Introduction

Microbiology : is the science that dealing with the study of microorganisms .

Types of biological relationships in the environment:

Mutualism : one organism get all benefits from the relationship ,the other organism get nothing with no harm (Commensalism).

Symbiosis : both organisms get benefits from the relationship .

Parasitism : all the benefits go to one party , the harm would go to other organism

Living organisms :

Prokaryotes	Eukaryotes
1-Relatively small cell size (1 µm in diameter). 2-Absence of nuclear membrane. 3- Almost (in bacteria) have circular DNA (1mm in length). 4-The region of condensed DNA is called nucleoid. 5- The genetic material containing genes almost responsible for the : a-Energy generation b-Cellular replication c-Molecular synthesis 6- Prokaryotes include: A-Bacteria (Eubacteria) B-Archaebacteria (primitive bacteria) , include: -Halophiles -Thermoacidophiles -Methanogenes	1-Relatively large cell size. 2-Presence of nuclear membrane. 3-Have linear DNA . 4- Including: a-Algae b-Protozoa c-Fungi d-Slime molds

Evolution of microbiology:

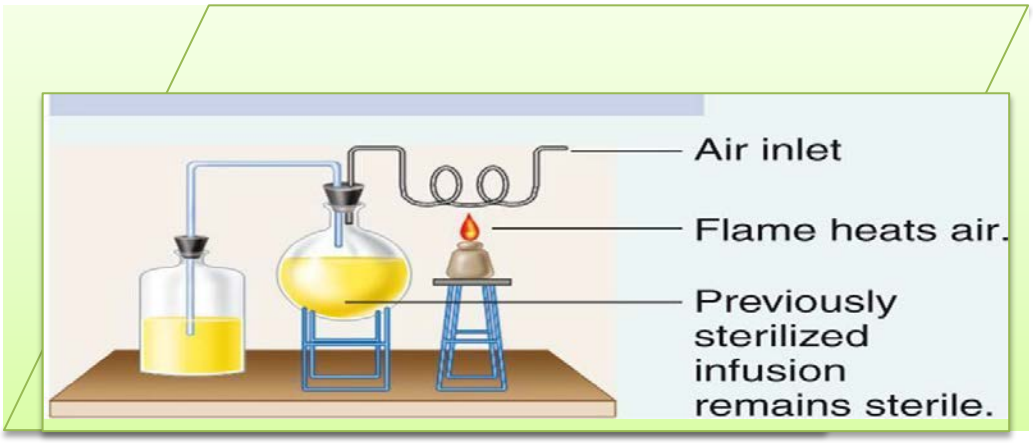
1-Van leevenhock (1677) : First observations.

2-Redi: Spontaneous Generation.

3-Spallazani(1729-1799) : Sterile Culture Medium : Meat infusion -→

→Boiled→Sealed→Remain Clear For A Long Time

4-Schwan(1837):



5-Schroder and van dusch: Introduce the use of cotton plug which is still used up to day.

6-Louis Pasture(1822-1895): Used swan-Neck flask.

7-John tyndall(1877): The problem of spores (could not achieve sterility in his lab by boiling).

8-Ferdinand Cohn (1877): Discovered the spores in *Bacillus subtilis*, and the invention of autoclave .

9-Winogradsky and Beijerinck :

⇒ Development of Soil Microbiology.

⇒ The Biochemical Role of Soil Microorganisms in Demineralization Of Organic Matter

Organic C → CO_2

Organic N → NH_3 or NO_3^-

Organic S → SO_4^{-2} or S^{-2}

10-Robert koch (1843-1910):

- The discovery of Anthrax bacteria (***Bacillus anthracis***).
- Development of the solid culturing methods .
- The use of staining techniques .
- The Identification of Tubercle bacillus in 1882 (***Mycobacterium tuberculosis***)

The Development of Koch´s Postulates:

1.The organism is found in the lesions of a disease.

2.The organism can be isolated in a pure culture.

3.Introducing the pure culture in an experiment organism (Animal) , will produce similar disease lesions and symptoms.

4. The mo. can be isolated from the lesions in a pure culture.

11-The golden era of medical bacteriology (1879-1889) when various members of the german school isolated :

1.The Cholera Vibrio (*Vibrio cholerae*)

2.The Typhoid Bacillus (*Salmonella typhi*)

3.The Diphtheria Bacillus (*Corynebacterium diphtheriae*)

4.The Pneumococcus (*Diplococcus pneumoniae*)

5.Boil Causing Bacteria (*Staphylococcus aureus*)

6.The Streptococci (*Streptococcus pyogenes)*

7.The Meningococci (*Neisseria meningitidis*)

8.Gonococci (*N. gonorrhoeae).*

8.The Tetanus Bacillus (*Clostridium tetani*).

-Lewis Pasteur and microbiology :

1-In 1857 his work on alcoholic fermentation and lactic fermentation

2-His work about microbial metabolism : the discovery of anaerobic microorganisms and the fact that "life is possible without air".

3-Fermentation is much less efficient than respiration in terms of growth rate (yield)/ unit substrate consumed .

4-The development of selective cultivation .

5-The development of pasteurization.

6-The development of vaccination.

Bacterial cell groupings (arrangement)

1-cocci

1.1.Chains : *Streptococcus pyogenes*

1.2.Pairs : *Diplococcus pneumoniae*

1.3.Cubical bundles :*Sarcina leutea*

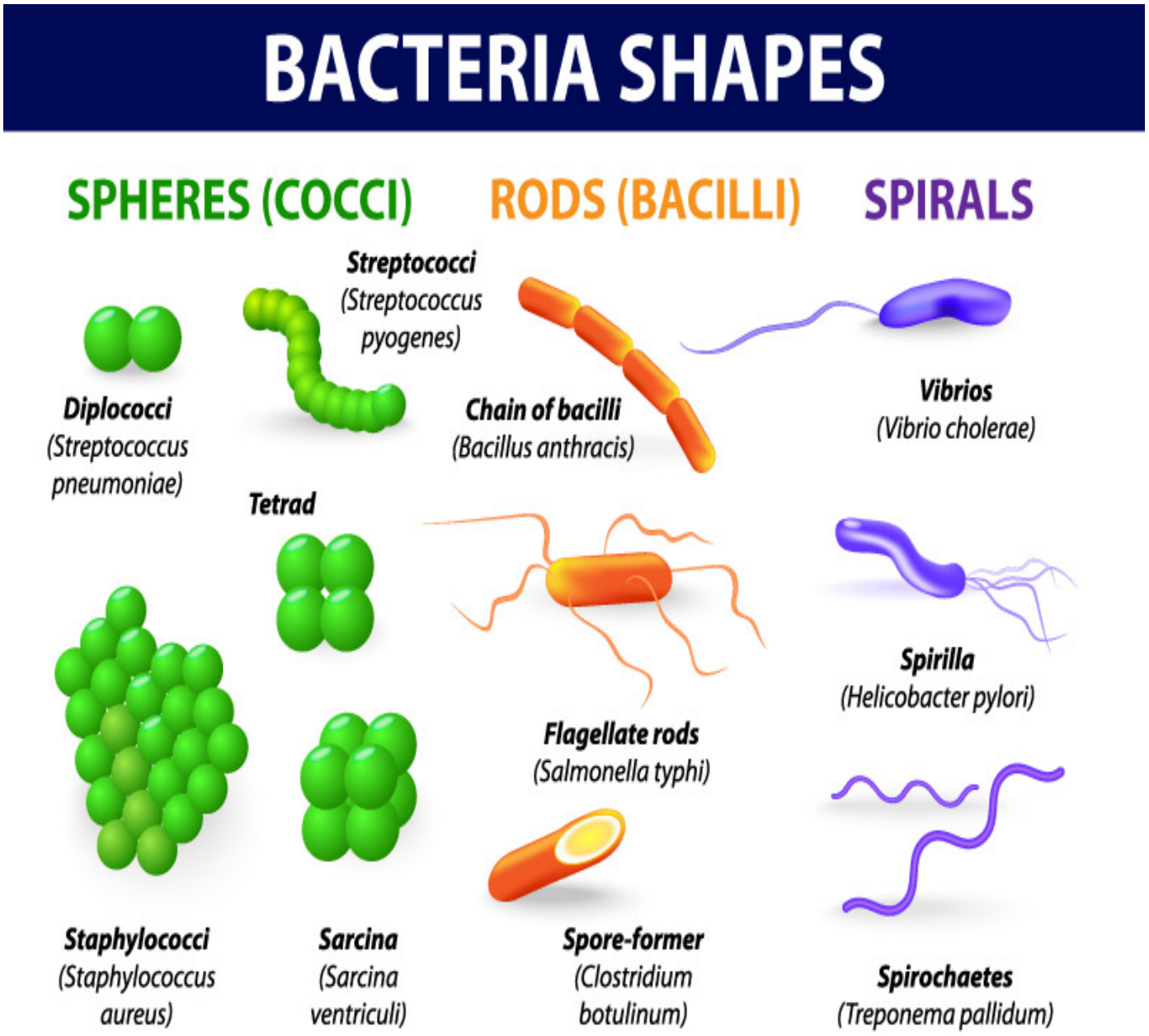
1.4.: Clusters : *Staphylococcus aureus*

2-Rods (bacilli) :

1.1.Pairs : *Bacillus*

1.2.Chains : *Streptobacillus ; Streptomyces*

3-Spiral –form : *Treponema pallidum*


BACTERIA SHAPES
SPHERES (COCCI)
RODS (BACILLI)
SPIRALS
Streptococci
(Streptococcus pyogenes)
Diplococci
(Streptococcus pneumoniae)
Chain of bacilli
(Bacillus anthracis)
Vibrios
(Vibrio cholerae)
Tetrad
Spirilla
(Helicobacter pylori)
Flagellate rods
(Salmonella typhi)
Staphylococci
(Staphylococcus aureus)
Sarcina
(Sarcina ventriculi)
Spore-former
(Clostridium botulinum)
Spirochaetes
(Treponema pallidum)

Classification of bacteria

Taxonomy: (Gr. Taxon = arrangement); is the science that dealing with the classification , identification and nomenclature of organisms.

Classification: is the categorisation of org.s into taxonomic groups; so that, biochemical, physiological, genetical, and morphological properties are necessary for establishing a taxonomic rank.

Identification: is the isolation and distinguishing of a specific mo. among a mixed microbial flora.

Nomenclature: is the naming of an org. by an established group of scientists.

The taxonomic ranks, are:

1. Kingdom
2. Phylum
3. Class
4. Order
5. Family
6. Genus
7. Species
8. Subsp. , biotype , strain , serotype.

Criteria for bacterial identification:

1. Growth on nutrient media:

A. General use complex media; e.g. (N. agar ; N. broth).

B. Non-selective N. media; e.g. (Blood agar ; Chocolate agar).

C. Selective N. media;

i. Na-azide, selects for G+ve over G-ve bacteria.

ii. Bile salts (Na-deoxycholate), selects for G-ve enteric bac.

Over mucosal and most G+ve bac.

iii. Colistin and nalidixic acid medium; inhibits the growth of many G-ve bac.

D. Differential media; used to differentiate between 2 groups of Mo.s ; e.g. EMB agar and McConkey agar.

2. Microscopy:

Examination of stained bacterial cells under the microscope, to determine the following traits :

(i) **cellular shape** (cocci, bacilli or spiral form)

(ii) **groups** (diplo- , tetrads , staph. Or strepto.)

(iii) **The cells** stained **G+ve** or G-ve

(iv) Acid-fast or Non-acid-fast.

3. Biochemical tests:

e.g. oxidase test, catalase test, IMViC tests , coagulase test; and many other reactions.

4. Immunological tests:

To differentiate serotypes, serogroups and serovars (all of these levels are below the species level).

The serological methods used in such differentiation are called:

i. biotyping

ii. serotyping

iii. bacteriophage typing

5. Genetic diversity:

The bacteria are wether:

i. carrying plasmid(s) or not.

ii. carrying bacteriophage or not.

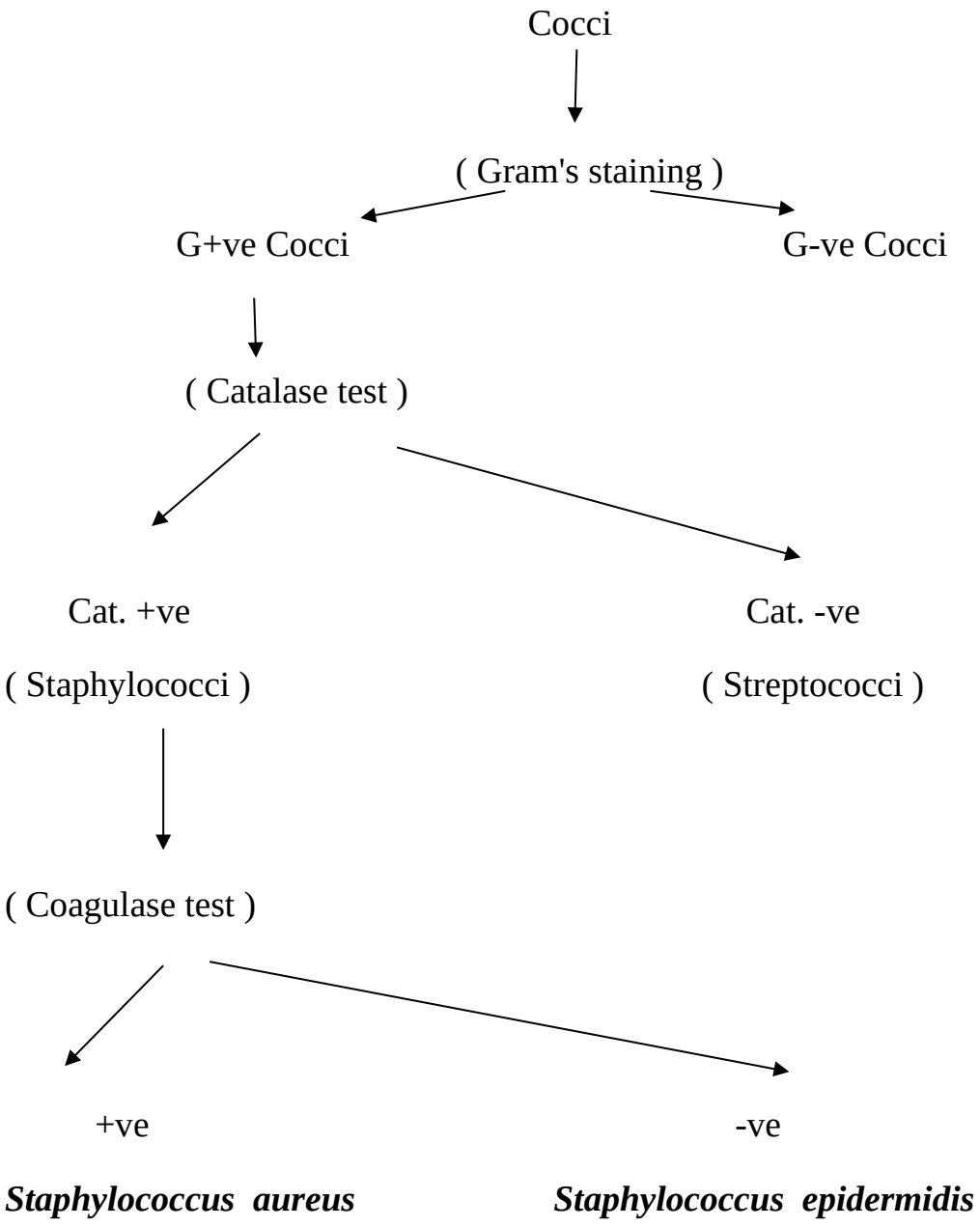
iii. Patterns of antibiotic resistance(s).

iv. Genes encoding certain enzymes (like lactose utilization).

Classification systems:

1. Dichotomous keys:

Depends on the presence (+ve) or absence (-ve) of a certain trait :



2. Numerical taxonomy:

Based on biochemical measures of activity; the best example is using the **" Analytical Profile Index " (API) System** ; which facilitate the use of num. taxonomy to identify a wide range of mo.s.

API system depends on biochemical and physiological traits. Identification of mo.s depends on levels of similarity (> 80% of trait similarity).

3. Nucleic Acid based taxonomy:

Includes:

i. Plasmid analysis

ii. Restriction endonuclease analysis

iii. Genomic analysis

iv. Repetitive sequence analysis

v. Ribosomal RNA analysis

The major groups of mo.s :

Based on **"Bergy's Manual of Determinative Bacteriology"**:

1. Bacteria (Eubacteria) : include;
   i. Green-filamentous bac.
   ii. Spirochetes.
   iii. G+ve bac.
   iv. G-ve bac.
   v. Eubacteria lacking cell wall

2. Archaea (primitive bac.):

i. Halophiles

ii. Methanogenes

iii. Thermoacidophiles

3. Eucarya (Eucariotic mo.s):

i. Fungi

ii. Slime molds

iii. Algae

iv. Protozoa

Bacterial cell structure

I-The Nucleoid :

∅ Concentrated DNA filament can be seen in stained cells by light microscope.

∅ Histone – like proteins can be associated with DNA.

∅ No nuclear envelope.

∅ The DNA can be considered as a single haploid chromosome , approx.. 1mm in length (supercoiled) .

Π-Cytoplasmic structures:

⇒ No plastids , no mitochondria or chloroplasts , no microtubular structures.

⇒ There are photosynthetic pigments localized in membrane–like arrangements in cyanobacteria known as thylakoids.

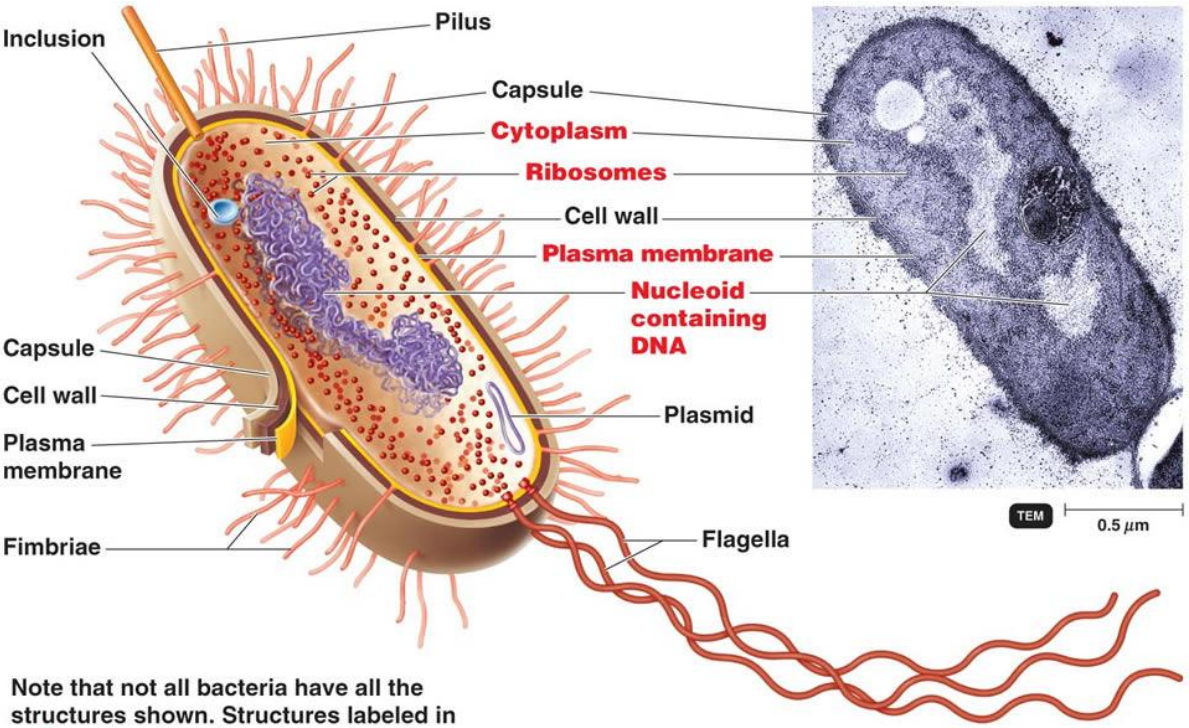
⇒ Many bacteria can accumulate granules of polyphosphate , that can be used in ATP synthesis , called (volutin granules or metachromatic granules) which can be seen in corynebacteria as red granules.

