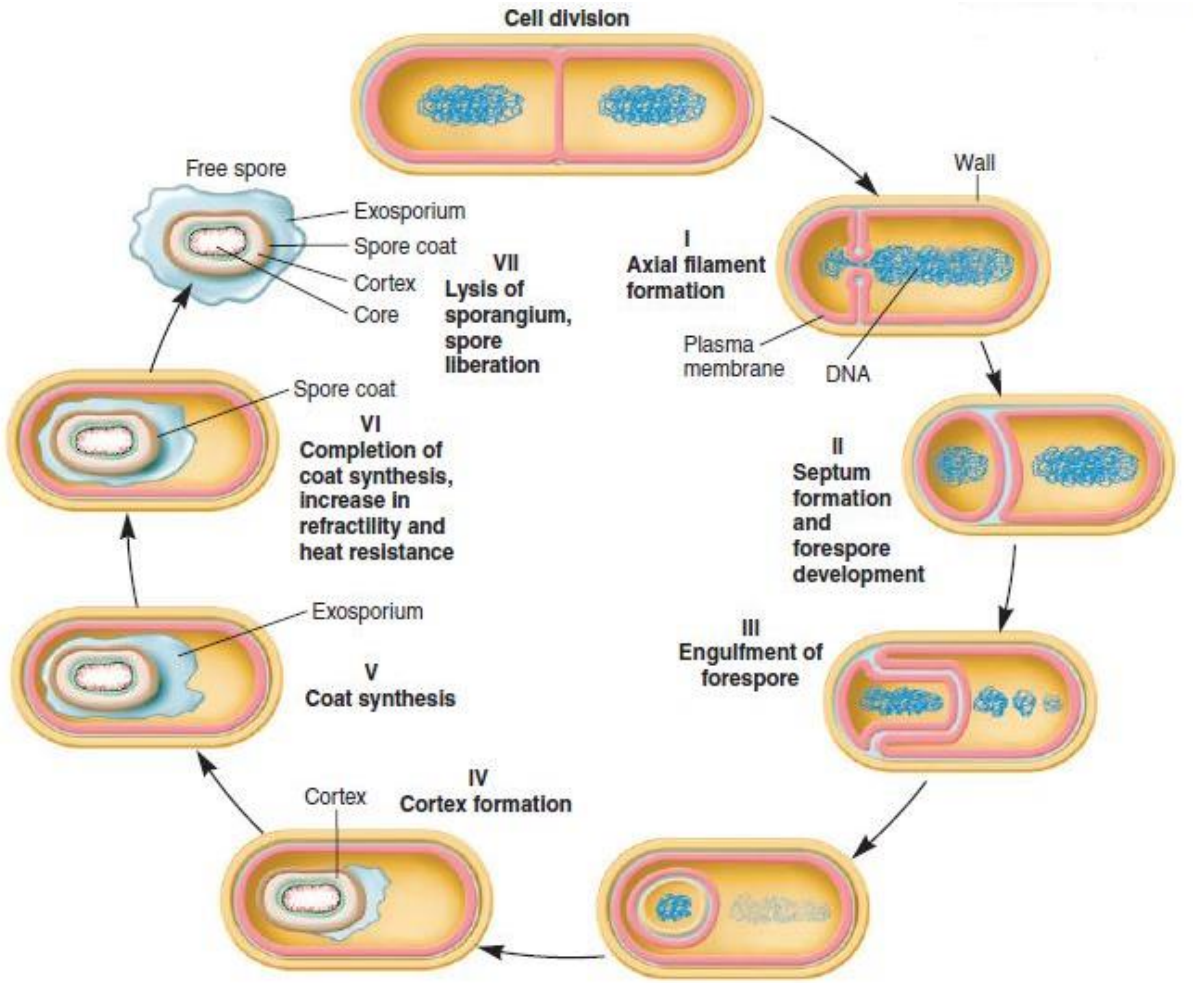


2. **Spore wall**—The innermost layer surrounding the inner spore membrane is called the spore wall.

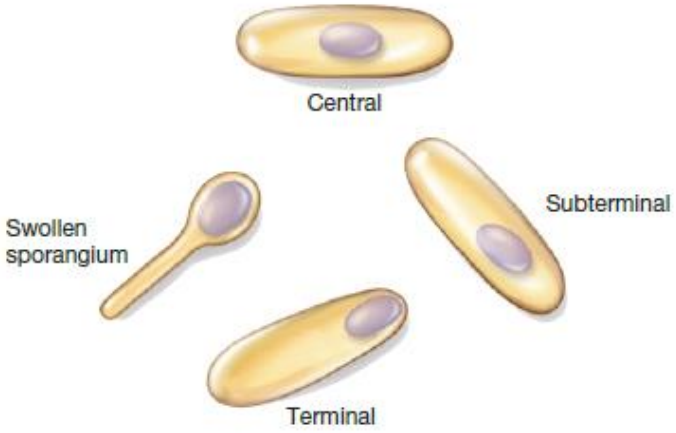
3. **Cortex**—The cortex is the thickest layer of the spore envelope. It contains an unusual type of peptidoglycan, with many fewer cross-links than are found in cell wall peptidoglycan. Cortex peptidoglycan is extremely sensitive to lysozyme, and its autolysis plays a role in spore germination.

4. **Coat.** The coat is composed of a keratin-like protein containing many intramolecular disulfide bonds.

5. **Exosporium**—The exosporium is composed of proteins, lipids, and carbohydrates.



The mature endospore occupies a characteristic location in the mother cell (referred to as the sporangium), depending on the species of bacteria. Endospores may be centrally located, close to one end (subterminal), or terminal. Sometimes an endospore is so large that it swells the sporangium.

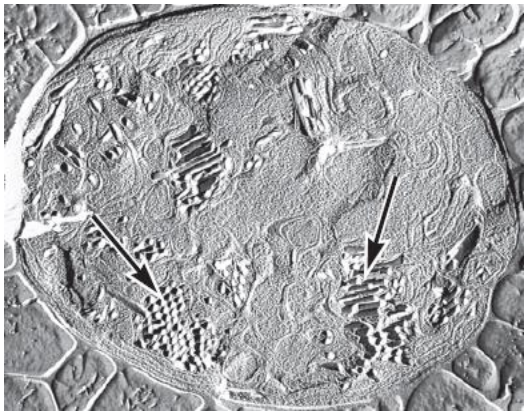


Inclusions

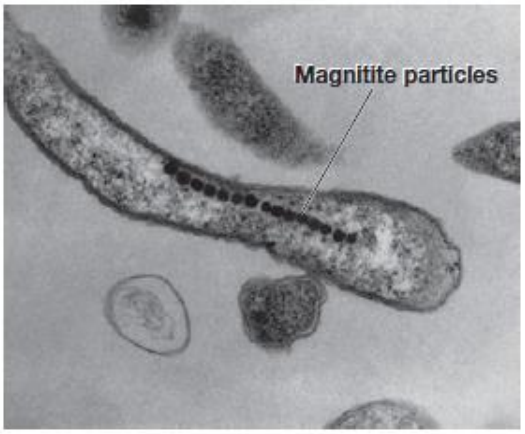
Inclusions are common in all cells. They are formed by the aggregation of substances that may be either organic or inorganic. Inclusions can take the form of granules حبات صغيرة, crystals بلورات, or globulesقطرات; some are amorphous.

Several important inclusions are found:

1. **Carbon granules**.
2. **Polyphosphate granules**.
3. **Carboxysomes**.
4. The **gas vacuole** provides buoyancy طفو to some aquatic bacteria.
5. Aquatic magnetotactic bacteria use **magnetosomes** to orient themselves in Earth's magnetic field.
6. **Metachromatic granules**.
7. **Polysaccharide Granules**.
8. **Lipid Inclusions.**
9. **Sulfur Granules.**



Gas Vacuoles are Clusters of Gas Vesicles. A freezefracture preparation of Anabaena flosaquae (389,000) showing gas vesicles and gas vacuoles. Both longitudinal and cross-sectional views of gas vesicles are indicated by arrows.



Magnetosomes. Transmission electron micrograph of the magnetotactic bacterium *Magnetospirillum magnetotacticum*.

Nucleoid

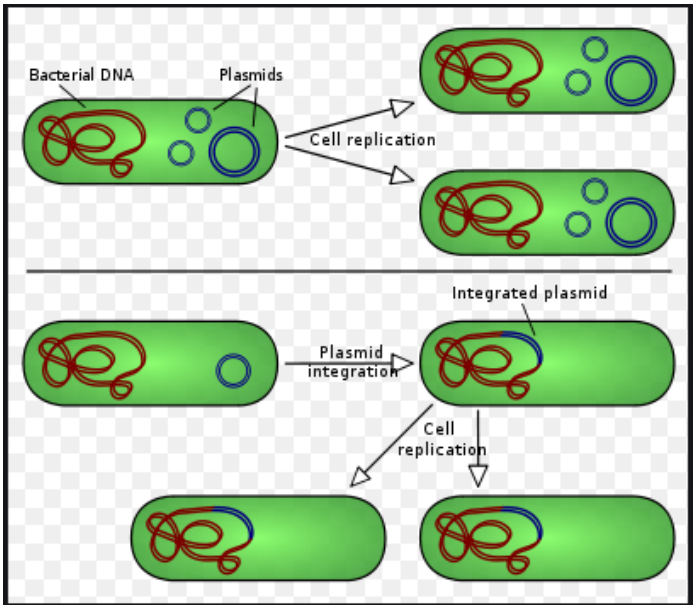
The nucleoid is an irregularly shaped region that contains the cell's chromosome and numerous proteins. The chromosomes of most bacteria are a single circle of doublestranded deoxyribonucleic acid (DNA), but some bacteria have a linear chromosome, and some bacteria, such as *Vibrio cholerae* and *Borrelia burgdorferi* (causative agents of cholera and Lyme disease, respectively), have more than one chromosome. A few bacteria (e.g., the very large bacteria *Thiomargarita* are polyploid.

Bacterial chromosomes are longer than the length of the cell. An important and still unanswered question is: how these microbes manage to fit their chromosomes into the relatively small space occupied by the nucleoid?

Answer: **Supercoiling** is thought to be important. It produces a dense, central core of DNA (*DNA is a polymer of deoxyribonucleotides*)with loops of DNA extending out from the core. Several nucleoid-associated proteins (NAPs) help compact the chromosome by causing the chromosome to bend and fold.

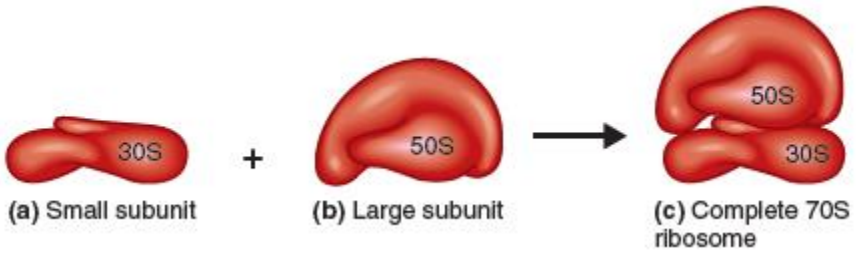
Plasmids

In addition to the genetic material present in the nucleoid, many bacteria contain **extrachromosomal DNA** molecules called plasmids. Plasmids use the cell's DNA-synthesizing machinery to replicate, but their replication is not linked to any particular stage of the cell cycle. Thus regulation of plasmid and chromosomal replication are **independent**. However, some plasmids are able to **integrate into the chromosome**. Such plasmids are called **episomes** and when integrated are replicated as part of the chromosome. Plasmids are inherited stably during cell division, but they are not always equally apportioned into daughter cells and sometimes are lost. The loss of a plasmid is called **curing**.



Ribosomes

All eukaryotic and prokaryotic cells contain ribosomes, where protein synthesis takes place. Prokaryotic ribosomes differ from eukaryotic ribosomes in the number of proteins and rRNA molecules they contain; they are also somewhat smaller and less dense than ribosomes of eukaryotic cells.



Microbial Growth

The requirements for microbial growth can be divided into two main categories: physical and chemical. Physical aspects include temperature, pH, and osmotic pressure. Chemical requirements include sources of carbon, nitrogen, sulfur, phosphorus, oxygen, trace elements, and organic growth factors.

Physical Requirements

Temperature

Most microorganisms grow well at the temperatures that humans favor. However, certain bacteria are capable of growing at extremes of temperature that would certainly hinder the survival of almost all eukaryotic organisms.

Microorganisms are classified into three primary groups on the basis of their preferred range of temperature: **psychrophiles** (cold-loving microbes), **mesophiles** (moderate-temperature– loving microbes), and **thermophiles** (heat-loving microbes).

Each bacterial species grows at particular **minimum**, **optimum**, and **maximum** temperatures. The minimum growth temperature is the lowest temperature at which the species will grow. The optimum growth temperature is the temperature at which the species grows best. The maximum growth temperature is the highest temperature at which growth is possible.

pH

pH refers to the acidity or alkalinity of a solution. Most bacteria grow best in a narrow pH range near neutrality, between pH 6.5 and 7.5. Very few bacteria grow at an acidic pH (acidophiles) below about pH 4.

Osmotic Pressure

Microorganisms obtain almost all their nutrients in solution from the surrounding water. Thus, they require water for growth. High osmotic pressures have the effect of removing necessary water from a cell. This osmotic loss of water causes **plasmolysis**, or **shrinkage** of the cell's cytoplasm.

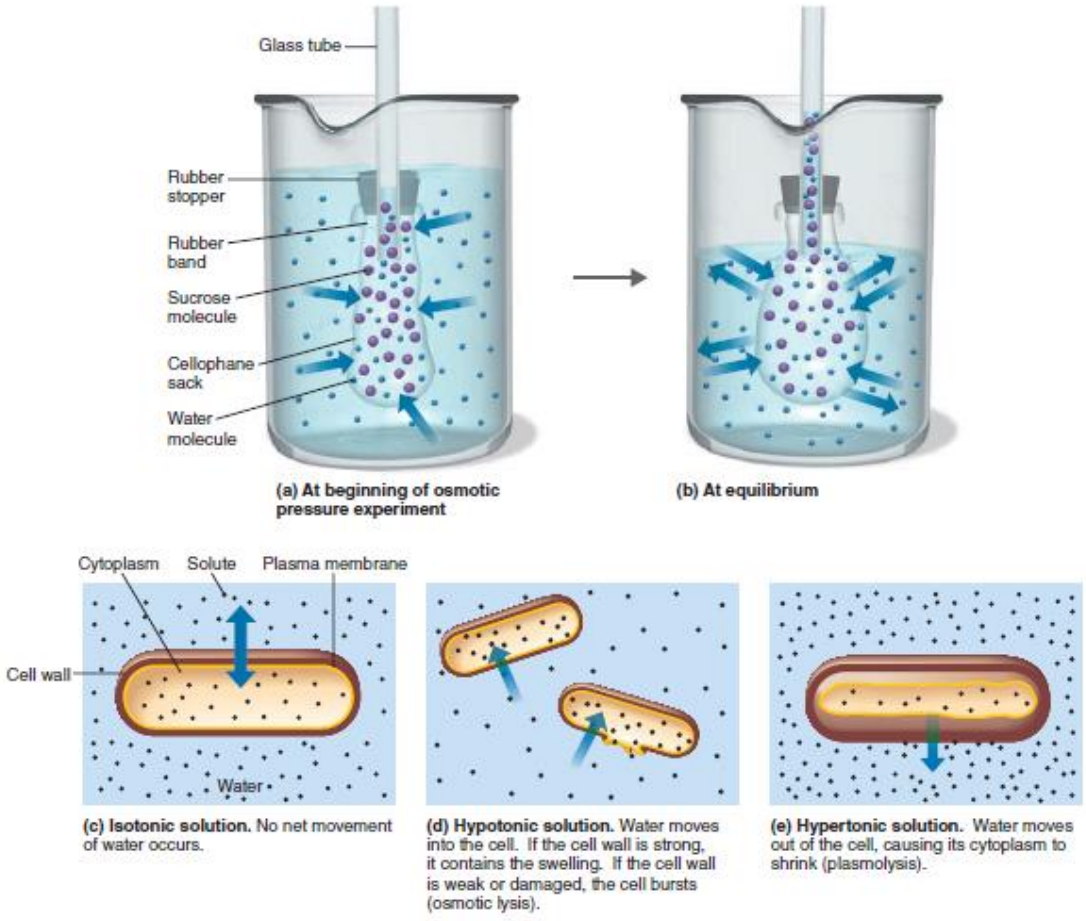
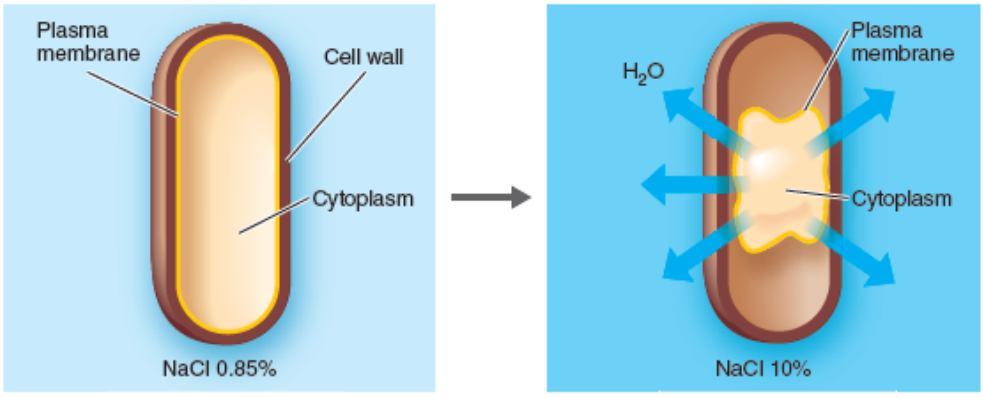


Figure 4.1 The principle of osmosis. (**a**) Setup at the beginning of an osmotic pressure experiment. Water molecules start to move from the beaker into the sack along the concentration gradient.
(**b**) Setup at equilibrium. The osmotic pressure exerted by the solution in the sack pushes water molecules from the sack back into the beaker to balance the rate of water entry into the sack. The height of the solution in the glass tube at equilibrium is a measure of the osmotic pressure. (**c**)–(**e**) The effects of various solutions on bacterial cells. (Tortora, an introduction to Microbiology, page, 92).



(a) Cell in isotonic solution. Under these conditions, the solute concentration in the cell is equivalent to a solute concentration of 0.85% sodium chloride (NaCl). See Figure 4.18.

(b) Plasmolyzed cell in hypertonic solution. If the concentration of solutes such as NaCl is higher in the surrounding medium than in the cell (the environment is hypertonic), water tends to leave the cell. Growth of the cell is inhibited.

Plasmolysis (Tortora, an introduction to Microbiology, page, 157).

Chemical Requirements

Carbon

Besides water, one of the most important requirements for microbial growth is carbon. Carbon is the structural backbone of living matter; it is needed for all the organic compounds that make up a living cell.

Nitrogen, Sulfur, and Phosphorus

In addition to carbon, microorganisms need other elements to synthesize cellular material. For example, protein synthesis requires considerable amounts of nitrogen as well as some sulfur.

The syntheses of DNA and RNA also require nitrogen and some phosphorus, as does the synthesis of ATP, the molecule so important for the storage and transfer of chemical energy within the cell.

Sulfur is used to synthesize sulfur-containing amino acids and vitamins such as thiamine and biotin.

Phosphorus is essential for the synthesis of nucleic acids and the phospholipids of cell membranes. Among other places, it is also found in the energy bonds of ATP.

Potassium, magnesium, and calcium are also elements that microorganisms require, often as cofactors for enzymes.

Trace Elements

Microbes require very small amounts of other mineral elements, such as iron, copper, molybdenum, and zinc; these are referred to as **trace elements.**

Oxygen

In table 4.1 the lecture summarize the effect of oxygen on the growth of various types of bacteria.

Table 4.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

Culture media

A nutrient material prepared for the growth of microorganisms in a laboratory is called a culture medium. Some bacteria can grow well on just about **any culture medium**; others require **special media**, and still **others cannot grow** on any nonliving medium yet developed. Microbes that are introduced into a culture medium to initiate growth are called an **inoculum**. The microbes that grow and multiply in or on a culture medium are referred to as a **culture**.

Culture medium must contain the **right nutrients** for the specific microorganism. It should also contain sufficient **moisture**, a properly adjusted **pH**, and a suitable level of **oxygen**, perhaps none at all.

The medium must initially be **sterile**—that is, **it must initially contain no living microorganisms**—so that the culture will contain only the microbes (and their offspring) we add to the medium. Finally, the growing culture should be incubated at the **proper temperature**.

When it is desirable to grow bacteria on a solid medium, a solidifying agent such as agar is added to the medium. A complex polysaccharide derived from a marine alga, **agar**. **Agar** liquefies at about **100**°C (the boiling point of water) and at sea level remains liquid until the temperature drops to about **40**°C. For laboratory use, agar is held in water baths at about **50**°C.

Agar media are usually contained in **test tubes** or **Petri dishes**. The test tubes are called **slants** when their contents are allowed to solidify with the tube **held at an angle** so that a large surface area for growth is available. When the agar solidifies in a **vertical** tube, it is called a **deep**.

