Introduction
Classification of Bacteria

The treating physician often identifies the disease causing organism on the basis of his clinical findings and accordingly treats the patient. There are many clinical conditions which are manifested only by fever and can be caused by a large number of organisms. The laboratory comes to the aid of physician in all such cases. Many a times even when clinical syndrome is diagnosed such as sore throat, urinary tract infection and acute diarrhea, it becomes difficult to prescribe most appropriate chemotherapeutic agent because of large number of organisms which can cause these syndromes. Laboratory investigation provides the information regarding organisms as well as the drug to be used.

Medical microbiology is the biological study of bacteria, viruses, fungi and algae which are collectively called as microorganisms, and unlike macroscopic organisms that are readily visible, these require magnification to be seen with the help of a microscope. It is concerned with etiology, pathogenesis, laboratory diagnosis and treatment of infections in an individual and with the epidemiology and control of infection in the community. Medical microbiology plays an important role in the diagnosis, prevention, treatment and control of infectious diseases.

All living organisms on earth are composed of one or the other of two types of cells: **prokaryotic** and **eukaryotic** cells based on differences in cellular organization and biochemistry.

There are many differences between the two major divisions: prokaryotes and eukaryotes, of cellular organisms. These include the following:

In prokaryotes:

• A distinct nucleus is absent.
• DNA is in the form of a single circular chromosome.
• Additional ‘extra-chromosomal’ DNA is carried in plasmids.
• Transcription and translation can be carried out simultaneously.

In eukaryotes:

• DNA is carried on several chromosomes within a nucleus.
• The nucleus is bounded by a nuclear membrane.
• Transcription requires formation of messenger RNA (mRNA) and movement of mRNA out of the nucleus into the cytoplasm.
• Translation takes place on ribosomes.
• The cytoplasm is rich in membrane-bound organelles (mitochondria, endoplasmic reticulum, Golgi apparatus and lysosomes) which are absent in prokaryotes (Fig.1).

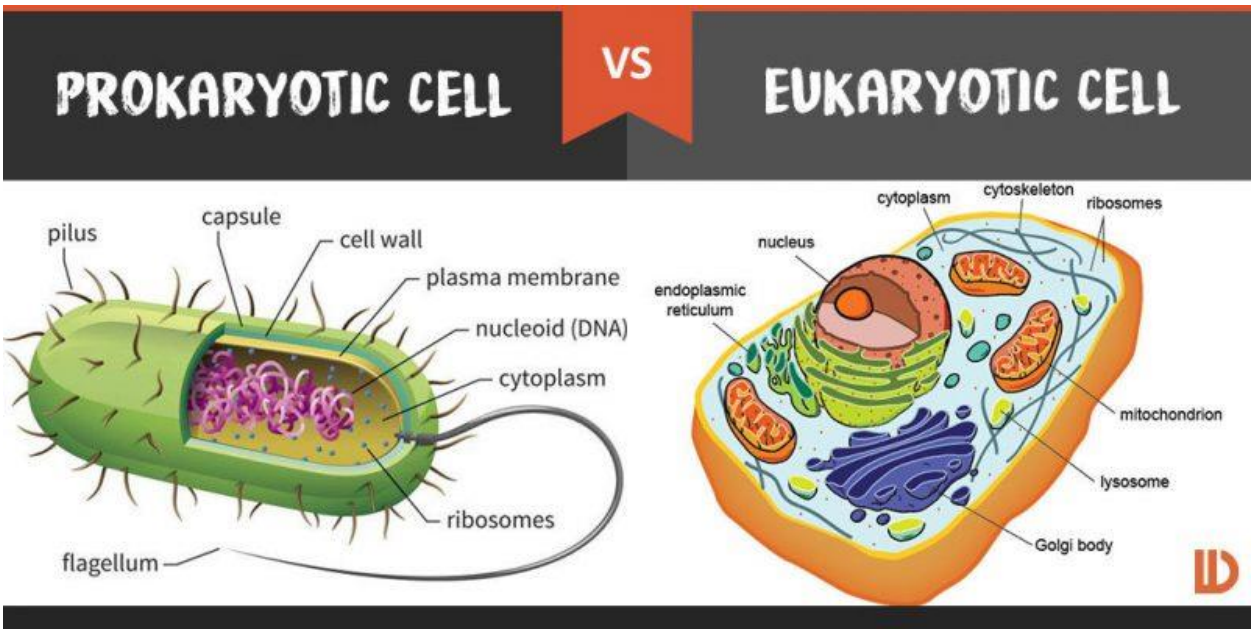

Figure 1: Prokaryotic cell vs. Eukaryotic cell differences

Bacteria are prokaryotes cells usually unicellular, in structure and vary in sizes, measure approximately 0.1 to 10.0 μm. The bacteria are single-celled organisms that reproduce by simple division, i.e. binary fission. Most are free living and contain the genetic information (DNA) free within the cytoplasm, energy-producing and biosynthetic systems necessary for growth and reproduction.

The bacteria were inactivated by:

• boiling
• at 120°C under pressure (autoclaved)
• at 170°C (hot air oven).

Microbes are widely spread over the surface of the Earth and play a crucial role in ecology. Soil and water contain high concentrations of bacteria and molds (two types of microbes), and the surface of every human body is covered with a unique microbial flora.

Bacterial Classification

Bacteria can be classified by their wall structure, intracellular/extracellular invasive, cell morphology, growth characteristics and finally by their genotype (Phylogenetic tree).

A: Wall structure

The bacterial cell wall is complex, consisting of one of two basic forms: a gram positive cell wall with a thick peptidoglycan layer (***Staphylococcus, Streptococcus, Clostridium, Bacillus***), and a gram-negative cell wall with a thin peptidoglycan layer and an overlying outer membrane such as Enteric rods (***Escherichia***, ***Shigella***, ***Salmonella*** and ***Enterobacter***). Gram staining is not a dependable test for

bacteria that are starved (e.g., old or stationary-phase cultures) or treated with antibiotics. Bacteria that cannot be classified by Gram staining include ***mycobacteria***, which have a waxy outer shell and are distinguished with the acid-fast stain, and ***mycoplasmas***, which have no peptidoglycan.

- ***Mycobacterium*** contain large amounts of lipid substances within their cell walls called mycolic acids. These acids resist staining by ordinary methods such as a Gram stain. It can also be used to stain a few other bacteria, such as *Nocardia*.
- ***Mycoplasma*** are a mollicute genus of bacteria that lack a cell wall around their cell membranes.

B: Intracellular/extracellular invasion

Pathogenic bacteria can be grouped into two categories on the basis of their invasive properties for eukaryotic cells.

1. Extracellular bacteria
2. Obligate intracellular bacteria

- Extracellular bacteria: Extracellular bacterial pathogens do not invade cells and proliferate instead in the extracellular environment which is enriched with body fluids. Some of extracellular bacteria even don't penetrate body tissues (e.g. *Vibrio cholera*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*), but adhere to epithelial surfaces and cause disease by secreting potent toxins.

- Obligate intracellular

This group of bacteria can't live outside the host cells. For e.g. *Chlamydial* cells are unable to carry out energy metabolism and lack many biosynthetic pathways, therefore they are entirely dependent on the host cell to supply them with ATP and other intermediates. Because of this dependency *Chlamydiae* were earlier thought to be virus (All viruses are obligate intracellular parasites). Obligate intracellular bacteria cannot be grown in artificial media (agar plates/broths) in laboratories but requires viable eukaryotic host cells (e.g. cell culture, embryonated eggs and susceptible animals).

C: Cell morphology

1- Shape of bacteria

Based on the shape of the bacterial cell, bacteria can be mainly classified into four major categories namely (fig. 2):

- Spherical bacteria or Coccus
- Rod-shaped bacteria or Bacillus
- Spiral bacteria
- Filamentous bacteria.

2- Arrangement of bacteria

i. **Diplococci:** Cocci may be arranged in pairs (diplococci) when cocci divide and remain together.

ii. L**ong chains:** Long chains (*Streptococcus* and *Enterococcus*) when cells adhere after repeated divisions in one plane.
iii. **Grape like clusters:** Grape like clusters (*staphylococci*) when cocci divide in random planes.
iv. **Tetrads:** Square groups of four cells (tetrads) when cocci divide in two planes as in members of the genus *Micrococcus*.
v. **Cubical packets:** Cubical packets of eight of cells (genus *Sarcina*) when cocci divide in three planes.

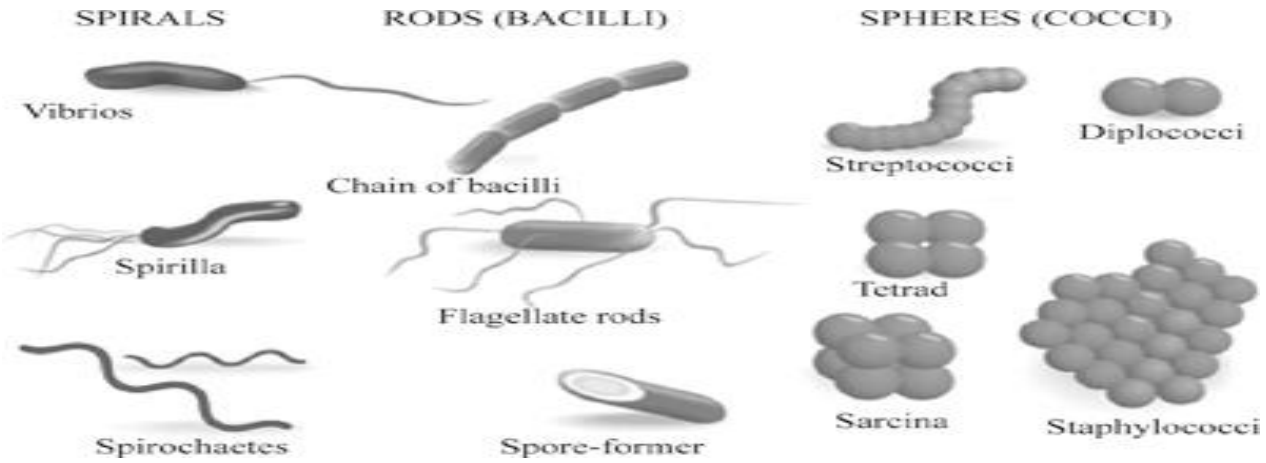

Figure 2: Shape and arrangement of bacteria

D: Growth characteristics

A. Oxygen requirement

Oxygen requirements of bacteria (fig: 3) reflect the mechanism used by them, to satisfy their energy needs. On the basis of oxygen requirements, bacteria can be divided into following different categories:

1- Aerobes: Aerobe grow in ambient air, which contains 21% oxygen and small amount of (0.03%) of carbon dioxide. Aerobes require molecular oxygen as a terminal electron acceptor so cannot grow in its absence (e.g., *Bacillus*)

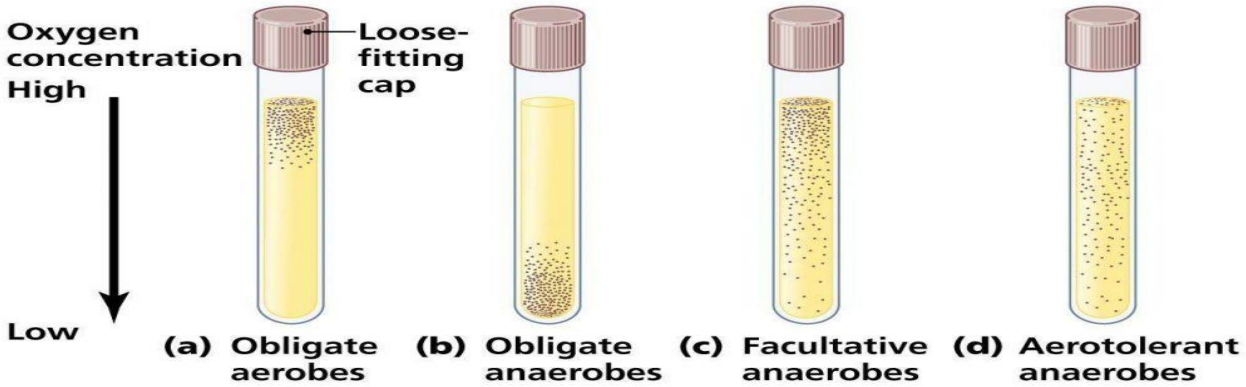

Figure 3: Oxygen requirements for pathogenic bacteria

2- Obligate aerobes: They have absolute requirement for oxygen in order to grow (*Pseudomonas aeruginosa* and *Mycobacterium tuberculosis*).

3- Anaerobes: Usually bacteria of this group cannot grow in the presence of oxygen, oxygen is toxic for them. They use other substances as terminal electron acceptor. Their metabolism frequently is a fermentative type in which they reduce available organic compounds to various end products such as organic acids and alcohols.

4- Obligate anaerobes: These bacteria grow only under condition of high reducing intensity and for which oxygen is toxic (*Clostridium perfringens* and *Clostridium botulinum*).

5- Facultative anaerobes: They are versatile organisms, capable of growth under both aerobic and anaerobic conditions. They preferentially use oxygen as terminal electron acceptor (e.g. *Enterobacteriaceae* group and *Staphylococcus aureus*).

6- Aerotolerant anaerobes: Are anaerobic bacteria that are not killed by exposure to oxygen.

B. Spore formation

Spore forming bacteria are tougher than the average microscopic unicellular organism. These species, which include the genera *Bacillus* and *Clostridium*, can surround themselves with durable coats of protein that allow them to survive in hostile environmental conditions. As spores, bacteria can remain dormant for years, protected from stresses such as chemicals, heat, radiation and dehydration. When revived, however, these bacteria can cause a number of diseases, including botulism, anthrax, tetanus and acute food poisoning.

C. Fastidious/non-fastidious

The difference between fastidious and nonfastidious bacteria is that fastidious bacteria require special nutritional supplements and conditions to grow while nonfastidious bacteria do not need such special nutritional supplements or conditions. Furthermore, fastidious bacteria show slow growth in contrast to nonfastidious bacteria, which grow fast.

E: Universal Phylogenetic Tree

Developing a “universal phylogenetic tree” for bacteria, based on a comparison of 16s ribosomal RNA sequences. These sequences are highly conserved and undergo change at a slow, gradual and consistent rate.

Structure and Function of Bacteria

Bacteria are distinguished from eukaryotes by their smaller size (0.2-10μm), their lack of internal organelles (*e.g*., mitochondria), the presence of a cell wall and their cell division by binary fission rather than mitosis. They lack introns and have single-stranded circular DNA rather than multiple discrete chromosomes.

Bacteria share a number of common structures that are briefly described below:

1) **Slime** (extracellular polysaccharide): This is extracellular material, loosely associated with the bacteria, that is elaborated by some bacterial species that facilitates colonization of smooth surfaces.