⇒ Some photosynthetic bacteria can oxidize S^{-2} from (H2S) producing S (sulfur) granules , deposited intracellularly.

⇒ Gas vesicles in aquatic microorganisms can be found .

⇒ Protein-bounded vesicles can be found in the cytoplasm (could be filled with proteins and/or enzymes).

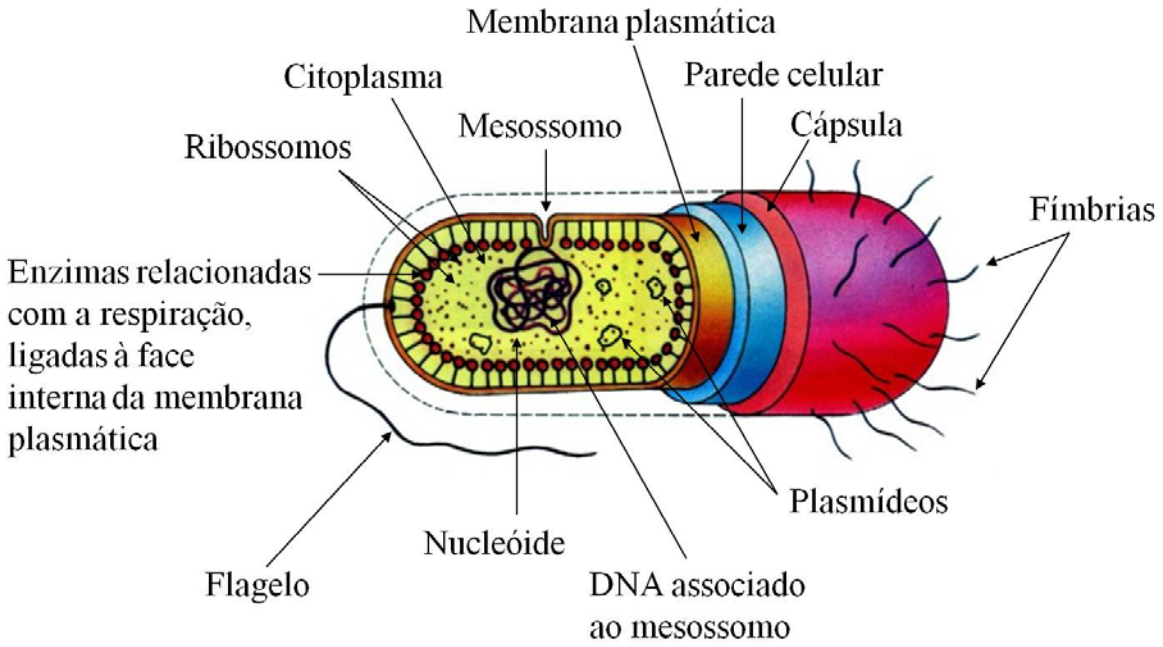
⇒ Ribosomes are found in the cytoplasm with different kinds of proteins and enzymes .



Note that not all bacteria have all the structures shown. Structures labeled in **red** are found in all bacteria. Both the drawing and the micrograph show a bacterium sectioned lengthwise to reveal the internal composition.

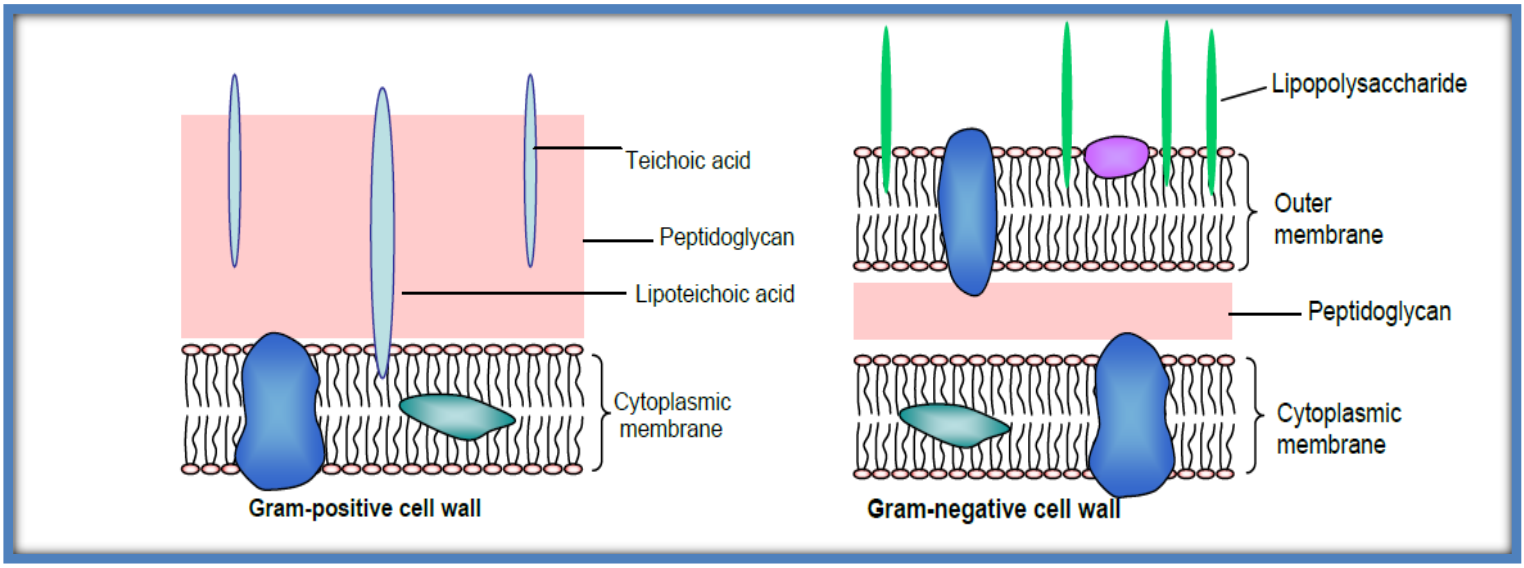
Key Concept

Prokaryotic cells lack membrane-enclosed organelles. All bacteria contain cytoplasm, ribosomes, a plasma membrane, and a nucleoid. Almost all bacteria have cell walls.



ΙΙΙ-The cell envelope

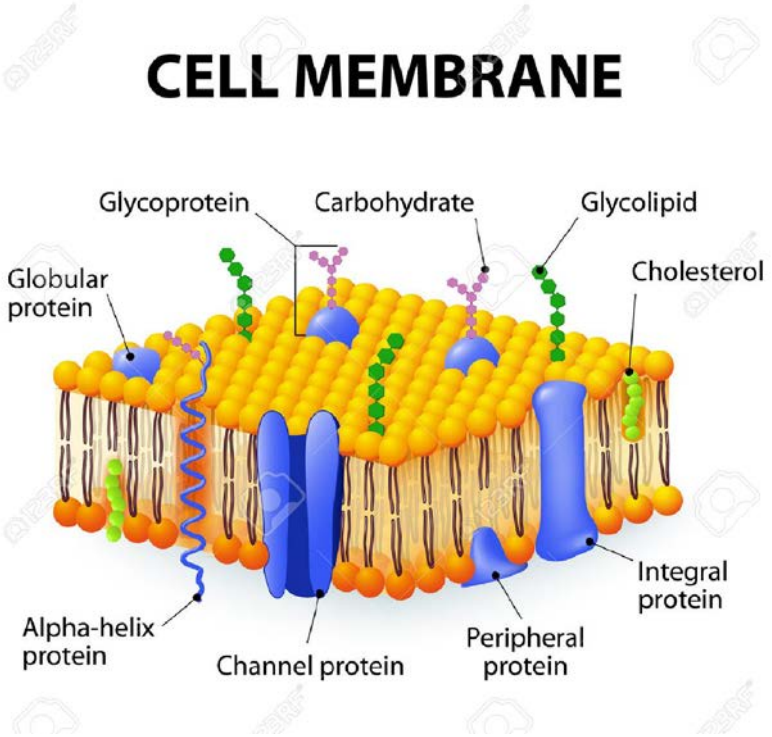
Gram positive bacteria (G+ve)	Gram negative bacteria (G-ve)
1-Cytoplasmic membrane 2-Thick peptidoglycan layer 3-Outer layer (capsule or S-layer) composed of Glycoproteins	More complex multilayered structure: 1-Cytoplasmic membrane (inner membrane). 2-Thin peptidoglycan layer (within the periplasmic space). 3-Outer membrane 4-Outermost capsule or S-layer composed of LPS (lipopolysaccharides)



1-The cytoplasmic membrane :

a) Composed of bilayered phospholipid and proteins with the absence of sterols (cholesterol) .

b) Presence of mesosomes (invaginations inside cytoplasm) lateral and septal mesosomes : function in the formation of cross -walls during cell division.



The functions of cytoplasmic membrane:

1-Permability and transport of nutrients.

2-Electron transport and oxidative phosphorylation.

3-Excretion of hydrolytic exoenzymes and pathogenecity proteins (toxins).

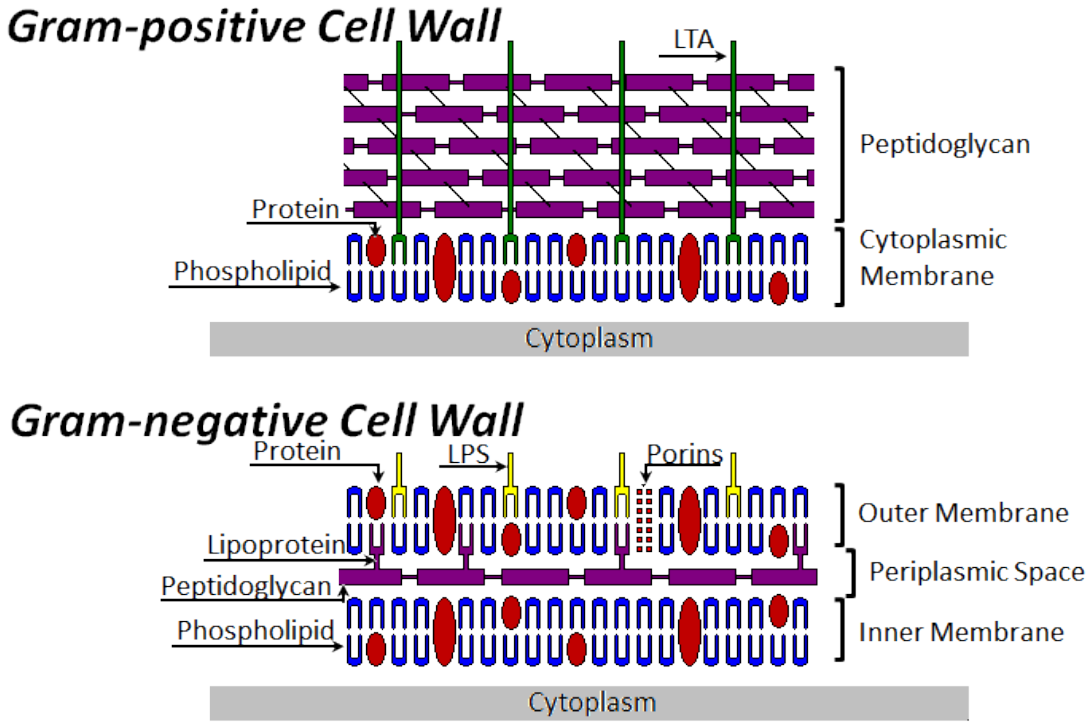
4-Biosynthetic functions : some proteins and enzymes of DNA replication , and enzymes of phospholipid synthesis.

5-Chemotactic systems : specific receptors for chemicals and other nutrients .

2-The cell wall :

-In Gram positive (+ve) : Peptidoglycan and teichoic acid.

-In Gram negative (G –ve): The peptidoglycan and outer membrane.



Peptidoglycan = murein = mucopeptide

Gram´s staining (differential stain) by Hans Christian Gram:

1-Crystal violet 1-2min (primary stain).

2-Iodine (mordant) 1 min.

3-Acetone or alcohol (decolorizer) 10-30 sec.

4-Washing with water.

5-Safranin or carbol fuchsin (counter stain) 1 min (secondary stain).

6- Washing with water

Gram +ve = blue (bluish purple) **Gram –ve = red**

Functions of the cell wall :

1-Gives osmotic protection to the cell.

2-Plays an essential role in cell division.

3-The site of many antigenic determinants of cell surface.

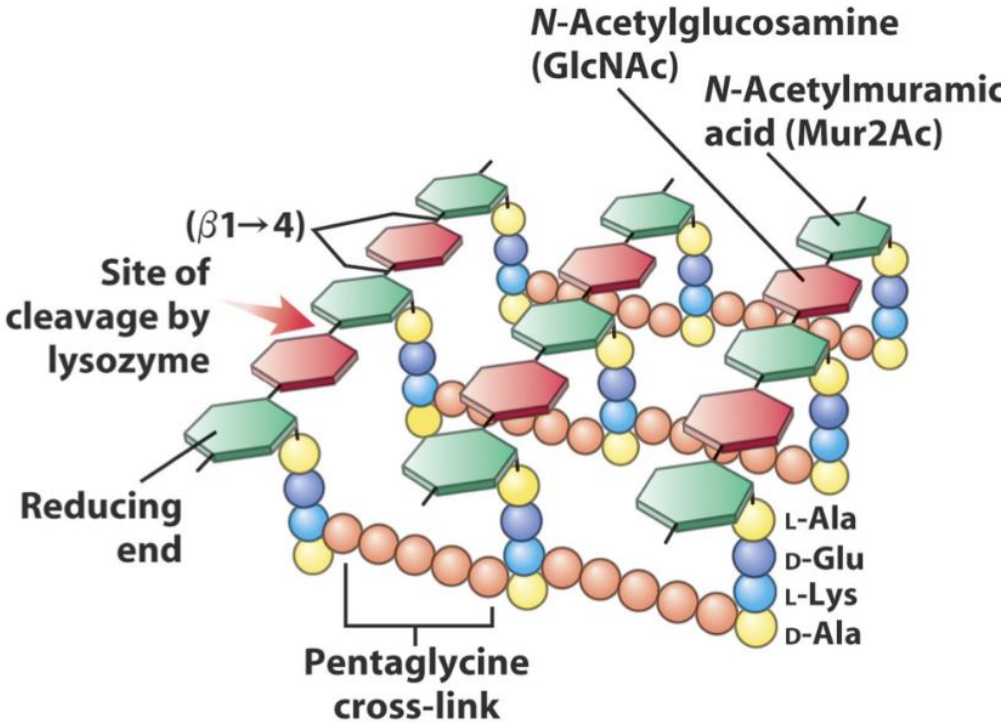
The peptidoglycan layer:

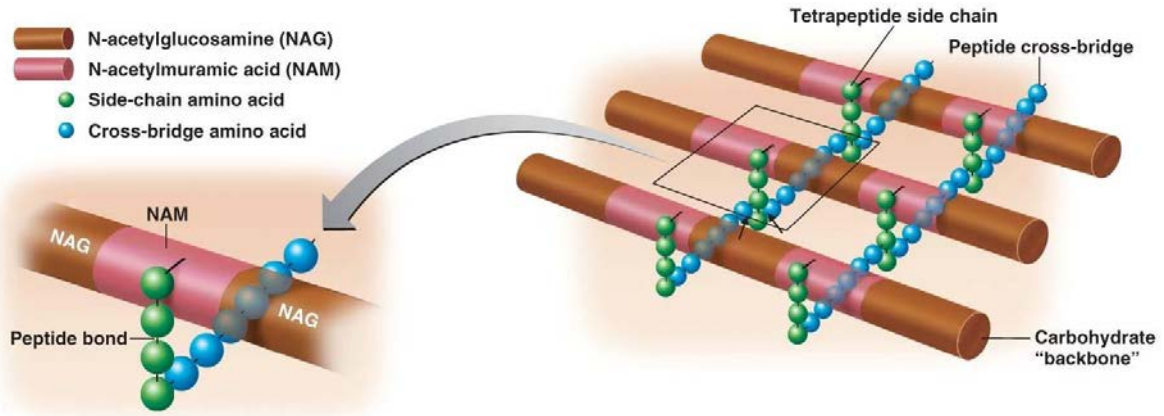
Is a complex polymer consisting of 3 parts:

1-Backbone : composed of N-acetylglucosamine and N-acetylmuramic acid.

2-Tetra peptide cross-linked.

3-Identical pentapeptide cross-bridge.





(a) Structure of peptidoglycan in gram-positive bacteria

-There are some components in G+ve cell walls attached to peptidoglycan and to cell membrane called :

A.Teichoic acid and teichuronic acid :

(in cell membrane lipoteichoic acid) , which are responsible for antigenic characteristics of G+ve cell wall .

B.polysaccharides : which representing the outer layer in G+ve cell.

3.Special components in –ve envelope :

A.Lipoprotein : function to stabilize outermembrane.