A medium in which all **chemical components** are known is a **defined** or **synthetic medium.** Media that contain some ingredients of **unknown chemical composition** are **complex media.**

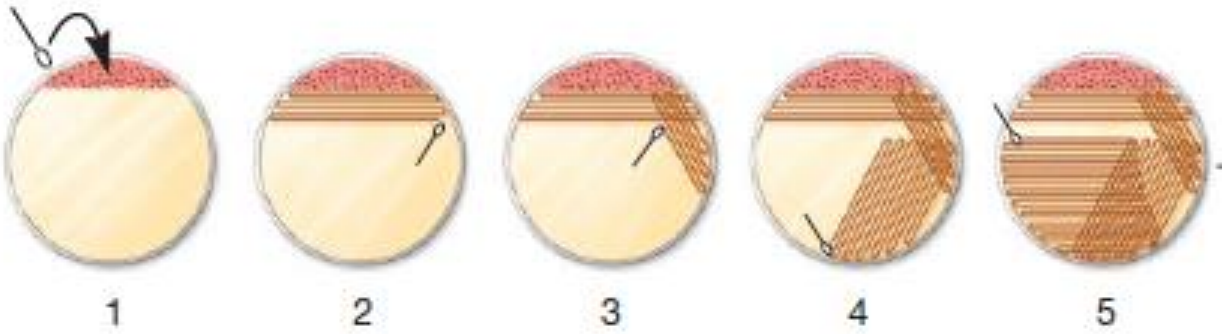
Functional Types of Media

- Media such as **tryptic soy broth** and **tryptic soy agar** are called general purpose or **supportive media**. because they sustain the growth of many microorganisms.
- Blood and other nutrients may be added to supportive media to **encourage** the growth of **fastidious** microbes. These fortified المعززmedia (e.g., blood agar) are called **enriched media**.
- **Selective media** allow the growth of particular microorganisms, while inhibiting the growth of others, such as **Eosin methylene blue agar** and **MacConkey agar**
- **Differential media** are media that distinguish among different groups of microbes. Blood agar is both a differential medium and an enriched one. It distinguishes between hemolytic and nonhemolytic bacteria, MacConkey agar is both differential and selective.

Cultivation of the Microorganisms

1. Streak plate technique

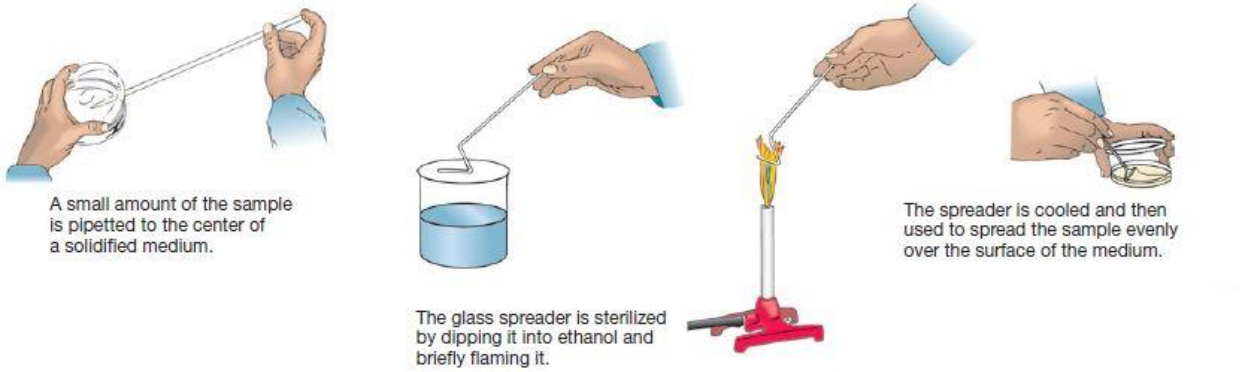
One method for separating cells is the streak plate. In this technique, cells are transferred to the edge of an agar plate with an inoculating loop or swab and then streaked across the surface in one of several patterns. After the first sector is streaked, the inoculating loop is sterilized and an inoculum for the second sector is obtained from the first sector. A similar process is followed for streaking the third sector, except that the inoculum is from the second sector.



Streak-Plate Technique. A typical streaking pattern (Prescott's Microbiology, 159)

Spread Plate and Pour Plate

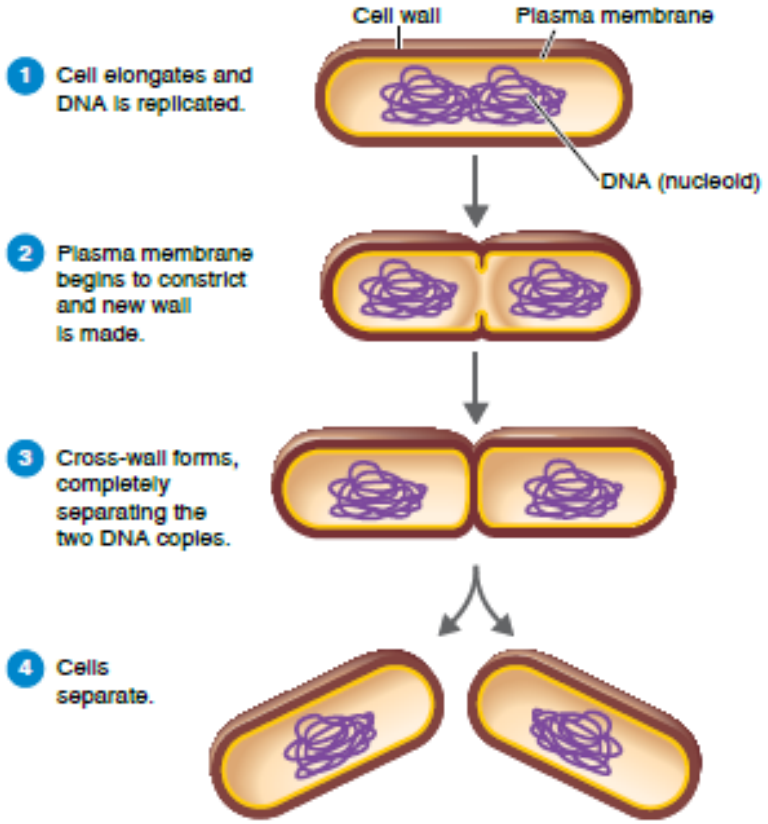
Spread-plate and **pour**-plate techniques are similar in that they both **dilute** a sample of cells before separating them spatially مكانيا. They differ in that the spread plate spreads the cells on the surface of the agar, whereas the **pour plate** embeds the cells **within** the agar.



Spread-Plate Technique. The preparation of a spread plate. (Prescott's Microbiology, 159)

Bacterial Growth Curve

Bacterial growth refers to an increase in bacterial numbers, not an increase in the size of the individual cells. Bacteria normally reproduce by binary fission.



A diagram of the sequence of cell division (Tortora, p. 165)

Bacterial population obtained after inoculation of the bacterium into a new culture medium.

The normal bacterial growth curve has four phases.

1. Lag phase

The period of **adaptation** with active macro-molecular synthesis like **DNA**, **RNA**, **various enzymes** and other structural components. It is the preparation time for reproduction; **no increase** in cell number.

2. Exponential(log) phase

The period of **active multiplication** of cells. Cell division precedes at a **logarithmic** rate, and determined by the medium and condition of the culture.

3. Maximal stationary phase

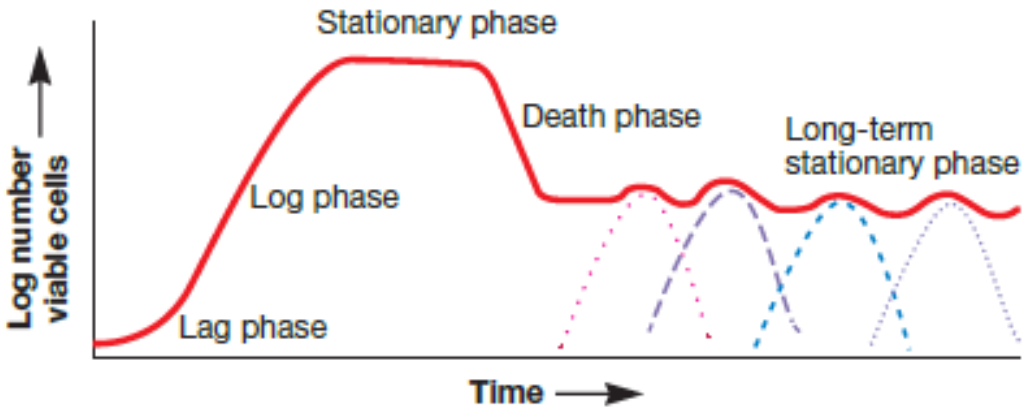
The period when the bacteria have achieved their maximal cell density or yield.

There is no further increase in viable bacterial cell number. The growth rate is exactly equal to the death rate. A bacterial population may reach stationary growth when one of the following conditions occur:

1. The required nutrients are exhausted.
2. Inhibitory end products are accumulated.
3. Physical conditions do not permit a further increase in population size.

4. Decline phase

The period at which the rate of death of bacterial cells exceeds the rate of new cell formation. There is drastic decline in viable cells. Few organisms may persist for so long time at this period at the expense of nutrients released from dying micro-organisms.



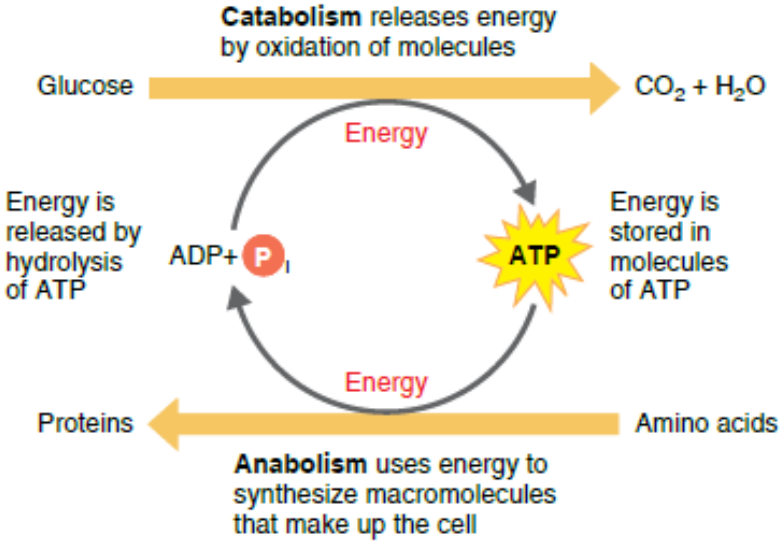
Microbial Growth Curve in a Closed System. (Prescott's, p161)

Bacterial Metabolism

The term **metabolism** refer to the sum of all chemical reactions within a living organism. Because chemical reactions either release or require energy, metabolism can be viewed as an energy-balancing act. Accordingly, metabolism can be divided into two classes of chemical reactions: those that release energy and those that require energy.

In living cells, the enzyme-regulated chemical reactions that release energy are generally the ones involved in **catabolism**, the breakdown of complex organic compounds into simpler ones. These reactions are called *catabolic*, or *degradative*, reactions. Catabolic reactions are generally hydrolytic reactions (reactions which use water and in which chemical bonds are broken), and they are exergonic (produce more energy than they consume). An example of catabolism occurs when cells break down sugars into carbon dioxide and water.

The enzyme-regulated energy-requiring reactions are mostly involved in **anabolism**, the building of complex organic molecules from simpler ones. These reactions are called *anabolic*, or *biosynthetic*, reactions. Anabolic processes often involve dehydration synthesis reactions (reactions that release water), and they are endergonic (consume more energy than they produce). Examples of anabolic processes are the formation of proteins from amino acids, nucleic acids from nucleotides, and polysaccharides from simple sugars. These biosynthetic reactions generate the materials for cell growth.



The role of ATP in coupling anabolic and catabolicreactions. (Tortora, p. 110)

Catabolic reactions provide building blocks for **anabolic reactions** and furnish the energy needed to drive anabolic reactions.

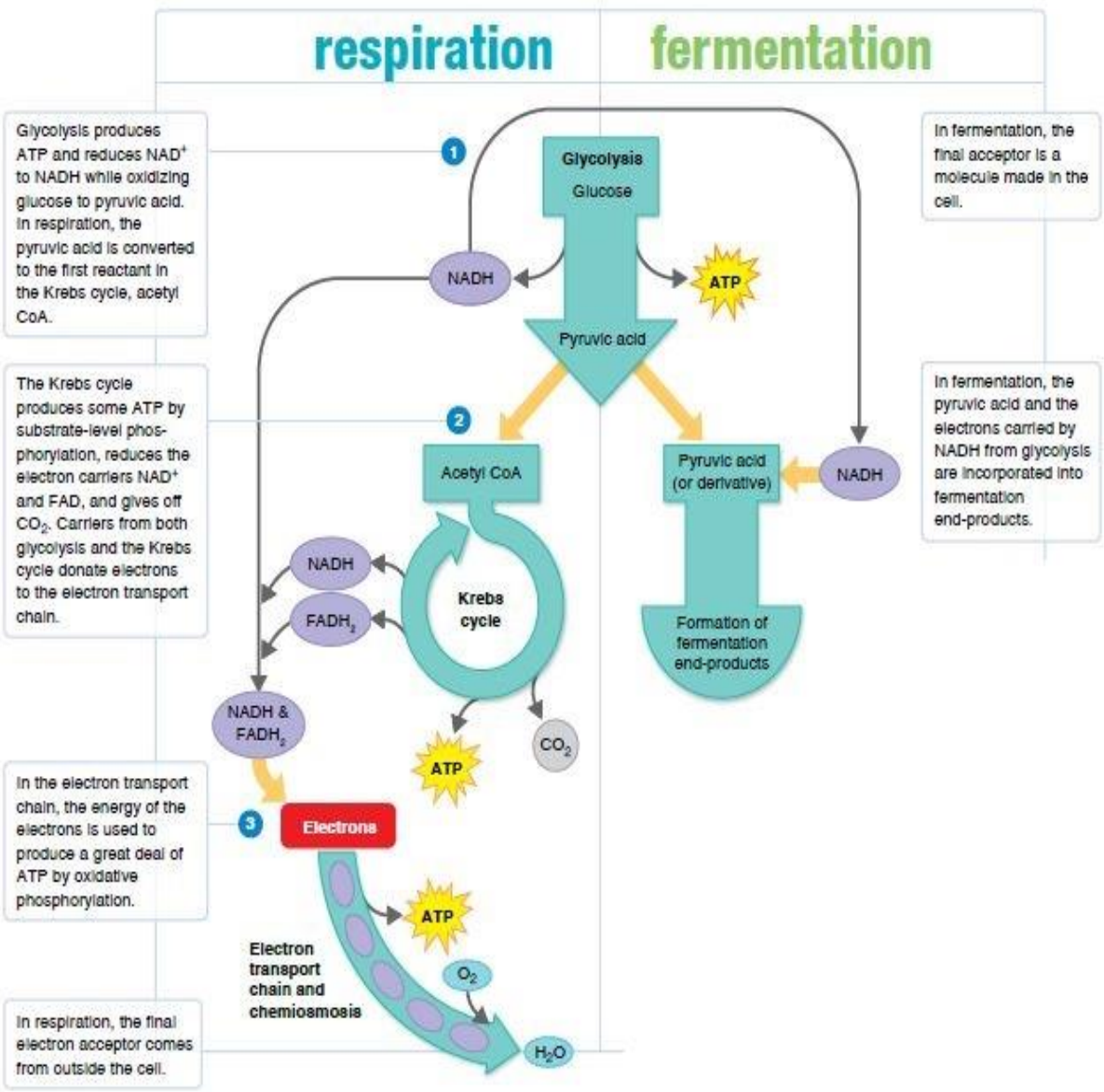
$$ATP \rightarrow ADP + P_i + energy$$

$$ADP + P_i + energy \rightarrow ATP$$

Metabolic Pathways of Energy Production

Organisms release and store energy from organic molecules by a series of controlled reactions rather than in a single burs.

An Overview of Metabolism



Bacterial Genetics

Genetics is the study of inheritance. Bacterial inherited characteristics are encoded in DNA. Bacteria have two types of DNA that contain their genes. These are :

- **Chromosome**
- **Extra chromosome: Plasmid.**

The bacterial chromosome is circular, double stranded DNA attached to bacterial cell membrane. DNA replication in bacteria is semi-conservative i.e. each strand of DNA is conserved intact during replication and becomes one of the two strands of the new daughter molecules.

Plasmids are self-replicating extra chromosomal DNA molecules. It multiplies independent of the host cell. Multiple copies of the same plasmid may be present in each bacterial cell.

Different plasmids are also often present in the same bacterial cell.

Plasmid types

There are many types of plasmid types. The following are examples.

a. R factors: Plasmids which contain genes that code for antibiotic resistance.

b. Col factors: Plasmids which contain genes that code for extracellular toxin (colicines) production that inhibit strains of the same and different species of bacteria.

c. F(fertility) factors: Plasmids that can recombine itself with the bacterial chromosome.

It promotes transfer of the chromosome at a high frequency of recombination into the chromosome of a second (recipient) bacterial cell during mating.

Genetic variation in Bacteria

Mechanisms: Mutation and Gene transfer

1. Mutation: It is due to a chemical alteration in DNA.

It could be spontaneous or induced by chemical and physical meanses. Mutants are variants in which one or more bases in their DNA are altered; which are heritable and irreversible

Types of mutation

1. Substitution: Change of a single base.

2. Deletion: Los of a base.

3. Insertion: Addition of a base.

2. Gene transfer

There are three types of gene transfer that alter the DNA gene content of bacteria.

These are:

- Transformation
- Transduction
- Conjugation

1. Transformation occurs when fragments of exogenous bacterial DNA are taken up and absorbed into recipient bacterial cells.

Transformation of genes from one bacterium to another results in Change in pathogenicity of the bacterium. Change in antibiotic sensitivity pattern of bacterium.

Competence: The recipient bacterium must be competent to absorb the exogenous fragments of bacterial DNA.

Frequency: The frequency of transformation is low.

2. Transduction occurs when fragments of chromosomal DNA is transferred or transduced into a second bacterium by phage.

During phage replication, the bacterial DNA may be accidentally enclosed instead of the normal phage DNA, and when this particle which enclosed the bacterial DNA infects a second bacterial cell, the DNA from the first bacterium is released and incorporated into The chromosome of the second bacterium.

3. Conjugation occurs when plasmid DNA is transferred from donor to recipient bacterium by direct contact via a sex pilus.

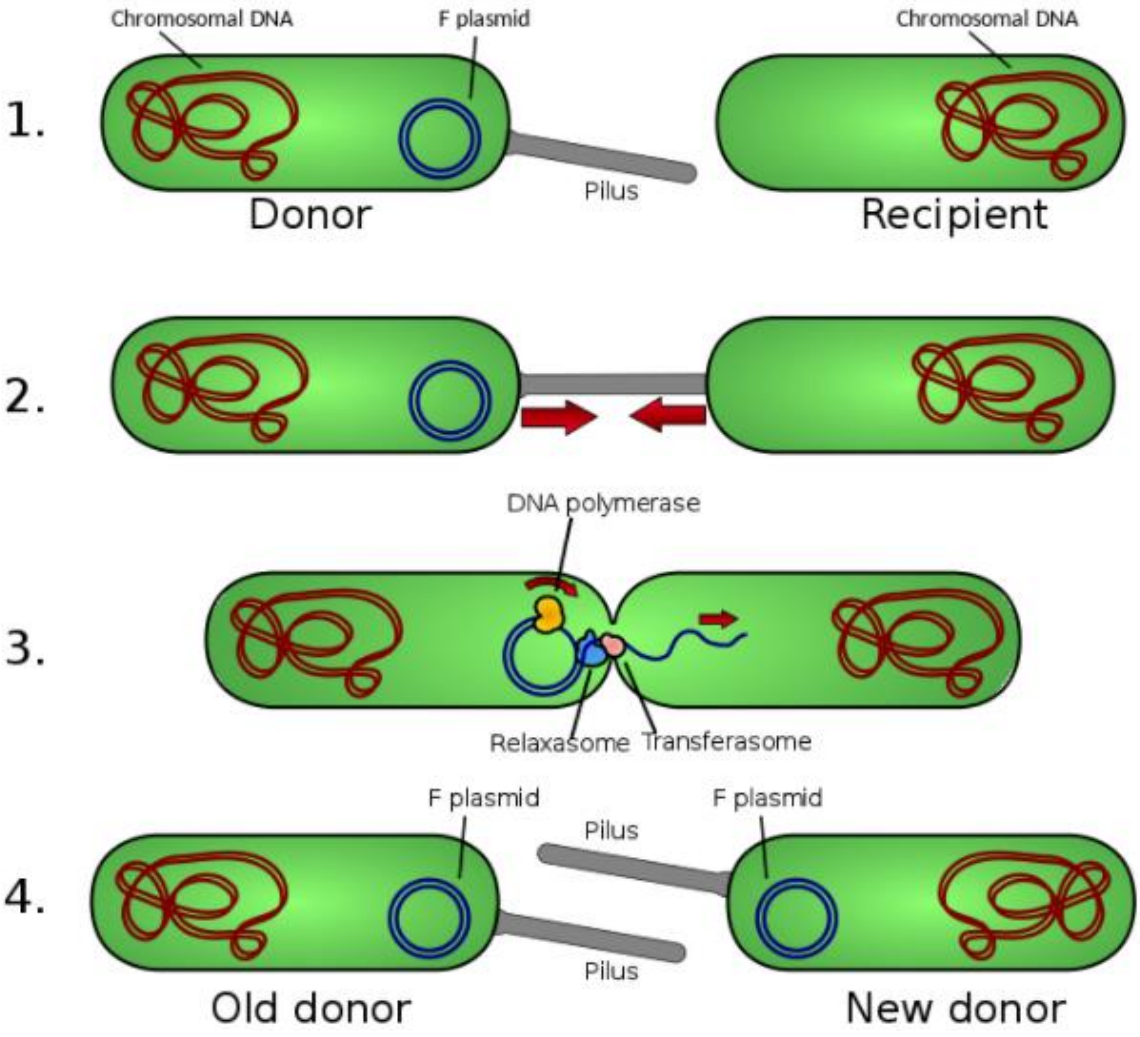
4. Transposition

Mechanism which enhances genetic flexibility among plasmids and bacterial chromosomes.

Transposons(Jumping genes) are segments of DNA that can transpose or move extremely readily, from plasmid to plasmid or from plasmid to chromosome(and viceversa).In this way, plasmid genes become part of the chromosomal component of genes.

When transposons transfer to a new site, it is usually a copy of the transposon that moves, the original transposon remaining in situ.

Transposons code for toxin production, resistance to antibiotics as wellas other fuctions.