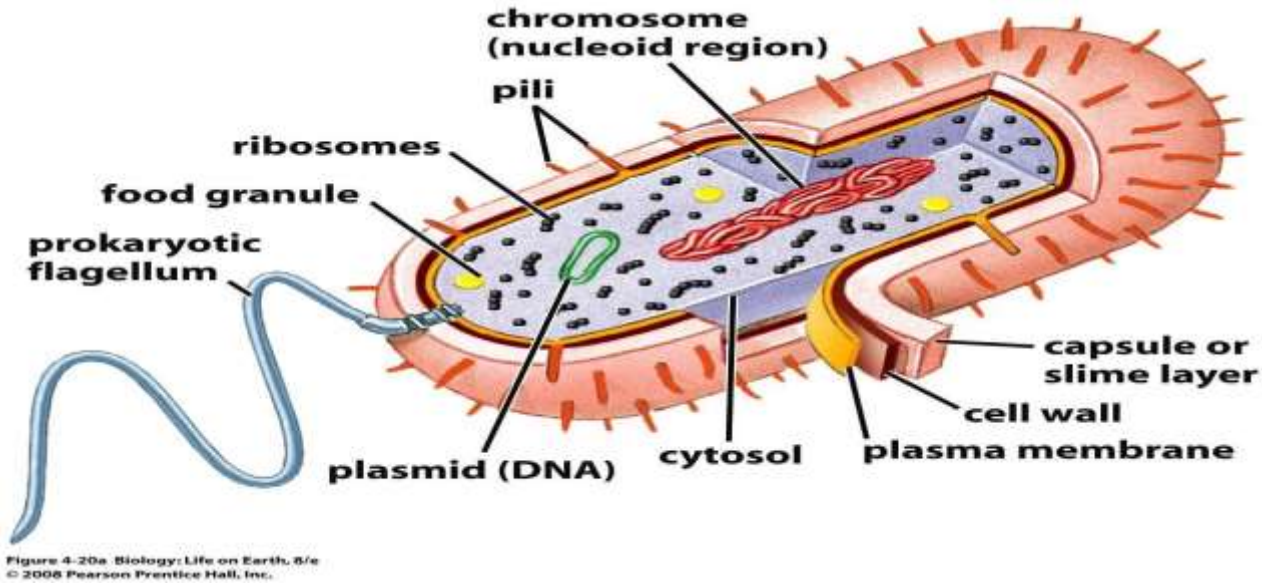

Figure 1: Bacterial structures

2) **Capsule:** This polysaccharide outer coating of the bacterial surface often plays a role in preventing phagocytosis of bacteria (fig.1). The main function of capsular material appears to be protection of the bacterium from adverse environmental conditions such as desiccation. In the body, capsules of pathogenic bacteria may facilitate adherence to surfaces.

3) **Peptidoglycan (cell wall):** The tough, rigid cell walls of bacteria protect them from mechanical damage and osmotic lysis. Provides bacterial shape and rigidity. The cell wall consists of alternating units of N-acetylglucosamine and N-acetylmuramic acid. The polysaccharide chains are cross-linked by a peptide bridge. It is a primary target of antimicrobial therapy – because it is specific to prokaryotes.

4) **Cytoplasmic membrane:** The cytoplasmic membranes of bacterial cells are flexible structures composed of phospholipids and proteins. Two major functions of the cytoplasmic membrane, the active transport of nutrients into the cell and the elimination of waste metabolites, require the expenditure of energy.

5) **Flagella:** Flagella are usually several times longer than the bacterial cell and are composed of a protein called flagellin. They consist of a filament, hook and basal body (fig. 1). The hook functions as a universal joint between the filament and the basal body. The basal body is anchored to the cell wall and to the cytoplasmic membrane.

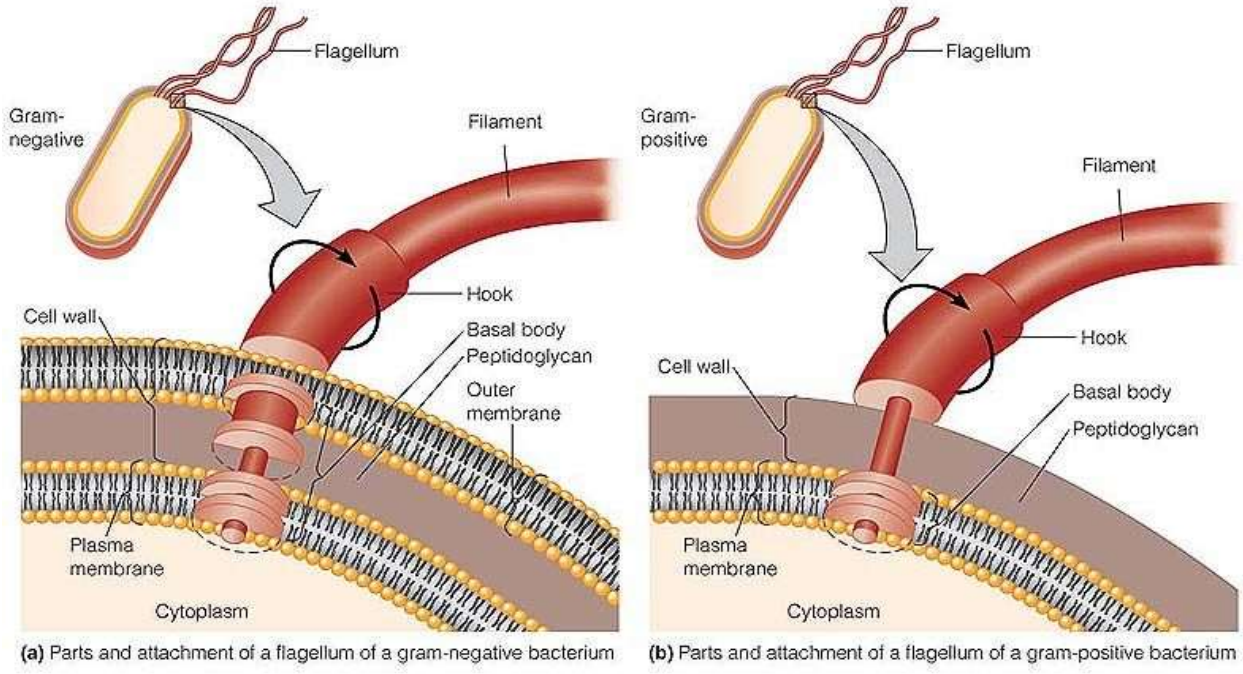

Figure 1: flagella of bacteria

The positions at which flagella are inserted into the bacterial cell vary and may be characteristic of a genus or family (fig. 2). Flagella provide bacteria with the capacity for locomotion. They vary in number and location.

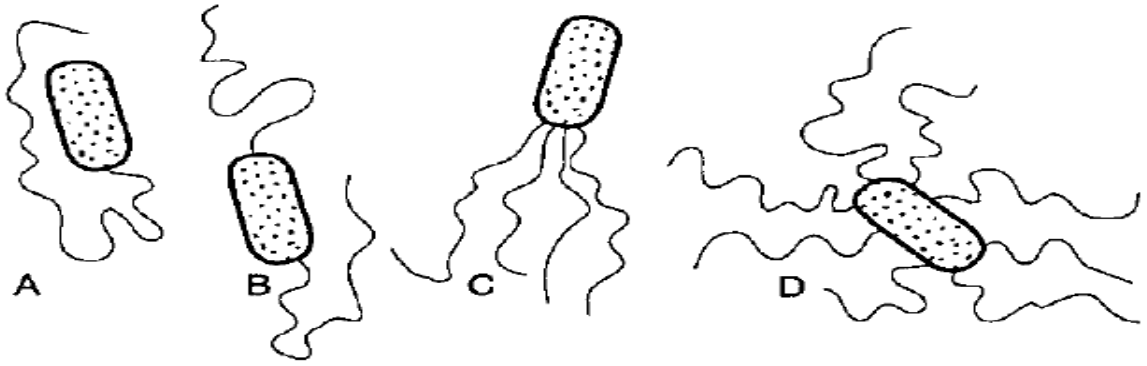

Figure 2: Bacterial flagella

A. Monotrichous (polar) flagellum

B. Amphitrichous flagella

C. Lophotrichous flagella

D. Peritrichous flagella

6) **Pili:** Fine, straight, hair-like appendages called pili or fimbriae, composed of the protein pilin, are attached to the cell wall of many bacteria. They are most common on Gram-negative bacteria and they may have different functions. In pathogenic bacteria, pili function as adhesions for receptors on host cells and transfer of DNA between bacteria during conjugation.

7) **Nuclear material:**

The bacterial genome is composed of a single haploid circular chromosome containing double-stranded DNA. Small amounts of protein and RNA are also associated with nuclear material. The genes in the bacterial chromosome code for all the vital functions of the cell.

Plasmids, small circular pieces of DNA which are separate from the genome, are capable of autonomous replication. Plasmid DNA may code for characteristics such as antibiotic resistance and exotoxin production.

8) **Ribosomes:**

All protein synthesis takes place on ribosomes. These structures are composed of ribonucleoproteins. They consist of two subunits, a larger 50s subunit and a smaller 30s subunit. Ribosomes may be present either in the cytoplasm or associated with the inner surface of the cytoplasmic membrane. During active bacterial growth and rapid protein synthesis.

9) **Endospores:**

Dormant highly resistant bodies, termed endospores, are formed by some bacteria to ensure survival during adverse environmental conditions (fig. 3). The only genera of pathogenic bacteria which contain endospore-forming species, are ***Bacillus*** and ***Clostridium.*** Endospores, which are produced inside the bacterial cell, show species variation shape, size and position within the mother cell. The primary **function** of most **endospores** is to ensure the survival of a bacterium through periods of environmental stress. When an endospore is reactivated, germination occurs in three stages namely activation, initiation and outgrowth. Activation may occur in response to factors such as brief exposure to heat, abrasion of the endospore coat or environ mental acidity.

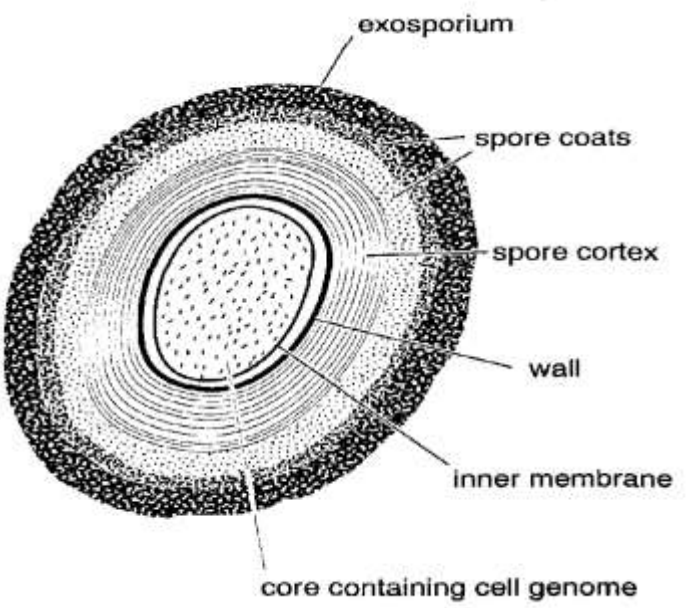

Figure 3: Structural features of a mature bacterial endospore.

Distinguishing Features between Gram Positive and Negative Bacteria

Gram-positive and **Gram-negative** bacteria are classified based on the ability to retain the gram stain. **Gram positive bacteria** will **stain** purple because of their thick peptidoglycan cell wall. **Gram negative** cell walls contain a thin peptidoglycan layer (without teichoic acids) that is surrounded by a thick plasma membrane (tab.1 and fig. 4).

Table: Comparative characteristics of Gram positive and Gram negative bacteria

Table 4.1 Some Comparative Characteristics of Gram-Positive and Gram-Negative Bacteria

Characteristic	Gram-Positive	Gram-Negative
		
Gram Reaction	Retain crystal violet dye and stain blue or purple	Can be decolorized to accept counterstain (safranin) and stain pink or red
Peptidoglycan Layer	Thick (multilayered)	Thin (single-layered)
Teichoic Acids	Present in many	Absent
Periplasmic Space	Absent	Present
Outer Membrane	Absent	Present
Lipopolysaccharide (LPS) Content	Virtually none	High
Lipid and Lipoprotein Content	Low (acid-fast bacteria have lipids linked to peptidoglycan)	High (because of presence of outer membrane)
Flagellar Structure	2 rings in basal body	4 rings in basal body
Toxins Produced	Exotoxins	Endotoxins and exotoxins
Resistance to Physical Disruption	High	Low
Cell Wall Disruption by Lysozyme	High	Low (requires pretreatment to destabilize outer membrane)
Susceptibility to Penicillin and Sulfonamide	High	Low
Susceptibility to Streptomycin, Chloramphenicol, and Tetracycline	Low	High
Inhibition by Basic Dyes	High	Low
Susceptibility to Anionic Detergents	High	Low
Resistance to Sodium Azide	High	Low
Resistance to Drying	High	Low

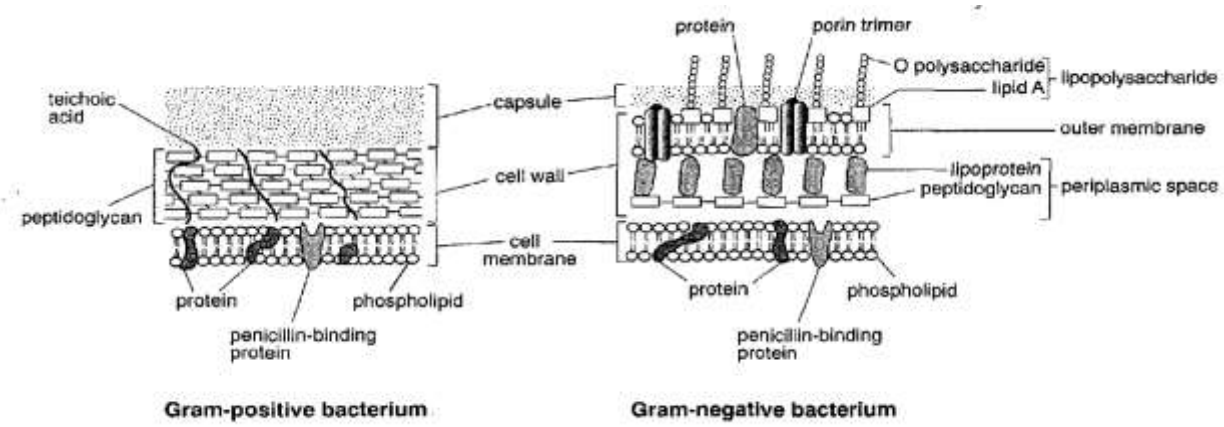

Figure 4: Comparison of the capsule, cell wall and cell membrane of a Gram-positive bacterium and a Gram-negative bacterium. Structures of importance in staining, virulence and toxicity, antigenicity, and susceptibility to antibiotics are illustrated.

Growth and death of bacteria

Bacterial growth

Growth of bacterial is defined as an increase in the number of bacteria in a population rather than in the size of individual cells. The time required for the formation of a parental cell into two daughter cells known as **generation time** or **doubling time** or **replication time**.

Bacterial growth curve

In the presence of fresh growth medium bacteria show following four phases during their growth:

- The lag phase
- The log phase
- The stationary phase
- The decline phase (fig.1).