B.Outer membrane :

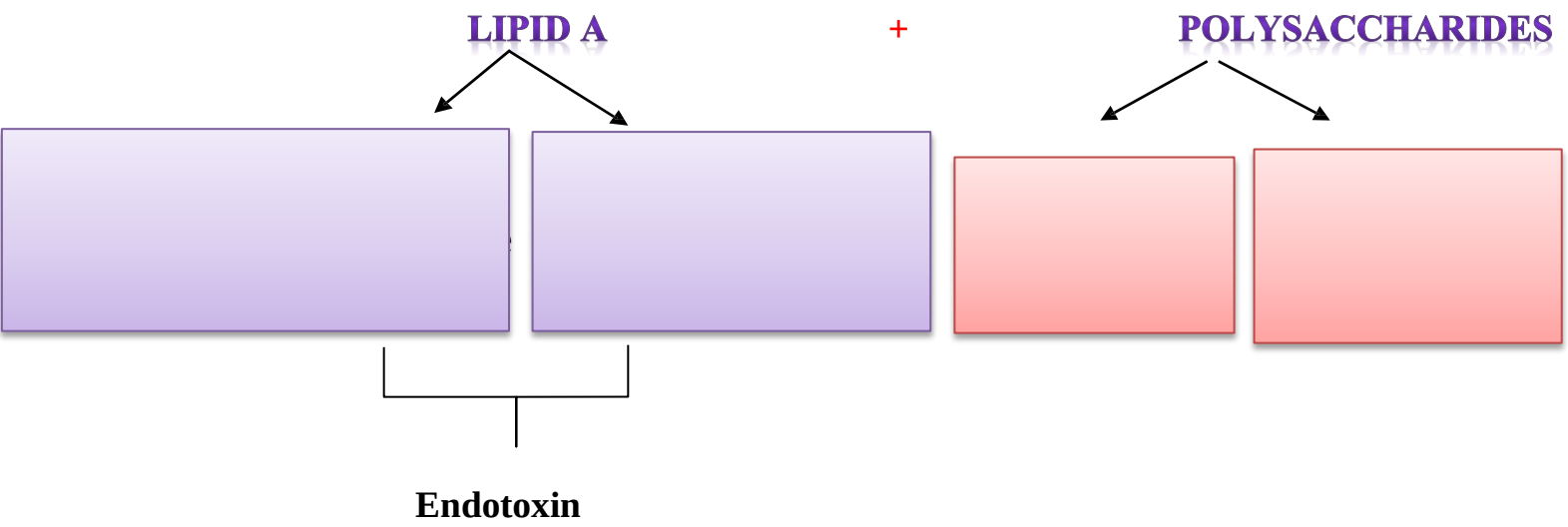
composed of bilayered phospholipid structure:

⇒ Inner layer : resemble the cytoplasmic membrane

⇒ Outer layer: The phospholipids are replaced by LPS

This kind of structure gives G-ve bacteria less permeability and higher selectivity to large molecules like antibiotics, and consequently more microbial resistance.

C.lipopolysaccharides (LPS) composed of :



Endotoxin

4- The periplasmic space : Located between the inner and outer membranes (contain high amounts of proteins and active enzymes).

Molecules and enzymes attacking cell membrane and cell wall :

A-Cell membrane :

1.Detergents : Disruption of the membrane like EDTA

2.Antibiotics :

-Disruption of the membrane , e.g. polymyxin

-Inhibit DNA synthesis and teichoic acid synthesis , e.g. : nalidixic acid , novobiocin

-Discharge membrane potential (ionophores) e.g. valinomycin

B-Cell wall :

1.Lysis by lysozyme enzyme that attack peptidoglycan layer ; treatment produce protoplasts of G+ve cells.

2.EDTA : disrupt outer membrane of G-ve cells ; treatment with EDTA + lysozyme produce spheroplasts of G-ve cells.

3.Autolysins in bacterial cells , causing autolysis .

4.Penicillins cause blocking of cell wall biosynthesis ; treatment of G+ve cells produce L-form cells.

5.Capsule (Glycocalyx):

An extracellular polysaccharides forming a layer surrounding the cell entirely , its role is in adherence and pathogenecity , example :

Streptococcus mutans , Diplococcus pneumoniae and Klebsiella pneumoniae.

Flagella :

Thread –like appendages , protein in structure , they are organs of locomotion

Arrangement of flagella :

1.Monotrichous : Single polar flagellum e.g Vibrio

2.Lophotrichous :Multiple polar flagella e.g Spirillum

3.Peritrichous : Flagella distributed over the entire cell e.g Proteus vulgaris

4.Amphitrichous : Having a single or multiple polar flagella at each end of the cell e.g: Alcaligenes faecalis.

Flagellar protein is called Flagellin (H-antigen), which is highly antigenic.

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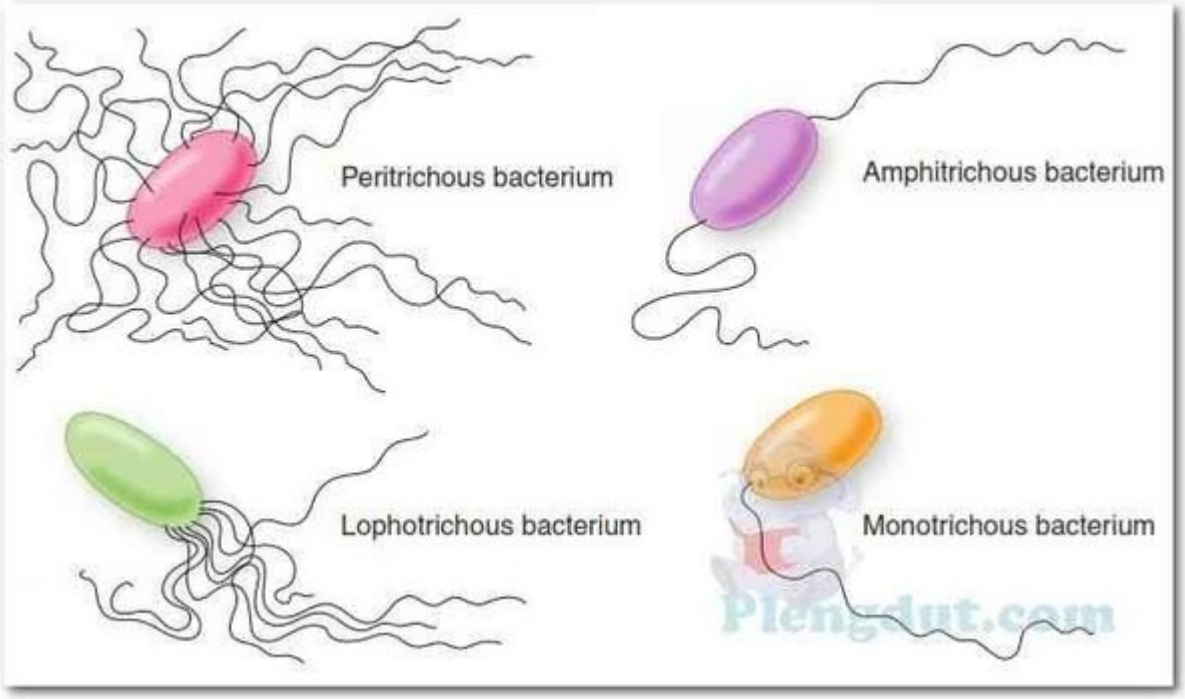
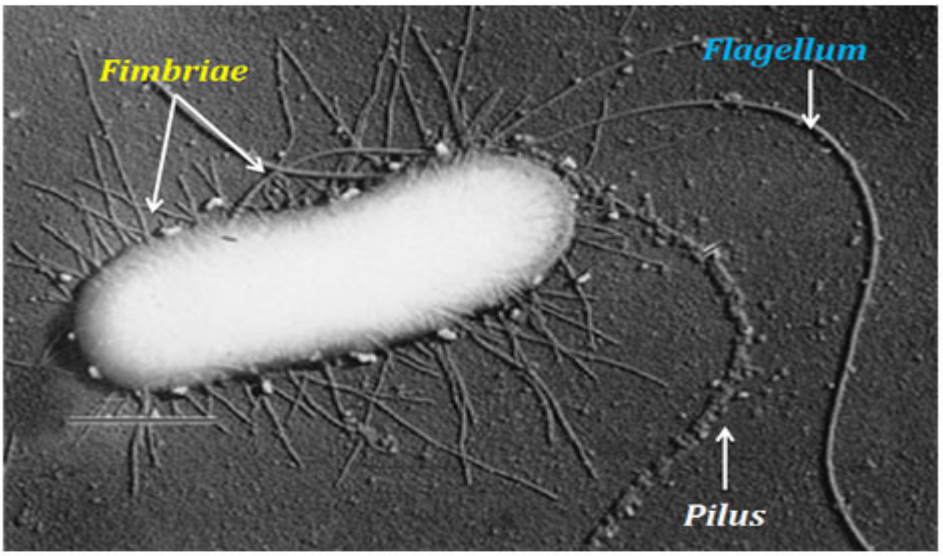


Motility of the bacterial cell is a response to a chemical substance (chemotaxis) , air (aerotaxis) , light (phototaxis) which **depends on cellular receptors** (Repellants and Attractants).

Pilli (Fimbriae): They are appendages found in many G-ve bacteria , shorter and finer than flagella , composed of protein subunits called pillin.



Pilli can be classified into 2 types:

1.Ordinary pilli : their role in adherence of symbiotic and pathogenic bacteria to host cells , which is called colonization Ag.

2.Sex pilli ; which are responsible for attachment of donor and recipient cells in bacterial conjugation.

Endospores :

1-The most common bacterial genera that form spores are Bacillus, Clostridium and the bacterial group Actiomycetes.

2-Endospores are forms of cellular differentiation undergo as a response to environmental conditions e.g. nutritional depletion.

3-The spore is a resting cell highly resistant to desiccation , heat and chemical agents.

4- At favorable environmental conditions, spores are activate and germinate producing single vegetative cells.

Structure of the spores :

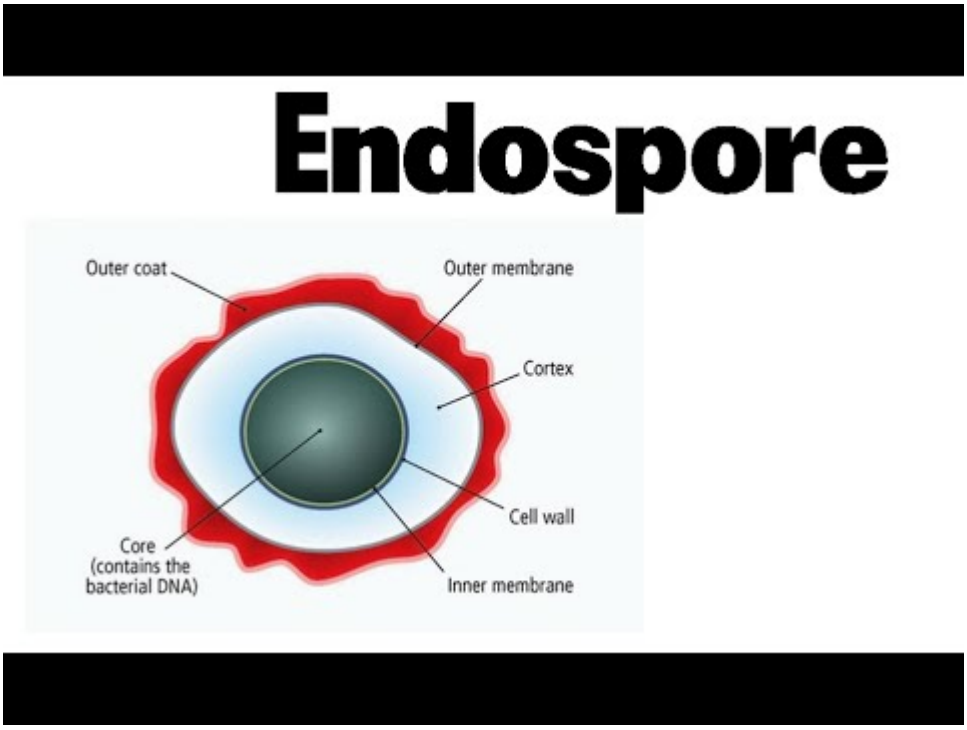
1.Core (spore protoplast) : contain DNA , proteins , enzymes of glycolysis , Ca-dipicolinate (which involves in spore heat resistance as enzyme stabilizers).

2.Spore wall ; consist mainly of peptidoglycan.

3.Cortex : represent the thickest layer of the spore envelope , composed of special type of peptidoglycan.

4.Coat : composed of keratin-like protein , pocessing high impermeability to antibacterial and chemical agents.

5.Exosporium : is a lipoprotein membrane with some carbohydrates.



Spore germination :

There are 3 main stages:

1.Activation : after resting period (days) , they can be activated by rich nutrient media, heat , abrasion , acidity or compounds containing free sulphydry groups .

2.Intiation : spores are containing receptors to recognize different effectors (signaling factors) , e.g L-alanine or adenosine (initiation triggers) .

3.Outgrowth : degradation of cortex and and outer layers releasing the protoplast with its cell wall as a new vegetative cell .

Biosynthesis period is started to build up the growing cell inside rich nutritional media to support cellular growth.

Bacterial staining:

- **Basic stains :** Consist of a colored cation and colorless anion , e.g. methylene blue+ & Cl^-.
- **Acidic stains:** Na+ and eosinate $^-$, e.g. safranine and carbol fuchsin.
- **Bacterial DNA :** are negatively charged → combine with basic dyes (positively charged).

Acidic dyes do not stain bacterial cells, and be used as a contrasting color as they stain background material .

A)The Gram´s stain : called as a differential stain (compound stain):

Procedure:

1.Crystal violet (1 min) → blue cells

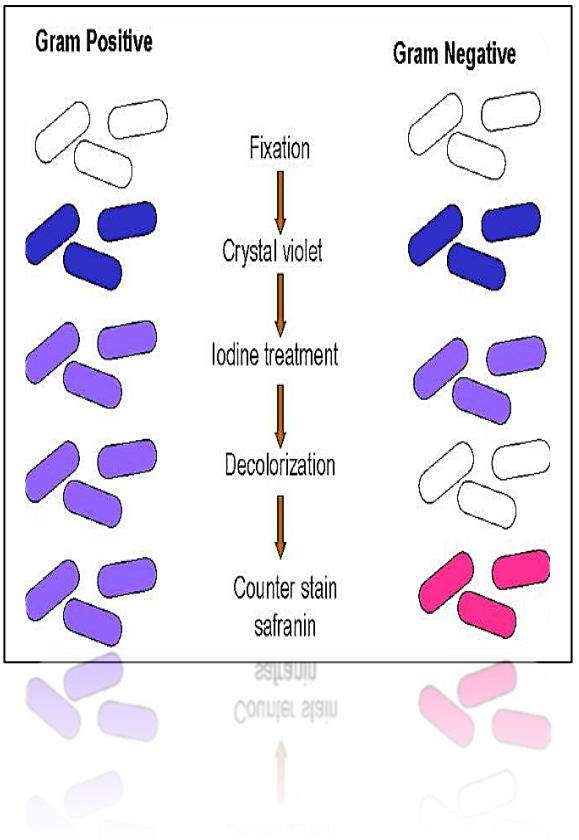
2-Iodine solution (2min) →mordant (fixative) blue cells.

3-Alcohol : (10-30 sec.)

→ Decolorize

blue cells= G+ve

→Colorless cells =G-ve



4-Counter stain (safranin) (1-2 min)

→ Bluish purple cells =G+ve

→Red cells = G-ve

5.Examining under the microscope

B)The Acid-fast stain: To stain and differentiate acid-fast bacteria , as these cells retain carbol fuchsin red dye after treatment with strong decolorizer (like acid alcohol).

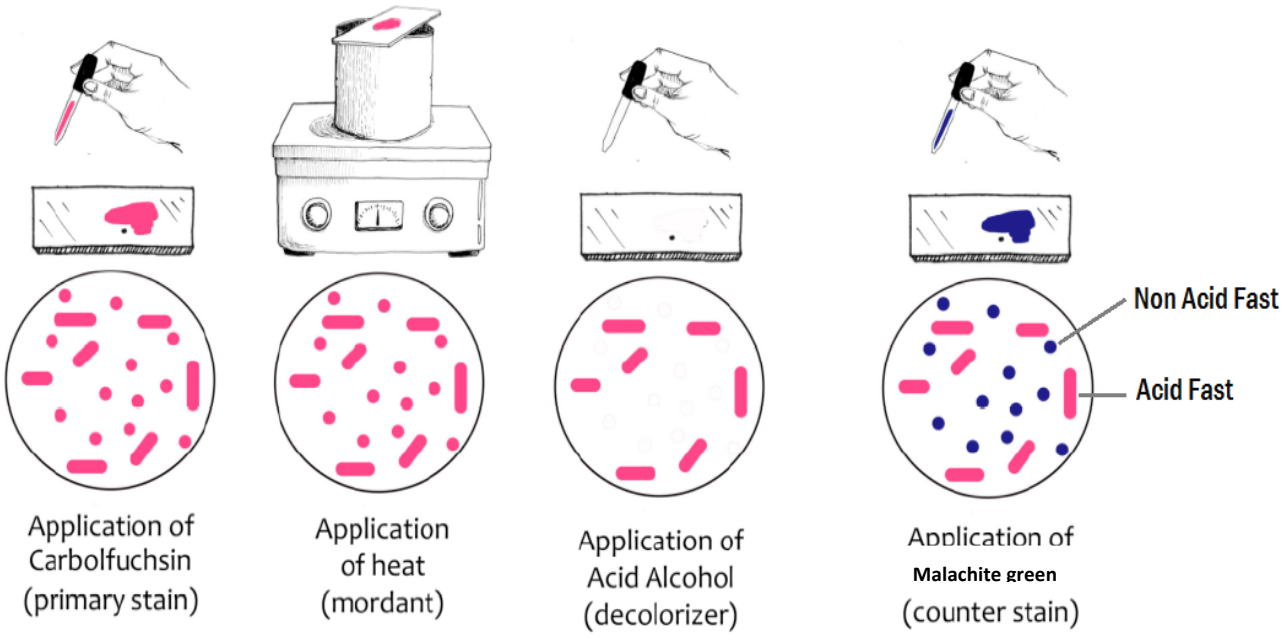
Procedure :

1.Carbol fuchsin (flooding smear) and steam bath (5 min.).

2.Acid- alcohol (decolorization) (15-20 sec.).

3.Malachite green (contrast) counter stain (1-2 min.).

- A-F bacteria (red) ;e.g. Mycobacteria and related Actinomycetes.
- Non A-F (green) … other bacteria.



c)Negative stain :staining the background with an acidic dye , leaving the cells contrastingly colorless .

Nigrosin black dye is commonly used (China ink) (India ink).

D) The flagellar stain : As flagella are too tiny and fine (12-30 ηm) , so that such structures:

"can be treated with colloidal suspension of tannic acid solution " causing heavy precipitation on flagella, which can be visualized by staining with basic fuchsin .

E) The capsular stain:

1.Using Negative stain

2.Welch's method :

- Prepare smear by air drying without heat fixation.
- Crystal violet (2 min.).
- Washing with CuSO4 solution (20%) (do not rinse with water).

F) DNA (nucleoid) stain:

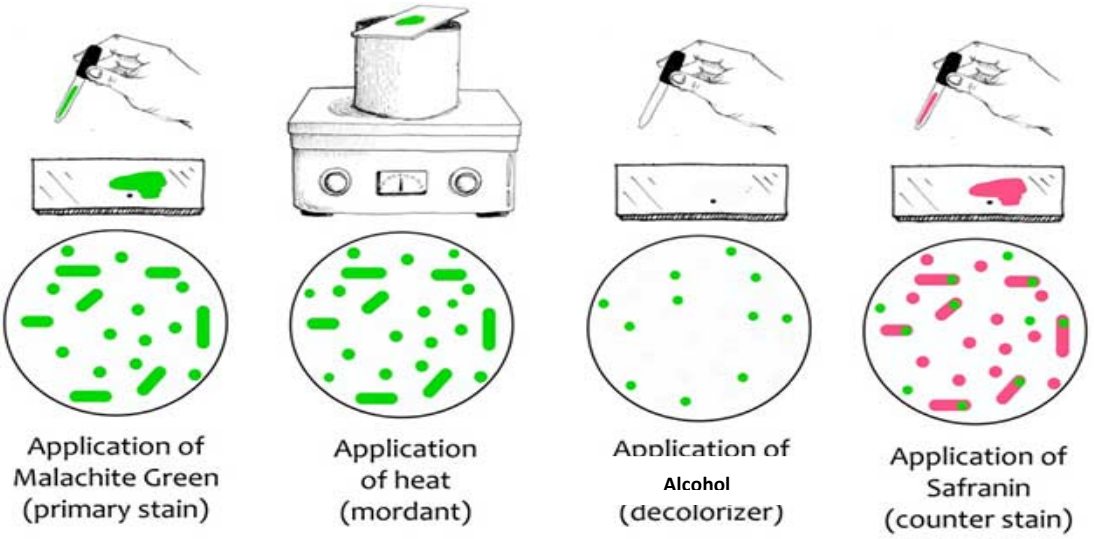
Can be stained with the specific Feulgen stain.