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available at: https://commons.wikimedia.org/wiki/File:Conjugation.svg

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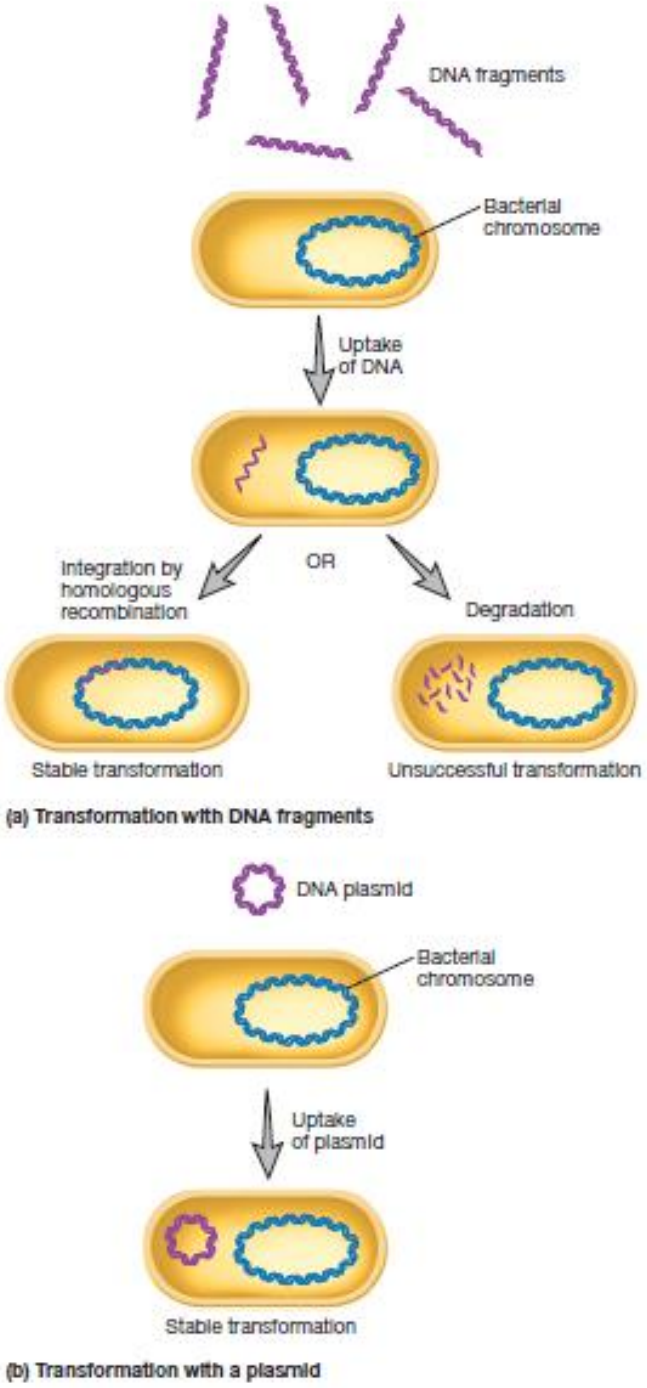
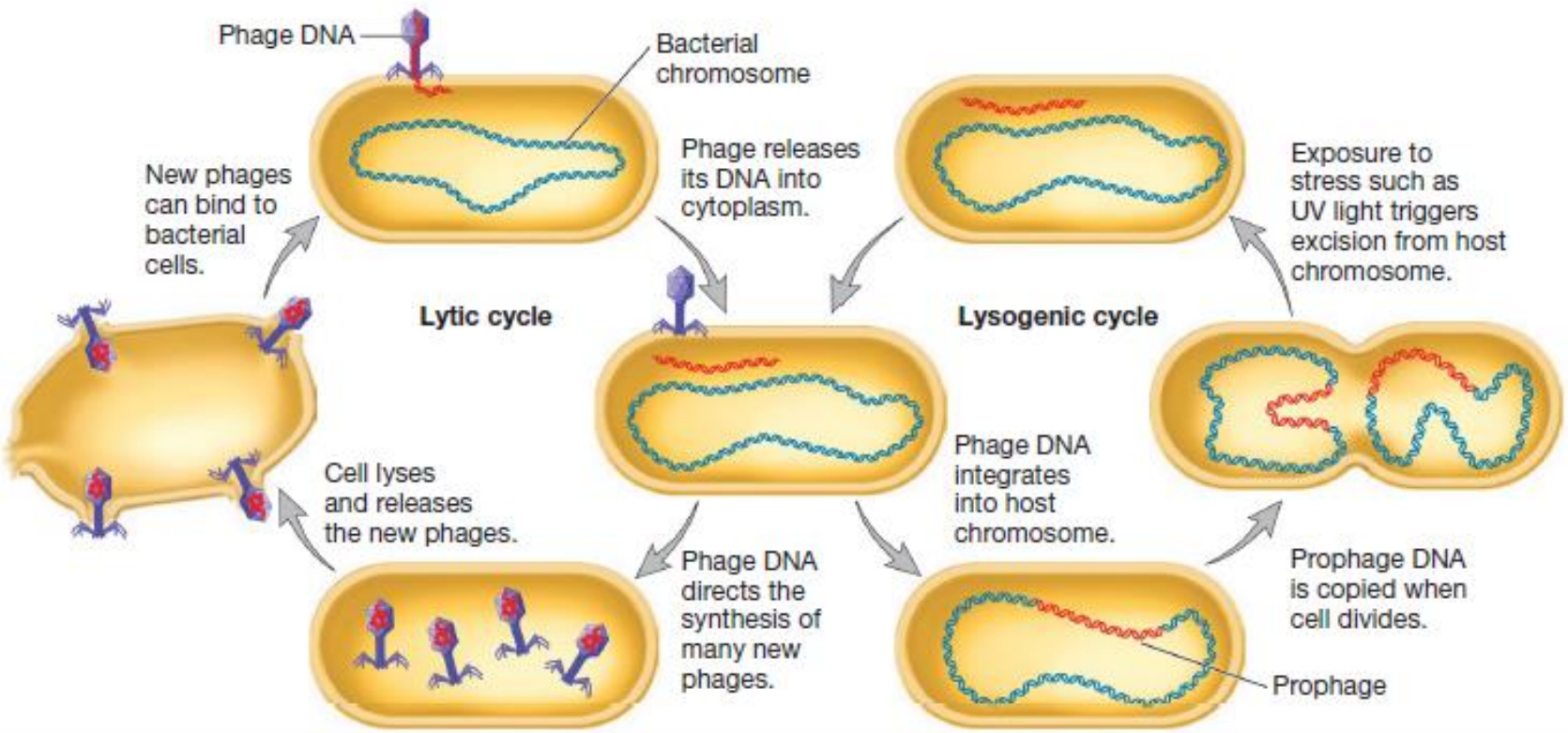


Bacterial Transformation. (Prescotts, p390)



Lytic and Lysogenic Cycles of Temperate Phages (العاثيات المعتدلة (او المتكيفة . Virulent phages undergo only the lytic cycle. Temperate phages have two phases to their life cycles. The lysogenic cycle allows the genome of the virus to be replicated passively as the host cell's genome is replicated. Certain environmental factors such as UV light can cause a switch from the lysogenic cycle to the lytic cycle. In the lytic cycle, new virions are made and released when the host cell lyses.(Prescott's, p.392)

Normal Flora (Microflora or Microbiota)

Introduction

Normal flora (normal microbial flora) is the term used to describe the population of microorganisms that inhabit the skin and mucous membranes of healthy normal persons at certain body sites. The human body is continuously inhabited mostly by bacteria and fungi. Viruses and parasites (protozoa and helminths), which are the other major groups of microorganisms, are usually not considered members of the normal flora but humans can be carriers of some of these organisms). The normal flora organisms are often referred to as commensals. Commensals are organisms that derive benefit from another host but do not damage that host.

The normal microbiota is new term used recently instead of Normal flora. The genomes of these microbial symbionts are collectively defined as the microbiome. Normal microbiota provides a first line of defense against microbial pathogens, assists in digestion, plays a role in toxin degradation, and contributes to maturation of the immune system. Although the normal flora extensively populates many areas of the body (especially the skin, oropharynx, colon, and vagina), the internal organs usually are sterile. Areas such as the central nervous system, blood, lower bronchi and alveoli, liver, spleen, kidneys, and bladder are generally considered to be sterile.

The human body harbors a variety of microorganisms that can be arranged into two groups: (1) the resident microbiota consists of relatively fixed types of microorganisms regularly found in a given area at a given age; if disturbed, it promptly reestablishes itself; and (2) the transient microbiota consists of nonpathogenic or potentially pathogenic microorganisms that inhabit body sites for hours, days, or weeks. The transient microbiota is derived from the environment, does not produce disease, and does not establish itself permanently. There is a distinction between the presence of these organisms and the carrier state. The term carrier implies that an individual harbors a potential pathogen and therefore can be a source of infection of others.

Distribution of normal flora in the body

The most common sites of the body inhabited by normal flora are those in contact or communication with the outside world, namely, the skin, eye, and mouth as well as the respiratory, gastrointestinal, and urogenital tracts, as summarized in Table 1.

1- **Skin.** The predominant member of the normal flora of the skin is *S. epidermidis*. It is an important cause of infections of prosthetic heart valves and prosthetic joints. *C. albicans*, a yeast also found on the skin, can enter the bloodstream and cause disseminated infections, such as endocarditis in intravenous drug users. *S. aureus* is also present on the skin, but its main site is in the nose. It causes abscesses in the skin and in many other organs.

2- **Eye** :The conjunctiva of the eye is colonized primarily by *S. epidermidis*, followed by *S. aureus*, aerobic corynebacteria (diphtheroids), and *Streptococcus pneumoniae*. Other organisms that normally inhabit the skin are also present but at a lower frequency. Tears, which contain the antimicrobial enzyme lysozyme, help limit the bacterial population of the conjunctiva.

3- **Oropharynx.** The main members of the normal flora of the mouth and throat are the viridans streptococci, such as *S. sanguinis* and *S. mutans*. Viridans streptococci are the most common cause of subacute endocarditis.

4- **Gastrointestinal tract.** The stomach contains very few organisms because of the low pH. The colon contains the largest number of normal flora and the most diverse species, including both anaerobic and facultative bacteria. There are both gram-positive and gram-negative rods and cocci. The members of the colonic normal flora are an important cause of disease outside of the colon. The two most important members of the colonic flora that cause disease are the anaerobe *B. fragilis* and the facultative *E. coli. E. faecalis*, a facultative, is also a very important pathogen.

5- **Vagina.** Lactobacilli are the predominant normal flora organisms in the vagina. They keep the pH of the vagina low, which inhibits the growth of organisms such as *C. albicans*, an important cause of vaginitis.

6- **Urethra.** The outer third of the urethra contains a mixture of bacteria, primarily *S. epidermidis*. The female urethra can become colonized with fecal flora such as *E. coli*, which predisposes to urinary tract infections.

Table 1: Summary of the members of normal flora and their locations

Location	Important Organisms[1]	Less Important Organisms[2]
Skin	*Staphylococcus epidermidis*	*Staphylococcus aureus, Corynebacterium* (diphtheroids), various streptococci, *Pseudomonas aeruginosa,* anaerobes (e.g., *Propionibacterium*), yeasts (e.g., *Candida albicans*)
Nose	*S. aureus*[3]	*S. epidermidis, Corynebacterium* (diphtheroids), various streptococci
Mouth	Viridans streptococci	Various streptococci, *Eikenella corrodens*
Dental plaque	*Streptococcus mutans*	*Prevotella intermedia, Porphyromonas gingivalis*
Gingival crevices	Various anaerobes (e.g., *Bacteroides, Fusobacterium,* streptococci, *Actinomyces*)	
Throat	Viridans streptococci	Various streptococci (including *Streptococcus pyogenes* and *Streptococcus pneumoniae*), *Neisseria* species, *Haemophilus influenzae, S. epidermidis*
Colon	*Bacteroides fragilis, Escherichia coli*	*Bifidobacterium, Eubacterium, Fusobacterium, Lactobacillus,* various aerobic gram-negative rods, *Enterococcus faecalis* and other streptococci, *Clostridium*
Vagina	*Lactobacillus, E. coli,*[3] group B streptococci[3]	Various streptococci, various gram-negative rods. *B. fragilis, Corynebacterium* (diphtheroids), *C. albicans*
Urethra		*S. epidermidis, Corynebacterium* (diphtheroids), various streptococci, various gram-negative rods (e.g., *E. coli*)[3]

[1]Organisms that are medically significant or present in large numbers.

[2]Organisms that are less medically significant or present in smaller numbers.

[3]These organisms are not part of the normal flora in this location but are important colonizers.

BENEFICIAL FUNCTIONS OF NORMAL FLORA Normal flora provides considerable benefits to the host.

➢ The members of the normal flora occupy receptor sites on the skin and mucosal surfaces, thereby preventing pathogens from binding to those receptors. (Also known as Colonization resistance, Figure 1)

➢ some bacteria of the bowel produce antimicrobial substances that is harmful to pathogenic bacteria

➢ Third, bacterial colonization of a newborn infant acts as a powerful stimulus for the development of the immune system. The microbiome plays an important role in "educating" the immune system.

➢ Bacteria of the gut provide important nutrients, such as vitamin K, and aid in digestion and absorption of nutrients.

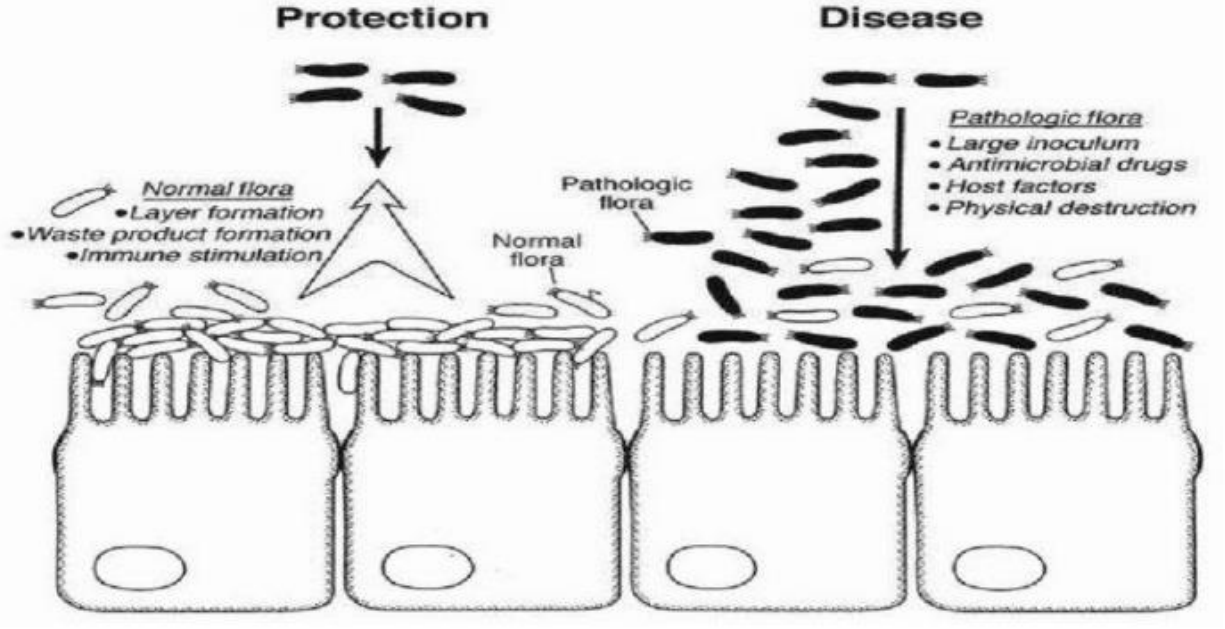


Figure 1: The mechanisms by which normal flora compete with gut pathogens

HARMFUL EFFECTS OF NORMAL FLORA Clinical problems caused by normal flora arise in the following ways:

➢ The organisms are displaced from their normal site in the body to an abnormal site. An example already mentioned is the introduction of the normal skin bacterium, S. epidermidis, into the bloodstream where it can colonize catheters and artificial joints.

➢ Potential pathogens gain a competitive advantage because of diminished populations of the microbiome. For example, normal bowel flora is depleted by antibiotic therapy leading to overgrowth by antibiotic-resistant Clostridium difficile, which can cause severe colitis.

➢ Harmless, commonly ingested food substances are converted into carcinogenic derivatives by bacteria in the colon. A well-known example is the conversion by

bacterial sulfatases of the sweetener cyclamate into the bladder carcinogen cyclohexamine.

➢ When individuals are immunocompromised, normal flora can overgrow and become pathogenic.

10. Bacterial virulence factors

Pathogenicity: is the ability to cause disease by overcoming host defenses, whereas virulence is the degree of pathogenicity**.** To cause disease, most pathogens must gain access to the host, adhere to host tissues, penetrate or evade host defenses, and damage the host tissues. However, some microbes do not cause disease by directly damaging host tissue. Instead, disease is due to the accumulation of microbial waste products.

Some microbes, such as those that cause dental caries and acne, can cause disease without penetrating the body.

Portals of Entry

1. Mucous Membranes

Many bacteria and viruses gain access to the body by penetrating mucous membranes lining the respiratory tract, gastrointestinal tract, genitourinary tract, and conjunctiva, a delicate membrane that covers the eyeballs and lines the eyelids.

Skin

The skin is the largest organ of the body, in terms of surface area and weight, and is an important defense against disease. Unbroken skin is impenetrable by most microorganisms. Some microbes gain access to the body through openings in the skin, such as hair follicles and sweat gland ducts. Larvae يرقة of the hookworm actually bore through intact skin, and some fungi grow on the keratin in skin or infect the skin itself.

Penetration of Host Defenses

Several factors that contribute to the ability of bacteria to invade a host.

1. Capsules: The capsule resists the host's defenses by impairing phagocytosis, the process by which certain cells of the body engulf and destroy microbes.

2. Cell Wall Components:

- **M protein=** mediates attachment of the bacterium to epithelial cells of the host and helps the bacterium resist phagocytosis by white blood cells.

- Some microorganisms use **fimbriae** and an outer membrane protein called **Opa** to attach to host cells. (Bacteria that produce Opa form *opa*que colonies on culture media.)
- The **waxy lipid** (mycolic acid) that makes up the cell wall of *Mycobacterium tuberculosis* also increases virulence by resisting digestion by phagocytes, and the bacteria can even multiply inside phagocytes.

3. **Enzymes**

- **Coagulases** are bacterial enzymes that coagulate (clot) the fibrinogen in blood.
- Bacterial **kinases** are bacterial enzymes that break down fibrin and thus digest clots formed by the body to isolate the infection.
- **Hyaluronidase** is another enzyme secreted by certain bacteria, such as streptococci. It hydrolyzes hyaluronic acid, a type of polysaccharide that holds together certain cells of the body, particularly cells in connective tissue.
- **Collagenase**, produced by several species of Clostridium, facilitates the spread of gas gangrene. Collagenase breaks down the protein collagen, which forms the connective tissue of muscles and other body organs and tissues.

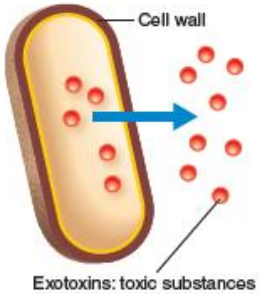
Production of Toxins

Toxins are poisonous substances that are produced by certain microorganisms. They are often the primary factor contributing to the pathogenic properties of those microbes. The capacity of microorganisms to produce toxins is called **toxigenicity**. Toxins transported by the blood or lymph can cause serious, and sometimes fatal, effects. Some toxins produce **fever**, **cardiovascular disturbances** اضطراب الجهاز القلبي الوعائي , **diarrhea**, and **shock** صدمة.

- **Exotoxins:** Exotoxins are produced inside some bacteria as part of their growth and metabolism and are secreted by the bacterium into the surrounding medium or released following lysis. (*Exo*- refers to "outside,").
- **Endotoxins:** Endotoxins differ from exotoxins in several ways. Endomeans "within," in this context referring to the fact that the endotoxins are located within the bacterial cells. Endotoxins are part of the outer portion of the cell wall of gramnegative bacteria**.** The lipid portion of LPS, called **lipid A**, is the endotoxin. Thus, endotoxins are lipopolysaccharides, whereas exotoxins are proteins.

exotoxins

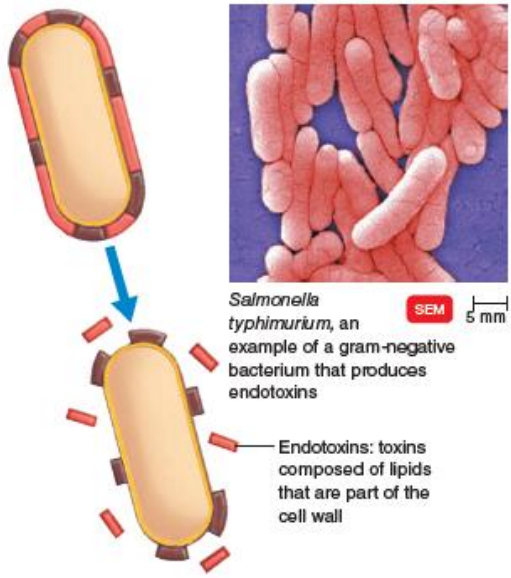
Proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted into the surrounding medium during log phase.



Clostridium botulinum, an example of a gram-positive bacterium that produces exotoxins

endotoxins

Lipid portions of lipopolysaccharides (LPS) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A). The endotoxins are liberated when the bacteria die and the cell wall lyses, or breaks apart.

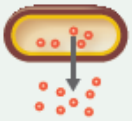


Salmonella typhimurium, an example of a gram-negative bacterium that produces endotoxins

KEY CONCEPTS

- Toxins are of two general types: exotoxins and endotoxins.
- Bacterial toxins can cause damage to host cells.
- Toxins can elicit an inflammatory response in the host, as well as activate the complement system.
- Some gram-negative bacteria may release minute amounts of endotoxins, which may stimulate natural immunity.