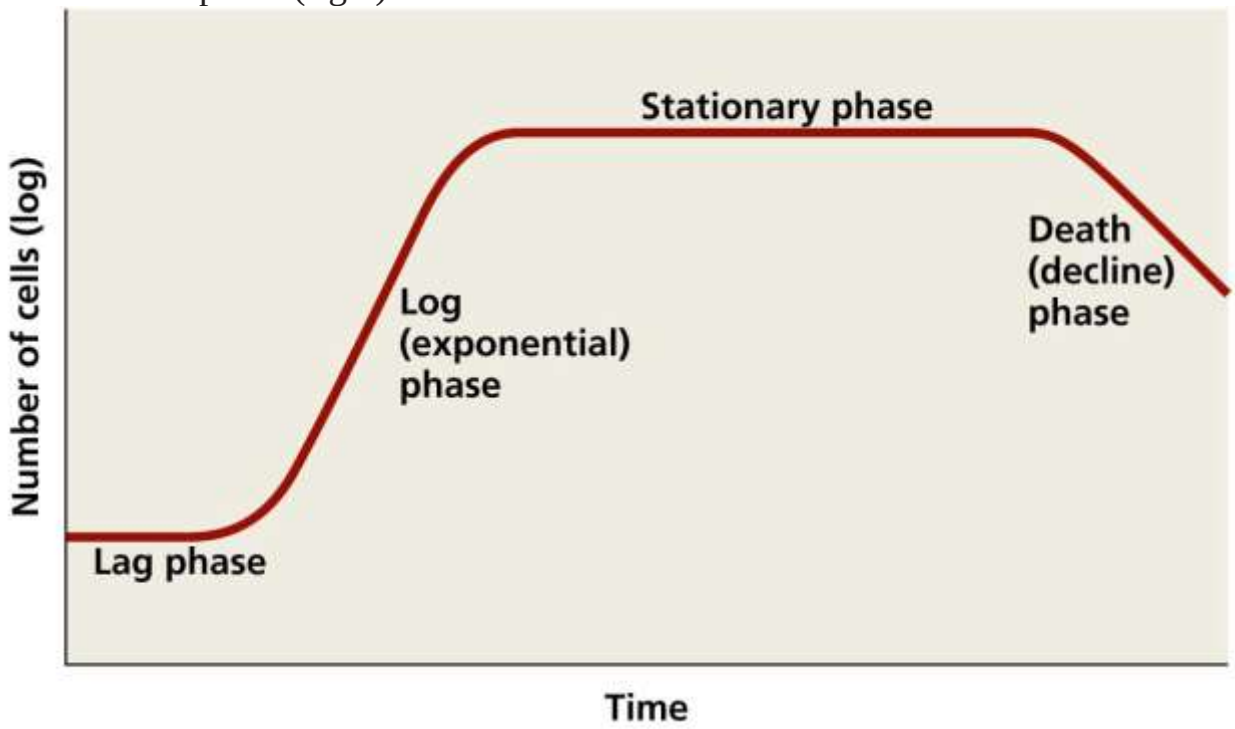

Figure 1: Bacterial growth curve

The Lag Phase:

Lag phase is short duration in which bacteria adapt themselves to new environment. This phase represents a period of active growth during which bacteria prepare for reproduction, synthesizing DNA, enzymes, and other macromolecules needed for cell division. Therefore, during this phase, there may be increase in size (volume) but no increase in cell number and near the end of this phase some cells may double or triple in size.

The lag phase is necessary before the initiation of cell division due to variety of reasons. If the cells are taken from an old culture or from a refrigerated culture, it might be possible that the cells may be old and depleted of ATP, essential cofactors and ribosomes.

The duration of lag phase depends on the type of bacteria, quality of culture medium, size of inoculum and several environmental factors such as CO2, temperature and PH. The average time of lag phase is 2 hours, although it varies from species to species (1-4 hours).

The Log Phase (Exponential Phase):

Regular growth of bacteria occurs and undergo division and their population (number) increase exponentially at a logarithmic rate in this phase. The nutrients present in the medium are utilized by the bacteria and daughter cells. Once exhaustion of nutrients occurs, slowing down of growth also takes place and bacterium passes onto stationary phase. The morphology of bacteria is best developed in this phase and organisms manifest typical biochemical characters. This phase will continue as long as cells have adequate nutrients and the environment is favorable. Hence, this phase can be prolonged by continuous supply of fresh nutrients at a constant and predefined rate. The average time of log phase is 8 hours, though it varies in different species.

The Stationary Phase:

In this phase, the growth i.e., cell division, almost ceases due to exhaustion of nutrients and also the accumulation of toxic products. At this stage the cell death starts at a slow rate and is compensated by the formation of new cell through cell division. The duration of this phase is variable which ranges from few days to few hours. The bacterial cells start dying and the number of such cells balances the number of new born cells, and the bacterial population stabilizes. This state of growth, during which the total number of viable cells remains constant because of no further net-increase in cell number and the growth rate is exactly equal to the death rate, is called stationary phase. The transition between the log and exponential and stationary phases involves a period of unbalanced growth during which the various cellular components are synthesized at unequal rates. Consequently, cells in the stationary phase have a different chemical composition from those in the exponential phase.

The Decline Phase:

The phase of decline is also called as *death phase*. Due to depletion of nutrients and accumulation of toxic end products the number of bacteria dying is much more than those dividing and hence there is a gradual decline in the total number of organisms. The growth curve now dips downwards. For a microbial cell, death means the irreversible loss of the ability to reproduce (growth and divide). The empirical test

of death is the culture of cells on solid media; a cell is considered dead if it fails to give rise to a colony on any medium, provided a suitable culture medium is chosen.

Requirements for microbial growth:

The requirements for microbial growth can be divided into two main categories: (i) chemical and (ii) physical.

I. **Chemical requirements:**

Chemical requirements include sources of carbon, nitrogen, sulfur, phosphorus, trace elements, oxygen, and organic growth factors. Growth factors some bacteria require certain organic compounds in minute quantities known as growth factors or bacterial vitamins. A **growth factor** is an organic compound which a cell must contain in order to grow, but which it is unable to synthesize. Growth factors are called **'essential**' when growth does not occur in their absence, or '**accessory**' when they enhance growth without being absolutely necessary for it.

II. Physical requirements:

Certain physical conditions affect the type and amount of microbial growth. There are physical actors influencing microbial growth such as:

• **Temperature:** Organisms can be grouped as psychrophiles, psychrotrophs, mesophiles, thermophiles, or hyperthermophiles based on their optimum growth temperatures.

• **Oxygen (O2) Requirements:** Organisms can be grouped as obligate aerobes, obligate anaerobes, facultative anaerobes, microaerophiles based on their oxygen (O2) requirements.

• **CO2:** Microbes that grow better at high CO2 concentrations are called ***capnophiles***.

• **PH**

• **Light**

• **Osmotic effect**

Culturing of bacteria and media types

Cultivation /Culturing of Bacteria is a method of multiplying microorganisms by letting them reproduce in predetermined culture media under controlled laboratory conditions. Cultivation is the process of growing microorganisms in culture by taking bacteria from the infection site (i.e. *in vivo* environment) by some means of specimen collection and growing them in the artificial environment of the laboratory (i.e. the *in vitro* environment). By appropriate procedures they have to be grown separately (isolated) on culture media and obtained as pure cultures for study. Bacteria have to be grown for their identification and investigating other properties like antimicrobial susceptibility, test for enzyme production or test for virulent genes.

Indications for culture

In the clinical laboratory, the indications for culture are mainly to:
1. To grow and isolate all bacteria present in an infection in pure culture
2. Demonstrate their properties
3. Obtain sufficient growth for preparation of antigens and for other tests
4. Determine sensitivity to antibiotics
5. Estimate viable counts
6. Maintain stock cultures
7. Identification and characterization: To obtain sufficient growth of clinically relevant bacteria to allow identification and characterization.

Culture media

A different essential nutrient material prepared for the growth of microorganisms in a laboratory is called a culture medium. 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. Bacterial culture media can be classified in at least three ways; Based on consistency, based on nutritional component and based on its functional use.

1. Classification based on consistency

A- Liquid media
B- Semi-solid media
C- Solid media

A) Liquid media: Liquids are usually broth contained in tubes, and the bacteria will grow suspended in this liquid. Media that contain only meat infusion, peptone, salt and water. There is some disadvantage: growth not exhibit characteristic appearance

and when there is more than one type of organisms they cannot be separated by growing in liquid media such as **nutrient broth** and **brain heart infusion broth**.

B) Semisolid media: Semisolid or solid consistency usually contains a thickening agent called agar. It is used to separate a mixture of a motile and non-motile organisms contain 0.2-0.5% agar such as motility media.

C) Solid media: To study colonial characteristics, culture organisms on solid media. Solidification may be achieved by adding agar-agar (2-3%), gelatin, and serum or egg albumin to other ingredients. Solid media is the consistency of a very firm gelatin type desert, but the bacteria will tend to grow only where it is inoculated on the media surface such as Blood agar.

2. Media can be classified according to composition

Synthetic media: are composed only of pure chemicals with defined quantity and quality (such as salts, sugars) e.g. glucose inorganic salt phosphate media for *E. coli.*

Non-synthetic (complex) media: are composed of complex materials, e.g. yeast extract, beef extract and peptone (partially digested protein), therefore their chemical composition is poorly defined. On the other hand, these materials are rich in nutrients and vitamins. e.g. Nutrient broth, Nutrient agar, McConkey and EMB.

3. Media can be classified according to the function as

1- Basic (Ordinary media).
2- Enriched media.
3- Differential and Selective media.
4- Special media.

Basic (Ordinary media):

Are simplest media, containing only some meat extract or other simple infusion, peptone, salt, and water. The meat extract or infusion supplies the organisms with amino acids, vitamins, salts, and traces of carbon, nitrogen, and other elements. Salts, usually, NaCl serve to obtain the required isotonicity for the maintenance of constant osmotic pressures (nutrient agar, nutrient broth and Brain heart infusion broth).

Enriched Media:

Enriched media as basic media that are supplemented with body fluid, specific vitamins, amino acids, proteins, or any other nutrients like blood, egg, serum such as Blood agar. It may by adding (5-10) % sterile blood to any basic agar media (use to detect hemolytic activity of bacteria, Chocolate agar (when blood agar is heated to 80 °C for 10 minutes, the media change to chocolate brown color. **According to hemolytic activity of bacteria, there are three types of hemolysis:**

1- Complete hemolysis (β-hemolysis) cause complete lysis of the red blood cells and there is a clear zone around the colony.

2- Partial hemolysis (α-hemolysis) lyse the red blood cells and reduce the hemoglobin to methemoglobin which produces a green zone around the colony.
3- No hemolysis (γ-hemolysis) no hemolysis or change in the red blood cells.

Differential and Selective media:

Differential media are used to differentiate closely related or groups of organisms. Due to the presence of certain dyes or chemicals in the media, the organisms will produce characteristic changes or growth patterns that are used for identification or differentiation.

Selective media allow certain types of organisms to grow, and inhibit the growth of other organisms. Selective inhibition of some types of microorganisms can be achieved by adding dyes, antibiotics, salts or specific inhibitors which affect the metabolism or enzyme systems of the organisms. Media containing potassium tellurite, sodium azide or thallium acetate will inhibit the growth of Gram-negative bacteria and supplements such as penicillin or crystal violet will inhibit the growth of Gram-positive bacteria. There are more common selective and differential media included moderately selective and differential medium (MacConkey agar), differential medium (Eosin Methylene Blue or EMB agar), and selective medium (Salt Manitol agar).

MacConkey agar: used to isolate and differentiate members of the ***Enterobacteriaceae*** based on the ability to ferment lactose**.**

Eosin Methylene blue agar (EMB): used for the detection and isolation of Gram-negative intestinal pathogens.

Salt Manitol agar: used for the isolation of pathogenic *staphylococci*. The medium contains mannitol, a phenol red indicator, and sodium chloride (inhibits the growth of most bacteria other than *staphylococci*.

Special media: media that cannot be easily grouped under one of the foregoing heading will be discussed here. Most of these are used to ascertain one or more biochemical characteristics.

A) Triple sugar iron agar (TSI): will detect an organisms ability to ferment glucose, lactose, sucrose produce H2S and gas. **Used** to differentiate enterics based on the ability to reduce sulfur and ferment carbohydrates (*Salmonella* and *Proteus***).**

B) Simmons citrate agar: used for differentiating gram-negative bacteria on the basis of **citrate** utilization (*Klebsiella* spp).

Transport media: are devised to maintain the viability of pathogen and to avoid overgrowth of the contaminants during transit from the patient to the Lab.

Storage media: to available condition for the maintenance of bacterial culture for long time.

Bacterial Physiology (Bacterial Metabolism)

Metabolism is the series of changes (chemical reactions) of substance (carbohydrate, protein or fat) within the bacterial cell from absorption to elimination (fig.1). Metabolism can be divided into two classes of chemical reactions: those that release energy and those that require energy. Because chemical reactions either release or require energy, metabolism can be can be subdivided into: **catabolism** refers to chemical reactions that result in the breakdown of more complex organic molecules into simpler substances that usually release energy and **anabolism** that refers to chemical reactions in which simpler substances are combined to form more complex molecules that usually require energy (fig.2). Anabolism is needed for growth, reproduction, and repair of cellular structure, while catabolism provides an organism with energy for its life processes, including movement, transport, and the synthesis of complex molecules – that is, anabolism. Catabolic reactions or sequences produce energy as adenosine triphosphate (ATP), which can be utilized in anabolic reactions to build cell material from nutrients in the environment. All bacteria obtain energy by oxidizing preformed organic molecules (carbohydrates, lipids and proteins) from their environment.

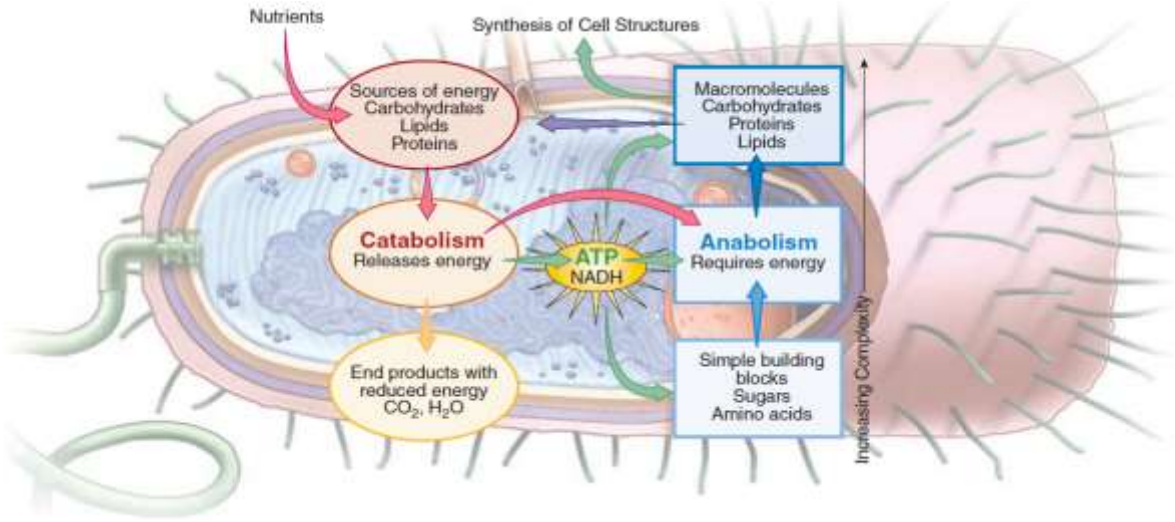

Figure 1: Metabolism of bacteria

Energy Production

Like all other living things, bacteria need to acquire energy in order to survive. Energy is required:

1. To maintain the structural integrity of the cell by repairing any damage to its constituents
2. To synthesis new cellular components such as nucleic acids, polysaccharides and enzymes

3. To transport certain substances into the cell from its surroundings
4. For the cell to grow and multiply
5. For cellular movement.