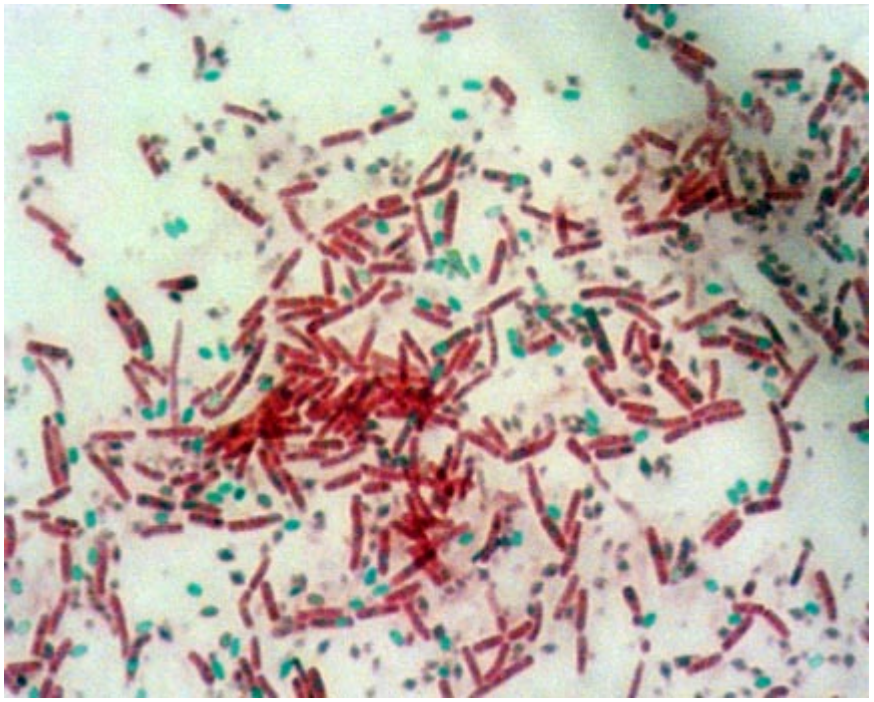
G) The spore stain:

1.Malachite green (steaming) (4-5 min).

2. Decolorization with water for few seconds.

3.Safranin (counter stain) (1-2) min.


Application of
Malachite Green
(primary stain)
Application
of heat
(mordant)
Application of
Alcohol
(decolorizer)
Application of
Safranin
(counter stain)



Bacterial physiology

Growth : Is the orderly increase in the sum of all components of an organism .

Such that ; cell multiplication and increase in cell number making up a population or culture, is a growth of unicellular organisms.

A.The measurement of microbial growth :

Depends on :

1-Measurment of viable cell number per unit volume of culture .

Or

2-Measurment of biomass concentration (dry weight of cells per unit volume of culture).

I-Cell concentration:

a.Viable cell count (plate count).

b.Measuring turbidity of a culture (photoelectric means) with standard curve.

II-Biomass concentration:

a.Dry weight of a microbial culture.

b.Estimation of cellular protein content.

c.PCV (packed cell volume).

B)The growth curve :

1.Lag phase

Cells adapt to the new environment , enzymes and other metabolites formed and accumulate to

permit cellular growth and multiplication.

2.Exponential phase (log phase) :

Cell number (biomass) increase in an exponential manner , until the exhaustion of one or more

nutrients in the medium or the accumulation of toxic products and inhibit growth .

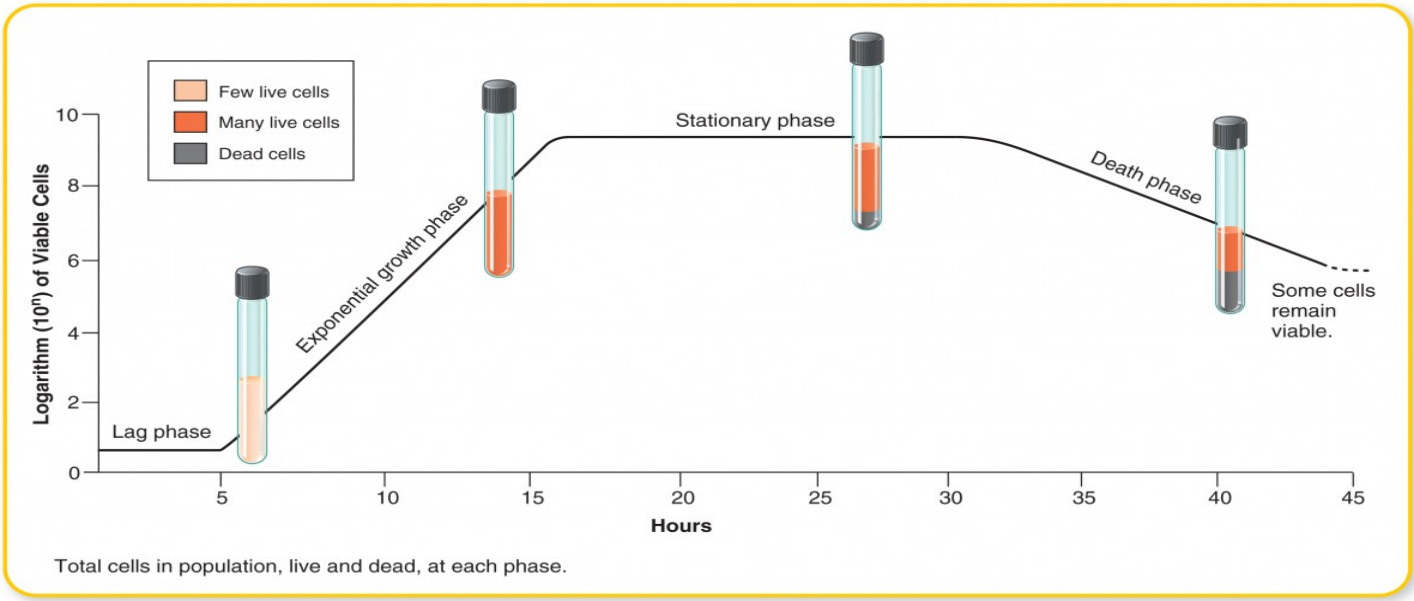
3.Stationary phase :

Exhaustion of nutrients and accumulation of toxic byproducts ceasing growth completely , such that number of new cells = number of dead cells.

4.Decline phase : (death phase)

The death rate increases , such that :

Number of dead cells > number of viable cells (survived cells).



C-Cultivation and nutrition of mo.s :

Cultivation : is the process of propagating organisms by providing the proper environmental conditions.

Environmental condition for microbial growth (Requirements of growth):

1-Temperature
2-Nutrients
3-pH of the medium
4-Aeration
5-Salt concentration
6-Ionic strength of the medium

1-Nutrition :

Nutrients in growth media should contain all the elements necessary for synthesis of new organisms:

1.1) C-source :

Autotrophs : organisms that do not require organic-nutrient (carbon for growth).

Heterotrophs : organisms that require organic carbon for growth.

Chemolithotrophs: organisms that require inorganic substrate (e.g.H2) as reductant and CO2 as C-source.

1.2) N-source :

Nitrogen is the major component of proteins and nucleic acids (≈ 10% of microbial dry weight) e.g. (NO_3^- , NO_2^- , NH_4^+ , N_2 , R-NH_2).

1.3) P-source :

PO_4^{3-} is a component of ATP , Nucleic acids and Coenzymes (NAD and

NADP) , flavins , phospholipids , teichoic acid .

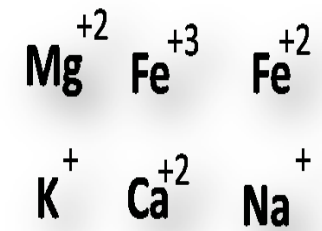
C : N : P ratio 1 : 0.1 : 0.01

1.4) S-source :

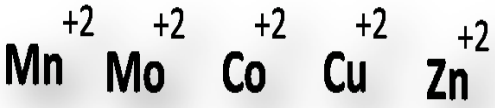
S° is not utilizable , however some autotrophs can oxidize S°→ SO_4^{2-}.

In nutrient media the usual chemical form for sulfur is SO_4^{2-} , that can be utilized and reduced to H_2S.

1.5) Mineral sources :



Major elements



Minor elements

1.6) Growth factors :

They are organic compounds necessary for cell growth , but can be able to synthesized:

Examples :

1-amino acids 2-purines 3-pyrimidines

4-vitamins 5-pentoses 6-some carbohydrates and fatty acids

2- Environmental factors affecting growth:

2.1) pH :

a.Neutralophiles : microorganisms that grow best at pH≈ 6,0 – 8,0.

b.Acidophiles : microorganisms that grow best at low pH (about 5,0).

c.Alkaliphiles : microorganisms that grow best at high pH (about 9,0).

2.2)Temp. :

a.Mesophiles : microorganisms that grow best at temp. 30-40˚c.

b.Psychrophiles : microorganisms that grow best at temp. 15-20˚c.

c.Thermophiles : mo.s that grow best at temp. 50 - 60˚c.

2.3) Aeration : (O_2 Supply)

a.Obligate aerobes : Organisms requiring O_2 as hydrogen acceptor .

b.Facultative aerobes : Organisms able to live aerobically and anaerobically .

c.Obligate anaerobes : Microorganisms are sensitive to oxygen and require another substance as a hydrogen acceptor .

d.Microaerophiles : Microorganisms that can tolerate a trace of oxygen .

2.4 : Ionic strength :

a.Halophiles : Microorganisms that require high salt concentration.

b.Osmophiles : Microorganisms require high concentration of osmotic pressure.

c.Saccharophiles : Require high sugar concentration (e.g. yeasts).

3.Cultivation methods :

These methods depend on:

a. The suitable method.

b. The microorganism.

3.1) The medium : the choice of nutrient medium depends on :

a. Just to isolate a microorganism (isolation only).

b.Need to determine number and type of the microorganism.

c.Need to isolate a particular type of microorganism.

3.2) Microorganism and the pure culture :

A pure culture is a progeny raised from one cell or a group of cells , cultivated in a certain nutrient medium .

Methods of purification :

3.2.1) Plating methods: - Pour plate method.

-Streaking.

3.2.2) Dilution to extinction method.

Antimicrobial agents and chemotherapy

A. Definitions

1.**Antibiotic :** a naturally occurring or synthetic organic compound , that inhibit or destroy selective microorganisms in low concentration.

2.**Biocide :** a broad-spectrum chemical agent , that inactivates microorganisms including:

Disinfectants	Antiseptics	Preservatives
Formaline	Chlorohexidine	Benzoic acid
Phenol	Hexachlorophene	Propionic acid
Hg-compounds	Chlorine and iodine compounds	Lactic acid
Alcohols	Alcohols	Alcohols

3.**Disinfectant:** a biocide used to kill microorganisms on inanimate (nonliving) objects or surfaces.

4.**Antiseptic :** a biocide used to kill or inhibit the growth of microorganisms in or on living tissues.

5.**preservative:** a biocide used to prevent the multiplication of microorganisms in formulated products including foods and pharmaceuticals.

6.**Sepsis:** is the presence of pathogenic microbes in living tissues.

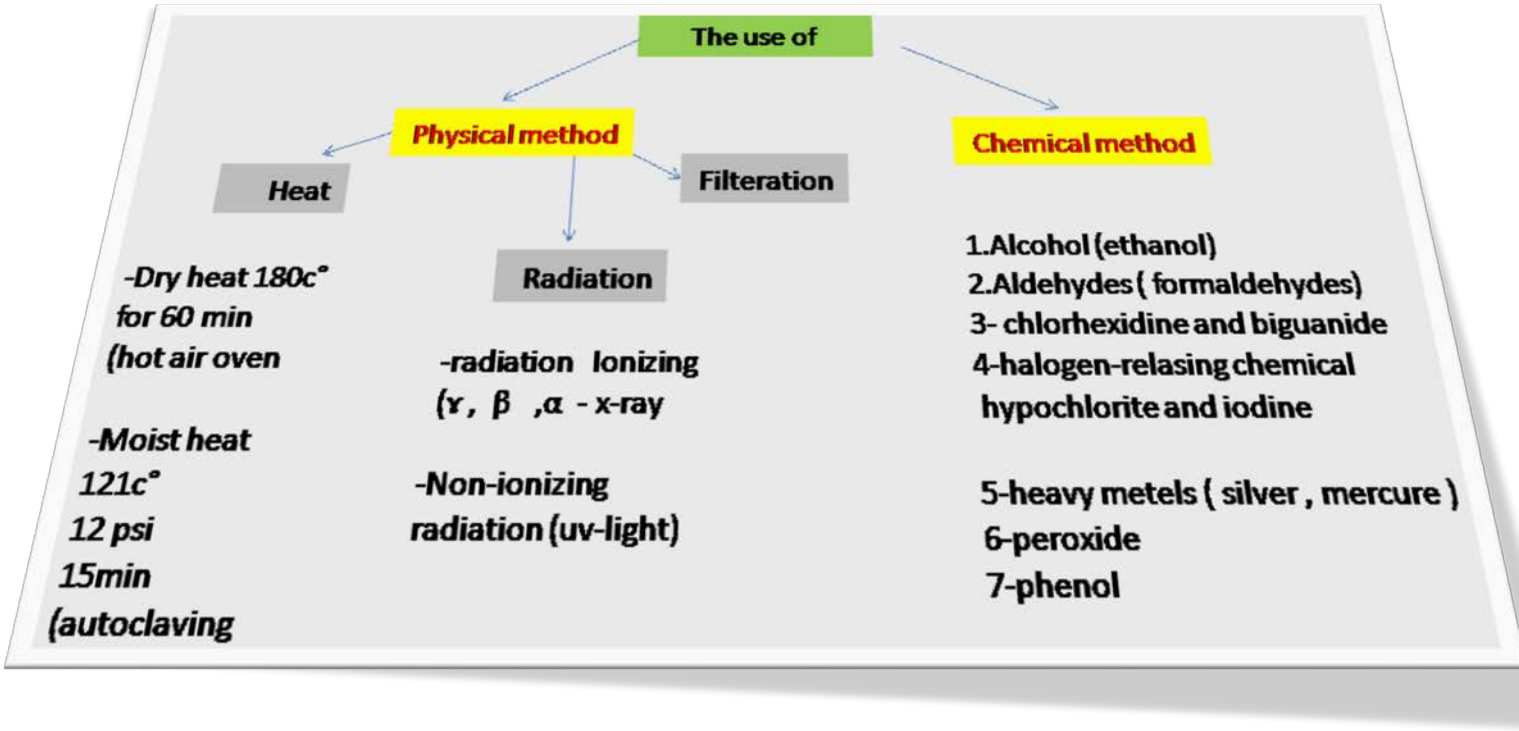
7.**Asepsis :** is the absence of pathogens.

8.**Bacteriostatic :** a biocide is able to inhibit bacterial multiplication (with reversible effect) , fungistatic , sporostatic

9.**Bactericidal :** a biocide is able to kill bacterial cells (with irreversible effect) Fungicidal , sporicidal , virucidal.

10.**sterlization :** a physical or chemical process that completely destroys or remove all microbial life including spores.

B.Methods of sterilization



C.Modes of action of antimicrobial agents :

1.Damage to DNA.

2.Protein denaturation.

3.Disruption of cell wall or membrane.

4.Removal of free sulfhydryl groups.

5.Chemical antagonism.

D. Antimicrobial chemotherapy

1.History :

-The use of herb Extracts.

- The use of quinine for malaria (since the 17^{th} century).

-The use of emetine for amebiasis.

-The discovery of penicillin by Alexander Fleming in 1929.

-The discovery of sulfonamides in 1935.

-The antibiotic era began with the discovery of streptomycin (by Salman Waxmann) , then tetracycline , chlormphenicol …. etc.

-Synthetic modification of these antibiotics were developed for the production of new drugs .

-The development of genetic engineering and production of novel antibiotics .

2.Chemotherapeutic agents:

a.Natural products : **e**.g. antibiotics produced by growing microorganisms.

b.synthetic products : e.g. sulfa drugs (chemically synthesized and produced in the lab.; or modified antibiotics (e.g. production of ampicillin from penicillin) .

Antibiotics :

-Broad-spectrum antibiotics : (against G+ve and G-ve bacteria).

-Narrow-spectrum antibiotics (against G+ve or G-ve bacteria only).

3.Mechanisms of action of antibiotics :

a.Inhibtion of cell wall synthesis : e.g. β –lactam antibiotics (penicillins and cephalosporins).

b.Inhibition of cell membrane functions : e.g. polymyxins , nalidixic acid , novobiocin , valinomycin, daptomycin , amphotericin B.

c.Inhibition of protein synthesis : e.g. erythromycin , lincomycin , tetracyclines , aminoglycosides , chloramphenicol .

d.Inhibition of nucleic acid synthesis : e.g. quinolones , rifampin , sulfonamides , trimethoprim and trimetrexate .

E.Microbial resistane to antimicrobial drugs :

1.Mechanisms of resistance:

a. Resistant mo.s produce enzymes to destroy the active drug; e.g. β-lactamases produced by staphylococci that resistant to penicillin –G .

b. Resistant mo.s change their permeability to the drug; e.g. resistance to tetracyclines and polymyxins.

c. Resistant mo.s develope an altered structural target for the drug; e.g. Resistance to erythromycin and cephalosporins.

d. Resistant mo.s develope altered metabolic pathway by-passing the reaction inhibited by the drug; e.g. resistance to some sulphonamides .

e. Resistant mo.s develope an altered enzyme to perform the metabolic function; e.g. resistance to trimethoprime.

2.Origin of drug resistance :

a.Non-genetic : e.g. L-form bacteria (cell wall deficient) are non-susceptible to penicillins and cephalosporins.

b.Genetic :

- Chromosomal .
- Plasmids (extrachromosomal elements).

Cross resistance : mo.s resistant to a certain drug may also be resistant to other drugs due to:

1.Drugs are closely related in chemical structure; e.g. resistance to aminoglycosides.

2.Drugs have a similar mode of action; e.g. resistance to penicillins and cephalosporins .