Mechanisms of Exotoxins and Endotoxins (Tortora, p. 425)

Property	Exotoxins	Endotoxins
		
Bacterial Source	Mostly from gram-positive bacteria	Gram-negative bacteria
Relation to Microorganism	Metabolic product of growing cell	Present in LPS of outer membrane of cell wall and released with destruction of cell or during cell division
Chemistry	Proteins, usually with two parts (A-B)	Lipid portion (lipid A) of LPS of outer membrane (lipopolysaccharide).
Pharmacology (Effect on Body)	Specific for a particular cell structure or function in the host (mainly affects cell functions, nerves, and gastrointestinal tract)	General, such as fever, weaknesses, aches, and shock; all produce the same effects
Heat Stability	Unstable; can usually be destroyed at 60–80°C (except staphylococcal enterotoxin)	Stable; can withstand autoclaving (121°C for 1 hour)
Toxicity (Ability to Cause Disease)	High	Low
Fever-Producing	No	Yes
Immunology (Relation to Antibodies)	Can be converted to toxoids to immunize against toxin; neutralized by antitoxin	Not easily neutralized by antitoxin; therefore, effective toxoids cannot be made to immunize against toxin
Lethal Dose	Small	Considerably larger
Representative Diseases	Gas gangrene, tetanus, botulism, diphtheria, scarlet fever	Typhoid fever, urinary tract infections, and meningococcal meningitis

11. Chemotherapy

- ✓ **Chemotherapy** is the chemical treatment of a disease.
- ✓ Two types of chemotherapeutic agents are **synthetic** drugs (chemically prepared in the laboratory) and **antibiotics** (substances produced naturally by bacteria and fungi that inhibit the growth of bacteria).
- ✓ Paul Ehrlich introduced an arsenic-containing chemical called salvarsan to treat syphilis (1910).
- ✓ Alexander Fleming observed that the Penicillium fungus inhibited the growth of a bacterial culture. He named the active ingredient penicillin (1928).
- ✓ Researchers are tackling the problem of drug-resistant microbes.

A sterilizing agent is called a **sterilant**. Liquids or gases can be sterilized by filtration.

Control directed at destroying harmful microorganisms is called **disinfection**. It usually refers to the destruction of vegetative (non–endospore-forming) pathogens, which is not the same thing as complete sterility.

In practice, the term is most commonly applied to the use of a chemical (a **disinfectant**) to treat an inert surface or substance. When this treatment is directed at living tissue, it is called **antisepsis**, and the chemical is then called an **antiseptic**.

Names of treatments that cause the outright death of microbes have the suffix **-cide**, meaning **kill**. A **biocide**, or **germicide**, kills microorganisms.

Other treatments only **inhibit** the growth and multiplication of bacteria; their names have the suffix **-stat** or **-stasis**, meaning to **stop** or to **steady**, as in bacteriostasis.

Sepsis, from the Greek for decay or putrid, indicates bacterial contamination.

Aseptic means that an object or area is free of pathogens.

Antimicrobial agents are often classified as **narrow-spectrum drugs**—that is, they are effective only against a **limited** variety of pathogens—or **broad spectrum** drugs that attack **many** different kinds of bacteria.

Some idea of the effectiveness of a chemotherapeutic agent against a pathogen 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. On the other hand, **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 more than the MIC, whereas a static agent kills at much higher concentrations, if at all.

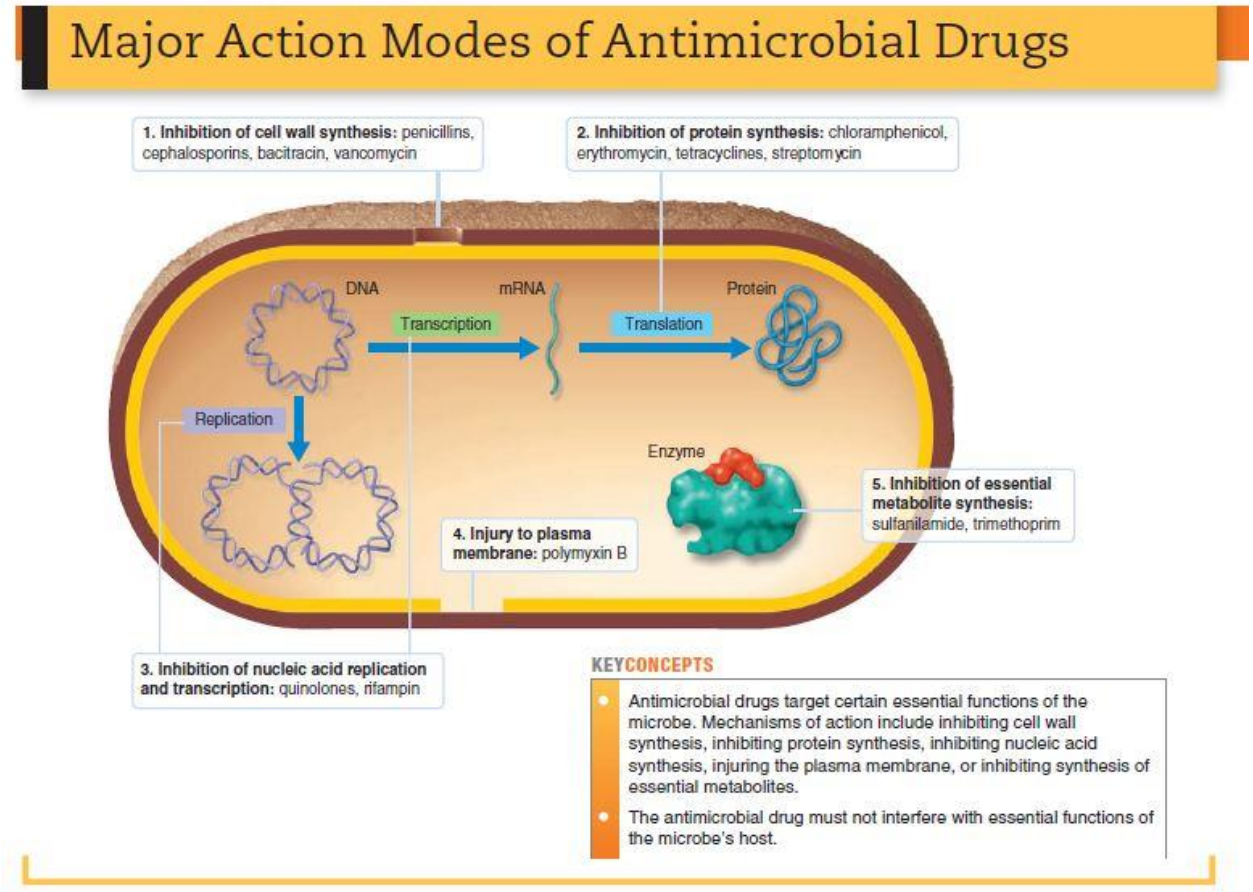
Properties of Some Common Antibacterial Drugs

Antibiotic Group	Primary Effect	Mechanism of Action	Example 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
Protein Synthesis Inhibition					
Aminoglycosides	Cidal	Bind to small ribosomal subunit (30S) and interfere with protein synthesis by directly 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 23S rRNA of large ribosomal subunit (50S) to inhibit peptide chain elongation during protein synthesis	Erythromycin	Broad (aerobic and anaerobic Gram-positive, some Gram-negative)	Gastrointestinal upset, hepatic injury, anemia, allergic reactions
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
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
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

Antimicrobial Activity Can Be Measured by Specific Tests….. Practical

There are different mode of action of antimicrobial drugs:

- Inhibition of cell wall synthesis.
- Inhibition of protein synthesis.
- Inhibition of nucliec acid replication and transcription.
- Injury of plasma membrane.
- Inhibition of essential metabolite synthesis.

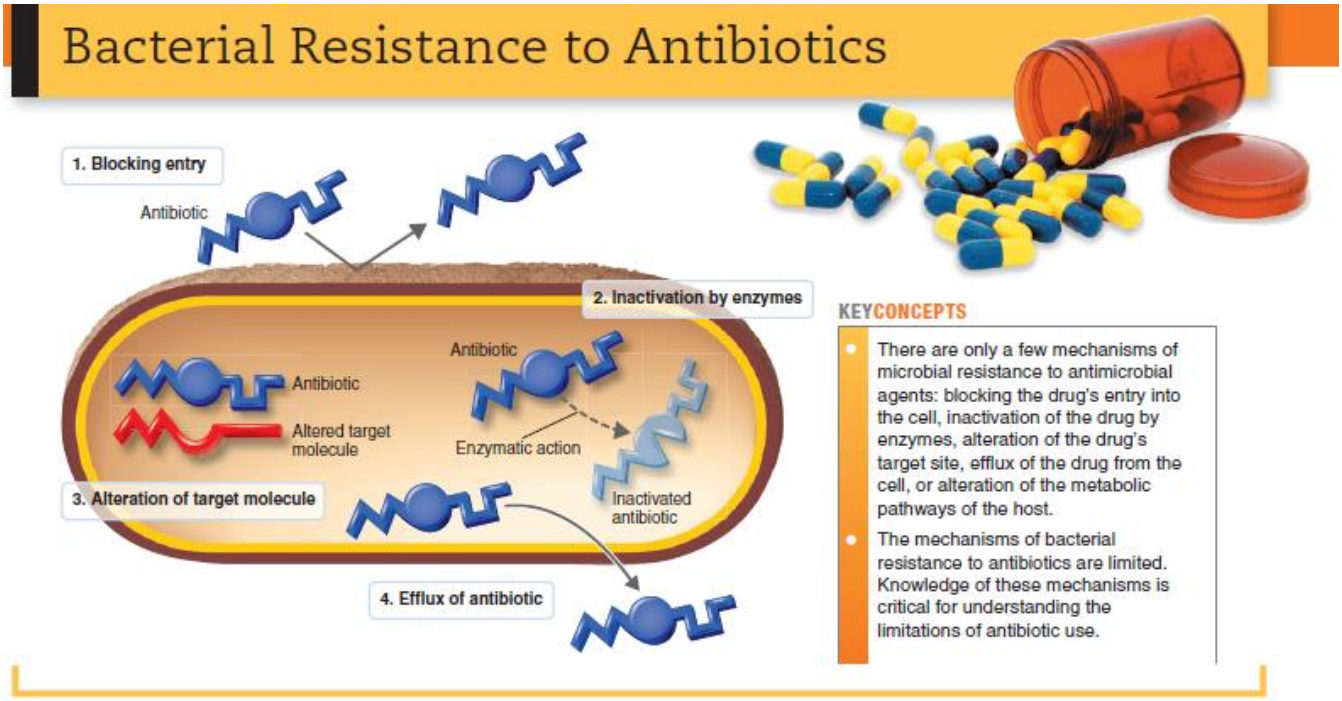


Tortora, 561

Bacterial cell (according to the species) developes different types of resistance,and this issue concidered the problem of the world.

The resistant mechanisms are:

- Blocking the antibiotic entry.
- Inactivation by enzymes.
- Alteration of the target molecule.
- Efflux of antibiotic.


Bacterial Resistance to Antibiotics
1. Blocking entry
Antibiotic
2. Inactivation by enzymes
Antibiotic
Antibiotic
Altered target molecule
Enzymatic action
Inactivated antibiotic
3. Alteration of target molecule
4. Efflux of antibiotic
KEYCONCEPTS
There are only a few mechanisms of microbial resistance to antimicrobial agents: blocking the drug's entry into the cell, inactivation of the drug by enzymes, alteration of the drug's target site, efflux of the drug from the cell, or alteration of the metabolic pathways of the host.
The mechanisms of bacterial resistance to antibiotics are limited. Knowledge of these mechanisms is critical for understanding the limitations of antibiotic use.

Tortora, 580

Staphylococci

The most clinically important species of Staphylococci include *Staphylococcus aureus*, *S. epidermidis* and *S. saprophyticus*. They are Gram-positive cocci; usually arranged in clusters; non-motile; catalase positive; non-sporing; grow over a wide temperature range (10–42 ˚C), with an optimum of 37 ˚C; aerobic and facultatively anaerobic; grow on simple media.

Classification

1 Colonial morphology: ***S. aureus*** colonies are **grey** to **golden yellow**; ***S. epidermidis*** and ***S. saprophyticus*** colonies are white. Staphylococci may produce haemolysins, resulting in haemolysis on blood agar.

2 Coagulase test: ***S. aureus*** possesses the enzyme **coagulase**, which acts on plasma to form a clot. Other staphylococci (e.g. ***S. epidermidis*** and ***S. saprophyticus***) do not possess this enzyme and are often termed, collectively, 'coagulase-negative staphylococci' (CoNS). There are three methods to demonstrate the presence of coagulase:

(a) tube coagulase test: diluted plasma is mixed with a suspension of the bacteria; after incubation, clot formation indicates S. aureus

(b) slide coagulase test: a more rapid and simple methodin which a drop of plasma is added to a suspension of staphylococci on a glass slide; visible clumping indicates the presence of coagulase.

(c) latex agglutination test: cells are mixed with coated latex particles; visible agglutination provides simultaneous detection of staphylococci containing coagulase and/or protein A.

3 Deoxyribonuclease (DNAase) production: ***S. aureus*** possesses an enzyme, DNAase, which depolymerises and hydrolyses DNA; other staphylococci rarely possess this enzyme.

4 Protein A detection: ***S. aureus*** possesses a cell-wall antigen, protein A; antibodies to proteinA agglutinate ***S. aureus*** but not other staphylococci.

5 Novobiocin sensitivity: useful for differentiating between species of coagulase-negative staphylococci; ***S. saprophyticus*** is novobiocin resistant and ***S. epidermidis*** is sensitive.

Table 3.1 Main characteristics of staphylococci

Characteristic	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Coagulase	+	–	–
Deoxyribonuclease	+	–	–
Novobiocin	S	S	R
Colonial appearance	Golden-yellow	White	White
Body sites which may be colonised	Nose Mucosal surfaces Faeces Skin	Skin Mucosal surfaces	Periurethra Faeces
Common infections	Skin (boils, impetigo, furuncles, wound infections) Abscesses Osteomyelitis Septic arthritis Sepsis Infective endocarditis Prosthetic device-related infections	Prosthetic device-related infections e.g. artificial valves, heart, intravenous catheters, CSF shunts	Urinary tract infections in sexually active young women

+, present; –, absent; CSF, cerebrospinal fluid, S, sensitive R, resistant.

Morphology and identification

On microscopy, ***S. aureus*** is seen as typical Gram-positive cocci in 'grape-like' clusters. It is both coagulase and DNAase positive. Other biochemical tests can be performed for full identification.


S. aureus colonies on a blood agar plate

Pathogenicity

S. aureus causes disease because of its ability to adhere to cells, spread in tissues and form abscesses, produce extracellular enzymes and

exotoxins, combat host defences and resist treatment with many antibiotics.

Adhesins

S. aureus has a wide range of adhesins known as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), which mediate adherence to host cells.

Pathogenicity factors produced by *S. aureus*

Factor	Effect
MSCRAMMs	Mediate adherence to host cells
Protein A	Evade host defence/inhibits phacocytosis
Fibronectin-binding protein	Mediates binding to fibronectin
Fibrinogen-binding protein	Clumping factors
Capsule	Evade host defences
Coagulase	Generates protective fibrin layer around *S. aureus*
Staphylokinase	Fibrinolysis
Proteases	Degrade antibacterial proteins and matrix proteins
Lipases	Promote interstitial spreading of microorganism
Hyaluronidase	Degrades hyaluronic acid
α-Haemolysin	Lyses erythrocytes, damages platelets
β-Haemolysin	Degrades sphingomyelin/toxic for cells
Leukocidin/leucotoxin	Lyse white blood cells
Exotoxins, e.g. enterotoxins	Food poisoning with profuse vomiting
Superantigens, e.g. TSST, exfoliative toxin	Toxic shock syndrome, scalded skin syndrome

NB: Toxin production varies between strains of *S. aureus*.

Associated infections

. **Skin:** boils, impetigo, furuncles, wound infections, staphylococcal scalded skin syndrome;

. **Respiratory:** pneumonia, lung abscesses, exacerbations of chronic lung disease;

. **Skeletal:** most common cause of osteomyelitis and septic arthritis;

. **Invasive:** bloodstream infection, infective endocarditis, deep abscesses (brain, liver, spleen), toxic shock syndrome;

. **Gastrointestinal:** toxin-mediated food poisoning;

. **Device related:** indwelling catheters, prosthetic joints and heart valves.

Laboratory diagnosis

Laboratory diagnosis is by microscopic detection of the microorganism in clinical samples, direct isolation from the infected site or blood cultures, and detection of serum antibodies to staphylococcal haemolysin and DNAase. *S. aureus* can also be genotyped by molecular methods,including pulsed field gel electrophoresis (PFGE). Typing of *S. aureus* is useful in epidemiological studies.

Treatment and prevention

Antimicrobial agents, such as flucloxacillin, remain the first-line treatment for sensitive strains of ***S.aureus***.

S. epidermidis

. *S. epidermidis* is both coagulase and DNAase negative and is present in large numbers on the human skin and mucous membranes.

. *S. epidermidis* is a cause of bacterial endocarditis. It is also a major cause of infections of implanted devices such as cerebrospinal shunts.

. The microorganism colonises implanted devices by attaching firmly onto artificial surfaces. Some strains also produce a slime layer (glycocalyx), which appears to facilitate adhesion and protect the microorganism from antibiotics and host defenses. The increased use of implanted devices, particularly central venous catheters, has resulted in S. epidermidis becoming one of the most frequently isolated microorganisms from blood cultures. *S. epidermidis* occasionally causes urinary tract infections, particularly in catheterised patients. When isolated from hospitalized patients, *S. epidermidis* is often resistant to antibiotics such as flucloxacillin and erythromycin, necessitating the use of glycopeptide antibiotics (e.g. vancomycin).

S. saprophyticus

S. saprophyticus is both coagulase and DNAase negative and is frequently associated with urinary tract infections in sexually active young women, occasionally resulting in severe cystitis with hematuria.

Streptococci

Gram-positive cocci, Non-spore forming, non-motile, facultative anaerobes, characteristically form pairs or chains during growth.

Classification of Streptococci

The classification of streptococci into major categories has
been based on

(1) colony morphology and hemolytic reactions on blood agar.

(2) serologic specificity of the cell walls group-specific substance (Lancefield antigens) and other cell wall or capsular antigens.

(3) biochemical reactions and resistance to physical and chemical factors.

(4) ecologic features.

(5) molecular genetics have replaced phenotypic methods.

A. Hemolysis

1. Complete disruption of erythrocytes with clearing of the blood around the bacterial growth is called β-hemolysis (the human-pathogenic species *Streptococcus pyogenes*).

2. Incomplete lysis of erythrocytes with reduction of hemoglobin and the formation of green pigment is called α-hemolysis (*Streptococcus pneumoniae*).

3. Nonhemolytic- Streptococci (sometimes called γ- hemolysis).

Streptococci grown in blood culture showing Gram-positive cocci in chains.

B. Group-Specific Substance (Lancefield Classification)

This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping into Lancefield groups **A–H** and **K–U.**

C. Capsular Polysaccharides

The antigenic specificity of the **capsular polysaccharides** is used to classify some streptococci.

D. Biochemical Reactions

Biochemical tests include sugar fermentation reactions, tests for the presence of enzymes, and tests for susceptibility or resistance to certain chemical agents.

STREPTOCOCCUS PYOGENES

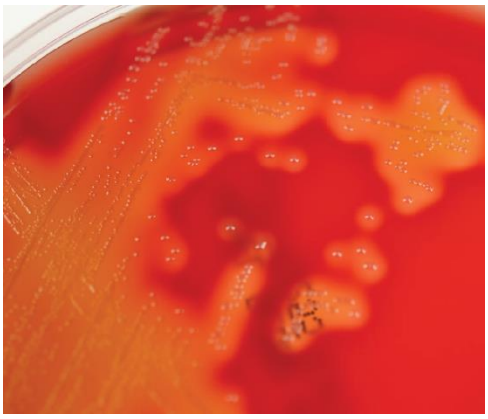
- important human pathogen.

- typically produces large (1 cm in diameter) zones of β-hemolysis around colonies greater than 0.5 mm in diameter.
- hydrolysis of L-pyrrolidonyl-β-naphthylamide.
- susceptible to bacitracin
- Streptococci are Gram-positive; however, as a culture ages and the bacteria die, they lose their Gram positivity and can appear to be Gram-negative; for some streptococci, this can occur after overnight incubation.
- Most group A strains produce capsules composed of hyaluronic acid.
- The hyaluronic acid capsule likely plays a greater role in virulence.
-*S. pyogenes* cell wall contains proteins (**M**, **T**, **R** antigens), carbohydrates (group specific), and peptidoglycans.
- Hairlike pili pili consist partly of **M** protein and are covered with **lipoteichoic acid**.

Culture

Growth of streptococci tends to be poor on solid media or in broth **unless** enriched with blood or tissue fluids. Nutritive requirements vary widely among different species.

Growth and hemolysis are aided by incubation in 10% CO2. Most pathogenic hemolytic streptococci grow best at 37°C. Most streptococci are facultative anaerobes and grow under aerobic and anaerobic conditions.



Group A β-hemolytic streptococci (*S. pyogenes*) after growth overnight on a 10-cm plate with 5% sheep blood agar. The small (0.5–1 mm diameter) white colonies are surrounded by diffuse zones of β-hemolysis 7–10 mm in diameter. (Courtesy of H Reyes.

Antigenic structure (M Protein)

This substance is a major virulence factor of *S. pyogenes*. **M** protein is a filamentous structure anchored to the cell membrane that penetrates and projects from the streptococcal cell wall.

Enzymes and Toxins

A. Streptokinase (Fibrinolysin)

Streptokinase is produced by many strains of group A β-hemolytic streptococci. It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins, allowing the bacteria to escape from blood clots.