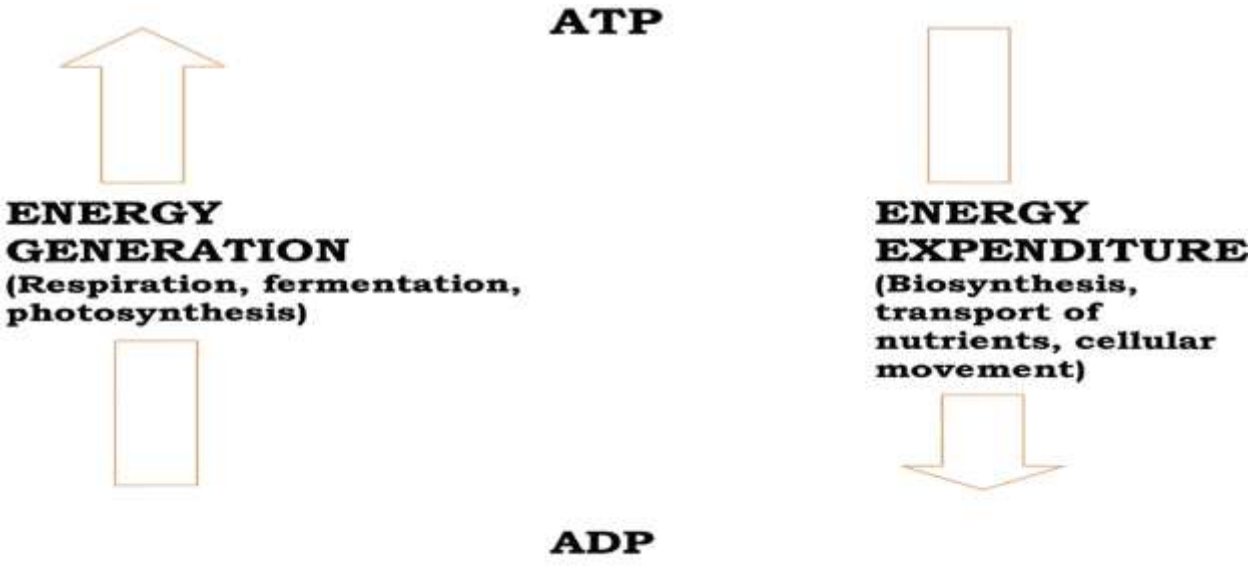

Figure 2: Generation and expenditure of energy in bacteria

Metabolism may be **aerobic**, where the final electron acceptor is oxygen, or **anaerobic**, where the final acceptor may be an organic or inorganic molecule other than oxygen (e.g. nitrogen).

There are three critical processes of bacterial energy production: **aerobic respiration, anaerobic respiration and fermentation**. Bacteria use most of their energy to synthesize substances needed for growth. These substances include structural proteins, which form cell parts, and enzymes, which control both energy production and synthetic reactions.

• In aerobic metabolism (i.e. aerobic respiration), complete utilization of an energy source such as glucose produces 38 molecules of ATP.

• Anaerobic metabolism utilizing an inorganic molecule other than oxygen as the final hydrogen acceptor (anaerobic respiration) is incomplete and produces fewer ATP molecules than aerobic respiration.

• Anaerobic metabolism utilizing an organic final hydrogen acceptor (fermentation) is much less efficient and produces only two molecules of ATP.

The main difference between fermentation and respiration is the energy yield, which can be greater from respiration than from fermentation. Summarizes the metabolic processes of bacteria showing in (tab.1 and fig.3).

Table1: Metabolic processes in bacteria

	Glycolysis	Fermentation	Krebs Cycle	Electron Transport Chain
Location	In cytoplasm	In cytoplasm	Prokaryotes: In cytoplasm Eukaryotes: in the mitochondrial matrix	Prokaryotes: in cell membrane Eukaryotes: in inner mitochondrial membranes
Oxygen Conditions	Anaerobic; oxygen is not required; does not stop, however, if oxygen is present	Without O2; presence of oxygen will cause it to stop	Aerobic	Aerobic
Starting Molecule(s)	1 glucose (6C)	Various substrate molecules go through glycolysis, yielding 2 pyruvic acid	2 pyruvic acid	6 O2
Ending Molecules	2 pyruvic acid (3C) 2 NADH	Various, depending on which form of fermentation occurs, e.g., ethanol, lactic acid, CO2, acetic acid	6 CO2 8 NADH 2 FADH	6 H2O
Amount of ATP Produced	2 ATP	Various, depending on which form of fermentation occurs, usually 2 or 3 ATP; always far less than is produced in aerobic respiration	2 GTP (=2 ATP)	34 ATP

Among all microorganisms, the bacteria are particularly versatile in the ways in which they obtain energy. The ways different microorganisms capture energy, and obtain carbon, can be classified as **autotrophy** (self-feeding) or **heterotrophy** (other-feeding). **Autotrophs** use carbon dioxide (an inorganic substance) to synthesize organic molecules. They include **photoautotrophs**, which obtain energy from light, and **chemoautotrophs**, which obtain energy from oxidizing simple inorganic substances such as sulfides and nitrites. **Heterotrophs** get their carbon from ready-made organic molecules, which they obtain from other organisms, living or dead. There are photoheterotrophs, which obtain chemical energy from light, and chemoheterotrophs, which obtain chemical energy from breaking down ready-made organic compounds.

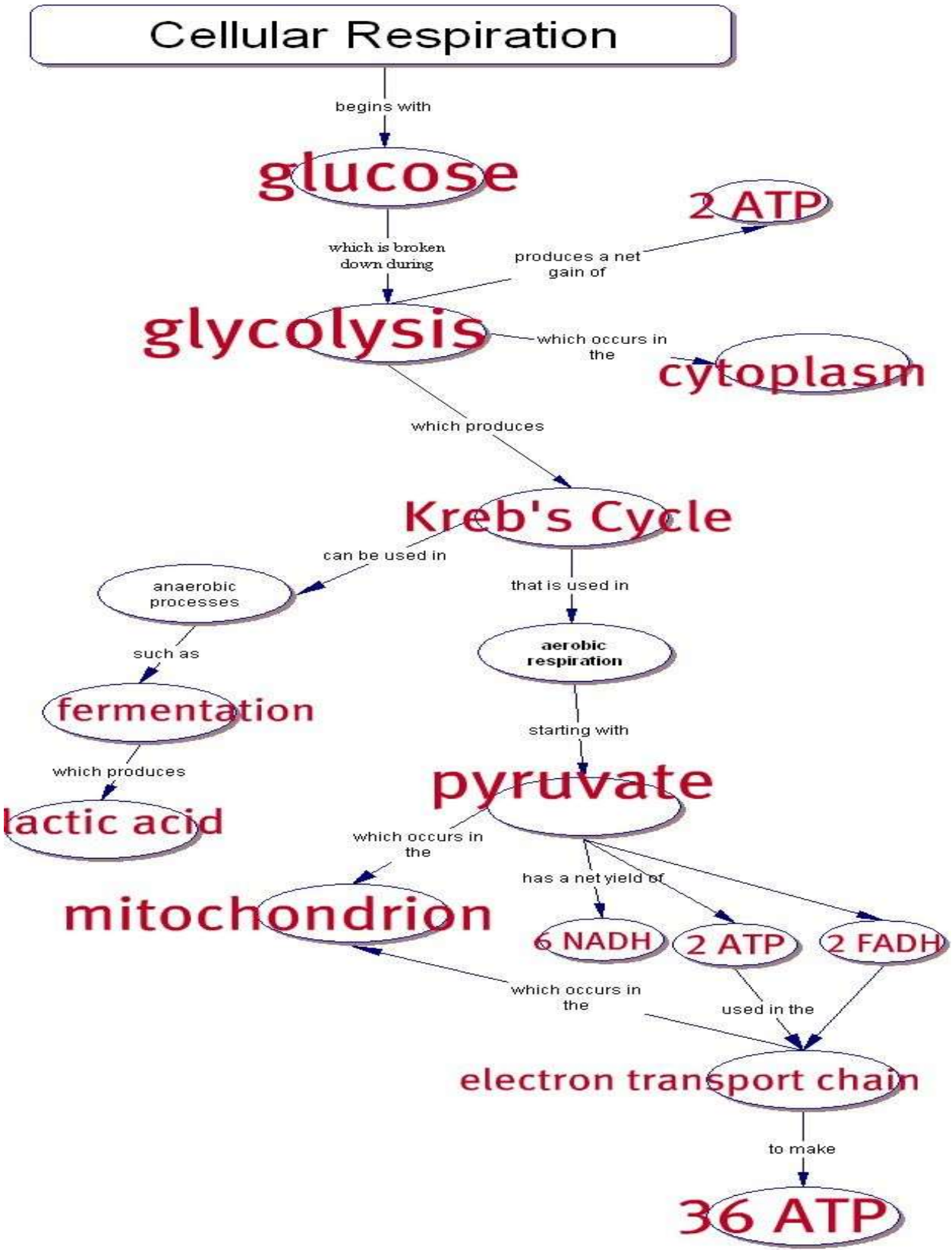

Figure 3: Metabolic processes

Nutrient cycles and regulation

The variety of nutrient and energy sources used by bacteria is reflected in the variation in metabolism. Bacteria must survive in response to a variety of environmental disorders. For this, living organisms sense environmental changes by detecting extracellular signals such as the concentrations of nutrients such as carbon, nitrogen, phosphate, sulfur, ion sources, and the growth conditions such as pH, temperature, oxygen availability or oxidative stress, osmotic stress, and solvent stress. These signals eventually feed into the transcriptional regulatory systems, which affect the physiological and morphological changes that enable organisms to adapt effectively for survival.

Adaptation to limited nutrients

Microorganisms adapt to limited nutrients in several ways:
1. Some synthesize increased amounts of enzymes for uptake and metabolism of limited nutrients. This allows the organisms to obtain and use a larger proportion of the few nutrient molecules that are available.
2. Others have the ability to synthesize enzymes needed to use a different nutrient. For example, if glucose is in short supply, some microorganisms can make enzymes to take up and use a more plentiful nutrient such as lactose.
3. Many organisms adjust the rate at which they metabolize nutrients and the rate at which they synthesize molecules required for growth to fit the availability of the least plentiful nutrient. Both metabolism and growth are slowed, but no energy is wasted on synthesizing products that cannot be used. Growth is as rapid as conditions will allow.

Regulation mechanism

All biological reactions are catalyzed by enzymes, the regulation of metabolism through control of gene expression, synthesis of enzymes and their activity, as well as the survival mechanisms used under starvation conditions and adapt to the ever-changing environment. The regulatory or control mechanisms operate in two levels. In one, the control operates at the genetic level. In this case, the synthesis of enzymes may be stopped or geared up. The other type of the activity of enzymes already synthesized and present in the cell is modulated, i.e. the enzyme activity is either accelerated or diminished (inhibited) according to the need of the cell. These mechanisms operate, therefore, at biochemical level. The mechanisms that control metabolism either regulate enzyme activity directly or regulate enzyme synthesis by turning on or off genes that code for particular enzymes.

In their evolution, cells of bacteria (and all other organisms) have developed mechanisms to turn reactions on and off in accordance with their needs. Of the various mechanisms that regulate metabolism, three have been extensively investigated in bacteria:

- Feedback inhibition
- Enzyme induction
- Enzyme repression

1- Feedback inhibition

In **feedback inhibition**, the end product of a biochemical pathway directly inhibits the first enzyme in the pathway. Enzymes subject to such regulation are generally allosteric. Feedback inhibition regulates the activity of existing enzymes and is a quick-acting control mechanism. In *feedback inhibition*, enzyme activity is regulated directly, and the control mechanism determines how rapidly enzymes already present will catalyze reactions.

2- Enzyme induction

In enzyme induction (Fig.1), the presence of a substrate activates an **operon**, a sequence of closely associated genes that include **structural genes** and **regulatory sites:** (1) in the absence of lactose, a **repressor**- a product of the **regulatory gene**- attaches to the operator and prevents transcription of the genes of the *lac* operon. (2) When lactose is present, it inactivates the repressor and allows transcription of the genes of the *lac* operon.

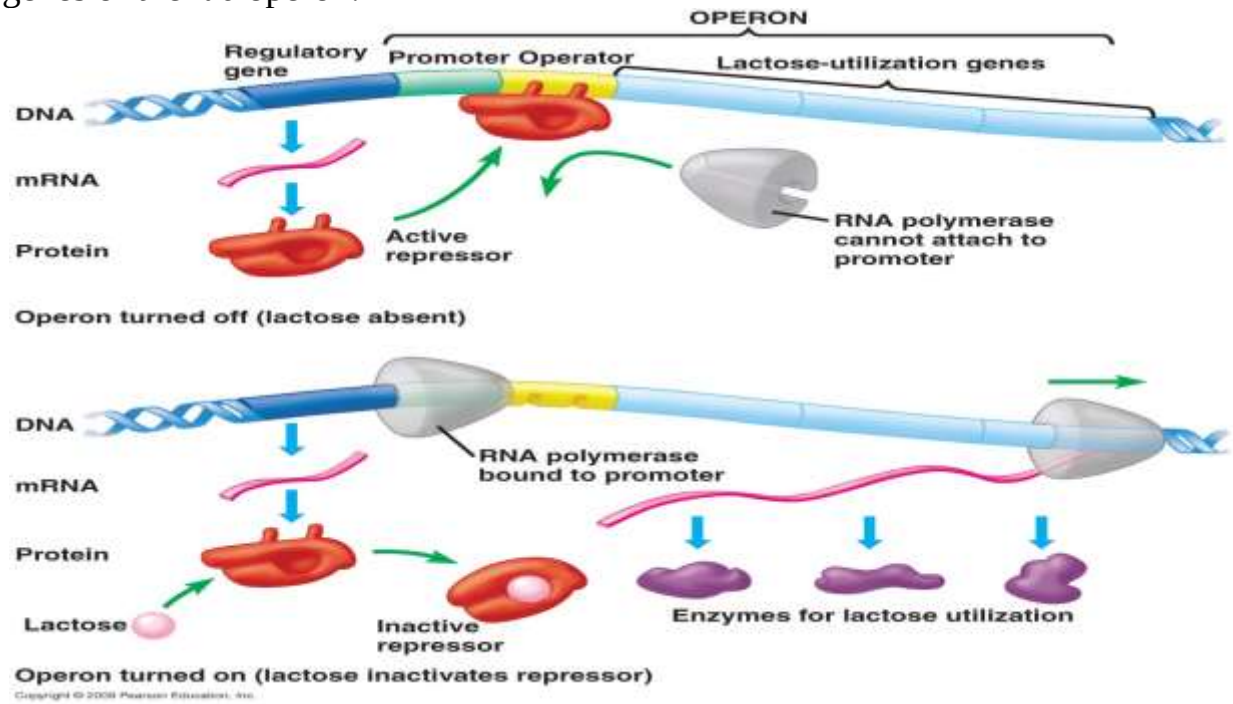

Figure 1: Lac operon

3- Enzyme repression

- In enzyme repression, the presence of a synthetic product inhibits its further synthesis by inactivating an operon: (1) When tryptophan is present (fig.2), it attaches to the repressor protein and represses genes of the *trp* operon. (2) In the absence of tryptophan, the repressor is not activated, and genes of the *trp* operon are transcribed.

- In **catabolite repression**, the presence of a preferred nutrient (often glucose) represses the synthesis of enzymes that would be used to metabolize some alternative substance.