Group of Antibiotics	Mechanism of action	Examples	Used against	Producer mo.
1. B-Lactam antibiotics	Inhibition of cell wall synthesis	a.Penicillin* : P.V, P.G, Amoxcillins ,Ampicillins b.Cephalosporins : 1st generation: cephalexiin , cephalothin 2nd generation : cephonicid , cefaclor 3rd generation : cefotaxime , ceftazidime 4th generation: cefepime , cefpirome 5th generation: ceftobiprole	G+ve bacteria (bactericidal)	Penicillium notatum*
2. Tetracyclines	Inhibition of protein synthesis	Tetracycline* , doxycycline , minocycline Glycylcyclines (synthetic tetracyclines))	G+ve and G-Ve (Bacteriostatic)	Streptomyces aureofaciens*
3. Chloramphenicol*	Inhibition of protein synthesis	_____	G+ve and G-ve (bacteriostatic	streptomyces venezuelae*
4. Erythromycins	Inhibition of protein synthesis	Erythromycin*, Clarithromycin Dirithromycin	G+ve	St. erythreus*
5. Clindamycin and Lincomycin	Resembling erythromycin in mode of action and antibacterial spectrum	Clindamycin Lincomysin*	Bacteroids and anaerobes	Streptomyces lincolnensis*
6. Glycopeptides	-inhibition of cell wall synthesis, and -disruption of cell membrane	Vancomycin , teichoplamin Daptomycin , bacitracin Polymyxin , gramicidin S*	G+ve and G-ve (bactericidal)	Bacillus brevis* ((Nephrotoxic))
7. Aminoglycosides	Inhibition of protein synthesis	Streptomycin* , neomycin, kanamycin , amikacin , gentamicin , tobramycin . Sisomycin	Minaly against G-ve (and some G+ve)	Streptomyces griseus* Nephrotoxic ((mainly ototoxic))
8. Quinolones	Inhibition of DNA synthesis synthesis	Nalidixic acid , Ciprofloxacin , norfloxacin	G-ve and G+ve	_____

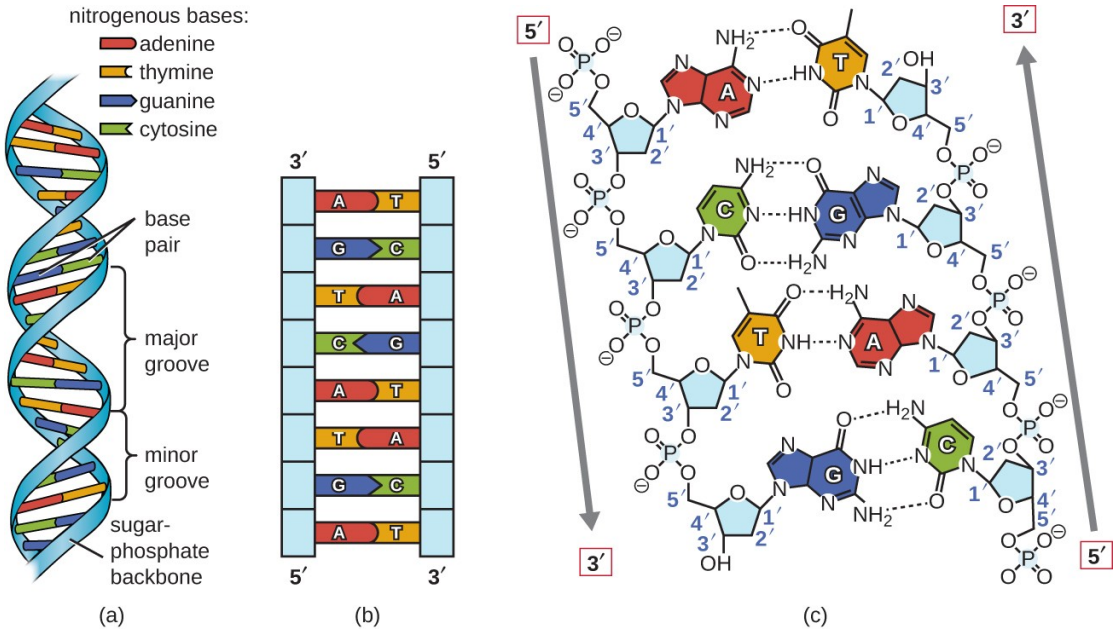
9. Sulfonamides and Trimethoprim	**Competitive utilization of PABA for synthesis of folic acid**	**Trimetroxate, Metronidazole (best for treatment of UTI urinary tract infections)**	**G+ve and G-ve (bacteriostatic) Also for chlamydia, nocardia and protozoa**	**_____**

Microbial Genetics

A-DNA structure :

A-T

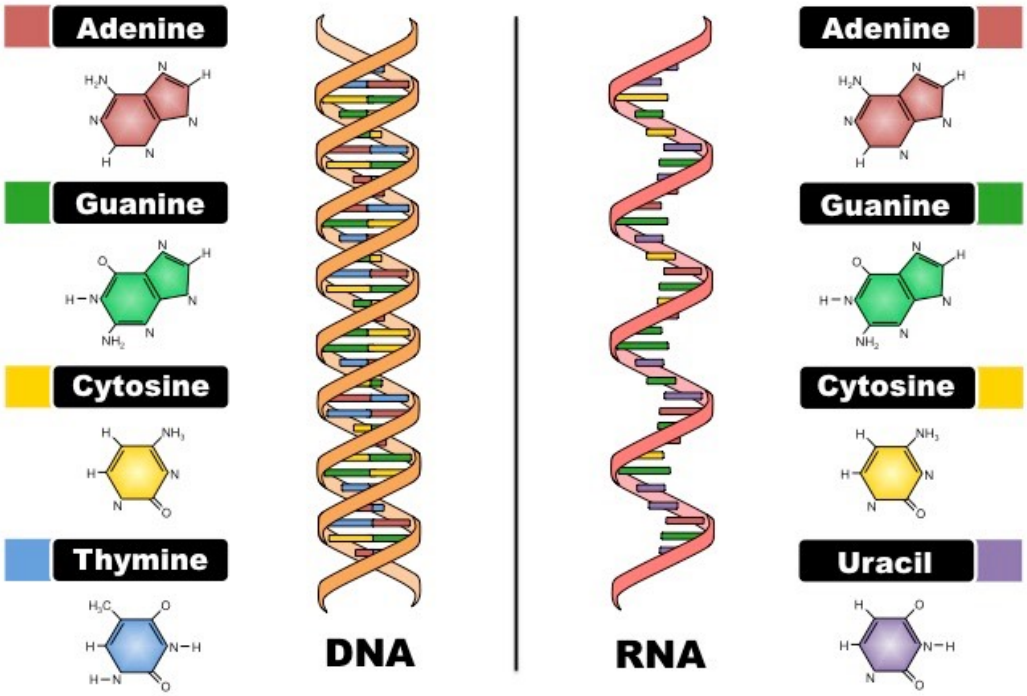
G-C



B-RNA structure : In mRNA , tRNA and rRNA

A-U

G-C



- bp(base pair)
- kbp(kilo base pair): is usually expressing the length of DNA molecules
- 5kbp=genome of a small virus
- 4639kbp=genome of E.coli

Genome : is the total genetic information in an organism

Eukaryotic genome :

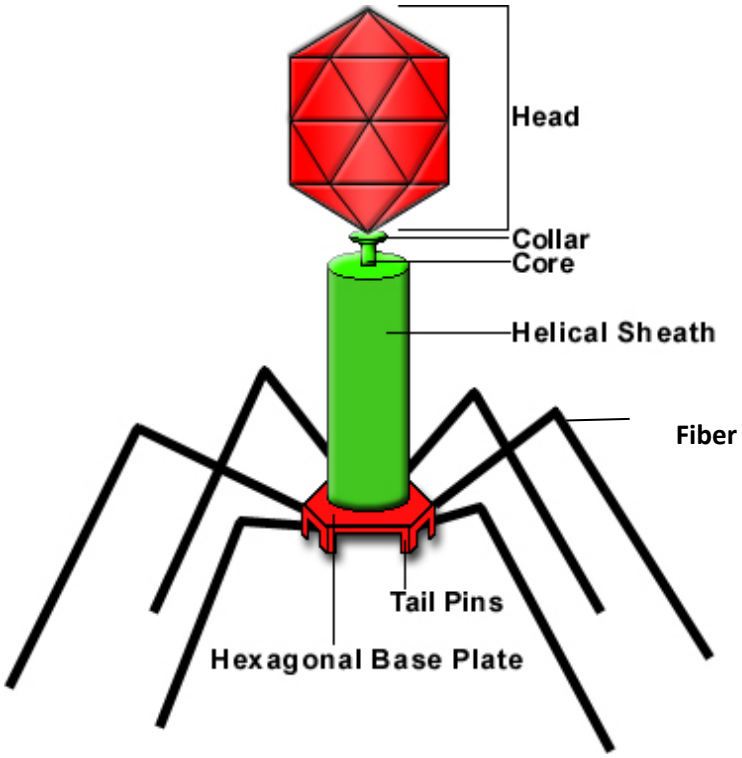
Is represented by two or more linear chromosomes separated from the cytoplasm by the nuclear membrane (nucleus). These chromosomes are in diploid number (2n) (contain 2 homologues chromosomes).

Yeast cells may contain plasmids which are small additional genetic elements (DNA) self–replicating, about 6kbp in size, encoding few minor cellular functions.

Prokaryotic genome:

In most bacteria genome is represented by a bacterial circular chromosome (haploid =1n). Replicons are exist on this genome (which are the necessary genetic informations for genome replication) .

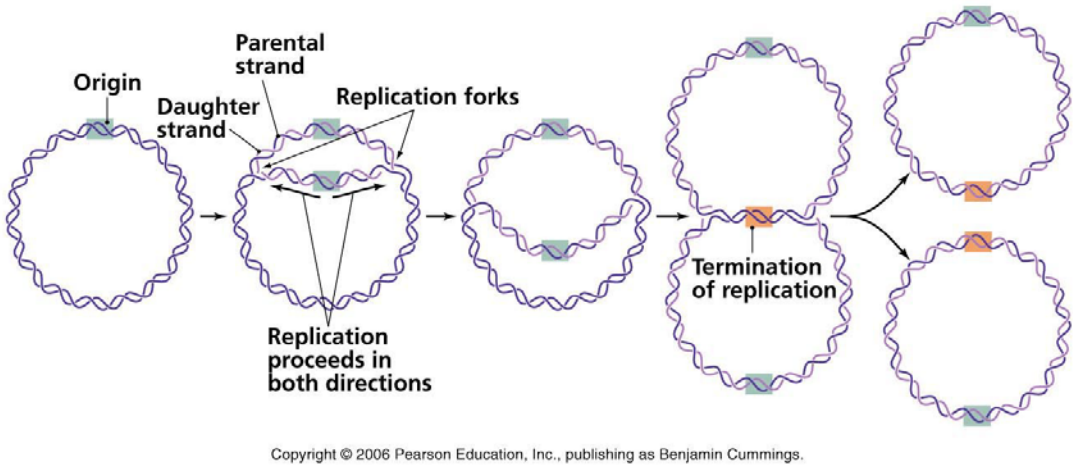
The viral genome :



Bacteriophage T2

DNA replication :

DNA recplicate by the semiconservative mechanism of replication. In bacteria, circular DNA molecule (chromosome or plasmid) replicate by the bidirectional replication.



Gene transfer :

DNA can be transferred from one organism to another by the mechanisms :

a.Conjugation

b-Transformation

c-Transduction

Mutations:

Mutation: is a change in DNA base sequence.

a-Spontaneons mutation : Occure in a frequency of $(10^{-6} - 10^{-8})$ in a population derived from one cell.

Mutations include :

1. Base substitution
2. Deletion
3. Insertion
4. Rearrangement of N.B.s

b- Other mutations : can be done using different mutagens :

1-Physical mutagens

i- U.V light

ii- g-ray

iii- X –ray

2-Chemical mutagens

i- HNO2 (nitrous acid)

ii- Acridine dyes

Gene expression :

Genetic informations are encoded in DNA molecules as sequences of N.B.s:

DNA

↓

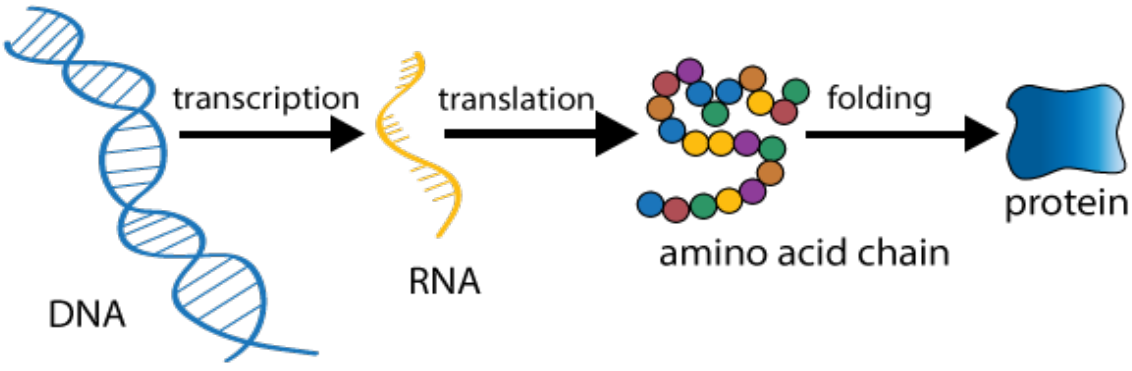
-----ATCCCGGAATTACTAGCCC----

↓

DNA is transcribed into mRNA (Transcription)

↓

Ribosome (m RNA translate genetic informations through tRNA into the structure of proteins) (Translation)



Normal Microbial flora of the human body

Normal microbial flora : is the population of mo.s that inhabit the skin and mucous membranes of a healthy person.

There are 2 types of microb. flora:

1- Resident microb. Flora (relatively fixed types of mo.s regularly found in a given area and a given age) .

2-Transient flora (pathogenic or non pathogenic mo.s that inhabit the skin and mucous mem.s for hours ,days ,weeks,……) .

General characteristics :

1- Normal flora depends in their existance on temp., moisture , nutrients and any inhibitory substances.

2-N. flora are not essential for life.

3-Some flora can produce useful substances (e.g. production of vitamin K by intestinal flora).

4-N. flora can prevent colonization of pathogens on skin and mucous membranes by competition for place , nutrients , production of antibiotics and/or bacteriocins , binding on host cells .

5-N. flora may become pathogenic and produce diseases or infections if they introduced into blood stream and other tissues.

I – N. flora of the skin :

The predominant resident mo.s are :

1- Aerobic and anaerobic diphtheroid bacilli (belongs to the genera *Corynebacterium* and *Propionibacterium*).

2-Nonhaemolytic staphylococci (*Staphylococcus epidermidis* and coagulase –negative *S.aureus*) .

3- G + ve ,aerobic ,spore –forming bacilli.

4- α-haemolytic *streptococci* (*Streptococcus viridans*).

5-Enterococci (*Enterococcus* sp.).

6-G-ve coliforms .

7- Fungi and yeasts (in skin folds).

8-Acid-fast nonpathogenic mycobacteria (in genitalia and external ear) areas rich in sebaceous secretions.

II. N.flora of the mouth and upper respiratory tract :

1-Nose: include

I-Corynebacteria.

II-Staphylococci (*S. epidermidis* ; *S. aureus*).

III-Streptococci.

2- Mouth and pharynx :

Sterile at birth ; within few hours after birth ,streptococci become predominant for life.

During life ,tens of bacterial forms inhabit buccal mucosa.

3-Pharynx and trachea :

Similar flora as above ; while small bronchi and alveoli are sterile.

III. N. flora of the intestinal tract :

1- At birth : the intestine is sterile .

2-In breast-fed children : large numbers of lactic acid streptococci and lactobacilli are developed.

(Lactic acid bacteria +*Bifidobacterium* produce acid from carbohydrates and lower pH down to pH 5).

3-In bottle–fed children : Lactobacilli are much less, and more mixed flora exists in the bowel.

4-Bowels of newbrons :Colonized by enterobacteriaceae members (*Klebsiella ,Citrobacter ,Enterobacter*).

5-Esophagus :Contains microorganisms arriving with saliva and food.

6-Stomach : Due to acidity, low numbers of mo.s $(10^3 - 10^5)$ cells/g of contents , including G+ve cocci and bacilli.

Low pH protects against infection with some enteric pathogens , e.g. *Vibrio cholerae* .

7- Intestine :keeping a relatively high pH :

i- Duodenum : $(10^3 - 10^6)$ bacteria /g of contents.

ii-Ileum : $(10^5 - 10^8)$ cells /g .

iii- Cecum and colon :$(10^8 - 10^{10})$ cells/g .

Lactobacilli and enterococci are predominant in upper intestine . While:

Fecal flora are dominant in lower ileum and cecum ,reaching up to (10^{11}) bacteria /g of contents.

8- In normal adult colon :

- 96 – 99% of microflora are anaerobes , e.g .*Bacteroides* ,*Fusobacterium* , *Bifidobacterium* , *Clostridium* , *Peptostreptococcus* .
- 1-4 % are facultative aerobes (G-ve coliforms and enterococci) .
- Small numbers of *Proteus* , *Pseudomonas* ,*Lactobacillus* , *Candida* and other *protozoa.*

9- Intestinal flora are important in :

I- Synthesis of vitamin K .

II- Conversion of bile pigments and bile acids .

III-Absorption of nutrients and breakdown products .

IV- Antagonism to microbial pathogens .

IV. Normal flora of the urethra :

The anterior part of urethra of both sexes contains small numbers of skin microflora. $10^2 - 10^4$ bacterial cells /ml urine is regularly appear in normal voided urine.

V . Normal flora of the vagina :

1- After birth : aerobic lactobacilli appear in vagina and persist causing the acidic pH of vaginal environment for several weeks .

2-When pH becomes neutral , a mixed flora of cocci and bacilli is present (remaining so until puberty).

3- At puberty : aerobic and anaerobic lactobacilli reappear in large numbers and maintain low pH through the production of lactic acid from fermented glycogen in vaginal mucosa.

4- After menopause :Lactobacilli again diminish in number and a mixed flora returns.

VI. N. flora of the conjunctiva :

The predominant mo.s are :

I- Diphtheroids.

II-*Staphylococcus epidermidis.*

III- nonhemolytic streptococci .

IV - Neisseriae .

V- G-ve bacilli (*Moraxella)* .

Conjunctival flora can be examined by the collection of tears which contain antibacterial lysozyme.

Spore – forming G+ve bacilli :

I- *Bacillus* sp. , *Clostridium* sp. , Actinomycetes and related bacteria.

General characteristics :

1- They are widely spread in the environment particularly in soil.

2-They can survive for many years.

3- *Bacillus* species are aerobes ,facultative anaerobes ; while *Clostridium* spp are anaerobes.

4- Most species of the two genera do not cause diseases; others like :

a- *Bacillus anthracis* causes anthrax.

b- *B. cereus* causes food poisoning.

c- *Clostridium tetani* causes tetanus.

d- *C. botulinum* causes botulism.

e- *C. perfringens* causes gas gangrene.

f- *C. difficile* causes pseudomembranous colitis.