B. Deoxyribonucleases

Streptococcal deoxyribonucleases A, B, C, and D degrade DNA (DNases) and similar to streptokinase facilitate the spread of streptococci in tissue by liquefying pus.

C. Hyaluronidase

Hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor).

D. Pyrogenic Exotoxins (Erythrogenic Toxin)

Pyrogenic exotoxins are elaborated by *S. pyogenes*. There are three antigenically distinct streptococcal pyrogenic exotoxins (Spe): A, B, and C.

The streptococcal pyrogenic exotoxins have been associated with **streptococcal toxic shock syndrome** and **scarlet fever**.

E. Hemolysins

The β-hemolytic group A *S. pyogenes* elaborates two hemolysins (streptolysins) that not only lyse the membranes of erythrocytes but also damage a variety of other cell types.

Streptolysin O

-protein.

- hemolytically active in the reduced state (available– SH groups) but rapidly inactivated in the presence of oxygen.
- responsible for some of the hemolysis seen when growth occurs in cuts made deep into the medium in blood agar plates.
- combines quantitatively with antistreptolysin O (ASO), an antibody that appears in humans after infection with any streptococci that produce streptolysin O.

-This antibody blocks hemolysis by streptolysin O.

Streptolysin S

- agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates.
- elaborated in the presence of serum—hence the name streptolysin S.

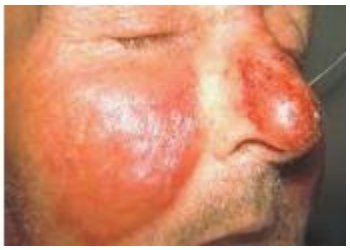
-not antigenic.

Most isolates of *S. pyogenes*produce both of these hemolysins. Up to 10% produce only one.

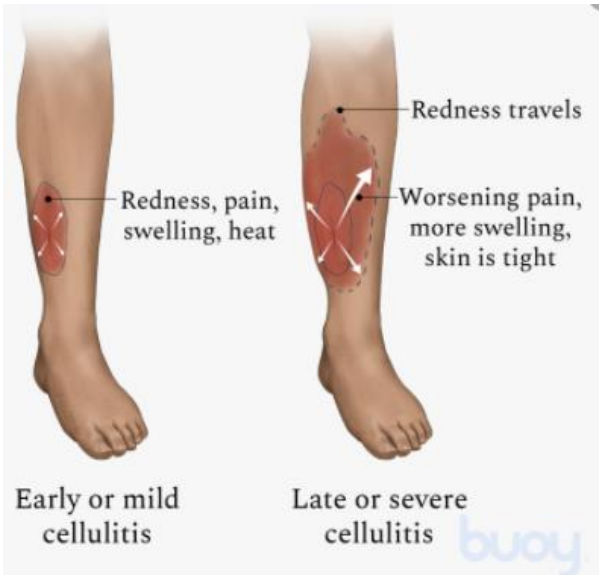
Pathogenesis and Clinical Findings

From the lymphatics, the infection can extend to the bloodstream.

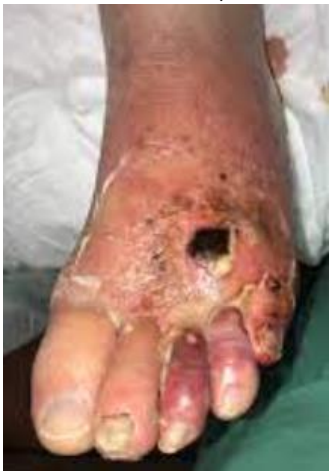
1. Erysipelas—If the portal of entry is the **skin**, erysipelas results. Lesions are raised and characteristically red.



2. Cellulitis—Streptococcal cellulitis is an acute, rapidly spreading infection of the skin and **subcutaneous tissues**.



3. Necrotizing fasciitis (streptococcal gangrene)—There is extensive and very rapidly spreading necrosis of the skin, tissues, and fascia.

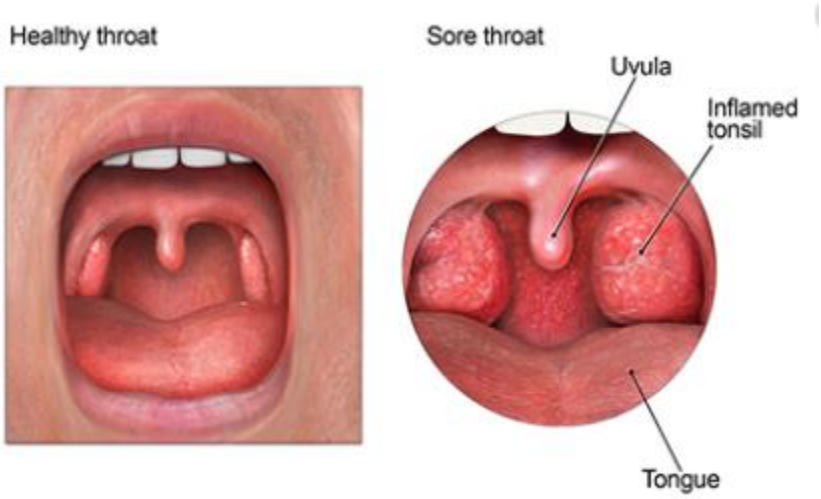


4. Puerperal fever—If the streptococci enter the uterus after delivery, puerperal fever develops, which is essentially a septicemia originating in the infected wound (endometritis).

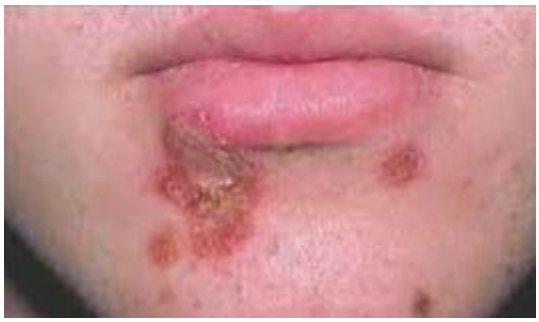
5. Bacteremia or sepsis—Infection of traumatic or surgical wounds with streptococci results in bacteremia, which can rapidly be fatal.

Local Infection

1. Streptococcal sore throat—The most common infection caused by β-hemolytic *S. pyogenes* is streptococcal sore throat or pharyngitis. *S. pyogenes* adheres to the pharyngeal epithelium by means of lipoteichoic acid-covered surface pili and by means of hyaluronic acid in encapsulated strains. *S. pyogenes* infection of the upper respiratory tract does not usually involve the lungs.



2. Streptococcal pyoderma—Local infection of superficial layers of skin, especially in children, is called impetigo. It consists of superficial vesicles that break down and eroded areas whose denuded surface is covered with pus and later is encrusted.



Streptococcal Toxic Shock Syndrome, and Scarlet Fever

Fulminant خاطف, invasive *S. pyogenes* infections with streptococcal toxic shock syndrome are characterized by shock, bacteremia, respiratory failure, and multiorgan failure. Death occurs in about 30% of patients.

Pyrogenic exotoxins A–C also cause **scarlet fever** in association with *S. pyogenes* pharyngitis or with skin or soft tissue infection.

Poststreptococcal Diseases (Rheumatic Fever, Glomerulonephritis)

1. Acute glomerulonephritis—This sometimes develops 1–5 weeks (mean 7days) after *S. pyogenes* skin infection (pyoderma, impetigo) or pharyngitis.

2. Rheumatic fever—This is the most serious sequela of *S. pyogenes* because it results in damage to heart muscle and valves. Certain strains of group A streptococci contain cell membrane antigens that cross-react with human heart tissue antigens.

Laboratory Diagnosis—Practical lecture.

Streptococcus pneumoniae

-is a member of the *S. mitis* group.

-Gram-positive diplococci, often lancet shaped or arranged in chains.

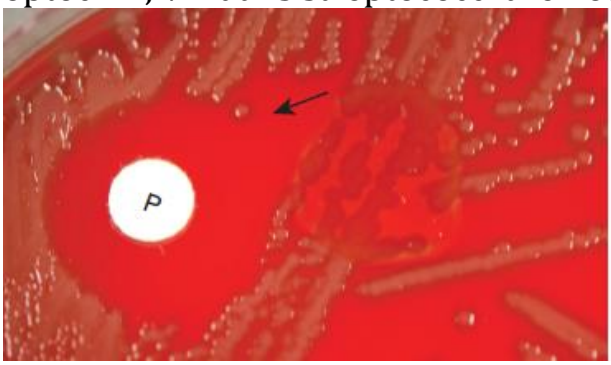
-possessing a capsule of polysaccharide that permits typing with specific antisera.

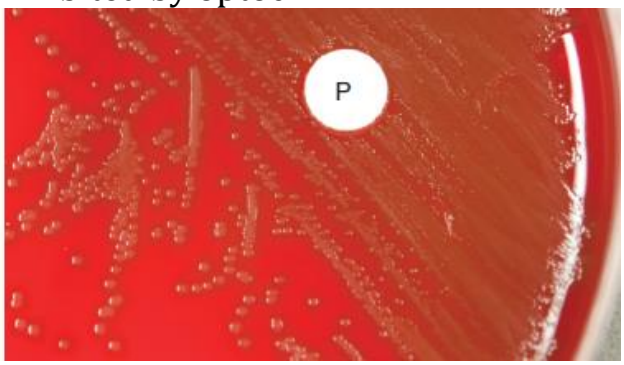
- In sputum or pus, single cocci or chains are also seen.

- With age, the organisms rapidly become Gram-negative and tend to lyse spontaneously.

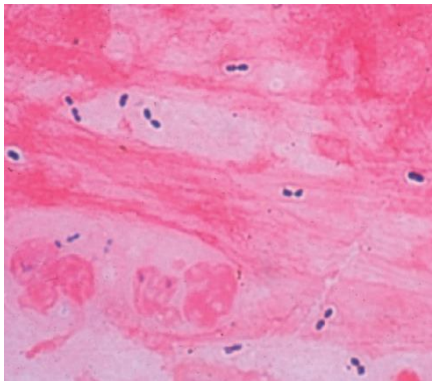
-Lysis of pneumococci occurs in a few minutes when ox bile (10%) or sodium deoxycholate (2%) is added to a broth culture or suspension of organisms at neutral pH. (Viridans streptococci do not lyse and are thus easily differentiated from pneumococci).

-On solid media, the growth of pneumococci is inhibited around a disk of optochin; viridans streptococci are not inhibited by optochin.





- capsule swelling test, or quellung reaction is very important in the diagnosis of this bacterium.



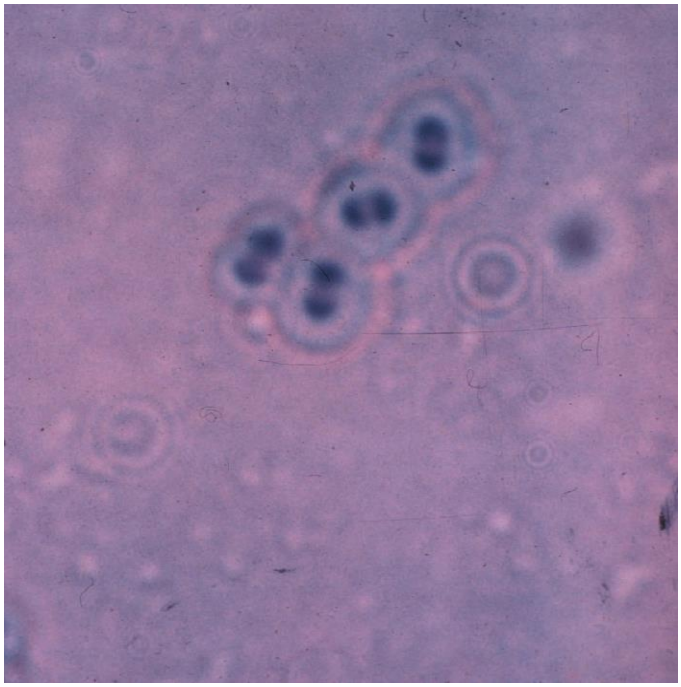
S. pneumoniae in sputum are seen as lancetshaped Gram-positive diplococci. Degenerating nuclei of polymorphonuclear cells are the large darker irregular red shapes (*arrow*). Mucus and amorphous debris are present in the background. Original magnification ×1000.

Culture

Pneumococci form small round colonies, at first domeshaped and later developing a central depression with an elevated rim. Other colonies may appear glistening because of capsular polysaccharide production. Pneumococci are α-hemolytic on blood agar. Growth is enhanced by 5–10% CO2.

Quellung Reaction

When pneumococci of a certain type are mixed with specific antipolysaccharide serum of the same type—or with polyvalent antiserum—on a microscope slide, the capsule swells markedly, and the organisms agglutinate by crosslinking of the antibodies.



Disease production

Pneumococci produce disease through their ability to multiply in the tissues. The virulence of the organism is a function of its capsule, which prevents or delays ingestion by phagocytes.

Enterobacteriaceae (part one)

The Enterobacteriaceae include the following genera: Escherichia, Enterobacter, Klebsiella, Proteus, Serratia, Shigella, Salmonella and Yersinia.

They are Gram-negative bacilli, which are found as commensals in the intestinal tract of mammals. They are also referred to as coliforms or enteric bacteria.

Definition

Aerobic and facultatively anaerobic growth; optimal growth normally at 37 C°; grow readily on simple media; ferment wide range of carbohydrates; oxidase-negative; some are motile; biletolerant and grow readily on bile-salt containing media, e.g. MacConkey's agar.

Morphology and identification

- Fermentation of lactose to produce pink colonies on MacConkey's agar is characteristic of *Escherichia*, *Enterobacter* and *Klebsiella*.

Salmonella, *Shigella*, *Serratia*, *Proteus* and *Yersinia* do not ferment lactose and form pale colonies on MacConkey's agar.

- Various tests are used for identification:

(a) oxidase (negative);

(b) carbohydrate fermentation reactions;

(c) production of urease (which splits urea with release of ammonia);

(d) hydrogen sulphide production;

(e) amino acid decarboxylation;

(f) indole production.

Commercial kits based on these biochemical tests are available for identification of the Enterobacteriaceae.

. Enterobacteriaceae possess a variety of antigens: these may include lipopolysaccharide ('O'), flagellar ('H') and capsular polysaccharide ('K')

antigens. These are used to subdivide (serotype) some genera and species, e.g. Escherichia, Salmonella.

Escherichia

The genus Escherichia currently contains several species. However, *E. coli* is the species most frequently isolated from humans.

Pathogenicity

. Specific fimbriae facilitate adherence to mucosal surfaces and colonisation of the intestinal and urinary tracts.

. The lipopolysaccharide (endotoxin) in the cell wall is liberated when Gram-negative bacteria lyse, resulting in production of inflammatory mediators (cytokines and nitric oxide) and complement activation. This results in endotoxic shock and intravascular coagulopathy.

Enterobacteriaceae infections

Genus/species	Common infections
Escherichia coli	Urinary tract infection
	Intra-abdomal infection
	Wound infection
Klebsiella spp	Urinary tract infection
	Pneumonia
	Intravascular catheter-related infection
Enterobacter spp	Hospital-acquired pneumonia
Serratia spp	Wound infection
Proteus spp	Urinary tract infection
Salmonella serotypes Typhi and Paratyphi	Enteric fever and bloodstream infection
Other salmonellae	Enteritis
Shigella spp	Enteritis
Yersinia enterocolitica	Enteritis
Yersinia pestis	Plague
Yersinia pseudotuberculosis	Mesenteric adenitis

The K1 capsular polysaccharide antigen is associated with neonatal meningitis.

- A number of distinct infections are mediated by the different protein toxins produced by *E. coli*.
- VTEC (verocytotoxin-producing *E. coli*, particularly the O157:H7 serotype, are an important cause of diarrhoea and hemolytic uremic syndrome (HUS). These are also referred to as enterohaemorrhagic *E. coli* (EHEC).
- Most are sporadic cases and have the following key features:

- zoonotic infections mainly from cattle, but also from vegetables washed in contaminated water;

- low infecting dose;

- acquired by eating undercooked contaminated meat and vegetables;

- damage gut endothelium, resulting in haemorrhagic colitis;

- HUS occurs in about 5% of patients, which results in renal failure, oliguria, thrombocytopenia (T**hrombocytopenia** is a condition characterized by abnormally low levels of platelets, also known as thrombocytes, in the blood).

Diarrhoea caused by other *E. coli*:

- Enteropathogenic (EPEC): cause of infantile diarrhoea;
- Enterotoxigenic (ETEC): travellers' diarrhoea, non-invasive;
- Enteroinvasive (EIEC): causes dysentery-like illness;
- Enteroaggregative (EAEC): watery diarrhoea without fever.

Genus: *Salmonella*

Introduction: The genus Salmonella belongs to the family Enterobacteriaceae. The organisms are named after the American veterinary pathologist Daniel Elmer Salmon in 1885. Currently, there are three recognized species: *S. enterica*, *S. bongori and S. subterranean*. Salmonella is found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses such as typhoid fever, paratyphoid fever, and foodborne illness.

Classification:

- The members of the genus Salmonella were originally classified on the basis of epidemiology; host range; biochemical reactions; and structures of the O, H, and Vi (when present) antigens.

- *Salmonella* spp. have both H and O antigens. There are over 60 different O antigens, and individual strains may possess several O and H antigens; the latter can exist in variant forms, termed 'phases'. *Salmonella* serotype Typhi also has a capsular polysaccharide antigen referred to as 'Vi' (for virulence), which is related to invasiveness

- Over 2500 serotypes are distinguished, most of which belong to the species *S. enterica*. However, many of these have been given binomial names (e.g. *Salmonella typhimurium* and *Salmonella enteritidis*), although they are not separate species. In clinical practice, laboratories identify microorganisms according to their binomial name.

Important Properties: Salmonellae are motile rods that characteristically ferment glucose and mannose without producing gas but do not ferment lactose or sucrose. Most salmonellae produce H2 S. They are often pathogenic for humans or animals when ingested.

Virulence Factors:

1. Type III secretion systems: which facilitate secretion of viru-lence factors of Salmonella into host cells.

2. Endotoxin: Endotoxin is responsible for many of the systemic manifestations of the disease caused by *Salmonella* spp.

3. Fimbriae: The species-specific fimbriae mediate binding of *Salmonella* to M (microfold) cells present in Peyer patches of the terminal part of the small intestine. These M cells typically transport foreign antigens, such as bacteria to the underlying macrophages for clearance.

4.Acid tolerance response gene: The acid tolerance response(ATR) gene protects *Salmonella* spp. from stomach acids and the acidic pH of the phagosome, thereby facilitating survival of bacteria in phagosomes 5. Enzymes: Catalase and superoxide dismutase are the enzymes that protect the bacteria from intracellular killing in macrophages.

Pathogenesis of Salmonella: The three types of Salmonella infections (enterocolitis, enteric fevers, and septicemia) have different pathogenic features.

(1) Enterocolitis: is characterized by an invasion of the epithelial and sub-epithelial tissue of the small and large intestines.

(2) In typhoid and other enteric fevers, infection begins in the small intestine, but few gastrointestinal symptoms occur.

(3) Septicemia accounts for only about 5−10% of Salmonella infections and occurs in one of two settings: a patient with an underlying chronic disease, such as sickle cell anemia or cancer, or a child with enterocolitis.

Laboratory Diagnosis:

In enterocolitis: the organism is most easily isolated from a stool sample in selective media e.g. XLD, DCA (deoxycholate citrate agar), salmonella-shigella

(SS) agar, and enrichment media, e.g. selenite broth; identification of Salmonella spp. by biochemical agglutination tests. Phage typing can be used for typing individual strain.

Salmonella Shigella (SS) Agar: salmonella colorless, transparent, with a black center if H2S is produced

XLD- Agar: *Salmonella* Typhi red Colonies, black centers.

TSI-Agar: Salmonella Alkaline slant/acidic butt (K/A); + H2S and Gas + In the enteric fevers: a blood culture is the procedure most likely to reveal the organism during the first weeks of illness. Stool cultures may also be positive, especially in chronic carriers in whom the organism is secreted in the bile into the intestinal tract. Urine culture results may be positive after the second week.

Serologic Methods:

I. Agglutination test: This test is particularly useful for rapid preliminary identification of cultures. There are commercial kits available to agglutinate and serogroup salmonellae by their O antigens: A, B, C1, C2, D, and E.

II. Tube dilution agglutination test (Widal test) : Serum agglutinins rise sharply during the second and third weeks of S serotype Typhi infection.

Genus: Shigella

Introduction: The genus Shigella belongs to the tribe Escherichia in the family Enterobacteriaceae. This organsim is named after Kiyoshi Shiga, who first discovered it in 1898. It is the causative agent of human shigellosis, which is naturally found only in humans and apes, but not in other mammals. **Classification:** There are more than 40 serotypes. The classification of shigellae relies on biochemical and antigenic characteristics (O antigens). The pathogenic species are *S sonnei, Shigella flexneri, S dysenteriae, and Shigella boydii*. **Important Properties**: Shigellae are short gram-negative rods , non–lactose-fermenting Resistant to bile salts, All shigellae have O antigens (polysaccharide) in their cell walls, and these antigens are used to divide the genus into four groups: A, B, C, and D.

Shigella can be distinguished from salmonellae by three criteria: • They produce no gas from the fermentation of glucose

• They do not produce H2S

• They are nonmotile.

Virulence Factors:

1. K. capsular antigen

2. O. antigen (HL)

3. Shiga toxin: with cytotoxic and neurotoxic

Pathogenesis of Shigella:

• Shigella causes bacillary dysentery , Low infective dose < 200 bacilli (can be transmitted easily unlike salmonella (More serious and virulent than salmonella

• Incubation period = 1-3 days

- Upon ingestion, the bacteria pass through the gastrointestinal tract until they reach the small intestine. There they begin to multiply until they reach the large intestine. In the large intestine, the bacteria cause cell injury and the beginning stages of Shigellosis via two main mechanisms: direct invasion of epithelial cells in the large intestine and production of enterotoxin 1 and enterotoxin 2. High fever, chill, abdominal cramp and pain accompanied by tenesmus, bloody stool with mucus & WBC and HUS are involved.