- Both enzyme induction and enzyme repression regulate by altering gene expression. The effect on enzyme synthesis in both cases depends on the presence or absence of the regulatory substance-lactose, tryptophan, or glucose in the preceding examples. In *enzyme induction* and *enzyme repression*, regulation occurs indirectly by enzyme synthesis, and the control mechanism determines which enzymes will be synthesized and in what amounts.

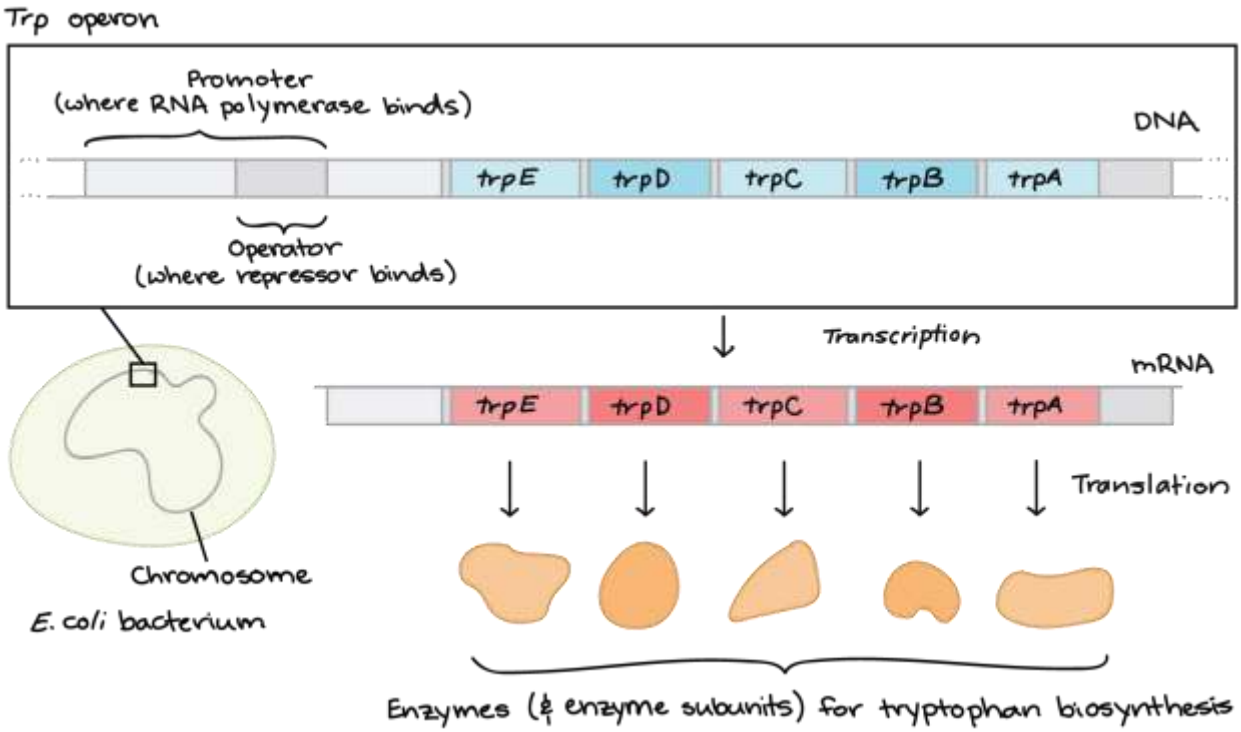

Figure 2: Trp operon

Bacterial Genetics

Genetics is the study of heredity and variation to understand the cause of resemblance and differences between parents and their progeny. The unit of heredity is the ***gene***, a segment of deoxyribonucleic acid (DNA) that carries in its nucleotide sequence information for a specific biochemical or physiologic property. Genes carry the information to code for all the necessary components and the actions of life. The genes at each cell division are replicated and a copy is transmitted to each daughter cell. Bacteria unlike eukaryotic cells are haploid, which means they have a single copy of each gene in contrast, eukaryotic cells are diploid.

Structure of DNA

The DNA is the key basic component of gene, which carries the genetic information that is transcribed onto ribonucleic acid and then translated as the particular polypeptide. The DNA molecule is composed of two strands of complementary nucleotides wound together in the form of a double helix.

DNA strand

Each DNA strand has a backbone of deoxyribose (sugar) and phosphate group residues arranged alternately. It has a sugar- phosphate backbone substituted with purine and pyrimidine bases. It contains four nitrogenous bases, two purines (adenine and guanine), and two pyrimidines (thymine and cytosine).

Gene

It is a segment of DNA that carries ***codons*** specifying for a particular polypeptide. A DNA molecule consists of a large number of genes, each of which contains hundreds of thousands of nucleotides.

Structure of RNA

Basically, the structure of RNA is similar to that of DNA except for two major differences:

1. In DNA, the sugar is deoxyribose; in RNA, the sugar is ribose.
2. The RNA contains the nitrogenous base uracil instead of thymine that is present in DNA.

On the basis of structure and function, the RNA can be differentiated into three types:

1. **Messenger RNA (mRNA):** Most genes encode proteins and transcribed into mRNA. These molecules are translated during protein synthesis.
2. **Ribosomal RNA (rRNA):** Ribosomes, composed of rRNA and proteins, also are central to translation and provide the site at which translation occurs.
3. **Transfer RNA (tRNA):** Specifically transfers the genetic information carried in the mRNA into functional proteins.

Mutations

Mutation is a random, undirected, and heritable variation seen in DNA of the cell. This is caused by a change in base sequence of DNA due to addition, deletion, or substitution of one or more bases in the nucleotide sequence of DNA. It can involve any of the genes present in the bacterial chromosome. Mutation results in insertion of a different amino acid into a protein, resulting in the appearance of an altered phenotype.

Types of Mutations

Mutations are a natural event occurring in dividing cells. These occur spontaneously or are enhanced by different mutagens. **Mutations are of three types:** (*a*) base substitution, (*b*) frame-shift mutation, and (*c*) mutations due to transposons or insertion sequences.

Mutation due to base substitution

This type of mutation occurs when one base in the nucleotide sequence is inserted in place of another. This occurs during replication of DNA either due to an error in the function of DNA polymerase or due to a mutagen that alters the hydrogen bonding of the base being used as a template in such a manner that the wrong base is inserted. The base substitution mutation may be of tree types: missense mutation, nonsense mutation and silent mutation.

A. Missense mutation: It is one in which the base substitution results in a codon that specifies a different amino acid to be inserted.

B. Nonsense mutation: It is another type of mutation in which the base substitution produces a terminal codon that stops synthesis of protein prematurely. Entire protein function is destroyed during the process of nonsense mutation.

C. Silent mutation: Is base substitutions that result in no change of the amino acid or amino acid functionality when the altered mRNA is translated.

Frame-shift mutation

It is the second type of mutation. This occurs when one or more base pairs are added or deleted in the DNA. This, therefore, leads to shifting of the reading frame of the ribosome that results in incorporation of the wrong amino acids downstream from the mutation. Result of the frame-shift mutation ends in production of an inactive protein.

Mutation due to transposons or insertion sequence

This is the third type of mutation that occurs when transposons or insertion sequences are integrated into the DNA. These newly inserted pieces of DNA cause profound changes in the gene into which they are inserted and also causes changes in the adjacent genes.

Transposons are a type of mobile DNA of 2000–20,000 bp. They can transfer DNA from one site of the bacterial chromosome to another site or to a plasmid.

Causative Agents of Mutation

Mutation can be caused by (*a*) viruses, (*b*) radiation, or (*c*) chemicals.

Viruses

Bacterial viruses (*mutator bacteriophage*) are an example of viruses that cause a high frequency of mutation by inserting their DNA into the bacterial chromosome. Mutations can occur in various genes as viral DNA can insert bacterial chromosome at many different sites. The mutations caused by these viruses may be either frame-shift mutations or deletions.

Radiations

X-rays and ultraviolet light are the examples of radiation that can cause mutation in chromosomal DNA.

1. **X-rays:** X-rays damage DNA in many ways. They cause damage by producing free radicals that can attack the bases or alter them in the strand, thereby changing their hydrogen bonding. They also damage DNA by breaking the covalent bonds that hold the ribose phosphate together.
2. **Ultraviolet light:** Ultraviolet radiation causes damage in DNA by cross-linking of the adjacent pyrimidine bases to form dimers. For example, the cross-linking of adjacent thymine to form thymine dimers results in the inability of DNA to replicate properly.

Chemicals

Various chemicals, such as nitrous acid, alkylating agents, benzpyrene, and base analogs.

Effects of Mutations

Mutations in the bacteria cause a lot of changes in their various properties. Mutation alter drug susceptibility, antigenic structure, and virulence of mutant bacteria. It also alter susceptibility of bacteria to bacteriophages, alter their colony morphology and pigment productions, and affect their ability to produce capsule or flagella. Development of drug resistance due to mutations in bacteria is a major health concern.

Extra-chromosomal DNA Substances

Plasmids

Plasmids are extra-chromosomal DNA substances. They are replicons that are maintained as discrete, extra-chromosomal genetic elements in bacteria. They are usually much smaller than the bacterial chromosome. Plasmids are circular and double stranded DNA molecules that encode traits that are not essential for bacterial viability. They are capable of replicating independently of the bacterial chromosomes. Plasmids are present in both Gram-positive and Gram-negative bacteria.

Plasmids, depending on transmissibility are of two types: (*a*) transmissible plasmids and (*b*) nontransmissible plasmids.

1. Transmissible plasmids: They can be transferred from cell to cell by a process of genetic transfer known as conjugation.

2. Nontransmissible plasmids: These cannot be transferred from cell to cell, because they do not contain the transfer genes.

Functions of plasmids

Many plasmids control medically important properties of pathogenic bacteria. These include (*a*) resistance to one or several antibiotics, (*b*) production of toxins, and (*c*) synthesis of cell surface structures required for adherence or colonization.

Transfer of DNA between Bacterial Cells

The genetic information can be transferred from one bacterium to another. **There are three general methods for genetic exchange in bacteria:** (*a*) transformation, (*b*) transduction, and (*c*) conjugation.

Transformation

Transformation is a process of the transfer of DNA itself from one bacterium to another. In nature, DNA is released from a bacterium by lysis, which may be taken up by recipient bacterium that must be competent.

Transduction

The transfer of a portion of DNA from one bacterium to another mediated by a bacteriophage is known as transduction. During replication of virus within the cell, a piece of bacterial DNA is incorporated into the bacteriophage and is carried into the recipient bacterium at the time of infection. The phage DNA within the recipient bacterial cell integrates into the cell DNA during a process called lysogenic conversion. Transduction is of two types: (*a*) generalized transduction and (*b*) specialized transduction.

Conjugation

Conjugation is a process of transfer of DNA from the donor bacterium to the recipient bacterium during the mating of two bacterial cells.

Recombination

After the DNA is transferred from one donor bacterium to the recipient through transformation, transduction, or conjugation, it combines with the chromosome of the bacterium by a process called ***recombination***. Recombination is of two types: homologous and nonhomologous. Homologous recombination takes place between two pieces of DNA showing extensive homologous regions. The nonhomologous recombination takes place between two pieces of DNA showing little or no homology.

Replication of DNA

The replication process requires many enzymes, including an enzyme (helicase) to unwind the DNA at the origin to expose the DNA, an enzyme (primase) to synthesize primers to start the process, and the enzyme or enzymes (DNA-dependent DNA polymerases) that synthesize a copy of the DNA, but only if there is a primer sequence to add onto and only in the 5′ to 3′ direction. New DNA is synthesized semiconservatively, using both strands of the parental DNA as templates. New DNA synthesis occurs at growing forks and proceeds bidirectionally. One strand (the leading strand) is copied continuously in the 5′ to 3′ direction, whereas the other strand (the lagging strand) must be synthesized as many pieces of DNA using RNA primers (Okazaki fragments).The lagging-strand DNA must be extended in the 5′ to 3′ direction as its template becomes available. Then the pieces are ligated together by the enzyme DNA ligase.

Microbial Virulence factors and pathogenesis of bacterial infection

Pathogenicity and Virulence

Virulence

The term virulence denotes the ability of a strain of a species to produce disease. Virulence provides a quantitative measure of pathogenicity, or the likelihood of causing disease. For example, *Escherichia coli* that express Shiga-like toxins are more virulent than those that do not express these toxins. The virulence of a strain is not constant and may undergo spontaneous or induced variation.

Virulence Factors

Virulence factors refer to the properties (i.e. gene products) that enable a microorganism to establish itself on or within a host of a particular species and enhance its potential to cause disease. **Virulence is determined by three characteristics of the pathogens:** invasiveness, infectivity, and pathogenic potential. A major aspect of pathogenic potential is toxigenicity. Virulence factors help bacteria to (1) invade the host, (2) cause disease, and (3) evade host defenses.

Types of Virulence Factors:

Adherence Factors: Many pathogenic bacteria colonize mucosal sites by using *pili* (fimbriae) to adhere to cells.

Invasion Factors: Surface components that allow the bacterium to invade host cells can be encoded on plasmids, but more often are on the chromosome.

Capsules: Many bacteria are surrounded by capsules that protect them from opsonization and phagocytosis.

Endotoxins: The lipopolysaccharide endotoxins on Gram-negative bacteria cause fever, changes in blood pressure, inflammation, lethal shock, and many other toxic events.

Exotoxins: Exotoxins include several types of protein toxins and enzymes produced and/or secreted from pathogenic bacteria. Major categories include cytotoxins, neurotoxins, and enterotoxins.

Siderophores: Siderophores are iron-binding factors that allow some bacteria to compete with the host for iron, which is bound to hemoglobin, transferrin, and lactoferrin.

Pathogenicity

Mechanism by which disease develops despite host resistance mechanisms. The mechanisms used by bacteria to evade host protective responses **include:** (1) the inhibition of phagocytosis and intracellular killing in the phagocyte, (2) inactivation of complement function, (3) cleavage of IgA, (4) intracellular growth (avoidance of antibody), and (5) change in bacterial antigenic appearance.

Stages of Pathogenesis for Bacterial Infections

The outcome of infection depends on a variety of factors of the microbe and host as follows:

1. The ability of the organism to break host barriers and to evade destruction by innate local and tissue host defenses.

2. The ability of the organism to replicate, to spread, to establish infection, and to cause disease.

3. The ability of the organism to transmit to a new susceptible host.

4. The innate and adaptive immunologic ability of the host to control and eliminate the invading microorganism.

The infection process involves the following stages: (*a*) transmission of infection, (*b*) entry of the organisms and evasion of the local defenses, (*c*) adherence to cell surfaces, (*d*) growth and multiplication of the bacteria at the site of adherence, (*e*) manifestations of disease, and (*f*) termination of disease.