I.A – *Bacillus* sp :

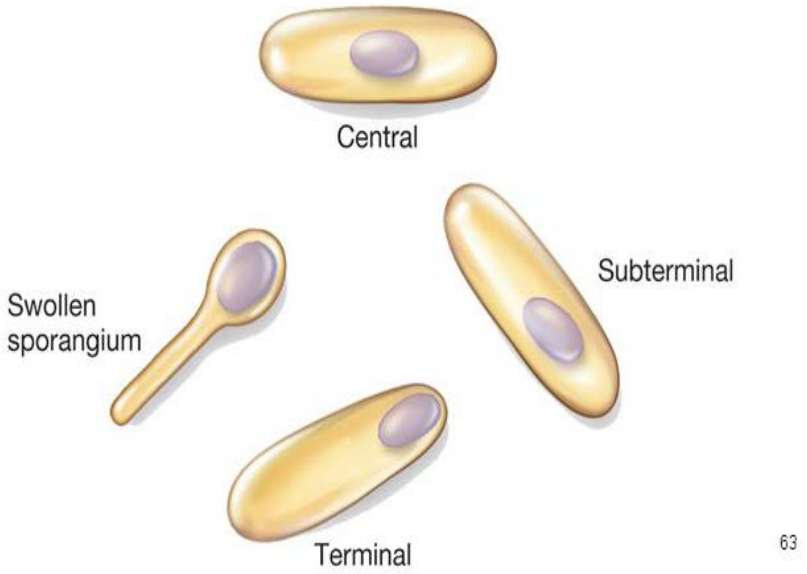
1- Large ,aerobic ,G+ve rods in chains , forming spores ,most members are saprophyte in soil , water ,air and on vegetation , e.g. *Bacillus subtilis.*

2- Some are insect pathogens , e.g . *B. thuringiensis*.

3-Some are pathogens to human and animals , e.g. *B. cereus* producing enterotoxin when grow in foods , causing food poisoning .

General morphology and identification :

a- Typical cells : measuring 1x3-4 µm with square ends ,usually arranged in long chains, spores are located in the center of the nonmotile bacilli.



b- Saprophyte members utilize simple sources of C&N for energy and growth. The spores are resistant to environmental changes , but can be sterilized by autoclaving

Bacillus arthracis

Pathogenesis :

Humans become infected by contact with infected animals or their products, the infection is usually acquired by the entery of spores through:

I- Injured skin causing **cutaneous anthrax**.

II-Mucous membranes causing **gastrointestinal anthrax**.

III- Inhalation of spores into the lung causing **inhalation anthrax** .

The spores germinate in the tissues producing veg. cells, **the virulent cells produce:**

1-**Capsular antigen** : (composed of poly –D – glutamic acid) which is highly antigenic (antiphagocytic) inducing anthrax disease.

2-**Anthrax toxin** : which is composed of 3 different proteins :

a-Protective antigen

b-Edema factor

c.Lethal factor

It is called Lethal toxin (which is the major virulence factor causing death of infected animals).

In inhalation anthrax (woolsorter's disease):

I-Spores from dust of wool , hair or hides inhaled to the lungs.

II- Taken by phagocytes (engulfed), and transported by lymphatic circulation to lymph nodes.

III- Spores germinate there ; vegetative cells produce toxin, causing hemorrhage and sepsis , which are usually fatal.

IV -In such cases: number of bacterial cells in blood stream exceeds 10^7 /ml.

Diagnostic lab. tests :

a-Specimens :

1-Fluid or pus from local lesions.

2- Blood.

3- Sputum.

b-Stained smears : chains of G+ve rods.

c-Arthrax bacilli can be identified in dried smears by immuno-fluorescence staining technique.

d- Bacterial growth on blood agar: nonhemolytic, gray to white colonies with a rough texture.

e- Carbohydrate fermentations : are not useful.

f- Anthrax bacilli are nonmotile in semisolid medium.

g-Virulent cultures kill mice or guinea pigs upon intraperitoneal injection.

h- Bacterial cultures isolated from infected lab. animals, **demonstrating capsule**.

Treatment:

1-Ciprofloxacin.

2- Penicillin G + Gentamicin.

3-Penicillin G + Streptomycin.

Bacillus cereus

Causing 2 types of **food poisoning** :

1- **Emetic type :** poisoning is accompanied with nausea ,vomiting ,abdominal cramps and diarrhea ; recovery occurring within 24 h.

2- **Diarrheal type** : poisoning is accompanied with profuse diarrhea and abdominal pain and cramps ; fever and vomiting are uncommon.

- ∅ The **enterotoxin,** which causing food poisoning, may exist in food or produced in the intestine .
- ∅ *B. cereus* also causing **eye infections**, **severe keratitis**, **endophthalmitis**. This organism can associate with **endocarditis** , **meningitis , osteomyelitis and pneumonia.**

Five *Bacillus* species are insect pathogens:

- ∅ *B. thuringiensis* .
- ∅ *B. popilliae* .
- ∅ *B. sphaericus* .
- ∅ *B. larvae* .
- ∅ *B .lentimorbus* .

I.B – genus *Clostridium*

General characteristics of genus members :

1-Large , anaerobic , G+ve , motile, spore-forming rods.

2-Many members **decompose proteins** or **form toxins** , and some do both.

3- Their natural habitat is the soil or the intestinal tract of animals and humans.

4-They live as saprophytes.

5- Few are causing sereous diseases or poisoning ; e.g. botulism , tetanus , gas gangrene and pseudomembranous colitis.

Cellular morphology :

1-Spores of clostridia are usually wider than rods, located **centrally** , **subterminally** or **terminally.**

2- Most clostridia are **motile** with **peritrichous** flagella.

Cultural and growth characteristics :

1-**Anaerobes** (grow under anaerobic conditions); few members are microaerophiles .

2- **Grow well on blood agar** ; many members produce a zone of hemolysis.

3- Some clostridia form large raised colonies (like *C. perfringens*) ; others form small colonies (like *C. tetan*i); some are spreading on the agar surface (**swarming**) .

4- Clostridia can **ferment sugars** , **digest proteins** ; milk turned acid by some; or digested by others (called stormy fermentation by *C. perfringens*).

Clostridium botulinum

1- This organism causes **botulism** **(food poisoning).**

2- The bacteria found in soil and animal feces.

3- Spores are highly resistant to heat , withstanding 100°c for several hours.

Botulinum toxin :

1- Toxin is produced by growing cells ; liberated from autolysed dead cells.

2-There are 7 different antigenic varieties of the toxin (A - G) are known.

3- Toxin type C produces **paralysis in birds** ; **type D causes botulism in mammals.**

4- The toxin is absorbed from the gut and binds to **receptors of presynaptic membranes of motor neurons** , **inhibiting the release of acetylcholine** at the synapse ;**resulting in lack of muscle contraction and paralysis**.

5-The lethal dose for a human is ≅ 1-2 µg ; the toxins are destroyed by heating for 20 min.s at 100°c.

6- The illness caused by *C. botulinum* is not an infection; it is rather intoxication resulting from the ingestion of food contaminated with the bacteria, grown and produced the toxin.

Clinical findings :

Symptoms begin 18 -24 h.s after ingestion of the toxic food , with :

1- Visual disturbances(double vision).

2-Inability to swallow.

3- Speech difficulty.

4- Progression of bulbar paralysis.

5- **No fever , no gastrointestinal symptoms** ; the patient remains fully conscious until shortly before death ; death occurs from respiratory paralysis or cardiac arrest.

6- The mortality rate is high in botulism.

Treatment

1- Trivalent (A, B, E) antitoxin must be promptly administered intravenously.

2-Adequate ventilation must be maintained by mechanical respirator ,if necessary.

These measures reduce the mortality rate from 65 % to below 25% .

Prevention & control

Canned foods , home- canned foods, vacuum-packed fresh fish, beans , corn , olives , peas , **all should be** :

1-Boiled for 20 min.s before consumption.

2- Swollen cans , rancid and spoiled food , innocuous appearance (**must be destroyed**) .

3- **Toxoids** are used for **active immunization** (for cattle).

Clostridium tetani

- It's normal habitat is the soil and animal feces, particularly horses .

- All types of *C. tetani* produce the same antigenic type of **neurotoxin** (**tetanospasmin**).

Tetanus toxin :

1- **Tetanospasmin** is produced by vegetative cells inside the wounds (molecular weight of 150 000 daltons) .

2-The toxin affects on **spinal cord** and **brain stem** , leaving **muscles in contineous contraction with muscular spasm and spastic paralysis**.

Pathogenesis :

1- The infection is strictly localized in area of destroyed tissues **(punctured wounds ,burns ,injury ,umbilical stump ,surgical suture).**

2- Spores of C. tetani can contaminate the tissue , and germinate to vegetative cells and produce the toxin under low oxidation –reduction potential .

3- The toxin released from veg .cells , **reaching the CNS and fixed to receptors in the spinal cord and brain stem** , and exerts the actions described.

Prevention & treatment:

1- Active immunization with tetanus toxoid.

2- Proper care of wounds contaminated with soil (particularly punctured wounds).

3- Prophylactic use of antitoxin (250 - 500 units of human tetanus antitoxin =human tetanus immunoglobulin).

4-Administration of β- lactam antibiotics.

Patients with developed symptoms of tetanus (locked jaw), should receives:

1-Muscle relaxants.

2- Sedatives.

3- Assisted ventilation .

4- Large doses of antitoxin (3000 – 10000 units of HTI) intravenously.

5- Surgical removement of all necrotized tissue .

6- Penicillin injections.

7- Dose of tetanus toxoid for previously immunized persons.

Control : is by the administration of " Triple Vaccine " tetanus toxoid + diphtheria toxoid +pertussis vaccine (*Bordetella pertussis).*

Clostridium perfringens **(formerly *C. welchii*) and related species**

1- A group of different spp . (about 30 members), that ,if introduced into damaged tissues, can produce invasive infection (causing myonecrosis and gas gangrene).

2- 90% of such cases caused by *C.perfringens* and some by *C .septicum* , *C.novyi* , *C. histolyticum* .

3- *C. perfringens* produce an enterotoxin , **which is a common cause of food poisoning** (similar to diarrhoeal food poisoning caused by *Bacillus cereus).*

Toxins :

1- α-toxin (lecithinase).

2- θ-toxin (hemolytic & necrotizing effects).

3- DNAase (digest DNA).

4-Hyaluronidase (digest hyaline of cartilage tissue).

5- Collagenase (digest collagen of subcutaneous tissues and muscles).

6- Enterotoxin.

Gas gangrene :

1- Spores reach to tissues by contamination with soil or feces , or from intestinal tract.

2- Spores germinate at low oxidation –reduction potential ; veg. cells multiply , ferment carbohydrates present in tissues, swollen tissues interfere with blood supply.

3-Secretion of necrotizing toxin and hyaluronidase , permit the spread of infection.

4-Tissue necrosis extends and increasing bacterial growth, causing haemolytic anemia ,and severe toxemia , and death.

5- Wound like compound bone fracture ,is distinguished with foul-smelling discharge, rapidly progressing necrosis , fever , hemolysis , toxemia , shock and death.

Diagnostic lab tests

1-Specimens (material from wounds ,pus and tissues) would:

2-Gram staining: presence of large G+ve rods in smears (spores are not regularly present).

3-Inoculation of material into cooked meat-glucose medium; thioglycholate medium and blood – agar plates , incubated anaerobically.

4- Growth from one of these cultures is transferred into litmus milk broth , stormy fermentation is appeared within 24 h.s , suggesting the presence of *C. perfringens*.

5- Hemolysis and lecithinase activities ensure identification.

Treatment of gas gangrene

1-Surgical amputation of tissues or organs.

2- Administration of penicillin.

3- Administration of antitoxins (polyvalent antitoxin).

Clostridium difficile

1-This bacteria causing **pseudomembranous colitis** ,which is a diarrhea caused by colitis due to the administration of antibiotics (antibiotic associated diarrhea) .

2- The illness is diagnosed by :

I - Detection of one or both *C. difficile* toxins in stools of the patient.

II -Endoscopic observation of pseudomembranes or abscesses in patient's colon who have diarrhea and have been given antibiotics.

3- Symptoms of the disease :

I – The diarrhea may be watery or bloody.

II- the patient frequently has associated abdominal cramps , leukocytosis and fever .

4- Ampicillin and clindamycin are the most common antibiotics associated with p.m.c , due to the proliferation of drug resistant *C. difficile* .

5- The organism produces 2 toxins :

I – Toxin A enterotoxin (also cytotoxic).

II – toxin B cytotoxin.

Non – spore forming G+ve bacilli

Including the genera :

Corynebacterium, Propionibacterium , Listeria, Erysipelothrix

General characteristics :

1-Non –spore forming G+ve bacilli.

2- Many members of the group are anaerobes , some are aerobes.

3- Mostly their natural habitats are skin and mucous membranes of humans, and can be found in animals and plants.

4- Some can produce a powerful **exotoxin** , e.g **diphtheria toxin** in humans.

5-Some members have high G+C content

6-The most medically important member is *C . diphtheriae.*

Corynebacterium diphtheriae

1- Aerobe ,G+ve , irregular shape rods, with high G+C content.

2- Characterized by possessing irregular swellings at one end of the rod (club- shaped), 0.5-1 µm in diameter or longer

3- Irregularly distributed metachromatic granules stained depply with aniline dyes.

4- Due to these morphological characteristics , *Corynebacterium* and other related bacterial genera are called Coryneform bacteria, including:

Corynebacterium, Brevibacterium, Mycobacterium, Rhodococcus, Arethrobacter and some other bacteria.

5- On blood agar, colonies are small, granular and gray , may have small zones of hemolysis.

6- Can grow on agar containing potassium tellurite ; colonies appear brown to black (with brown –black halo) due to the precipitation of tellurite intracellularly.

7- **There are 4 biotypes of *C. diphtheriae* :**

I- gravis

II- mitis

III- intermedius

IV- belfanti

8- the bacteria can grow on Loeffler's serum medium.

9- Rods of *C. diphtheriae* tend to show pleomorphism (rod –coccus cycle).

10- toxigenic strains of *C. diphtheriae* are lysogenic.

Pathogenesis :

1-Cells of *C.diphtheriae* occurs in the respiratory tract , wounds , or skin of infected persons or normal carriers

2- Cells can spread by droplets or contact to susceptible individuals.

3- Bacilli can grow on mucous membranes or in skin abrasion.

4- Toxigenic bacteria capable of producing an exotoxin causing diphtheria.

5- Produced diphtheria toxin , is a heat –labile protein , with a lethal dose of 0.1 µg/kg body wt. ; it's lethal effect is the inhibition of protein synthesis in the ribosomes.

Pathology :

1- Diphtheria toxin is absorbed into the mucous membranes and causes destruction of epithelium and inflammatory response.

2- Necrotized epithelium embeded with exuding fibrin and RBCs and WBCs; and forming a grayish pseudomembrane over the tonsils ,pharynx or larynx. Any attempt to remove the pseudomembrane causing bleeding.

3- The regional lymph nodes in the neck enlarged and could be swollen due to edema.

4- Continuous production of the toxin by growing bacteria; **the toxin distribute to other parts and organs causing damages to** heart ,muscles , liver , kidneys , adrenal gland and gross hemorrhage ; as well as to nerve damage **resulting in paralysis of eye muscles , soft palatte or extremities**.

Clinical findings

1- The diphtherial inflammation in the respiratory tract begins with sore throat and fever.

2- Prostration and dyspnea because of the obstruction caused by the membrane. This obstruction may cause suffocation.

3- Irregularity of cardiac rhythm indicate damage to the heart.

4- Later , there may be difficulties with vision ,speech , swallowing or movement of the arms or legs.

Diagnostic lab . tests:

1- swabs from the nose , throat and other lesions are obtained (before administration of antibiotics).

2- Swabs should be placed in semisolid medium.

3- Smears stained with alkaline methylene blue or Gram's stain , showing beaded rods.

4- Inoculate on blood agar plates ,Loeffler's agar slants and Tellurite agar plates ; incubate at 37° C to recognize *C.diphtheriae*.

5- Diffusion plate assay is performed for the detection of toxigenic strains.

6- PCR is recommended to the detection of diphtheria toxin gene (*tox*).

7-ELISA technique for detection of the toxin .

Treatment :

1- Injection of diphtheria antitoxin (20000 -100000 units) given intramuscularly or intravenously.

2- Penicillin or erythromycin injections , to inhibit the growth of diphtheria bacilli.

Listeria monocytogenes

1- Short, G+ve, non –spore forming rods.

2- Motile at temp . 22-28°c ,but not at 37°c, with tumbling movement.

3- Grows on Mueller–Hinton's agar and blood agar (showing zone of hemolysis).

4- The organism is facultative anaerobe , catalase +ve.

5- Produce acid (partial fermentation) but not gas (complete fermentation) in carbohydrates.

Pathogenesis :

1-Causing listeriosis by entering the gastrointestinal tract (due to eating contaminated cheese or vegetables).

2- Bacterial cells are phagocyted by the epithelial cells.

3- After phagocytosis ,the bacterial cells produce listeriolysin O enzyme ,causing lysis the membrane of lysosomes (of phagocytes), escaping into the cytoplasm of the cell and rupturing it.

4- The cycle begins again , and *L. monocytogenes* move from cell to cell spread the infection , with other bacteria like *Shigella* and rickettsiae.

5-Causing other infections in man ; e.g . meningitis in neonates ; encephalitis and bacterimia in adults.

6-In sheeps may cause meningoencephalitis ; in rabbits and chickens causing septicemia and abscesses in liver and heart muscles.

7-**Listeriosis** can be treated with ampicillin ,erythromycin or trimethoprim-sulfamethoxazole.

- Clinically , ampicillin +gentamicin are often recommended for therapy.

Nocardia

1- **Nocardiosis** is the disease caused by *Nocardia asteroids* and other species ; the infection begins by inhalation of the bacterial cells causing subacute to chronic pulmonary infection , could spread to other organs (brain or skin).

2-***Nocardia*** species are **aerobic , G+ve ,catalase +ve ,acid –fast bacilli** ; able to grow on different nutrient media ,developing after days, **irregular ,waxy colonies with white , orange or red colored colonies , urease +ve.**

3- under microscope , cells form **filaments**.

Pathology :

Nocardiosis begins as **chronic lobar pneumonia** , **accompanied with fever, weight loss and chest pain** (**mimic tuberculosis**) with abscess formation , which cause the spread to brain ,skin and kidney.

Diagnostic lab . tests :

1-Specimens of sputum , pus , spinal fluid and biopsy material.

2-Gram stained smears show G+ve bacilli ,coccobacilli and branching filaments.

3- Acid-fast staining method show positive staining.

Treatment :

1- The drug of choice is trimethoprim + sulfamethoxazole.

2- Other antibiotics are amikacin , imipenem and cefotaxime.

Gram+ve Cocci

Include :

Staphylococci (*Staphylococcus*)

Streptococci (*Streptococcus*)

Sarcinae (*Sarcina*)

Diplococci (*Diplococcus*)

Micrococcus

The Staphylococci :

1-G +ve ,spherical cells , usually arranged in grape-like irregular clusters .

2-Fermenting carbohydrates and producing pigments (white , yellow and deep yellow).

3-Some are members of normal flora of the skin and mucus membranes of human .

4- Others cause suppuration , abscess formation , pyogenic infections, fatal septicemia.

5-Pathogenic staphylococci hemolyze blood ,coagulate plasma , and producing a variety of extracellular enzymes and toxins .

6- Enterotoxic strains causing the most common type of food poisoning by a **heat – stable staphylococcal enterotoxin** .

7-Staphylococci rapidly develope resistance to many antimicrobial agents.

Genus *Staphylococcus*

This genus has at least 35 species ; the three main clinically important spp. are:

S. aureus ; S. epidermidis ; S. saprophyticus

S. aureus

Is coagulase+ve differentiating it among other spp.