Laboratory Diagnosis:

Specimens: include fresh stool, mucus flecks, and rectal swabs for culture. Large numbers of fecal leukocytes and some red blood cells often are seen microscopically. Culture : The materials are streaked on differential media (eg, MacConkey or EMB agar) and on selective media (Hektoen enteric agar or xylose-lysine-deoxycholate agar), which suppress other Enterobacteriaceae and gram-positive organisms. Colorless (lactose-negative) colonies are inoculated into TSI agar. Organisms that fail to produce H_2S, that produce acid but not gas in the butt and an alkaline slant in TSI agar medium.

Salmonella Shigella (SS) Agar: Shigella Clear, colorless, transparent.

XLD- Agar: *Shigella flexneri* Red Colonies

TSI-Agar: Salmonella Alkaline slant/acidic butt (K/A); - H_2S and Gas-

Pseudomonads* and *Acinetobacter Pseudomonads The pseudomonads are Gram-negative, motile, aerobic rods, some of which produce water-soluble pigments. The pseudomonads occur widely in soil, water, plants, and animals. *P aeruginosa* is frequently present in small numbers in the normal intestinal flora and on the skin of humans, and is the major pathogen of the group. Other pseudomonads infrequently cause disease.

***Pseudomonas aeruginosa*.**

It is widely distributed in nature and is commonly present in moist environments in hospitals. It can colonize normal humans; in whom it is a saprophyte. It causes disease in humans with abnormal host defenses, especially in individuals with neutropenia.

Morphology and Identification:

A. Typical Organisms *P. aeruginosa* is motile and rod shaped, measuring about 0.6 ×2 μm (Figure 16-1). It is Gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains.

B. Culture *P aeruginosa* is an obligate aerobe that grows readily on many types of culture media, sometimes producing a sweet or grape-like or corn taco–like odor. Some strains hemolyze blood. *P aeruginosa* forms smooth round colonies with a fluorescent greenish color. It often produces the non-fluorescent bluish pigment pyocyanin, which diffuses into the agar. Other *Pseudomonas* species do not produce pyocyanin. Many strains of *P. aeruginosa* also produce the fluorescent pigment pyoverdin, which gives a greenish color to the agar Some strains produce the dark red pigment pyorubin or the black pigment pyomelanin.
C. Growth Characteristics P aeruginosa grows well at 37–42°C; its growth at 42°C helps differentiate it from other *Pseudomonas* species that produce

fluorescent pigments. It is oxidase positive. It does not ferment carbohydrates, but many strains oxidize glucose.

Antigenic Structure and Toxins:

- Pili: Adhere to epithelial cells
- Exopolysaccharide: Anti-phagocytic property/ inhibit pulmonary clearance.
- Lipopolysaccharide: Endotoxic effect Enzymes
- Elastases: Digests protein (elastin, collagen, IgG)
- Proteases
- Hemolysins
- Phospholipases C (heat labile): Degrade cytoplasmic membrane components

Exotoxin A: Cytotoxic by blocking protein synthesis Endotoxin: like that of other gram-negative bacteria, causes the symptoms of sepsis and septic shock. **Pathogenesis**: *Pseudomonas aeruginosa* is primarily an opportunistic pathogen that causes infections in hospitalized patients (e.g., those extensive burns),with in whom the skin host defenses are destroyed; in those with chronic respiratory disease (e.g., cystic fibrosis), in whom the normal clearance mechanisms are impaired; in those who are immunosuppressed;

- Urinary tract infection- chronic, complicated Urinary tract infection and associated with indwelling catheter.
- Wound infection of burn sites, pressure sores and ulcers.
- Septicaemia- “Ecthyma gangrenosum” skin lesion (haemorrhagic skin necrosis)
- Otitis externa- Malignant external ear infection in poorly treated diabetic patients.
- Pneumonia- Infection of the lung in patients with cystic fibrosis.

- Eye infection- Secondary to trauma or surgery.

Laboratory diagnosis: Specimen: pus, urine, sputum, blood, eye swabs, surface swabs Smear: Gram-negative rods Culture: Obligate aerobe, grows readily on all routine media over wide range of temperature (5-42 ^{O}C). Bluish-green pigmented large colonies with characteristic "fruity" odor on culture media.

In **Centrimide** agar: *Pseudomonas aeruginosa* colonies (greenish-blue in color) are medium sized and characterized by an irregular growth

In blood agar: Colonies of Pseudomonas *aeruginosa* surrounded by a wide zone of beta-hemolysis. Cultivation 48 hours in an aerobic atmosphere, 37°C. **Biochemical reactions:** Oxidase positive Catalase positive Citrate positive Indole negative Produce acid from carbohydrate by oxidation, not by fermentation.

Acinetobacter *Acinetobacter* species are aerobic, Gram-negative bacteria that are widely distributed in soil and water and can occasionally be cultured from skin, mucous membranes, secretions, and the hospital environment. *A baumannii* is the species most commonly isolated. *Acinetobacter lwoffii* and other species are isolated occasionally.

A. Morphology and Identification: Acinetobacters are usually coccobacillary or coccal in appearance; they resemble neisseriae on smears, because diplococcal forms predominate in body fluids and on solid media. Rod-shaped forms also occur, and occasionally the bacteria appear to be Gram-positive.

B. Culture: *Acinetobacter* grows well on most types of media used to culture specimens from patients. *Acinetobacter* recovered from patients with meningitis, bacteremia, female genital, sputum, skin, pleural fluid, and urine, usually.

Bacillus

The Bacillus genus contains numerous species, many of which are not of clinical importance. Two important human pathogens – *Bacillus anthracis* and *Bacillus cereus* cause anthrax and food poisoning, respectively.

Definition

Gram-positive bacilli often arranged in chains; aerobic (some species are obligate aerobes and some facultative anaerobes); spore-forming; most species are motile; usually catalase-positive; some species are capsulate; grow over a wide temperature range on simple media.

B. anthracis

Epidemiology

Anthrax is principally a zoonotic disease and is common in some parts of the developing world.

Human infections can be classified as: non-industrial (direct human contact with infected animals) or industrial (processing of animal products by humans). Spores can survive in the soil for long periods of time and are relatively resistant to chemical disinfectants and heat.

Infection with *B. anthracis* in the UK is rare but is normally associated with handling imported animal products. Recent UK cases have occurred in intravenous drug users, probably as a result of contaminated heroin. Anthrax has been used as a biological weapon.

Morphology and identification

Can grow under anaerobic conditions; its nonmotility allows it to be distinguished from other Bacillus species; virulent strains are capsulate; do not produce a zone of haemolysis on blood nor a zone of precipitation on egg yolk agar (i.e. does not produce lecithinase). Identification is normally confirmed by morphological and biochemical tests.

Pathogenicity

Virulent strains of *B. anthracis* possess a protein capsule, which prevents phagocytosis. This microorganism also produces a plasmid-encoded exotoxin, which is composed of three proteins: protective antigen, oedema factor and lethal factor.

Protective antigen is concerned with receptor binding and therefore the attachment and translocation of oedema factor and lethal factor into the cell. Oedema factor causes impairment of macrophage function and lethal factor lysis of macrophages.

Associated infections

Types of anthrax infection include:

- Skin and soft tissues: cutaneous anthrax is the predominant clinical manifestation. Development of a necrotic skin lesion (malignant pustule) occurs.
- Respiratory: ('wool-sorter's disease'): spores are inhaled (often from wool fibres) causing pulmonary oedema, haemorrhage and commonly, death.
- Gastrointestinal: consumption of contaminated meat results in haemorrhagic diarrhorea. This type of anthrax can also result in death.

Laboratory diagnosis

Normally by direct isolation of the microorganism from infected sites, i.e. sputum or specimens from skin lesions. A safety cabinet should be used to handle such specimens. (practical lecture)

Treatment and prevention

B. anthracis is sensitive to many antibiotics. Common therapeutic agents used are penicillin, erythromycin, ciprofloxacin or doxycycline. Ciprofloxacin or doxycyclinemaybe given for post-exposure prophylaxis. Anthrax vaccinations are available for individuals at high risk, e.g. military personnel, veterinary

practitioners and farm workers. Livestock in endemic areas may also be vaccinated.

B. cereus

B. cereus can grow under anaerobic conditions, is motile and it does not produce lecithinase; however, unlike *B. anthracis*, it produces a zone of haemolysis on blood agar. This microorganism is an important cause of food poisoning, particularly associated with rice dishes and cereals. Pathogenicity is related to the production of enterotoxins.

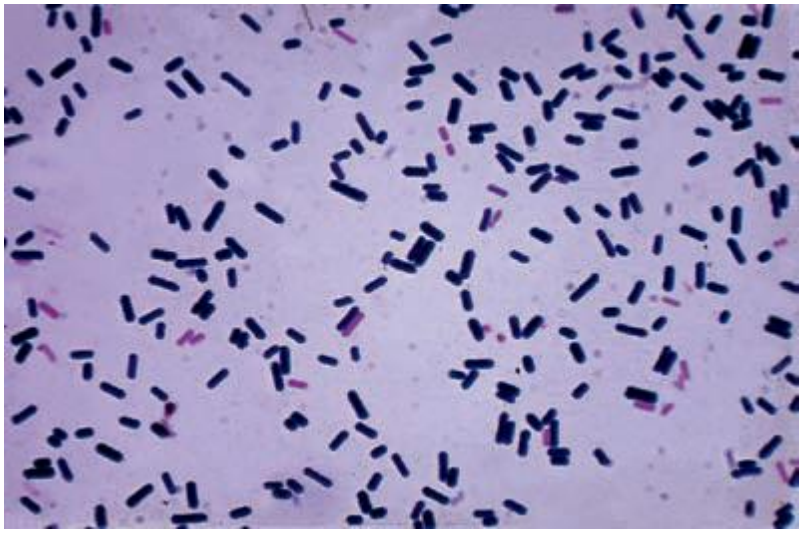
Food poisoning can present as the emetic form, which has a short incubation period (typically 1–6 hours) and is characterised by nausea and vomiting, or as the diarrhoeal form, for which the incubation period is longer (typically 8–16 hours).

Food-poisoning is self-limiting, therefore antimicrobial therapy is not normally required. *B. cereus* is an infrequent cause of non-gastrointestinal infections, including ocular infections, pneumonia and endocarditis.

Clostridia

Definition

Large Gram-positive bacilli; strictly anaerobic; spore-forming; fermentative. Whilst many Clostridium species exist, only some are of medical importance, including *C. perfingens, C. difficile, C. tetani, C. botulinum, C. septicum* and *C. tertium*.



Epidemiology

Clostridria are widely distributed in the environment and in the gastrointestinal tract of mammals. They produce highly resistant, transmissible spores, which can survive desiccation, ultraviolet and gamma radiation, extreme temperatures, starvation and disinfection. Spores of clostridia are the vector of infection.

Classification

Based on morphology, biochemical activity, fatty acid production and gene sequencing techniques.

C. perfringens

Morphology and identification

Non-motile, sub-terminal spores. Forms irregular, spreading colonies on blood agar surrounded by a double zone of b-haemolysis (inner zone of complete lysis

due to q-toxin and wider outer zone of partial haemolysis due to a-toxin). Five types (A to E) of *C. perfringens* are recognised based on surface antigens and the types of toxin produced:

1 Type A strains: commonly found in human infections; produce only α-toxin

2 Type B to E strains: commonly found in animals (lambs, goat, cattle); produce α- and other toxins.

Pathogenicity

Toxin and enzyme production: α-toxin (phospholipase C) is associated with toxaemia seen in gas gangrene; hyaluronidase breaks down cellular cement facilitating spread; collagenase/proteinase– liquefaction of muscle; lipase-lipid breakdown.

Associated infections

- Skin and Soft tissue: gas gangrene, cellulitis;
- Gastrointestinal: necrotising enteritis, food poisoning;
- Gynaecological: septic abortion.

Laboratory diagnosis

Gram stained smears and culture of clinical samples, e.g. blood, pus and tissue may provide evidence of clostridial infection. Recovery of *C. perfringens* on simple or selective agar provides a definitive diagnosis. Identification of *C. perfringens* is determined through biochemical tests (e.g. API 20A, Rapid ID 32A kits). Confirmation of α-toxin and lipase production is established on egg-yolk agar (Nagler plate); toxin-producing strains generate a zone of opalescence around the colonies, which can be inhibited by specific antitoxin to α-toxin.

Treatment and prevention

Gas gangrene: surgical debridement, immediate antibiotic therapy with high dose benzylpenicillin and/or metronidazole and supportive measures.

Co-infecting microorganisms may be present, therefore additional antibiotics may be required.

Prophylactic benzylpenicillin may be given for dirty wounds and lower limb amputation.

Food poisoning: self limiting; no antimicrobial therapy warranted.

C. *difficile*

Morphology and identification

Motile; sub-terminal spores. Forms colonies with irregular edges and a ground glass appearance on blood agar. Colonies have a typical 'horse manure' odour.

Epidemiology

Ubiquitous in the environment and colonises the intestine of 50% of healthy neonates and 4% of healthy adults. A major cause of healthcare-associated infection in the 21st century; patients taking antibiotics, e.g. cephalosporins, clindamycin are at increased risk of developing *C. difficile* antibiotic associated diarrhoea. This is due to suppression of the normal bowel flora and subsequent overgrowth of *C. difficile*. Infection may be endogenous or exogenous (through ingestion of environmental spores).

Pathogenicity

Produces two major toxins: Toxin A (enterotoxin) and Toxin B (cytotoxin). A further binary toxin is present in some strains. Toxin A induces cytokine production with hypersecretion of fluid. Toxin B induces depolymerisation of actin with loss of cytoskeleton. Adhesin factor and hyaluronidase production are also associated virulence factors.

Hypervirulent, hypertoxin producing strains now recognised (e.g. ribotype 027, 078).

Associated infections

- Gastrointestinal: antibiotic associated diarrhoea, pseudomembranous colitis, fulminant colitis.

Laboratory diagnosis

Direct detection of toxin in faeces by various methods: cell toxicity neutralisation assay, commercial assays (e.g. ELISA, latex agglutination) and polymerase chain reaction (PCR). Culture of *C. difficile*

on selective agar (e.g. Cycloserine Cefoxatime Fructose Agar); genotyping of isolates by ribotying where necessary. Assay for glutamate dehydrogenase (GDH) and lactoferrin in faecal samples.

Treatment and prevention

Treatment: Oral Vancomycin or Metronidazole. Prevention of *C. difficile* is multifactoral and includes: clinical awareness, judicious use of antibiotics, infection control strategies, e.g. hand hygiene, environmental decontamination and cleanliness.

C. tetani

Morphology and identification

Motile; terminal spore ('drumstick' appearance); produces a thin spreading film of growth without discrete colonies on blood agar; motile via numerous peritrichous flagella.

Epidemiology

C. tetani is present in mammalian intestines and the environment (particularly manured soil).

Spores are ubiquitous in nature. Incidence of tetanus varies worldwide; more common in developing tropical and subtropical countries; infection is inversely related to living standards, preventative medicine and wound management.

Pathogenicity

Many strains are highly toxigenic, producing oxygen- labile haemolysin (tetanolysin) and a potent neurotoxin (tetanospasmin). Tetanospasmin blocks neurotransmitter release, resulting in the characteristic motor spasms associated with tetanus (e.g. lockjaw, arching of the back).

Associated infections

- Neurological: tetanus.

Laboratory diagnosis

Demonstration of characteristic 'drumstick' bacilli in clinical samples, followed by anaerobic culture on selective or blood agar; serological detection of circulating neurotoxin by enzyme immunoassay.

Treatment and prevention

Treatment includes administration of human tetanus immunoglobulin and benzylpenicillin or metronidazole. Surgical debridement and cleansing of wounds is important in successful treatment. Prevention includes administration of the tetanus toxoid vaccine.

C. botulinum

Morphology and identification

Toxin producing; subterminal spores; motile with peritrichous flagella. Seven main types of *C. botulinum* are recognised (A–G), based on antigenically distinct toxins with identical actions.

Epidemiology

Ubiquitous saprophyte occurring in soil, vegetation, fruit and manure. Infection arises through consumption of contaminated food or wound contamination. Human infection is commonly caused by types A, B and D. Infection in the UK is rare.

Pathogenicity

Production of potent neurotoxin which blocks the release of acetylcholine at neuromuscular junctions, resulting in flaccid paralysis.

Associated infections

- Neurological: botulism, wound botulism, infant botulism.

Laboratory diagnosis

Detection of the microorganism or its toxin in food; toxin may be demonstrated in patient's blood by toxin-antitoxin neutralisation assay.

Treatment and prevention

Treatment includes administration of human tetanus immunoglobulin and benzylpenicillin or metronidazole. Surgical debridement and cleansing of wounds is important. Prevention includes administration of the tetanus toxoid vaccine.

Other clostridial infections

C. septicum is associated with non-traumatic myonecrosis more often in immunocompromised patients. *C. tertium* is associated with traumatic wound infection.

Reference

Elliot, *et al*. Medical Microbiology and Infection "Lecture Notes". 2011 by Blackwell Publishing Ltd

Genus*: Corynebacterium* (Gram Positive Bacilli) *Corynebacteria* (from the Greek words koryne, meaning club, and bacterion, meaning little rod). Gram-positive, aerobic or facultative anaerobic, non-motile, and Catalase-positive rod-shaped bacteria. They have a cell wall with arabinose, galactose, and short-chain mycolic acids. They do not form spores or branch, they have club-shaped, or V-shaped arrangements in normal growth.

Corvnebacterium diphtheriae

C. diphtheriae is the most important species causing diphtheria. Diphtheria is an acute upper respiratory tract illness characterized by sore throat, low- grade fever, and an adherent membrane on the tonsil(s), pharynx, and/or nose.

Cell Wall Components and Antigenic Structure The cell wall contains neuraminidase, arabinose, galactose, mannose, corynemycolic acid, and corynemycolenic acid. The cell walls of the diphtheria bacilli are antigenically heterologous.

Pathogenesis Diphtheria is a classic example of toxin-mediated bacterial disease.

Diphtheria toxin: the exotoxin produced by *C. diphtheriae*, is the key virulence factor of the bacteria and have Biological functions (Neuro and cardiotoxin; inhibits protein synthesis by inactivating elongation factor).

Pathogenesis of diphtheria

1. *C. diphtheriae* usually enters the body through the upper respiratory tract but can also enter through the skin, genital tract, or eye.

2. Infection begins by adherence of the bacteria at the infected site.

3. The initial lesion usually occurs on the tonsils and oropharynx, and from this site it may spread to the nasopharynx, larynx, and trachea.

ϖ The organisms multiply rapidly in the epithelial cells, forming a local lesion and secrete exotoxins that cause necrosis of the cells in that area.

Host immunity

In diphtheria, immunity against clinical diseases depends on the presence of antitoxin in the blood stream, in response to clinical or subclinical disease or active immunization.

The immune status of the individuals is assessed by the presence of antitoxin levels or by Schick's test.

Schick's test: introduced by Schick in 1913 to assess the immunity among children. The test is performed by injecting 0.1 mL of highly purified toxin (1/50 minimum lethal dose) into one hour and 0.1 mL of heat-inactivated toxin into another hour as a control. This brings about four types of reactions:

A. Positive reaction

B. Negative reaction

C. Pseudoimmune reaction

D. Combined reaction.

Positive reaction: This is characterized by a local inflammatory reaction that reaches maximum intensity in 4-7 days in the test arm and then reduces gradually. This indicates absence of immunity to *C. diphtheriae*.

Negative reaction: Absence of any inflammatory reaction is suggestive of a negative reaction. This indicates the presence of antitoxin in the individual,

which neutralizes the toxin injected. Such an individual is immune to *C. diphtheriae* infection.

Pseudoimmune reaction: In endemic areas, allergy to the toxin is seen among children and adults. Even though the individual is immune, yet an allergic reaction is observed in both the test as well as the control arm. This reaction is called pseudoimmune reaction and it indicates that the individual is immune but hypersensitive.

Combined reaction: This is the condition in which an individual injected with the toxin **b** develops inflammation in the test arm, which increases in intensity by 4-7 days. In the control arm, the inflammation is seen for a maximum of 48-72 hours and then subsidies. It indicates the individual is not immune and is hypersensitive.

Clinical Syndromes

The clinical manifestations of diphtheria depend upon the following: (a) immune status of the patient, (b) virulence of the bacteria, and (c) the site of the infection.

Diseases caused by *C. diphtheriae*

A. Toxigenic strains (tox+) of *C. diphtheriae* cause:

1. Serious, sometimes fatal, disease in nonimmune patients.
2. Mild respiratory diseases in partially immune patients.
3. Asymptomatic colonization in fully immune individuals.

B. Nontoxigenic strains (tox-) cause a mild disease, such as cutaneous diphtheria.

Habitat

The upper respiratory tract of an infected host.

Reservoir, source, and transmission of infection

1. Humans are the only natural host of *C. diphtheriae* and thus are the only significant reservoirs of infection.

2. Infective droplets or nasopharyngeal secretions are the common sources of infection.

3. Direct human contact (droplets of nasopharyngeal secretion) facilitates transmission

of the disease.

Treatment

Treatment should be started immediately after the clinical diagnosis of diphtheria.

Treatment of diphtheria is based on:

1. Antitoxin therapy
2. Antibiotics therapy

Antitoxin therapy

Diphtheria antitoxin is a hyper-immune antiserum produced in horses, which is administered to neutralize the toxin responsible for diphtheria. The antitoxin neutralizes only free toxin before the toxin enters the cells, but is ineffective after toxin has entered into the cell.

- The dosage of antitoxin is dependent on the site of infection, patient's clinical picture, and duration of illness.

Antibiotics therapy

Antimicrobial therapy is useful in treatment of diphtheria.

Antibiotics:

1. Limit the production of toxin
2. kill diphtheria bacteria from infected hosts, and
3. Prevent transmission of the bacteria to patient contacts.