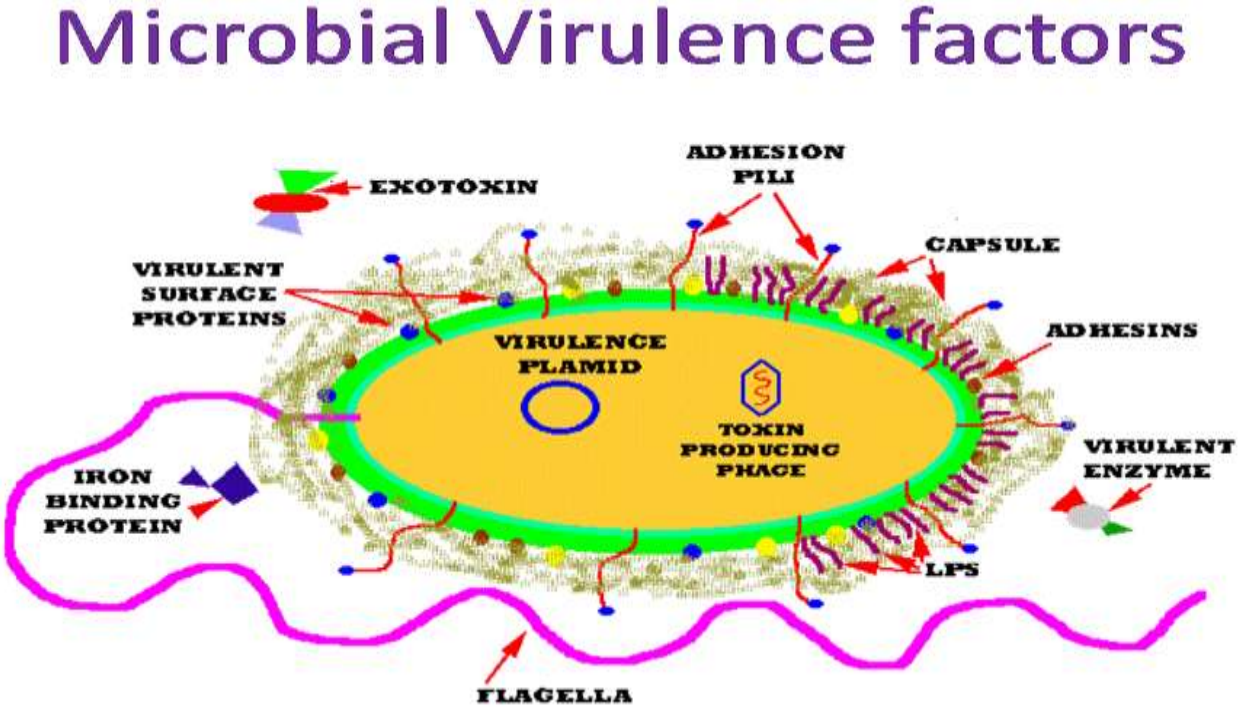

Figure 1: General virulence factors of bacteria

Microflora (Normal flora)

The term "normal microbial flora" denotes to the population of microorganisms that inhabit the skin and mucous membrane of normal healthy individuals. The majority of bacteria are present in the large bowel, which constitutes the normal flora. The organisms are present in parts of the body (the skin, nose, mouth, intestinal and urogenital tracts) that are exposed, or communicate with, the external environment (fig. 1).

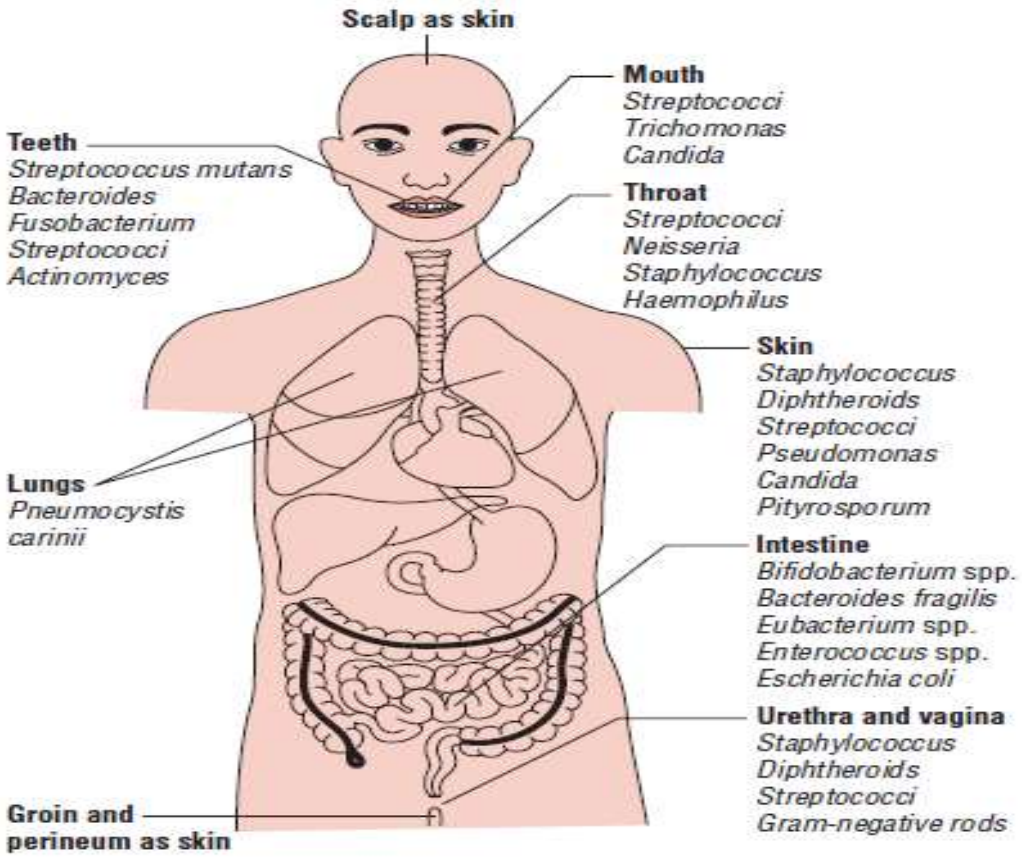

Figure 1: Sites of microbial flora

The human fetus, in pregnant mother, lives in a sterile environment protected from microbes except when pathogens like cytomegalovirus, rubella virus, or *toxoplasma gondii* cross the placenta for first 9 months of life. At the time of birth, the newborn is confronted with the mother's vaginal and environmental microbes. The infant's skin surface is initially colonized followed by the oropharynx, gastrointestinal tract, and mucosal surfaces.

The skin and mucous membranes always harbor a variety of microorganisms that can be arranged into two groups:

1. The resident flora consists of fixed types of microorganisms regularly found in a given area at a given age; if disturbed, it reestablishes itself.

2. The transient flora consists of non-pathogenic or potentially pathogenic microorganisms that inhabit the skin or mucous membranes for hours, days, or weeks; it is derived from the environment, does not produce disease, and does not establish itself permanently on the surface. However, if the resident flora is disturbed, transient microorganisms may colonize, proliferate, and produce disease.

Resident Flora:

It consists of organisms which are regularly present in a particular area and when disturbed it reestablishes itself like *Escherichia coli* is a normal inhabitant of the intestine.

Role of Resident flora:

Microorganisms that are constantly present on body surfaces are commensals. Relationships between organisms called symbiosis, which is permanent association between two different organisms (fig. 2). Growth flora in a given area depends upon physiologic factors like temperature, moisture, and the presence of certain nutrients and inhibitory substances. Members of the resident flora in the intestinal tract synthesis vitamin K and aid in the absorption of nutrients. On mucous membranes and skin, the resident flora may prevent colonization by pathogens through "bacterial interference". It has both advantages as well as disadvantages.

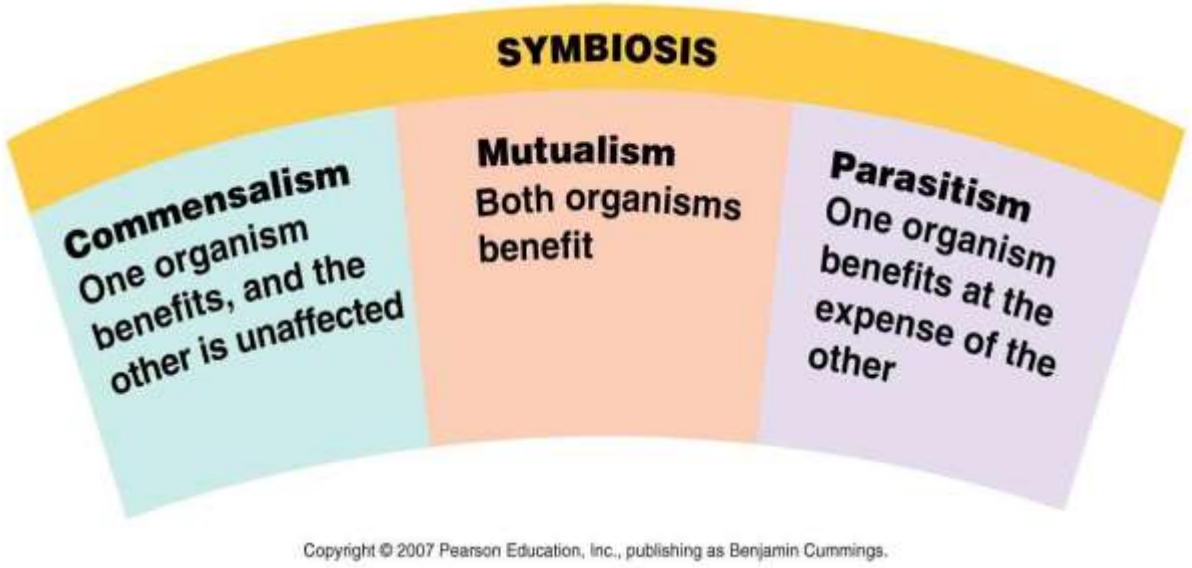

Figure 2: Relationship between normal flora and host

Advantages:

1. They prevent or suppress the entry of the pathogens.
2. Synthesis the vitamins especially vitamin K and several B group vitamins.
3. The normal flora evokes the antibodies production.
4. Endotoxins liberated by normal flora may help the defense mechanism of the body.
5. Colonies produced by some organisms of normal flora have a harmful effect on the pathogens.

Disadvantages:

1. They become pathogenic when the immunity is lowered.
2. They may act as pathogens in different issue (other than their normal habitat) e.g. normal flora of intestine may cause urinary tract infection (UTI).
3. Normal flora may cause confusion in diagnosis due to their ubiquitous presence in the body and their resemblance to some of the pathogens.

Transient Flora:

It consists of both non-pathogenic and potentially pathogenic bacteria that inhabit the body surface or mucous membranes for a limited period. Members of the normal flora form part of the host and include: saprophytes, commensals, facultative pathogens and true pathogens.

Normal Flora of the Skin:

Skin is constantly exposed and contact with the environment, the skin is particularly apt to contain transient microorganisms. The predominant resident microorganisms of the skin are *corynebacterium* and *staphylococcus*. Low pH, fatty acids in sebaceous secretions and presence of lysozymes are important factors for eliminating non-resident microorganisms from the skin.

Normal Flora of Gastrointestinal Tract:

The GI tract of the fetus in utero is sterile. It becomes contaminated with organisms shortly after birth. In breast fed infants, the intestine contains *lactobacilli*, *enterococci* and *staphylococci*. With the change of food, flora changes. Diet has a marked influence on the composition of the intestinal and fecal flora. In the stomach as pH is low, the stomach is sterile but as the pH increases in small intestine the number of bacteria increases progressively beyond the duodenum to the colon. The bacterial count is low in small intestine as compared to large intestine.

Lactobacilli and *entrococci* predominate in the duodenum and proximal ileum. The bacterial flora is similar in lower ileum, caecum and rectum. The anaerobic condition of colon is maintained by aerobic bacteria which utilizes the free oxygen.

Functions of Resident Flora:

The normal microbiota maintains a protected environment that prevents colonization with potentially pathogenic organisms. For example, *clostridium difficile* produces gastrointestinal disease when the normal intestinal flora have been reduced or removed by antibiotics. The production of proteolytic enzymes by microbes augments host factors in the digestion of food. Intestinal bacteria can also synthesis vitamins and other biological products (e.g., biotin, pantothenic acid, pyridoxine, riboflavin, vitamin K, etc.). Colicins produced by some bacteria of normal flora prevent harmful effects of the bacteria.

The interaction between microbes and humans can result in the following general outcomes: (*a*) disease, (*b*) transient colonization, and (*c*) prolonged colonization. The other outcome of microbe and host interaction is colonization, either transient or prolonged.

Factors Determining the Colonization by Microbes:

Factors that determine whether exposure to a microbe result in transient passage through a human host or prolonged colonization are complex and involve microbial properties, host characteristics, and environmental factors. The most important factors that determine colonization in human body are the properties of the specific organism. Various host factors determine the success of colonization with microbe. Nutritional and environmental conditions must favor the survival of microbes. The age of the host also influences microbial colonization. The hormonal secretions, alteration of dietary habits, person-to-person interaction, sexual activity, and many other factors determine the establishment of normal microbiota.

Chemotherapy and antibiotics resistance

Chemotherapy is the use of any drug to treat any disease. Antibiotics agents are widely employed to cure bacterial diseases. Antibiotics are substances that are derived from various species of microorganisms and can inhibit or destroy other microorganisms when administered in small concentrations.

Synthetic antimicrobial drugs are derived in laboratory from dyes or other organic compounds through chemical reactions. A ***semisynthetic antimicrobial drugs*** is a chemically modified derivative of a natural antibiotic. The chemical modifications are generally designed to increase the range of bacteria targeted, increase stability, decrease toxicity, or confer other properties beneficial for treating infections. Antimicrobial drugs may be bactericidal or bacteriostatic. A **bactericidal** drug kills bacteria, whereas a **bacteriostatic** drug inhibits the growth of bacteria, but does not kill them.

Antimicrobial drugs vary in their spectrum of activities. They may be broad-spectrum or narrow-spectrum antibiotics.

Broad-spectrum antibiotics are active against a wider range of different microbes. For example, tetracyclines are active against a variety of Gram-positive and Gram-negative bacteria, *rickettsiae*, *mycoplasmas*, and even protozoa.