Morphology and growth characteristics :

1-Spherical cells ≈ 1 µm in diameter , arranged in clusters , single cocci , pairs , tetrads and chains are seen in liquid cultures.

Young cultures stained G+ve; on aging , many cells become G –ve .

2- Non-motile , do not form spores ,susceptible cells lysed under the influence of β-lactam drugs like penicillin .

3- Staphylococci grow aerobically or microaerophilically on most lab. media at 37°c, forming pigments (S. aureus usually forms golden–yellow pigment) .

4- Catalase +ve , slowly ferment carbohydrates producing lactic acid but not gas .

5- Generally , resist drying and heat (50°c for 30 min.s) ; tolerate 10 % NaCl .

6 – Almost , producing β-lactamase and resist β-lactam antibiotics (penicillin G , ampicillin , ticarcillin , Piperacillin) .

Enzymes and toxins :

1-Catalase : $2H_2O_2 \longrightarrow 2H_2O + O_2$

To differentiate between *Staph* .(catalase +ve) ,and *Staph.(*catalase –*ve)*

2-Coagulase : Enzyme-like protein clots oxalated or citrated plasma

Coagulase + prothrombin ⟶ Fibrin polymerization

3-Other enzymes :

A – Hyaluronidase (spreading factor)

B-Staphylokinase (causing fibrinolysis)

C-Proteinases (lysing proteins)

D-Lipases (lysing lipids)

E. β –lactamases

4-Exotoxins :

A –α-toxin (potent hemolysin)

B- β-toxin (cytotoxic)

C-Delta -toxin (disrupt cell membrane)

D- Gamma - toxin (ϑ- hemolysin –lysis of WBCs)

5-Leukocidin : it is a toxic protein like ϑ- hemolysin ,killing WBCs

6- Exfoliative toxins:

Dissolve the muco-polysaccharide matrix of the epidermis composed of :

A –Epidermolytic toxin A (heat-stable protein ,resist boiling for 20 min.s)

B- Epidermolytic toxin B (heat –labile protein)

7-Enterotoxins :

A – *S. aureus* produce multiple enterotoxins (A,B,C,D,E,G,H,I,K,L,M) types. About 50% of *S. aureus* can produce one or more of enterotoxins. They are heat-stable and resist the enzymatic action of digestive tract.

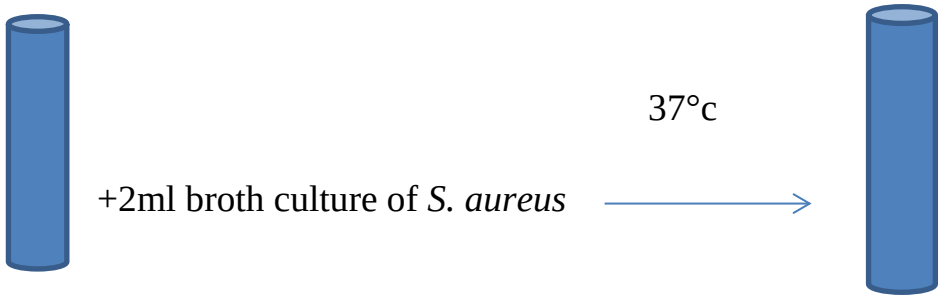
B- Enterotoxins are produced when *S. aureus* grows in carbohydrate and protein foods. Ingestion of 25 µg of enterotoxin B reslts in vomiting and diarrhea. This emetic effect is probably due to the stimulation of vomiting center in the brain.

Diagnostic lab. tests :

1- Specimens : surface swabs ,pus , blood ,tracheal aspirate ,spinal fluid…..all for culture.
2- Smears : typical staphylococci appear as G+ve in clusters.
3- On blood agar :typical colonies appear within 18 h.s at 37°c , hemolysis may seen after several days .
4- *S. aureus* **ferment mannitol**.
5- Specimens cultured on media containing 7.5% NaCl inhibits most other normal flora ,but not *S. aureus*; hence, **mannitol salt agar** can be used for the screening of *S.aureus*.
6- Catalase test :

$$2H_2O_2 \xrightarrow[\text{Oxidase enzyme}]{\text{Cytochrome}} 2H_2O + O_2$$

7- Coagulase test :



2ml of human or rabbit plasma Clot

1:5 Na-citrate buffer

Treatment:

1-Tetracyclines are used for long –term treatment in acne.

2-Prolonged intravenous therapy with a β –lactamase resistant penicillin is used in bacteremia ,endocarditis, pneumonia and other severe infections due to *S. aureus*.

3-Vancomycin is used for the treatment of infections caused by nafcillin – resistant staphylococci .

4-Because of high frequency of drug resistant strains of staphylococci, they should be tested for antimicrobial susceptibility **(antibiotic sensitivity test culture**) to seek for the drug of choice **using the multi-disc diffusion assay.** .

The Streptococci

1- G +ve, Spherical cells, forming pairs or chains during growth .

2-They are widely distributed in nature; some are members of the normal flora; others are associated with human diseases and infections.

3-They produce a variety of extracellular substances and enzymes .
4-Can be classified into several groups **, according to**;

A .Type of hemolysis ;

1. β-hemolytic streptococci
2. α- hemolytic streptococci
3. $^{\delta}$-hemolytic streptococci (no relative effect on RBCs)

B. Lancefield classification;

It is a serological grouping depends on the type of antigens (amino – sugars) that exist as a part of cell wall structure ;such that :

1-Group A streptococci : contains rhamnose –N-acetylglucosamine .

2-Group B streptococci : contains rhamnose-glucosamine.

3- Group C streptococci : contains rhamnose-N-acetylgalactosamine.

4-Group D streptococci :contains glycerol teichoic acid

Lancefield serological groups A-H and K-U (19 types); this is called (serological typing).

C. capsular polysaccharides:

According to the antigenic specificity of capsular antigens ,streptococci classified into over 90 types.

D. biochemical reactions : include

1-Sugar fermentation reactions.

2-Presence of certain enzymes.

3-Susceptibility (sensitivity) or resistance to certain chemicals.

4-Biochemical tests.

Groups of streptococci:

1-Group A ;e.g. *Streptococcus pyogenes* .

2-Group B ; e.g. *Streptococcus agalactiae* .

3-Group D ;e.g. *Streptococcus bovis*; *Enterococcus faecalis* (enterococci).

4-Viridans streptococci ;e.g. *S. mutans* (causing dental caries ,endocarditis).

5-*Streptococcus pneumoniae* (causing pneumonia,meningitis, endocarditis).

6-*Peptostreptococcus* (causing abscesses).

Toxins and enzymes:

1-Streptokinase (fibrinolysin):

Transform plasminogen of human plasma → plasmin (proteolytic enzymes digest fibrin) used for treatment of pulmonary emboli, coronary arteries and venous thrombosis.

2-Streptodornase:streptococcal DNAase .

3-Hyaluronidase:spreading factor .

4-Pyrogenic exotoxins (erythrogenic toxins): there are 3 antigenically streptococcal pyrogenic exotoxins A, B and C.

5- Hemolysins : 1-streptolysin O , 2-streptolysin S .

Diseases caused by : *S. pyogenes*

Skin infections:

a. Erysipelas

b. Cellulitis

c. Necrotizing fasciitis (streptococcal gangrene).

d. Impetigo (streptococcal pyoderma).

Other infections :

e. Puerperal fever

f. Bacteremia

g. Streptococcal sore throat (pharyngitis).

h. Scarlet fever and streptococcal toxic shock syndrome (associated with exotoxins A, B, or C).

i. Rheumatic fever

j. Acute glomerulonephritis.

Diagnostic lab. tests:

1- Specimens :throat swab , pus ,blood (for culture), serum (for determination of Ab.s) .

2- Smears of pus: show single, pairs or chains of G+ve cocci.

3- Culture on blood agar: incubation in 10% CO2 often enhance hemolysis(because O2 inactivates streptolysin O).

4- Antigen detection tests: commercial kits are available for rapid detection of streptococcal Ag.s from swabs ,with specificity up to 98-99% compared to culture methods.

5-Serological tests:

a. Determination the titre of antistreptolysin O (ASO)in respiratory infections.

b. Anti-DNAase and Anti-hyaluronidase in skin infections .

c. Anti-streptokinase.

d.Anti-M type–specific Ab.s.

Treatment :

All *S. pyogenes* are susceptible to penicillin G, most are susceptible to erythromycin .

Viridans streptococci

1-This group include the bacteria:

Streptococcus mitis , ***S. mutans*** , ***S. salivarius*** , ***S. sanguis*** and others

2- α –hemolytic ;their growth is not inhibited by **optochin;** colonies are not soluble in bile (desoxycholate).

3-They are the most prevalent members of the normal flora of the upper respiratory tract and buccal cavity ; So that ,they are important for the healthy state of the mucous membranes there .

4-May reach the blood stream and cause endocarditis on abnormal heart valves .

5-Some members (e.g. *S. mutans*) synthesize large polysaccharides like **dextrans** or **levans** from **sucrose** and contribute in the genesis of **dental caries .**

Enterococci

1-They are part of the normal enteric flora .

2-Usually non –hemolytic, but occasionally α-hemolytic .

3- Able to grow in the presence of **bile** and hydrolyze **esculin**.

4-Able to grow in **6.5% NaCl** .

5-Able to grow at between 10-45°c.

6-More resistant to penicillin G; many isolates are vancomycin –resistant .

7- ***Enterococcus faecalis*** is the most common pathogen that causes 85-90 % of entercoccal infections, while ***E. faecium*** causes 5-10% of other cases.

8-They are transmitted from one patient to another by hands and through hospital personnel, causing **nosocomial infections** **(hospital-acquired infections).**

9- Sites of infections are the **urinary tract ,wounds, biliary tract, and blood** ;may cause **meningitis and bacteremia in neonates** .

Enteric G–ve rods

(Enterobacteriaceae)

1-This family is a large, heterogeneous group of G–ve rods; the natural habitat is the intestinal tract of humans and animals .

2-The family includes many genera: *(Escherichia , Shigella , Salmonella ,Enterobacter , Klebsiella , Serratia , Proteus* , and others) .

3-Some members are part of the normal intestinal flora ,e.g. *E.coli* ; but incidentally cause diseases; while others are pathogens for humans or animals like *Salmonella* and *Shigella* .

4-They are aerobes or facultative anaerobes, ferment a wide range of carbohydrates .

5-Posses a complex antigenic structure , and produce a variety of toxins and other virulence factors.

6-They are commonly called : **enterobacteriaceae** , **enteric G –ve rods** ; **enteric bacteria** ; **enteric group** , and **coliforms** .

7-Motile with peritrichous flagella or nonmotile .

8- Able to grow on peptone or meat extract without any supplements or NaCl; grow well on MacConkey's medium .

9-Ferment glucose (rather than oxidize) with gas production; catalase +ve ; oxidase –ve ; reduce nitrate to nitrite($NO_3 \rightarrow NO_2$)

10-Have a 39-59 % G+C DNA content .

Rapid, presumptive identification of G-ve enteric bacteria :

1-Lactose fermentors

a. **Slow fer.s**:

1- ***Edwardsiella*** , 2- ***Serratia*** , 3- ***Citrobacter***, 4- ***Arizona*** ,5- ***Providencia*** , 6- ***Erwinia*** .

b. **Rapid fer.s** :

1- ***Escherichia coli*** (metallic sheen on EMB agar, motile , nonviscous growth).

2-***Enterobacter aerogenes*** (no metallic sheen , often motile , more viscous growth).

3-***Klebsiella pneumoniae*** (very viscous, mucoid growth , nonmotile).

2- Lactose non-fermentors

a. ***Shigella*** (nonmotile)

b. ***Salmonella*** (motile)

c. ***Proteus*** (swarming, urease activity with smell of ammonia).

e. ***Pseudomonas*** (pigmented growth ,sweetish smell).

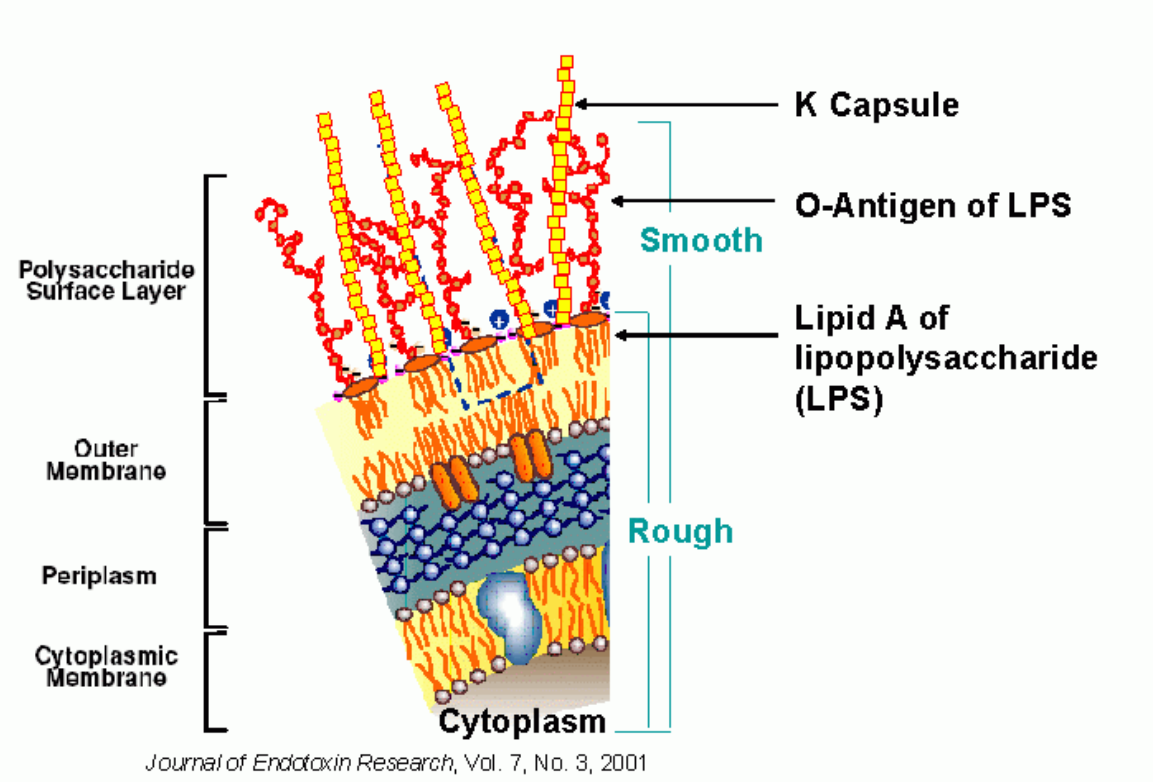
Antigenic structure :

Enterobacteriaceae members have a complex antigenic structures; such that:

1-More than 150 different heat-stable somatic O antigens (consist of LPS).

2- More than 100 heat –labile capsular K antigens .

3-More than 50 flagellar H antigens.



Somatic O Ag.s (lipopolysaccharides):LPS

1-The most external part of the cell wall ,consist of repeating units of polysaccharides and lipids.

2-Resist heat and alcohol .

3-Can be detected by bacterial agglutination with specific Ab.s (mainly lgM)

4-Each member of enteric group has specific O groups of Ag.s; i.e. a certain bacteria may carry several (different) O Ag.s .

5- Most members of enteric group share one or more O Ag.s ; e.g.

E. coli (some strains):

Cross –react with strains of ***Klebsiella , Salmonella*** and ***Providentia***

6- O Ag.s may be associated with specific human diseases; e.g. O types of ***E. coli*** are found in **diarrhea** and in **UTIs** .

Capsular K Ag.s :

1-Some are polysaccharides , as in ***E. coli*** ; others are proteins.

2-They may be associated with virulence ,e.g. ***E. coli*** strains produce K1 Ag in neonate meningitis ; in ***Salmonella typhi***, K Ag.s are called **Vi Ag.s (virulence Ag.s).**

3-Help in attaching bacteria to epithelial cell surfaces to invade urinary or gastrointestinal tracts.

4-***Klebsiella pneumoniae*** capsular types 1 and 2 causing respiratory tract infections; types 8,9,10 and 24 causing urinary tract infections (**UTIs**).

Flagellar H Ag.s :

1-Those Ag.s are located on flagella and are denatured or removed by heat or alcohol .

2-Can be agglutinated with anti –H Ab.s (mainly lgG) .

3-They may interfere with agglutination by Anti-O Ab.s .

Examples for serotyping :

E. coli O 75 : K 100 : H 5

E. coli O 55 : K 5 : H 21

Salmonella O 1, 4, 5, 12 : Hb 1, 2

schottmölleri

Colicins (Bacteriocins) :

1-Many G-ve bacteria produce bacteriocins , which are bactericidal substances produced by certain strains of bacteria active against some other strains of the same species.

2-Their production is controlled by plasmids.

3- ***E. coli*** strains producing **colicins** ; ***Serratia*** strains producing **marcescens** and ***Pseudomonas*** strains producing **pyocins**.

4-**Bacteriocin–producing strains are resistant to their own substances; thus can be used for typing of mo.s.**

Diseases caused by coliforms :

1- E. coli :

a- UTI : causing about 90% of infections in young women.

b- *E. coli*-associated diarrheal diseases:

i-Enteropathogenic ***E. coli*** (EPEC): common cause of diarrhea in infants.

ii-Enterotoxigenic ***E. coli*** (ETEC) :common cause of traveler's diarrhea, and in infants.

iii-Enterohemorrhagic ***E. coli*** (EHEC):associated with hemorrhagic colitis , and hemolytic uremic syndrome.

iv-Enteroinvasive ***E. coli*** (EIEC) : causes a disease very similar to shigellosis .

v-Enteraggregative ***E. coli*** (EAEC) :causes acute and chronic diarrhea (>14 days in duration).

c. Sepsis : may occure secondary to UTIs , or in newborns.

d. Meningitis : *E. coli* and group B streptococci are the leading causes of meningitis in infants.

2- Enterobacter aerogenes :

This organism has small capsule, may be found free-living and in the intestinal tract , may cause UTIs and sepsis.

3- *Klebsiella pneumoniae :*

a. Present in the respiratory tract and feces of about 5% of normal individuals.

b-It causes about 1% of bacterial pneumonias.

c- Can produce extensive hemorrhage of the lungs.

d- It occasionally produce UTI and bacteremia.

e. ***K. pneumoniae*** and ***K. oxytoca*** cause **hospital-acquired inf.s** .

f. Other strains may associated with inflammatory conditions of the upper respiratory tract

4- Proteus spp :

P. mirabilis , P. vulgaris , P. morganii

a- Causing UTIs, and bacteremia and pneumonia .

b- ***P. vulgaris*** & ***P. morganii*** are **important nosocomial pathogens** .

c. Due to ***Proteus*** high urease activity and liberation of NH_3, urine become alkaline and promote stone formation .

d. Rapid motililty of ***Proteus*** may contribute to its invasion of the urinary tract.

5- *Shigella spp :*

S. sonei , S. flexneri , S. boydii , S. dysenteriae

This organism produce :

A – **endotoxin** : cells autolyse and release their LPS, which contribute to the irritation of the intestine wall .

b. **dysentery exotoxin** : a heat-labile toxin affects both gut and CNS. It is a protein and stimulate the production of Ab.s , and lethal to lab . animals ; **acting as :**

-Enterotoxin : producing diarrhea.