Prevention and Control

1. Active immunization

Active immunization by vaccination with diphtheria toxoid is the key in preventing diphtheria. Vaccines consist of microorganisms or cellular components that act as antigens.

2. Passive immunization

Passive immunization is carried out by anti-diphtheric serum (ADS).

3. Combined immunization

Combined immunization is carried out by simultaneous administration of ADS and diphtheria toxoid. The ADS is given in one arm, while the toxoid is given in the other arm, followed by a complete schedule of vaccination with toxoid.

***Listeria monocytogenes*:** The genus currently contains ten species. But only this species is associated with human illness.

Important properties:

- It is belonging to Listeriaceae family.
- Gram-positive rod arranged in V-or L-shape.
- It does not form spores and capsule.
- Catalase positive.
- It produces beta-hemolysis.
- Tumbling motility by peritrichous flagella.
- Facultative intracellular.

Habitat and transmission:

L. monocytogenes primarily found in intestine of animals and rodents, and it is found in soil and plants. The bacteria can be found in intestine of healthy persons and in vagina of asymptomatic women. The infection usually transmitted by ingestion of contaminated food such as milk, cream, cheese, poultry, vegetables and fruits. The transmission of organism from mother to her fetus can occur across placenta (prenatal) or during delivery (perinatal). Nosocomial transmission occurring by hospital workers.

Antigenic structure and virulence factors:

Surface proteins: the organism has many outer membrane proteins (OMP) facilitate binding and endocytosis into epithelial cells and macrophages.

Adhesion proteins like; internalins (InlA and InlB) ,fbp, flagellin mediated adherence of the organism to target cell.

Endotoxin: An early study suggested that *L. monocytogenes* is unique among Gram-positive bacteria in that it might possess lipopolysaccharide, which serves as an endotoxin. Later, it was found to not be a true endotoxin. Listeria cell walls consistently contain lipoteichoic acids(LTA), in which a glycolipid moiety, such as a galactosyl-glucosyl-diglyceride, is covalently linked to the terminal phosphomono-ester of the teichoic acid. This lipid region anchors the polymer chain to the cytoplasmic membrane. These lipoteichoic acids resemble (endotoxin-like materials) the lipopolysaccharides of Gram-negative bacteria in both structure and function, being the only amphipathic polymers at the cell surface.

Listeriolysin-O(LLO) has hemolytic activity and pore-forming toxin. It also allows organism to escape from the endosome(phagosome).

Phospholipase-C(PLC) is mediates the passage of bacteria directly to neighboring cells.

Listeria is **non-exotoxin** producer.

Pathogenesis: *L. monocytogenes* has 13 serotypes bases on O and H antigens. The most human infections (more than 90%) caused by serotype 1a, 1b, and 4b. Few number of the organism require to cause disease, 10^3.

Pathogenesis is dependent on:

1. The organism has ability to survive and multiply inside phagocytic cells (intracellular in monocytes).

2. Ability of the organism to ovoid immune system by spreading within the cells or between the cells. Movement of organism from cell to cell by uses of cellular actin and contractile system.

If the organisms are taken by ingestion, it will attach to and enters epithelial cells of intestine cells by endocytosis. The bacteria have many adhesin surface proteins interact with cellular receptor (E-cadherin) on epithelium cells, promoting endocytosis into epithelium cells.

If the organisms become blood borne, can disseminated to brain and placenta. The receptor molecules (Cadherin) are also found in brain and placenta, this explain the affinity and ability of listeria to cause meningitis and abortion.

After phagocytosis(endocytosis), they invade mononuclear phagocytic cells. The bacteria are enclosed in phagolysosome, where low pH activates the bacteria to produce listeriolysin-O (membrane-damaging toxin). This enzyme with phospholipase lyses membrane of phagolysosome, and allows the organism to escape from phagosome vacuole into cytoplasm of epithelium cell. It grows in cytosol and stimulates changes in cell function that facilitate its direct passage from cell to cell.

The organism proliferates in cytoplasm, and ActA, another listerial surface protein, induces host cell actin polymerization, and reorganization of it. Pushing against the host cell membrane, they cause forming elongated protrusions acomet-like tail (filopods/pseudopods). These filopods are ingested by adjacent cells. The complex appears to propel the organism through pseudopods in contact with adjacent cell, then the listeria is released and the cycle begins again. *L. monocytogenes* aggregates actin filaments on its surface and is propelled in a "sling-shot" fashion, called actin rockets, from one host cell to another. The use of actin polymerization machinery to polymerize

cytoskeleton that give boost motility in intracellular space and it can move in the cell, and it also travel from cell to cell are called Zipper mechanism.

L monocytogenes can move from cell to cell without being exposed to antibodies, complement, or polymorphonuclear cells. *Shigella flexneri* and rickettsia also usurp the host cells' actin and contractile system to spread their infections. So the pathogenesis of Shigella, Rickettsia and Listeria in same mechanism.

Pathogenicity and clinical features (listeriosis)

1. Meningitis and sepsis

   In neonates; the organism causes two forms of syndrome disease; Earlyonset syndrome (1-5 days); neonatal infection is result of infection in uterine. The infection is acquired either during pregnancy (usually in third trimester) or during delivery. The disseminated form of the disease is characterized by sepsis, pustular lesions, and granulomas containing *L. monocytogenes* in multiple organs, so called granulomatous infantiseptica. Neonatal sepsis can cause malformation, abortion or still birth, or premature delivery. The death may occur before or after delivery. Late-onset syndrome (10-20 days); cause development of meningitis between birth and third week of life. It is often caused by serotype 4b. Newborns infected can have acute meningitis 1-4 weeks later. Listeriosis has significant mortality rate.

2. Meningoencephalitis: In adults, the listeriosis is most common as meningoencephalitis in some immunocompromised adults, especially in elderly, patients who receiving corticosteroids, renal transplant patients or patients with cancer.

3. Gastroenteritis (food poisoning) Usually associated with ingestion of contaminated dairy products. This disease develops after period 6-48 hours. Antacid and cimetidine are risk factors associated with this disease.

Epidemiology:

Listeria infections are most common in pregnant women, fetuses and newborns, and in some immunocompromised individuals, such as older adults and patients receiving corticosteroids.

The infection in pregnant women is typically asymptomatic (vaginal colonization) or the mother may have flu-like illness. 1-15% of healthy humans are asymptomatic intestinal carriers.

Spontaneous infection occurs in many domestic and wild animals.

Lab. Dx:

Specimens are stool, blood and CSF.

- Isolation of organism from cultures of materials on blood agar. Colonies display β-hemolysis.
- Gram-stain display gram-positive rods resembling diphtheroids.
- Tumbling motile organism at room temperature (at 20-25°C).
- Identification by biochemical tests like catalase +ve, oxidase –ve, glucose ferment, hemolysis (CAMP test +ve like GBS).

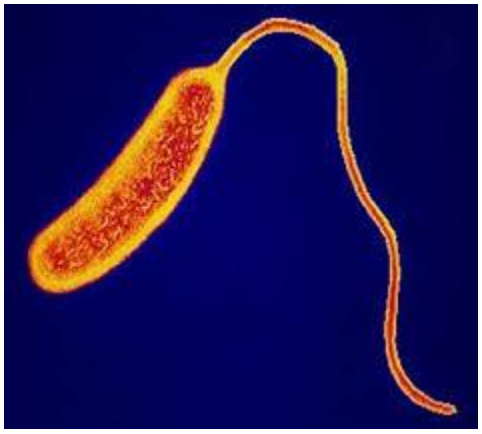
Control: Antibiotics including ampicillin and erythromycin.

- Listeriosis is zoonotic disease, so it can have prevented by good food handling and good cooking, in addition to pasteurization of dairy products.
- Because the organism has ability to grow at 4°C or less, thus refrigeration does not reliably suppress its growth in food, but enhancement of growth at cold condition.
- No vaccine available.

Vibrio cholerae

Morphology

These are gram-negative, short, curved, cylindrical rods, on prolonged cultivation, vibrios may become straight rods that resemble the gram-negative entericbacteria, about 1.5 µm × 0.2-0.4 µm in size, with rounded or slightly pointed ends. The cell is typically comma shaped (hence the old name *V. comma*) but the curvature انعطاف is often lost on subculture. Upon serial transfers, the organisms revert to straight forms. S shaped or spiral forms may be seen due to two or more cells lying end to end. In old cultures, they are frequently highly pleomorphic.



It is actively motile, by means of a single, polar sheathed flagellum. The motility is of the darting اندفاعtype. They are non-sporing, non-capsulated and non- acid fast.

Most important pathogenic members in man are: *Vibrio cholerae*. (classic, El Tor)

Specimens

Specimens for culture consist of mucus flecks بقع المخاطfrom stools.

Cultural Characteristics

The cholera vibrio is strongly aerobic, growth being scanty and slow anaerobically. It grows within a temperature range of 16-40°C (optimum 37°C). Growth is better in an alkaline medium and it occurs freely between pH 7.4 and 9.6 (optimum pH 8.2). *V. cholerae* is a non-halophilic vibrio.

On **nutrient agar**, after overnight growth, colonies are **smooth, convex**, **moist**, **translucent round disks**, about 1-2 mm in diameter, with a **bluish tinge** خضاب in transmitted light. The growth has a distinctive odor. On MacConkey agar, the colonies are smaller than those on nutrient agar and are **colorless**, but become **reddish** on prolonged incubation due to the **late fermentation** of **lactose**. Growth is poor on most other enteric selective media. On blood agar, colonies are **initially** surrounded by a zone of **greening**, which later becomes clear due to **hemo-digestion**.

Holding or Transport Media

V. cholerae is quite sensitive to drying, exposure to sunlight and extreme changes in pH. It is also inhibited by normal intestinal flora. The stool samples

should be transported in transport (holding) media if the cultures cannot be put up immediately.

Cary-Blair Medium

This is a buffered solution. It is a suitable transport medium for Salmonella and Shigella as well as for vibrios.


Cary-Blair Medium

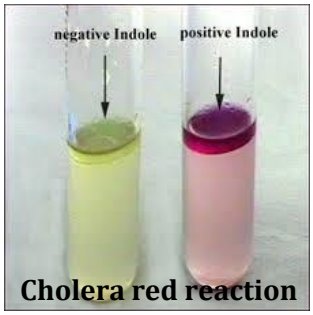
Enrichment Media such as Alkaline Peptone Water.

Plating Media such as Alkaline Bile Salt Agar (BSA); pH 8.2 and Thiosulphate-Citrate-Bile-Sucrose (TC BS) Agar.


TCBS

Biochemical Reactions

Cholera red reaction is tested by adding few drops of H_2SO_4 to 24 hour growth in peptone water. With *Vibrio cholerae* red pink color is produced. Vibrios are susceptible to the compound O/129 (2, 4- diamino- 6,7 - diisopropylpteridine) which differentiates them from *Aeromonas* species. Another difference between vibrios and aeromonads is that vibrios grow on media containing 6% NaCl, but Aeromonas does not.



Cholera red reaction

***Pseudomonas aeruginosa* (part 2)**

- Found in human and animal intestine, water, soil and moist environment
- ❖ **in hospitals.**
  - Primarily a nosocomial pathogen.
  - Invasive and toxigenic, produces infections in patients with

abnormal host defenses

Antigenic characteristic:

- Pili: Adhere to epithelial cells
- Exopolysaccharide: Anti-phagocytic property/ inhibit pulmonary clearance.
- Lipopolysaccharide: Endotoxic effect

Enzymes

- Elastases: Digests protein (elastin, collagen, IgG)
- Proteases
- Hemolysins
- Phospholipases C (heat labile): Degrade cytoplasmic membrane components

Exotoxin A: Cytotoxic by blocking protein synthesis

Clinical features:

Pathogenic only when introduced into areas devoid of normal defenses eg. Breached mucus membrane or skin, use of IV line or urinary catheterization, neutropenia of any cause.

- Urinary tract infection- chronic, complicated Urinary tract infection and associated with indwelling catheter.
- Wound infection of burn sites, pressure sores and ulcers.
- Septicaemia- "Ecthyma gangrenosum" skin lesion (haemorrhagic skin necrosis)
- Otitis externa- Malignant external ear infection in poorly treated diabetic patients.
- Pneumonia- Infection of the lung in patients with cystic fibrosis.
- Eye infection- Secondary to trauma or surgery.

Laboratory diagnosis:

Specimen: pus, urine, sputum, blood, eye swabs, surface swabs

Smear: Gram-negative rods

Culture:

- Obligate aerobe, grows readily on all routine media over wide range of temperature (5-42 ^{0}C).
- Bluish-green pigmented large colonies with characteristic "fruity" odor on culture media.

Biochemical reaction:

- Oxidase positive
- Catalase positive
- Citrate positive
- Indole negative
- Produce acid from carbohydrate by oxidation, not by fermentation.

NB: identification of the bacteria is based on colony morphology, oxidase-positivity, characteristic pigment production and groth at 42 C°.

Treatment: Ticarcillin or piperacillin and aminoglicosides

- Aztreonam
- Imipenem
- Ceftazidime
- Cefoperazone
- Flouroquinolones

Prevention and control:

Special attention to sinks, water baths, showers and hot tubs Polyvalent vaccine tohigh risk groups.

GENUS: VIBRIOS

- .Actively motile, gram-negative curved rods.
- Species of medical importance: *Vibrio cholerae*-O1

Vibrio cholerae

Characteristics:

- Found in fresh water, shellfish and other sea food.
- Man is the major reservoir of *V. cholerae*-01, which causes epidemic cholera.
- Readily killed by heat and drying; dies in polluted water but may survive in clean stagnant water, esp. if alkaline, or sea water for 1-2 weeks.

Antigenic structure:

- antigen

- Six major subgroups.
- All strains possess a distinctive O antigen and belong to subgroup I with subdivision into three serotypes; Ogawa, Inaba, Hikojima.

Any serotype can be either Classical or El-Tor biotype.

- El-Tor biotype is more resistant to adverse conditions than Classical biotype of *V. cholerae*.

H antigen

- Little value in identification

Clinical features:

Route of infection is fecal-oral route. After ingestion of the *V. cholerae*-O1, the bacteria adhere to the intestinal wall without invasion then produces an exotoxin causing excessive fluid secretion and diminished fluid absorption resulting in diarrhea (rice water stool) which is characterized by passage of voluminous watery diarrhea containing vibrios, epithelial cells and mucus; and result in severe dehydration.

Laboratory diagnosis:

Specimen: Stool flecks

Smear: Gram-negative motile curved rods.

Motility of vibrios is best seen using dark-field microscopy.

Presumptive diagnosis: Inactivation of vibrios in a wet preparation after adding vibrio antiserum.

Culture:

1. TCBS (thiosulphate citrate bile salt sucrose agar) media, Selective
2. media for primary isolation of *V. cholerae*.

Observe for large yellow sucrose-fermenting colonies after 18-24 hrs. of incubation.

2. Alkaline peptone water: Enrichment media for *V. cholerae*-O1

Growth on and just below the surface of peptone water within 4-6 hours at room temperature as well as 37 C˚.

Biochemical Reaction:

- Oxidase-positive.
- Ferment sucrose and maltose (acid; no gas).
- Do not ferment L-arabinose.

Treatment: Sensitive to tetracycline and chloramphenicol. Fluid and electrolyte replacement are the first line of management for cholera.

Brucella

Disease

Brucella species cause brucellosis (undulant fever, Malta Fever).

Important Properties

Brucellae are small gram-negative rods without a **capsule.** The three major human pathogens and their animal reservoirs are *Brucella melitensis* (goats and sheep) *Brucella abortus* (cattle), and *Brucella suis* (pigs).

Typical Organisms

The appearance in young cultures varies from cocci to rods 1.2 m in length, with short cocco-bacillary forms predominating. They are gram-negative but often stain irregularly, and they are aerobic, non-motile, and non-spore-forming.

Culture

Small, convex, smooth colonies appear on enriched media in 2–5 days.

Growth Characteristics

Fresh specimens from animal or human sources are usually inoculated on **trypticase-soy agar or blood culture media**. *B abortus* requires 5–10% CO_2 for growth, whereas the other three species grow in air.

Brucellae utilize carbohydrates but produce neither acid nor gas in amounts sufficient for classification. Catalase and oxidase are produced by the four species that infect humans. Hydrogen sulfide is produced by many strains, and nitrates are reduced to nitrites.

Virulence factors

lipopolysaccharide (LPS), T4SS secretion system and BvrR/BvrS system, which allow interaction with host cell surface

Pathogenesis

The common routes of infection in humans are the intestinal tract (ingestion of infected milk), mucous membranes (droplets), and skin (contact with infected tissues of animals). Cheese made from unpasteurized goats' milk is a particularly common vehicle

They localize in the **reticuloendothelial system,** namely, the lymph nodes, liver, spleen, and bone marrow. Many organisms are killed by macrophages, **but some survive within these cells, where they are protected from antibody.** The host response is granulomatous, with lymphocytes and epithelioid giant cells, which can progress to form focal abscesses.

Clinical Findings

After an incubation period of 1 to 3 weeks, nonspecific symptoms such as fever, chills, fatigue, malaise, anorexia, and weight loss occur. The onset can be acute or gradual. The undulating (rising-and-falling) fever pattern that gives the disease its name occurs in a minority of patients

Enlarged lymph nodes, liver, and spleen are frequently found. Pancytopenia occurs.

Note *:-Brucella melitensis* infections tend to be more severe and prolonged, whereas those caused by *B. abortus* are more self-limited.

Treatment

The treatment of choice is tetracycline plus rifampin.

Prevention

Prevention of brucellosis involves pasteurization of milk, immunization of animals, and slaughtering of infected animals. There is no human vaccine.

Haemophilus

Diseases

Hemophilus influenzae used to be the leading cause of meningitis in young children, it is still an important cause of upper respiratory tract infections (otitis media, sinusitis, conjunctivitis, and epiglottitis) and sepsis in children. It also causes pneumonia in adults. Most *H. influenzae* organisms in the normal flora of the upper respiratory tract are not encapsulated.

Typical Organisms

In specimens from acute infections, the organisms are short (1.5 m) coccoid bacilli, sometimes occurring in pairs or short chains. In cultures, the morphology depends both on age and on the medium. At 6–8 hours in rich medium, the small cocco-bacillary forms predominate. Later there are longer rods, lysed bacteria, and very pleomorphic forms. Organisms in young cultures (6–18 hours) on enriched medium have a definite capsule. The capsule is the antigen used for "typing" *H influenzae*.

Culture

1-On chocolate agar, flat, grayish-brown colonies with diameters of 1–2 mm are present after 24 hours of incubation.

2-IsoVitaleX in media enhances growth.

Growth Characteristics

Identification of organisms of the haemophilus group depends in part upon demonstrating the need for certain growth factors called X and V. Factor X acts physiologically as hemin; factor V can be replaced by nicotinamide adenine nucleotide (NAD) or other coenzymes. Colonies of staphylococci on sheep blood

agar cause the release of NAD, yielding the satellite growth phenomenon. The requirements for X and V factors of various

Note: - Un-encapsulated strains can also cause disease, especially mucosal diseases of the upper respiratory tract such as sinusitis and otitis media, but are usually noninvasive

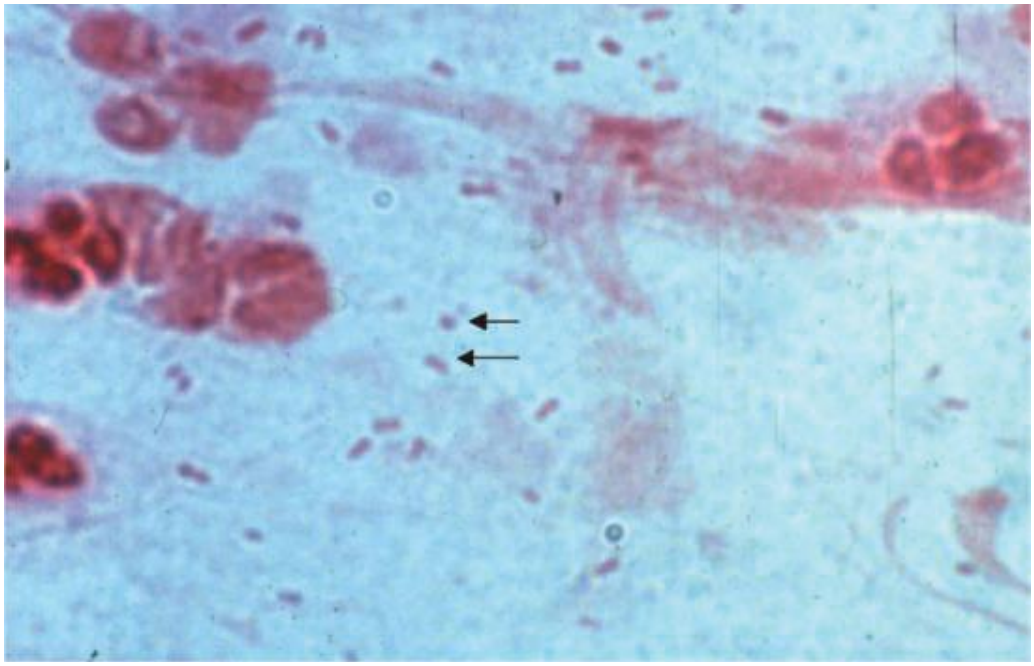


Figure (1): *Haemophilus influenzae*—Gram stain. Arrows point to two small "coccobacillary" gram-negative rods

Pathogenesis and spectrum of disease

Production of a capsule and factors that mediate bacterial attachment to human epithelial cells are the primary virulence factors associated with *Haemophilus* spp. In general, infections caused by *H. influenzae* are often systemic and life-threatening, whereas infections caused by non-typeable (do not have a capsule) strains are usually localized. Most serious infections caused by *H. influenzae* type b are biotypes I and II. The development and use of the conjugate vaccine in children since 1993 has reduced the infection rate by 95% in children younger than 5 years old in the United States. Most *H. influenzae* infections are now caused by non typeable strains (NTHi). Transmission is often via respiratory secretions. The

organism is able to gain access to sterile sites from colonization in the upper respiratory tract. Clinical infections include otitis media (ear infection), sinusitis, bronchitis, pneumonia, and conjunctivitis. Immuno-deficiencies and chronic respiratory problems such as chronic obstructive pulmonary disease may predispose an individual to infection with NTHi.