Narrow-spectrum antibiotics are effective against one or very few microbes. For example, vancomycin is active against certain Gram-positive bacteria (such as *staphylococci* and *enterococci*)

Mechanisms of action of antimicrobial drugs:

Antibiotics act against bacteria by the following mechanisms:

1. Inhibition of cell wall synthesis
2. Inhibition of protein synthesis
3. Inhibition of nucleic acid synthesis
4. Alteration of cell membrane function
5. Inhibition of metabolism (fig.1&2)

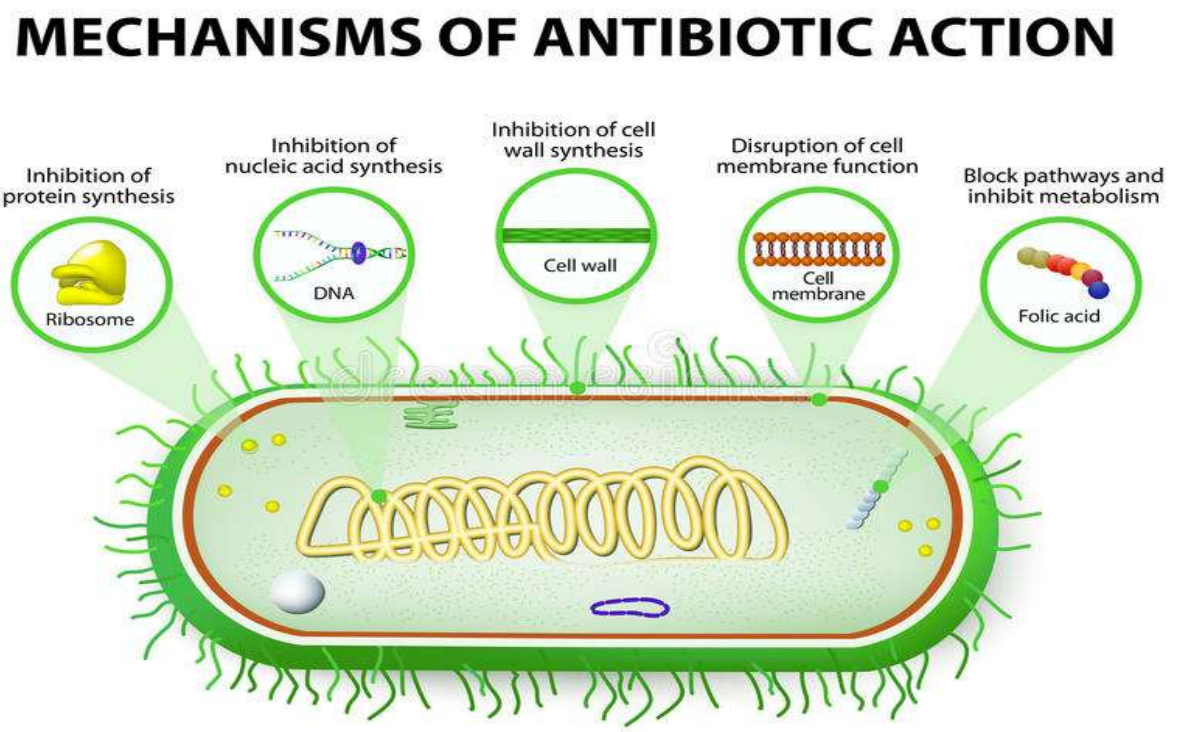

Figure 1: Mode of antibiotics action

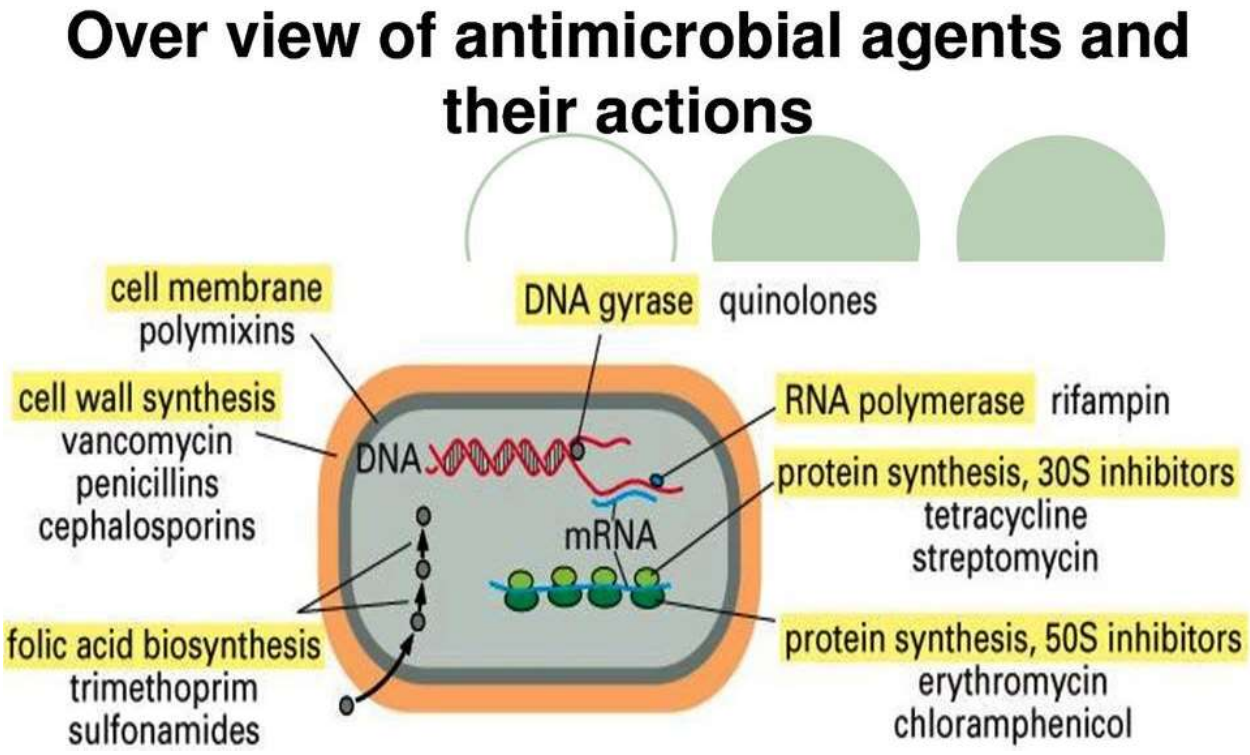

Figure 2: Antimicrobial agents and their site of action

Inhibition of cell wall synthesis:

Penicillins and cephalosporins are the major antibiotics that inhibit bacterial cell wall synthesis by binds and blocks peptidases involved in cross-linking the glycan molecules.

Inhibition of protein synthesis:

Antibiotics that block translation in bacteria by binding to the 30S ribosome are the aminoglycosides and tetracyclines.

Antibiotics that block translation by binding to the 50S ribosome include chloramphenicol and erythromycin.

Antibiotic that block transcription in bacteria is rifampin.

Aminoglycosides: binds the 30S ribosome and misreads mRNA

Tetracyclines: binds the 30S ribosome and blocks attachment of tRNA. Chloramphenicol: binds to the 50S ribosome and prevents peptide bond formation.

Inhibition of nucleic acid synthesis:

Antibiotics that are active against bacterial DNA are the quinolones (nalidixic acid, norfloxacin and ciprofloxacin), which inhibit DNA gyrase, and metronidazole, which fragments DNA.

Sulfonamides and chloroquine are examples of drugs that act by inhibition of nucleic acid synthesis.

Chloroquine: binds and cross-links the double helix.

Other quinolones (ciprofloxacin): inhibits DNA unwinding enzymes (gyrase) and block replication.

Alteration of cell membrane function:

Antibiotics that are active against the bacterial cytoplasmic membrane are polymyxin B and E (colistin). Action of polymyxin B on bacterial membranes by interact with membrane phospholipids and distorts the cell surface lead to leakage of proteins and nitrogen bases.

Inhibition of metabolism:

Antibiotics that act by inhibiting folic acid biosynthesis include the sulfonamides and trimethoprim.

Resistance to antimicrobial drugs

Bacterial resistance to drugs is a condition in which the bacteria that were earlier susceptible to antibiotics develop resistance against the same antibiotics and are not susceptible to the action of these antibiotics. Evolution of microbes towards resistance to antibiotics drugs, including multidrug resistance, is unavoidable because it represents a particular aspect of the general evolution of bacteria that is unstoppable.

Mechanisms of antibiotic resistance:

There are many different mechanisms by which microorganisms might exhibit resistance to drugs. These are (a) production of enzymes, (b) production of altered enzymes, (c) synthesis of modified targets, (d) alteration of permeability of cell wall, and (e) alteration of metabolic pathways.

Genetic basis of drug resistance:

The genetic basis of drug resistance, mediated by genetic change in bacteria, is most important in the development of drug resistance in bacteria. This is of three types as follows: (*a*) chromosome-mediated resistance, (*b*) plasmid-mediated resistance, and (*c*) transposons-mediated resistance.

Vaccination

Immunization is defined as the procedure by which the body is prepared to fight against a specific disease. It is used to induce the immune resistance of the body to a specific disease. Immunization is of two types: passive immunization and active immunization.

1- Passive immunization or immunity is produced without challenging the immune system of the body. It is done by administration of serum or gamma globulins from a person who is already immunized (affected by the disease) to a non-immune person. Passive immunization is acquired either naturally or artificially.

Passive natural immunization is acquired from the mother before and after birth. Before birth, immunity is transferred from mother to the fetus in the form of maternal antibodies (mainly IgG) through placenta. After birth, the antibodies (IgA) are transferred through breast milk.

Passive artificial immunization is developed by injecting previously prepared antibodies using serum from humans or animals. This type of immunity is useful for providing immediate protection against acute infections like tetanus and measles.

2- Active immunization or immunity is acquired by activating immune system of the body. Body develops resistance against disease by producing antibodies following the exposure to antigens. Active immunity is acquired either naturally or artificially.

Active natural immunization is acquired active immunity involves activation of immune system in the body to produce antibodies. It is achieved in both clinical and subclinical infections.

Active artificial immunization that is achieved by the administration of vaccines or toxoids.

A **vaccine** is a suspension of organisms or fractions of organisms that is used to induce immunity. The administration or distribution of vaccines is called vaccination that provides protection against infection by inducing antigen-specific humoral and cellular immunity after artificial antigen administration. Vaccine consists of dead pathogens or live but attenuated (artificially weakened) organisms. The vaccine induces immunity against the pathogen, either by production of antibodies or by activation of T lymphocytes. Vaccines are used to prevent many diseases like measles, mumps, tuberculosis, smallpox, rubella, yellow fever, rabies, typhoid, influenza and hepatitis B.

Types of vaccine:

There are two basic types of vaccines: live attenuated and inactivated. The characteristics of live and inactivated vaccines are different, and these characteristics determine how the vaccine is used.

1. **Live-attenuated (weakened) vaccines:** these vaccines contain modified strains of a pathogen (bacteria or viruses) that have been weakened but are able to multiply within the body and remain antigenic enough to induce a strong immune response. Oral poliovirus (OPV) vaccine and yellow fever virus vaccine are some examples of this type of vaccine.

2. **Heterologous vaccines:** are a sub-group of live attenuated vaccines produced from strains that are pathogenic in animals but not in humans. It is a vaccine that confers protective immunity against a pathogen that shares cross-reacting antigens with the microorganisms in the vaccine. Example cowpox virus that protects against smallpox in humans.

3. **Killed-inactivated vaccines:** to produce this type of vaccines, bacteria or viruses are killed or inactivated by a chemical treatment or heat. This group includes for example the inactivated poliovirus (IPV) vaccine, rabies vaccine and hepatitis A virus vaccine.

4. **Sub-unit vaccines:** instead of the entire microbe, subunit vaccines include only the antigens that best stimulate the immune system. In some cases, these vaccines use epitopes the very specific parts of the antigen that antibodies or T cells recognize and bind. Because subunit vaccines contain only the essential antigens and not all the other molecules that make up the microbe, the chances of adverse reactions to the vaccine are lower.

5. **DNA vaccine:** when the genes for a microbe antigens are introduced into the body, some cells will take up that DNA. The DNA then instructs those cells to make the antigen molecules. The cells secrete the antigens and display them on their surfaces. In other words, the body's own cells become vaccine-making factories, creating the anti gens necessary to stimulate the immune system.

6. **Recombinant vector vaccines:** are experimental vaccines similar to DNA vaccines, but they use an attenuated virus or bacterium to introduce microbial DNA to cells of the body. "Vector" refers to the virus or bacterium used as the carrier.

7. **Toxoid vaccines**: these vaccines are used when a bacterial toxin is the main cause of illness. When the immune system receives a vaccine containing a harmless toxoid, it learns how to fight off the natural toxin. The immune system produces antibodies that block the toxin e.g. vaccines against diphtheria and tetanus.

Toxoid is a substance which is normally toxic and has been processed to destroy its toxicity but retains its capacity to induce antibody production by immune system. Toxoid consists of weakened components or toxins secreted by the pathogens. Toxoids are used to develop immunity against diseases like diphtheria, tetanus and cholera.

8. **Gene deleted vaccines**: these are genetically engineered vaccines which involve the removal or mutation of virulence gene of the pathogen.

9. **Peptide vaccine:** these are the subunit vaccine prepared by chemical synthesis of short immunogenic peptides.

Routes of administration:

1. Deep subcutaneous or intramuscular route (most vaccines)
2. Oral route (oral BCG vaccine)
3. Intradermal route (BCG vaccine)
4. Scarification (small pox vaccine)
5. Intranasal route (live attenuated influenza vaccine).

Scheme of immunization:

Primary vaccination: one dose vaccines (measles, mumps and rubella). Multiple dose vaccines (hepatitis B). Booster vaccination: to maintain immunity level after it declines after some time has elapsed.

Periods of maintained immunity due to vaccines. Short period (months): cholera vaccine. Two years: TAB vaccine (typhoid-paratyphoid A and B vaccine). Three to five years: DPT vaccine (diphtheria and tetanus). Five or more years: BCG vaccine (Bacillus Calmette–Guérin is a vaccine against tuberculosis). Ten years: yellow fever vaccine.

Gram-positive cocci

Gram positive cocci are a group of bacteria which heterogeneous and can be identified by their shape and Gram stain color. Bacteria are broadly divided into two groups, rods and cocci, depending on the shape of the cells (fig.2). Cocci refers to bacteria which are spherical, whereas rods are rod-shaped. In an identification process known as Gram staining, the bacteria appear blue; this is classed as Gram positive. Gram positive bacteria have a thick layer of peptidoglycan which holds the crystal violet stain used and so appear blue. Gram negative bacteria have a thin layer of peptidoglycan held between two membranes which is not able to hold the stain and therefore appear pink following a counter-stain. There are medically important genera of gram-positive cocci: ***staphylococcus***, ***streptococcus*** and ***enterococcus***. Two of the most important human pathogens: ***staphylococci*** and ***streptococci*** are non-motile and do not form spores. Both *staphylococci* and *streptococci* are gram-positive cocci, but they are distinguished by two main criteria:

1. Microscopically, *staphylococci* appear in grapelike clusters, whereas *streptococci* are in chains.
2. Biochemically, *staphylococci* produce catalase (aerobic catalase-positive and they degrade hydrogen peroxide), whereas *streptococci* do not (aerobic catalase-negative) figure (1).

The *enterococcus* are aerobic catalase-negative and motile bacteria.

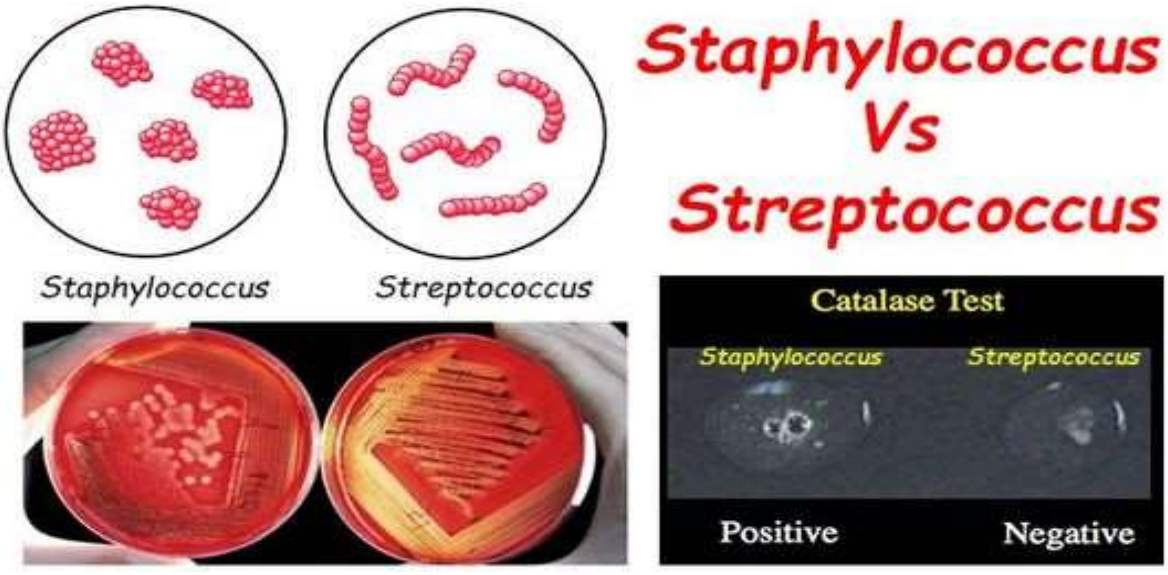

Figure 1: Characteristics of *staphylococcus* and *streptococcus*

Staphylococcus

The genus *staphylococcus* consists of 32 species, most of which are pathogens or commensals associated with skin, skin glands and mucous membranes. The bacteria belonging to this genus are aerobic and facultative anaerobic, catalase positive, oxidase negative, non-motile, non-spore forming and are arranged in clusters, pairs, or tetrads. The genus name *staphylococcus* refers to the fact that these gram-positive cocci grow in a pattern resembling a cluster of grapes. *Staphylococcus aureus* is the most important human pathogen. The other important human pathogens are coagulase negative, which include *staphylococcus epidermidis, staphylococcus saprophyticus,* and *staphylococcus haemolyticus.*

Properties of the bacteria

Morphology

Staphylococci show following features:

- They are Gram-positive cocci, measuring around 1 μm in diameter.
- They are non-motile and non-sporing.
- They are non-capsulated. They, however, contain a microcapsule, which can be visualized by electron microscope only, but not by a light microscope.