Virulence factors

- Capsule: Anti-phagocytic, type b most common.
- Additional cell envelope factors mediate attachment to host cells.
- Un-encapsulated strains: pili and other cell surface factors mediate attachment.

Laboratory Diagnosis

Direct Observation **Gram stain** is generally used for the direct detection of *Haemophilus* in clinical material. However, in some instances the **acridine orange** stain is used to detect smaller numbers of organisms that may be undetectable by Gram staining.

Antigen Detection *H. influenzae* type b capsular polysaccharide in clinical specimens, such as CSF and urine, can be detected directly using commercially available particle agglutination assays.

Molecular Methods Rapid screening procedures are very useful for patient therapy and evaluating outbreaks and have been developed for detection from CSF, plasma, serum, and whole blood.

Cultivation

Media of Choice *Haemophilus* spp. typically grow on chocolate agar as **smooth, flat or convex, buff or slightly yellow colonies.** Chocolate agar provides hemin (X factor) and NAD (V factor), necessary for the growth of *Haemophilus* spp. Most strains will not grow on 5% sheep blood agar, which contains protoporphyrin IX but not NAD. Several bacterial species, including *Staphylococcus aureus*, produce NAD as a metabolic byproduct. Therefore, tiny colonies of *Haemophilus* spp. may be seen

growing on sheep blood agar very close to colonies of bacteria capable of producing V factor; this is known as the **satellite phenomenon**. The blood used in chocolate agar is heated to inactivate nonspecific inhibitors of *H. influenzae* growth.

Treatment

The treatment of choice for meningitis or other serious systemic infections caused by *H. influenzae* is **ceftriaxone**

Prevention

The vaccine contains the capsular polysaccharide of *H.influenzae* type b **conjugated to diphtheria toxoid** or other carrier protein

Bordetella

Disease

Bordetella pertussis causes whooping cough (pertussis).

Typical Organisms

The organisms are minute gram-negative coccobacilli resembling *H influenzae.* With toluidine blue stain, bipolar metachromatic granules can be demonstrated. A capsule is present.

Culture

Primary isolation of *B pertussis* requires **enriched media**. **Bordet-Gengou medium** (potato-blood-glycerol agar) that contains **penicillin G**, 0.5 g/mL, can be used; however, a **charcoal-containing medium**. The plates are incubated at 35–37 °C for 3–7 days in a moist environment (eg, a sealed plastic bag). The small, faintly staining gram-negative rods are identified by immunofluorescence staining. *B pertussis* is non-motile.

Growth Characteristics

The organism is a strict aerobe and forms acid but not gas from glucose and lactose. It does not require X and V factors on subculture. Hemolysis of blood-containing medium is associated with virulent *B pertussis.*

The Virulence factors

1- Adhesins such as filamentous hemagglutinin, fimbriae.
2- Pertussis toxin.
3- Adenylate cyclase,
4- Tracheal cytotoxin.

Pathogenesis & Epidemiology

Bordetella pertussis, a pathogen **only for humans,** is transmitted by **airborne droplets** produced during the severe coughing episodes. The organisms attach to the ciliated epithelium of the upper respiratory tract but do not invade the underlying tissue. Decreased cilia activity and subsequent
death of the ciliated epithelial cells are important aspects of pathogenesis.

Clinical Findings

Whooping cough is an acute trachea-bronchitis that begins with mild upper respiratory tract symptoms followed by a severe paroxysmal cough, which lasts from 1 to 4 weeks. The paroxysmal pattern is characterized by a series of hacking coughs, accompanied by production of copious amounts of mucus, that end with an inspiratory "whoop" as air rushes past the narrowed glottis.

Laboratory Diagnosis

The organism can be isolated from nasopharyngeal swabs taken during the paroxysmal stage. Bordet-Gengou1 medium used for this purpose contains a high percentage of blood (20%–30%) to inactivate inhibitors in the agar.

Treatment

Azithromycin is the drug of choice.

Prevention

There are two types of vaccines: an acellular vaccine containing purified proteins from the organism and a killed vaccine containing inactivated *B. pertussis* organisms

Chlamydia

The *Chlamydia* spp. are members of the order Chlamydiales and the family Chlamydiaceae. Members of the family Chlamydiaceae Members of the order Chlamydiales are obligate intracellular bacteria that were once regarded as viruses because, like viruses, the chlamydiae require biochemical resources of eukaryotic host cells to fuel their metabolism for growth and replication by providing high-energy compounds such as adenosine triphosphate. *Chlamydia* spp. are similar to gram-negative bacilli in that they have lipopolysaccharide (LPS) as a component of the cell wall. The chlamydial LPS, however, has little endotoxic activity. The chlamydiae have a major outer membrane protein (MOMP) that is very diverse. Chlamydiae have a unique developmental life cycle reminiscent of parasites, with an intracellular, replicative form, the **reticulate body** (RB), and an extracellular, metabolically inert, infective form, the **elementary body** (EB). The EB cannot survive outside of a host cell for an extended period. After infection of a host cell, the EB differentiates into an RB. The RB divides by **binary fission** within vacuoles. As the numbers of RB increase, the vacuole expands, forming an intracytoplasmic inclusion. The RB then revert to EB, and 48 to 72 hours post infection, the EB are released from the host cell. In addition to the replicative cycle associated with acute chlamydial infections, there is evidence that *Chlamydia* can persist in an aberrant form in vitro, depending on the amount of interferon-gamma (IFN-g) and tryptophan in the host cell as well as function of the tryptophan synthase encoded by the organism. Removal of IFN-g or increase in tryptophan will result in differentiation of chlamydiae into an active EB infection. Therapeutic implications of this persistence in vivo have not yet been completely defined; however, evidence suggests that activity of the tryptophan synthase gene in *C. trachomatis* differs between isolates recovered from the eye versus the genital tract. *C. trachomatis, C. pneumoniae*, and *Chlamydia psittaci* are important causes of human infection.

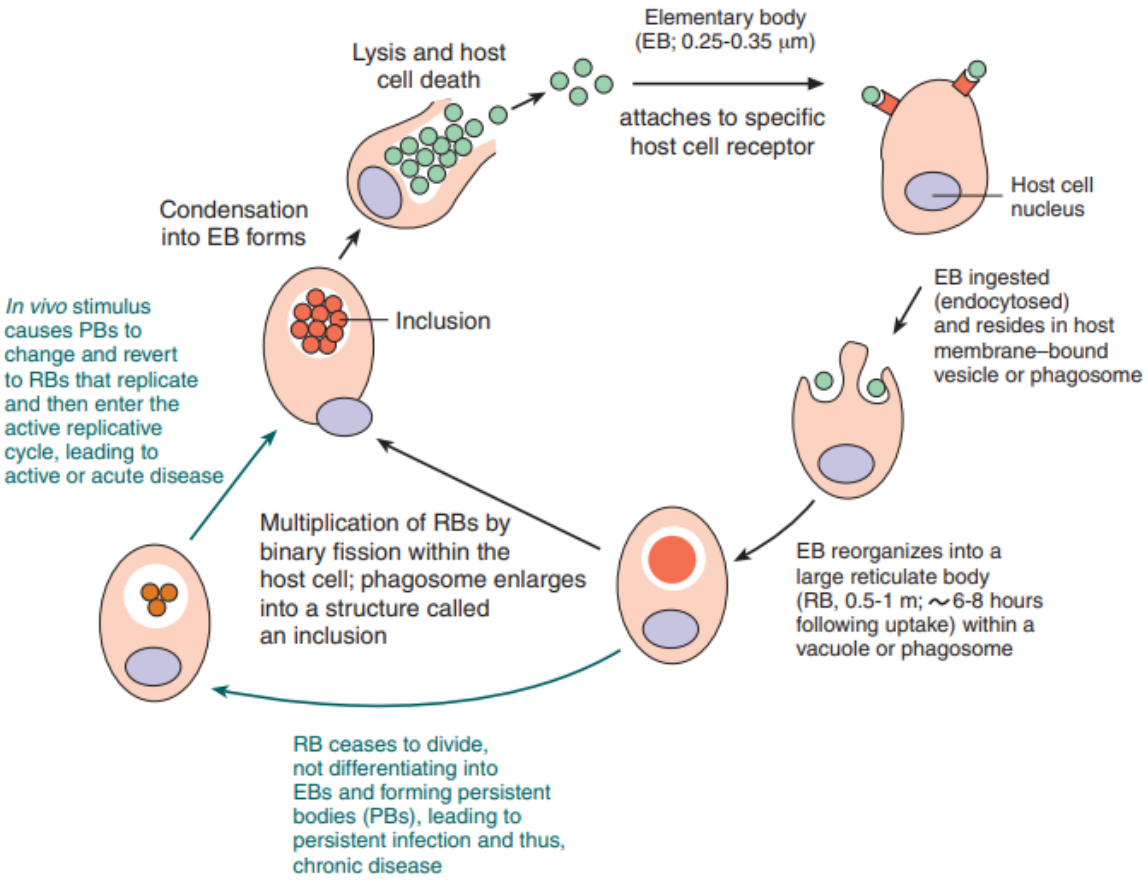


Figure 43-1 The life cycle of chlamydiae. The entire cycle takes approximately 48 to 72 hours.

Differential Characteristics Among Chlamydiae That Cause Human Disease

Property	*Chlamydia trachomatis*	*Chlamydia psittaci*	*Chlamydia pneumoniae*
Host range	Humans (except one biovar that causes mouse pneumonitis)	Birds, lower mammals, humans (rare)	Humans
Elementary body morphology	Round	Round	Pear-shaped
Inclusion morphology	Round, vacuolar	Variable, dense	Round, dense
Glycogen-containing inclusions	Yes	No	No
Plasmid DNA	Yes	Yes	No
Susceptibility to sulfonamides	Yes	No	No

DNA, Deoxyribonucleic acid.

Chlamydia trachomatis

General Characteristics *C. trachomatis* infects humans almost exclusively and is responsible for various clinical syndromes. Based on MOMP antigenic differences, *C. trachomatis* is divided into 18 different serovars that are associated with different primary clinical syndromes.

Spectrum of Disease

- Trachoma is manifested by a chronic inflammation of the conjunctiva and remains a major cause of preventable blindness worldwide.
- Lymphogranuloma venereum (LGV) is a sexually transmitted disease.
- Oculogenital Infections *C. trachomatis* can cause acute inclusion conjunctivitis in adults and newborns. The organism is acquired when contaminated genital secretions get into the eyes via fingers or during passage of the neonate through the birth canal.
- Perinatal Infections Approximately one fourth to one half of infants born to females infected with *C. trachomatis* develop inclusion conjunctivitis. Usually, the incubation period is 5 to 12 days after birth, but it may be as long as 6 weeks

Laboratory Diagnosis

1. Indirect method: Culture: Several different cell lines have been used to isolate *C. trachomatis* in cell culture, including McCoy, HeLa, and monkey kidney cells; cycloheximide-treated McCoy cells are commonly used. After shaking the clinical specimens with 5-mm glass beads, centrifugation of the specimen onto the cell monolayer (usually growing on a coverslip in the bottom of a vial, commonly called a “shell vial”) facilitates adherence of elementary bodies. After 48 to 72 hours of incubation, monolayers are stained with a fluorescein labeled monoclonal antibody.

2. Direct Detection Methods

- Cytologic Examination. Cytologic examination of cell scrapings from the conjunctiva of newborns or persons with ocular trachoma can be used to detect *C. trachomatis* inclusions, usually after Giemsa staining.
- Antigen Detection and Nucleic Acid Hybridization. To circumvent the shortcomings of cell culture, antigen detection methods are commercially available.

Mycobacterium tuberculosis

Table-1. Classification of mycobacteria

Tubercle bacilli
1. Human—*M. tuberculosis*
2. Bovine—*M. bovis*
3. Murine—*M. microti*
4. Aviam—*M. avium*
5. Cold blooded—*M. marinum*

Lepra bacilli
Human—*M. leprae*
Murine—*M. lepraemurium*

Mycobacteria causing skin ulcers
1. *M. ulcerans*
2. *M. balnei*

Atypical mycobacteria
1. Photochromogens
2. Scotochromogens
3. Nonphotochromogens
4. Rapid growers

Johne's bacillus
M. paratuberculosis

Saprophytic mycobacteria
M. butyricum, M. phlei, M. stercoris.

Morphology

M. tuberculosis is a slender, straight or slightly curved rod with rounded ends, about 3 µm × 0.3 µm, in pairs or as small clumps. The bacilli are non-motile, non-sporing, non-capsulated and acid-fast. They are gram-positive but are difficult to stain.

When stained with carbol fuchsin by the Ziehl-Neelsen method, they resist decolorization by 20 percent sulfuric acid and absolute alcohol for 10 minutes **(acid and alcohol fast).** With this stain, the *Tubercle bacilli* stain **bright red,** while the tissue cells and other organisms are stained blue (Fig. 1). Organisms in **tissue** and **sputum smears** often stain irregularly and have a beaded مخرزة or barred appearance, presumably because of their **vacuoles** and **polyphosphate** content.

Acid fastness has been ascribed يعزى to the presence in the bacillus of **mycoloic acid.** It is related to the **integrity of the cell** and appears to be a property of the lipid-rich waxy cell wall. Staining may be uniform متسق or granular. In *M. tuberculosis* beaded or barred مخطط forms are frequently seen,

but *M. bovis* stains more uniformly. *M. bovis* appear straighter, bolder and shorter with uniform staining.

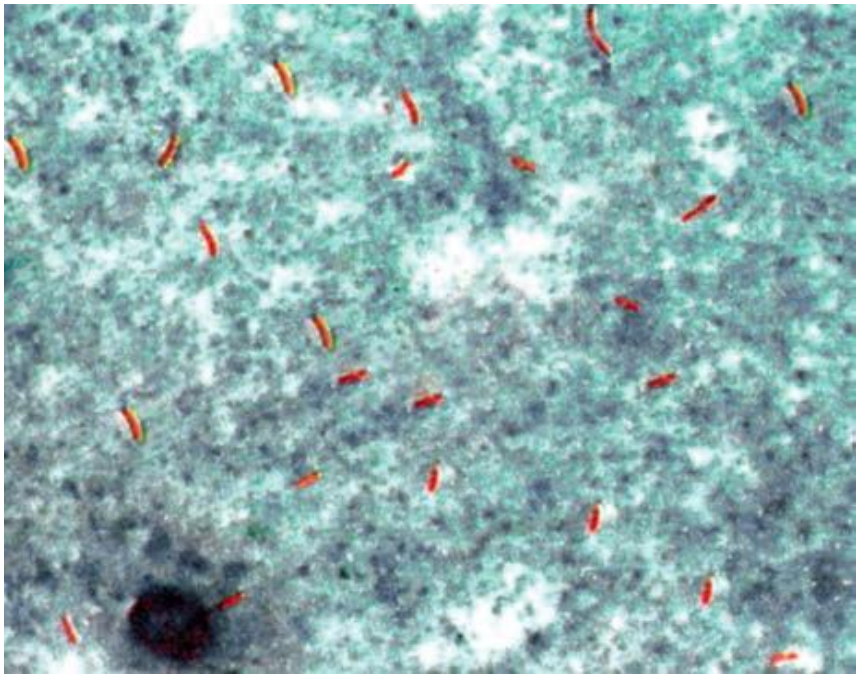


Fig. 1- *Mycobacterium tuberculosis* in Ziehl-Neelsen stained smear

Cultural Characteristics

M. tuberculosis is an **obligate aerobe** while *M. bovis* is **microaerophilic** on primary isolation, becoming aerobic on subculture. The optimal growth temperature of tubercle bacilli is 35 to 37°C but they fail to grow at 25°C or 41°C. Most other mycobacteria grow at one or other, or both, of these temperatures. Optimum pH is 6.4 to 7.0. The bacilli grow slowly, the **generation time** *in vitro* being **14** to **15** hours. Colonies appear in about **two weeks** and may sometimes take up to **eight weeks**.

The solid medium most widely employed for routine culture is **Lowenstein-Jensen (LJ) medium** without starch.

Human tubercle bacilli produce visible growth on LJ medium in about 2 weeks, although on primary isolation from clinical material colonies may take up to 8 weeks to appear. On solid media, *M. tuberculosis* forms **dry**, **rough**, **raised**, **irregular** colonies with a **wrinkled** مجعدsurface. They are **creamy white**, **becoming yellowish or buff** اصفر برتقالي**colored on further incubation**. They are tenacious دبق، متماسك and not easily emulsified يمكن استحلابه . *Mycobacterium tuberculosis* has a luxuriant وافر growth **(eugenic** محسن النسل

growth*)* as compared to *Mycobacterium bovis* which grows poorly on LJ glycerol medium **(dysgenic growth)** and colonies, in comparison are **flat** مسطح , **smooth**, **moist**, **white** and **break up** بسهولة **تتقطع** easily when touched. The growth of *M. bovis* is much better on LJ pyruvate medium (media containing sodium pyruvate in place of glycerol).

Antigenic Structure

Mycobactria contain many unique immune-reactive substances, most of which are components of the cell wall. Mycobacteria possess two types of antigens, **cell wall** (insoluble) and **cytoplasmic** (soluble) antigens.

1. Cell wall antigens

The basic structure of the cell wall is typical of gram-positive bacteria: an inner cytoplasmic membrane overlaid with a thick peptidoglycan layer and no outer membrane. The cell wall consists of **lipids**, **proteins** and **polysaccharides**. These lipids constitute **60%** of the cell wall weight and contributes to several biological properties. Lipids of the cell wall particularly **mycolic acid** fraction جزء are responsible for acid-fastness of bacteria and the cellular reaction of the body. The cell wall is made up of four distinct layers (Fig. 2).

(i) Peptidoglycan (murein) layer
(ii) Arabinogalactan layer
(iii) Mycolic acid layer
(iv) Mycosides (peptidoglycolipids or phenolic glycolipids)

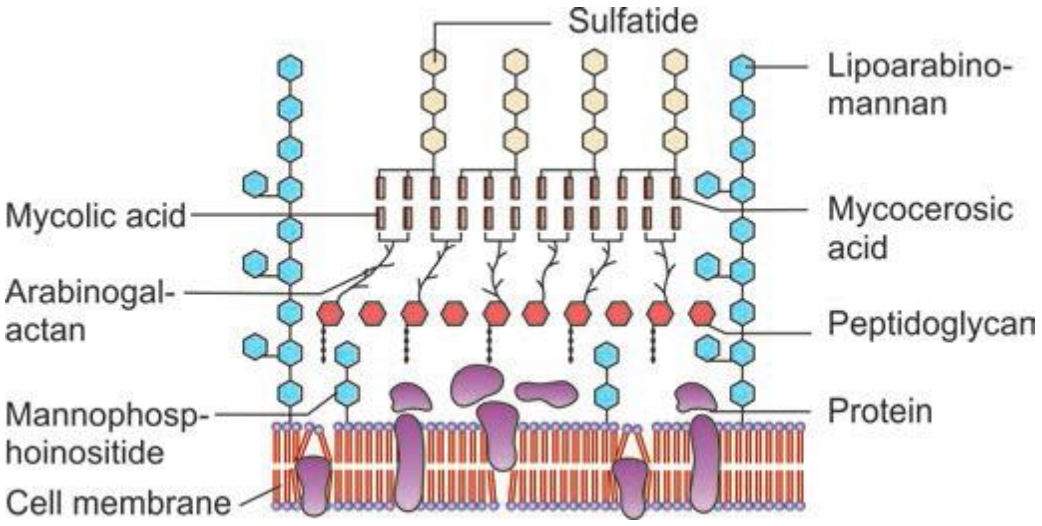


Fig.2- Cell wall of *Mycobacterium tuberculosis*

Test	M. tuberculosis	M. bovis	Atypical mycobacteria
Production of niacin	+	–	–
Binding of neutral red	+	+	+/–
Hydrolysis of Tween 80	–	–	+
Production of enzymes:			
• Nitrate reduction	+	–	+/–
• Arylsulphatase	–	–	–/+
• Catalase at room temp	–	–	+
at 68°C	–	–	+
• Catalase-Peroxidase	Weak +	Weak +	Strong +
Nicotinamidase	+	–	–
• Pyrazinamidase	+	–	+/–
Susceptibility to:			
• Pyrazinamide	+	–	–
Uptake of iron	–	–	–/+

Specimen Collection

Persons suspected of having pulmonary or laryngeal TB should have at least three sputum specimens examined by smear and culture. It is best to obtain a series سلسلة of early-morning specimens collected on 3 consecutive متعاقب days. Specimens should be obtained in an isolated, well-ventilated area or a sputum collection booth.

For patients unable to cough up sputum, deep coughing may be induced by inhalation of an aerosol of warm, hypertonic (5%-15%) saline. Patients should be given time — 15 minutes is usually sufficient — to produce sputum, which is usually brought up by a deep cough. Because induced sputum is very watery and resembles saliva, it should be labeled "induced" to ensure that the laboratory staff do not discard it.

Bronchoscopy can be done if there is suspicion مشكوك فيه of TB and the patient cannot cough up sputum.

Gastric aspiration can also be used to obtain specimens of swallowed sputum.

During specimen collection, patients produce an aerosol that may be hazardous to health care workers or other patients in close proximity. For this reason, precautionary measures for infection control must be followed during sputum induction, bronchoscopy, and other common diagnostic procedures.

Because TB can occur in almost any anatomical site, a variety of clinical specimens other than sputum (e.g., urine, cerebrospinal fluid, pleural fluid, pus, or biopsy specimens) may be submitted يخضع for examination when extra-pulmonary TB disease is suspected. Tissue specimens for the culture of *M. tuberculosis* should be placed in a transport medium (e.g., Dubos) or a normal saline solution. Formalin or other preservatives should not be used because these solutions kill or inhibit the growth of *M. tuberculosis*.