Culture

Staphylococci are aerobes and facultative anaerobes but can grow in the absence of oxygen also. They grow at a temperature range of 10 - 42°C (optimum temperature 37°C) and a pH range of 7.4 - 7.6 (optimum pH 7).

Blood agar: *Staphylococci aureus* produces a clear zone of hemolysis (beta-hemolysis) surrounding the colonies on blood agar.

Selective media: Mannitol salt agar is the commonly used selective medium for isolation of *S. aureus* from clinical specimens containing normal bacterial flora (e.g., stools). Most strains of *S. aureus* ferment mannitol with acid production, which gives rise to yellow zone formation around the colonies.

Hemolysins: *S. aureus* are golden yellow and produces four hemolysins: alpha, beta, gamma and delta hemolysins.

Laboratory diagnosis

Laboratory diagnosis of staphylococcal infections is based on the demonstration of *staphylococci*, in appropriate clinical specimens, by culture and microscopy.

Streptococcus

Streptococci are aerobic and facultative anaerobic Gram positive cocci, arranged in pairs, or chains. *Streptococci* are part of the normal flora in humans. They are spherical or ovoid cocci, and have hyaluronic acid capsules. *Streptococci* divide in one plane and thus occur in pairs or in chains of varying lengths, especially in liquid media and clinical specimens.

Properties of the bacteria

Morphology

- They are catalase negative by which they are distinguished from *staphylococci*.
- They are fastidious bacteria requiring enriched medium, such as blood agar for their growth.
- They are non-motile and non-spore.
- Some strains of *S. pyogenes* and some strains of group C *streptococci* produce capsule during the first 2- 4 hours of growth.

Enterococcus

Enterococcus species are enteric *streptococci* which are found naturally in the intestinal tract of human. The *enterococci* are facultative anaerobes. They require complex nutrients for their growth. They are opportunistic pathogens and differ from the *streptococcus* species in two important respects:

1. They tolerate bile salts and grow on MacConkey agar as red, pin-point colonies.
2. Some isolates are motile.

Characteristics of *enterococci*

The enterococci are gram-positive cocci typically arranged in pairs and short chains, and is non-motile and non-capsulate. The cocci are facultatively anaerobic and grow optimally at 35°C, although most isolates can grow in the temperature range l0ºC to 45°C. They grow readily on blood agar media, with large, white colonies appearing after 24 hours of incubation; the colonies are typically non-hemolytic but can be α-hemolytic or b-hemolytic.

Distinctive features of *enterococci*

The *Enterococci* possess several distinctive features separating them from *streptocooci*: The enterococci grow in the presence of 6.5 percent NaCl, 40 percent bile, at pH 9.6, at 45°C and in 0.1 percent methylene blue. It survives heating at 60°C for 30 min, a feature distinguishing it from *streptococci*, and also grows within a

wider range of temperatures (10-45°C). On MacConkey medium they produce deep pink colonies.

Identification

The identification of enterococcus species is made on biochemical characteristics. *E. faecalis* can be identified by its ability to ferment mannitol, sucrose and sorbitol, and to grow on tellurite blood agar producing black colonies with gas production.

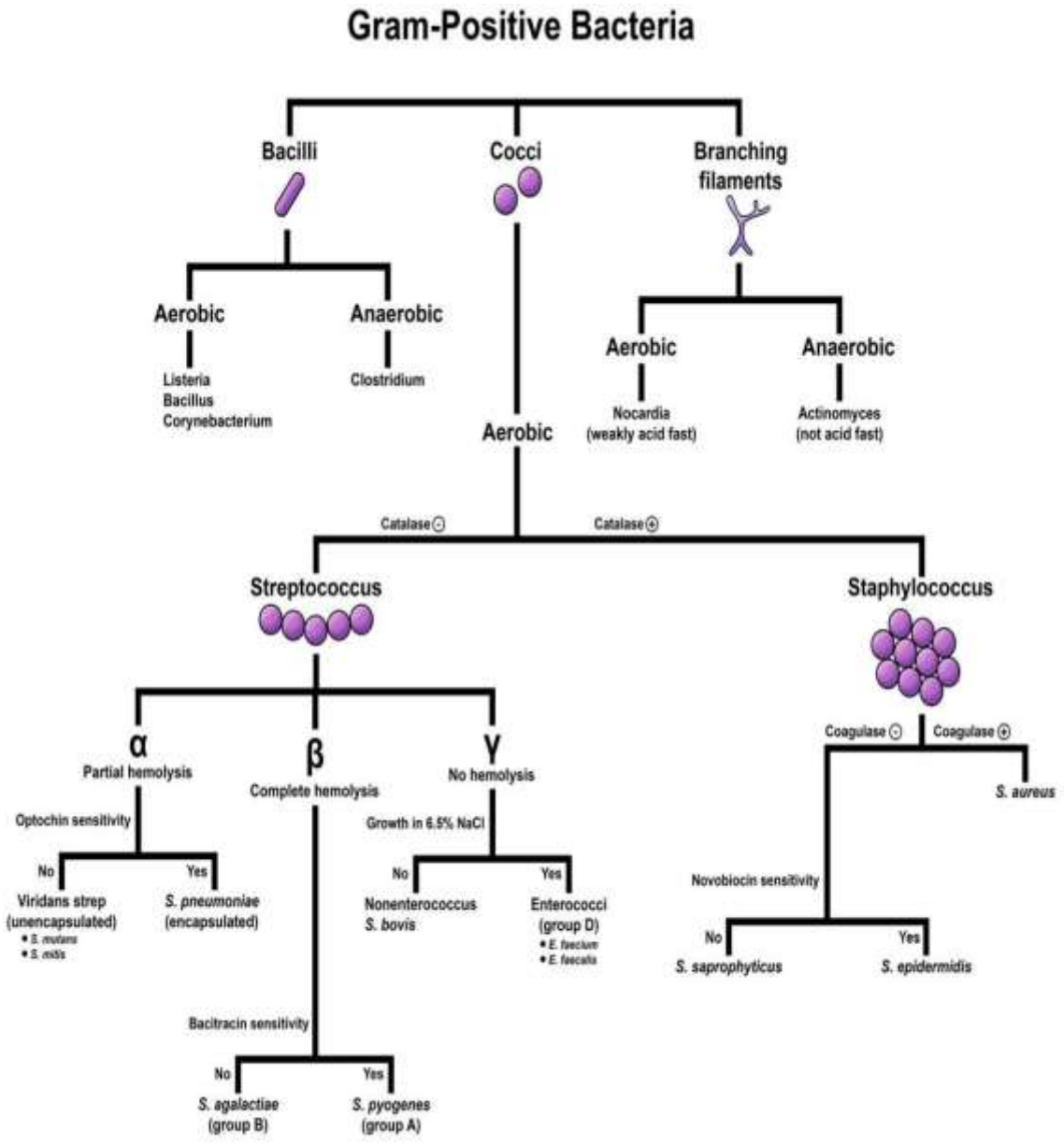

Figure 2: Gram positive bacteria

Gram positive non-spore forming bacilli (*Listeria* and *Corynbacterium*)

Listeria species

Listeria-Organisms of the genus *Listeria* contains eight species but almost all cases of human listeriosis are caused by *L. monocytogenes.*

- *Listeria monocytogenes* is a small, coccoid, gram positive bacillus and exhibits a characteristic, slow, tumbling motility when grown at 25 °C but at 37 °C is non-motile.
- The disease chiefly affects pregnant women, unborn or newly delivered infants, the immunosuppressed and elderly. It is predominantly transmitted by the consumption of contaminated food.

General characteristics of *Listeria*

- Small, Gram-positive rods
- Grow on non-enriched media
- Tolerates wide temperature and pH ranges
- Small hemolytic colonies on blood agar
- Facultative anaerobes, catalase-positive, oxidase-negative
- Tumbling motility at 25°C
- Aesculin hydrolyzed
- Environmental saprophytes

Morphology

Listeria monocytogenes is a small, coccoid, gram-positive bacillus. They occur singly or in pairs which are often angled at the point of contact and may resemble diphtheroids or diplococci. It exhibits a characteristic, slow, tumbling motility when grown at 25°C but at 37°C is non-motile. This is because peritrichous flagella are produced by the bacillus optimally at 20 to 30°C but only scantily or not at all at 37°C. They are non-capsulate, non-sporing and nonacid-fast.

Cultural characters

Listeriae are aerobes and facultative anaerobes. They can grow over a temperature range of 2 to 43°C, the optimum temperature for the growth is 35 to 37°C. They can grow on ordinary media containing fermentable carbohydrate but growth is better on blood agar or tryptose phosphate agar. After 24 hours incubation at 37°C,

colonies are smooth, translucent and non-pigmented. On blood agar, *L. monocytogenes* develops zones of slightly hazy β-hemolysis.

Pathogenicity

Listeria monocytogenes is commonly ingested in food and is usually a harmless transient in the intestinal tract. The disease chiefly affects pregnant women, unborn or newly delivered infants, the immunosuppressed and elderly. It is predominantly transmitted by the consumption of contaminated food. Human infection is believed to result from contact with infected animals, inhalation of contaminated dust or ingestion of contaminated milk or food. Outbreaks of food borne listeriosis have been known.

Differentiation of *Listeria* species

The pattern of haemolysis on sheep blood agar and acid production from a short range of sugars are useful differentiating laboratory methods for ***Listeria*** species. The colonies are small, smooth and transparent after incubation for 24 hours.

Laboratory diagnosis

1. Specimens

Blood, CSF, amniotic fluid, placenta, pus and biopsy material from the organs involved may be collected. Specimens may also be collected from neonate, stillbirth or products of conception.

2. Microcopy

If the gram stain shows organisms, they are intracellular and extracellular gram-positive coccobacilli. Care must be used to distinguish them from other bacteria, such as *S. pneumoniae, Enterococcus, Corynebacterium* and occasionally, *Hemophilus.*

3. Culture

Specimens should be inoculated on blood agar, chocolate agar and tryptose phosphate agar and incubated at 35 to 37°C for 1 to 3 days. Uncentrifuged CSF and blood may be added to nutrient broth and incubated at 35 to 37°C for 5 days followed by subculture on solid media. Greater success in isolation is achieved if the materials are stored in tryptose phosphate or thioglycollate broth at 4°C and subcultures are done at weekly intervals for 1 to 6 months (cold enrichment). Isolates are likely to be missed as nonpathogenic diphtheroids unless properly investigated. In case of listerial brain abscess, pus culture may be done. Blood agar shows small colonies surrounded by a narrow zone of b-hemolysis. The bacteria are actively motile when grown at 25°C. The isolate is identified by its morphology and biochemical tests.

Corynebacterium species

The *coryneform* group consists of *Corynebacterium* and related genera that are aerobic, non-sporing and irregularly shaped, non-spore forming and gram-positive rods. The genus *Corynebacterium* comprises 66 species; 38 of them associated with human disease. *Corynebacteria* are nonacid fast, non-motile rods with irregularly stained segments, and sometimes granules. They frequently show club shaped swellings and hence the name corynebacteria (from ***coryne,*** meaning club). Corynebacteria are closely related to mycobacteria and nocardiae. Some corynebacteria are found as part of the normal flora of humans in the skin, upper respiratory tract, and urogenital tract. *Corynebacteriurn* species are small, pleomorphic Gram positive bacteria which occur in coccoid, club and rod forms (coryneform morphology). In stained smears, they occur singly, in pallisades of parallel cells and in angular clusters resembling Chinese letters. The type species is *Corynebacteriurn diphtheriae,* the cause of diphtheria in children.

General characteristics of *Corynebacterium*

- Gram-positive, pleomorphic bacteria
- Fastidious, requiring enriched media
- Majority are commensals on mucous membranes
- Cause pyogenic infections
- *Corynebacteriurn* species:
  -non-motile facultative anaerobes
  -catalase positive, oxidase negative

Morphology

They are thin, slender **gram-positive bacilli** but are easily decolorized, particularly in old cultures. They have a tendency to **clubbing** at one or both ends. They are highly **pleomorphic.** Cells often show septa, and branching is infrequently observed. They are non-motile, non-spore forming, and nonacid fast. The bacilli are arranged in a characteristic fashion in smears. They are usually seen in pairs, palisades (resembling stakes of a fence) or small groups or as individual cells lying at sharp angles to another, resembling the letters V or L. This particular arrangement

with *C. diphtheriae* has been called the **Chinese letter or cuneiform arrangement** .This is due to the incomplete separation of the daughter cells after binary fission.

Cultural Characteristics

C. diphtheriae is an aerobe and facultative anaerobe; the optimum temperature for growth is 37°C (range 15-40°C) and optimum pH 7.2. Complex media are required for primary isolation and characterization. It can grow on ordinary nutrient agar, but its growth is improved by the presence of animal proteins such as blood or serum.

Two media are useful for this purpose:

1. Loffler's serum slope.
2. Blood agar containing fresh, lysed or heated blood.

Pathogenesis

In the upper respiratory tract, diphtheria bacilli elicit an inflammatory exudate and cause necrosis of the cells of the facial mucosa. The diphtheria toxin possibly assists colonization of the throat or skin by killing epithelial cells or neutrophils. Diphtheria is a toxemia. The organisms do not penetrate deeply into the mucosal tissue and bacteremia does not usually occur. The exotoxin is produced locally and is spread by the bloodstream to distant organs, with a special affinity for heart muscle, the peripheral nervous system and the adrenal glands.

Laboratory diagnosis

These include swabs from the nose, throat, pieces of pseudomembrane, if possible even from beneath the membrane, biopsy tissue, etc. The first swab is used to make a direct smear and the other is used for the culture. These are then transported to the laboratory in a sterile empty container or in silica gel sachets for immediate processing.

It depends upon microscopy, culture and virulence tests. Albert, Neisser, or Ponder stain of direct smears shows metachromatic granules. Virulence testing may be by *in vivo* or *in vitro* methods. Virulence tests demonstrate the production of exotoxin by bacteria isolated on culture. *In vivo* tests are: (i) Subcutaneous test; (ii) Intracutaneous test. *In vitro* test include (i) Precipitation test; (ii) Tissue culture test; (iii) Enzyme-linked immunosorbent assays (ELISA); (iv) Polymerase chain reaction (PCR).