-Neurotoxin : which may contribute to the extreme severity and fatal nature of the infection and to the CNS reactions (meningismus and coma) .

Diagnostic lab tests :

1-Specimens : include fresh stool , mucus flecks , and rectal swabs :

Large numbers of fecal leukocytes and some RBCs are seen microscopically.

2-Culture : the material streaked on **differential media** (**MacConkey agar** or **EMB agar**) ; and on **selective media** (**Hekton enteric agar** or **SS agar**).

Colorless lactose –negative colonis cultured on TSI agar slants:

-H_2S –ve

- Acid no gas (but)

- Alkaline (slant) } Presumptive ***Shigella***

-Non-motile)

Followed by slide agglutination with *Shigella* antisera .

6- The *Salmonella* group :

a. Salmonellae are often pathogenic for humans or animals when acquired by oral route.

b. They are transmitted from animals and their products to humans , causing enteritis , systemic infection and enteric fever .

c. Motile with peritrichous flagella ; able to grow on simple media ;lactose and sucrose nonfermentor ; producing H_2S; form acid and gas from glucose and mannose; survive freezing in water for long period.

d.Salmonellae are resistant to certain chemicals (brilliant green ,sodium tetrathionate , Na–desoxycholate) that inhibit other enteric bacteria ,and useful to isolate salmonellae from feces and other clinical and environmental samples.

e. Salmonellae are serogroup to more than 1400 type ,according to :

i. O Ag.s as A, B, C1 , C2, D and E.

ii. H Ag.s .

iii. Vi Ag.s .

f. They show a wide variation in serotyping ,as they may :

i. Lose H Ag.s and become nonmotile.

ii.Loss of O Ag.s associated with a change from smooth to rough colony form: S --------→ R

iii . Vi Ag.s may be lost partially or completely .

Diseases caused by *Salmonella* :

1-Enteric fevers (typhoid fever).

2-Bacteremia with focal lesions .

3- Enterocolitis .

4- ***Salmonella*** **enterotoxin causing food poisoning** .

Diagnostic lab. tests for *Salmonella* :

A: Clinical Specimens :

i. Blood for culture :

In Enteric fevers and septicemias, blood cultures are often positive in the first week of the disease.

ii. bone marrow : is useful for culture .

iii. urine cultures :may be +ve after the 2nd. week of infection.

iv. Stool culture .

v. Doudenal drainage from the biliary tract : +ve culture in carriers.

B: Cultures :

i. Differential media :

- **EMB**, **MacConkey** and **desoxycholate agar**: for detection of lactose nonfermentors.

- For rapid detection of salmonellae : **bismuth sulfite agar** is used ;black colonies for salmonellae indicate the production of H_2S.

ii. Selective media (selenite media):

Specimens plated on **SS-agar , Hektoen enteric agar , XLD agar** ,or **desoxycholate–citrate agar** , which favor growth of salmonellae and shigellae over other enterobacteriaceae members.

iii. Enrichment cultures :

Stool usually cultured in **selenite-F** or **tetrathionate broth** ,which inhibit the growth of normal intestinal flora and permit the multiplication of salmonellae. After incubation for 1-2 days, growth streaked on differential and selective media.

iv. Final identification:

Suspect colonies are identified by biochemical reaction patterns and slide agglutination tests with specific antisera .

C. Serological methods :

i. Slide agglutination test:

Known antisera mixed with unknown cultures. Commercial kits are available to agglutination and serogroup salmonellae by their O Ag.s (A,B,C1,C2, D & E).

ii.Tube dilution agglutination test (Widal's test):

- Serum agglutinins (Ab.s) rise during the 2nd. & 3rd. week of *Salmonella typi* infections .

- The widal test is designed to detect these antibodies against the O and H Ag.s .

- At least 2 serum specimens abtained at 7-10 days interval ,to detect the rise in antibody titre. Serial dilutions of unknown sera are tested against Ag.s from salmonellae .

- A titre against the O Ag.s of >1/320, and against the H Ag.s of >1/640 is considered positive.

Microbial Toxins

1-Toxin :

A poisonous substance , especially a **protein** , that is produced by living cells or organisms, capable of causing a **disease** when introduced into the body tissues ;**oftenly** capable to induce the immune system to produce **antibodies (antitoxin)** .

2- Microbial toxins are produced by:

A. Fungi (fungal toxins or mycotoxins).

B. Bacteria (bacterial toxins) .

3-Toxins can be classified according to :

I - Production :

a- Endotoxins :cell associated substances, produced and located in the cell envelope.

b-Exotoxins : usually secreted by growing cells.

II .Chemical structure (nature):

-proteins

-polypeptides

Usualy acting as enzymes (exotoxins) in G+ve bacteria

-LPS

-LOS (lipo-oligosaccharides)

-Lipoproteins

usualy acting as (endotoxins)in G-ve bacteria

III. Activity (mode of action):

-Enterotoxins

-Neurotoxins

-Enzymatic activity

-Enterotoxin + Neurotoxin

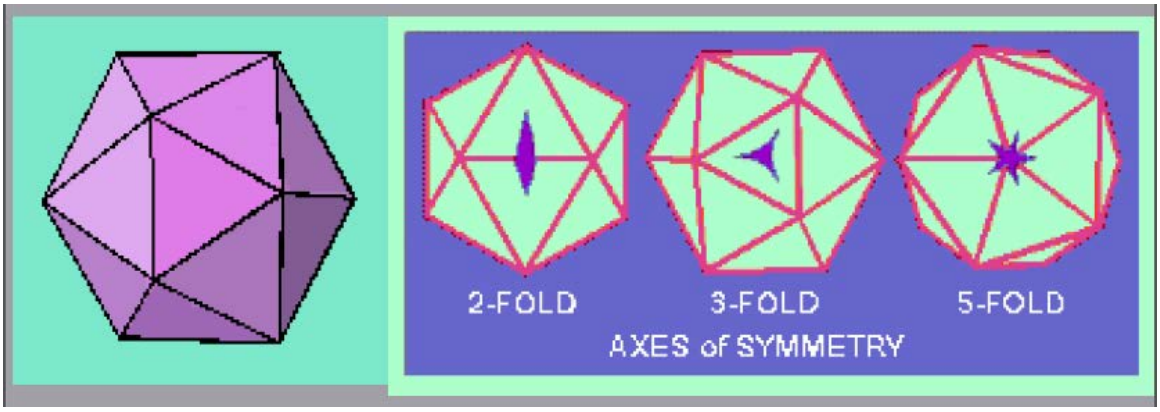
-Cytotoxins

Property	Exotoxin	Endotoxin
Chemical prop.s	proteins, mainly produced by G+ve bac., generally heat-labile	Complexed LPS-lipoproteins; produced due to cell lysis of G-ve bac.; heat-stable
Mode of action	Specific; either cytotoxic, enterotoxic or neurotoxic	General effects; fever ,diarrhea, vomiting
Toxicity	Highly toxic, often fatal	Weakly toxic , rarely fatal
Immunogenicity	Highly immunogenic; produced antibodies are neutralizing (antitoxin)	Relatively poor immunogen; antibodies not sufficient to neutralize toxin
Toxoid potential	Immunogenic	None
Fever potential	Do not produce fever in host	Pyrogenic

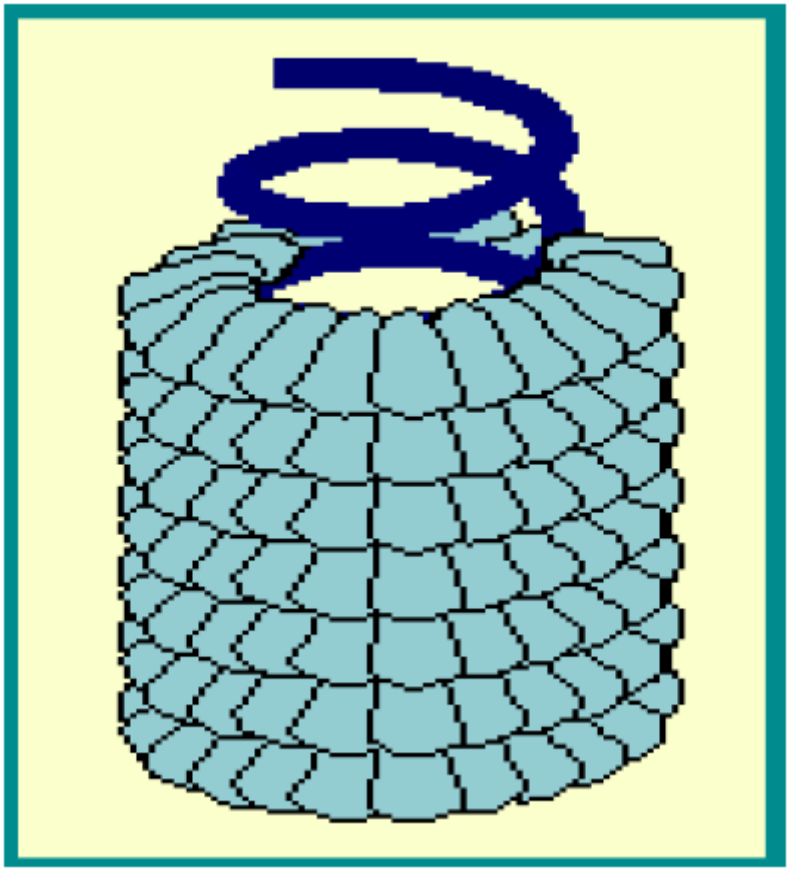
Virology

☒ **Virology**: is the study of viruses ; their structure ,classification and evolution , their ways to infect cells and the diseases they cause.

Geometrical symmetry (shapes) of viral particles:

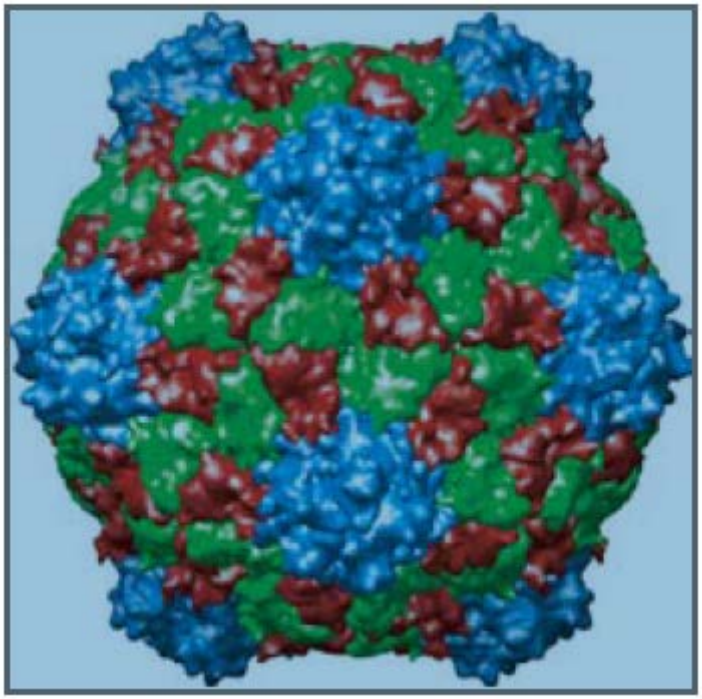


1-Virus with **icosahedral symmetry** (virion: the complete virus particle).



2- Virus with **helical symmetry (virion).**

Cowpea mosaic virus capsid



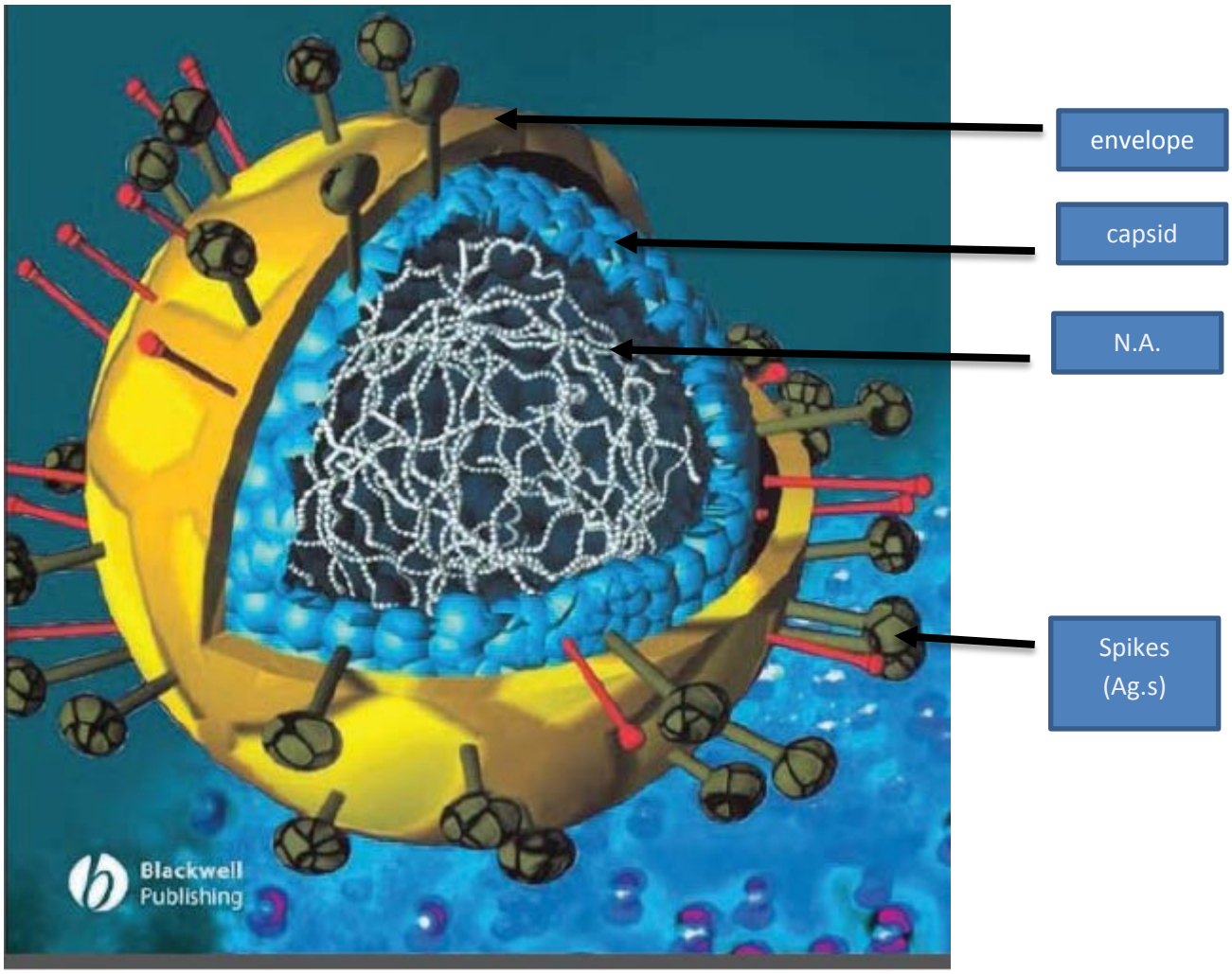
3- Virus with **complex symmetry**.

Viral particles are mainly composed of:

i. Envelope.

ii. Capsid.

iii. Neucleic acid (DNA or RNA).



Viruses can be classified according to :

1- Infection:

- Animal v.s
- Plant v.s
- Fungal v.s
- Bacterial v.s (bacteriophages: v.s infecting bacteria) .

2- Geometrical shape of the capsid :

-Helix (helical)

-Icosahedron

- Complex

3-Chemical structure :

-Presence of lipid envelope .

-Absence of lipid envelope .

4-The type of nuleic acid they use as a genetic material :

-DNA v.s :

1- Double-stranded DNA viruses

2-Single-stranded DNA viruses (much less)

-RNA v.s

1-DS-RNA viruses (much less)

2-SS-RNA viruses

-Reverse transcribing viruses

1-DS-RT DNA viruses

2-SS-RT RNA viruses (e.g. retroviruses) .

Viral diseases :

1-Common cold ,influenza ,rabies , measles.

2-Many forms of diarrhea ,hepatitis ,yellow fever ,polio ,small pox

3-AIDS (acquired immuno –deficiency syndrome)

4-Herpes simplex (causes cold sore and genital herpes)

5-Oncoviruses: are contributed to certain forms of cancer; like papillomavirus with **cervical cancer**, and hepatitis B & hep. C viruses which are associated with **liver cancer**.

Vibrio , Aeromonas , Plesiomonas , Campylobacter , and Helicobacter species :

Are :

1- G-ve rods ,widely distributed in nature .

2- *Vibrios* are found in marine and surface waters .

3-*Aeromonas* is found in fresh water and cold –blooded animals (e.g . fish) .

4-*Plesiomonas* exists in both cold –blooded and warm –blooded animals.

5- *Campylobacter* is found in many animal species including domesticated animals . *C. jejuni* is a common cause of enteritis in humans.

6- *Helicobacter pylori* associated with gastric and duodenal ulcer disease .

7- *Vibrio cholerae* produces an **enterotoxin** that causes **Cholera** , **which is a profuse watery diarrhea that , can rapidly lead to dehydration and death**.

The *Vibrios* :

1- Are the most common bacteria in surface waters worldwide.

2-Curved , aerobic , rods , motile with one polar flagellum .

3- ***V. cholerae* serogroups O1 and O139 cause cholera in humans** ; other vibrios may cause sepsis or enteritis ; e.g. *V. parahaemolyticus* causes gastroenteritis and perhaps extraintestinal infections.

Vibrio cholerae

Morphology and identification:

Typical organisms : comma-shaped , curved rods 2-4 µm long; actively motile with a single polar flagellum.

Culture :

1- Bacteria grown on solid media produce convex , smooth, round colonies which are opaque and granular in transmitted light .

2- Grow well at 37°c on different media , like **TCBS - agar** (thiosulfate – citrate – bile – sucrose) agar ,producing **yellow** colonies .

3- Oxidase +ve , to differentiates from other enteric G-ve bacteria.

4- Characterised by growing at pH 8.5 - 9.5 , and rapidly killed by acidic pH ; therefore cultures containing fermentable carbohydrates , quickly become sterile.

5- In areas where cholera disease is **endemic** :

Direct cultures of stool on selective media → For rapid characterization of *V. cholerae*:
- i. TCBS agar
- ii. Alkaline pepton water enrichment Medium

Growth characteristics :

1- Ferment sucrose and mannose ,but not arabinose.

2- Oxidase +ve (which is a key characteristic in preliminary identification of *V.cholerae* and other vibrios).

3- *Vibrio* species are **susceptible** to the compound O/129 (2,4 – diamino - 6,7 – diisopropylpteridine phosphate) , to differentiate them from *Aeromonas* spp . , which are resistant to O/129.

4- Most *Vibrio* species are **halotolerant** ; because NaCl oftenly stimulates their growth ; such that vibrios grow on media containing 6% NaCl , while *Aeromonas* dose not .**(this is to differentiate between the two genera).**

Antigenic structure &biological classification :

1- Many vibrios share a single heat – labile flagellar H Ag.

2-Therefore ; Anti –H Ag. (antibodies), probably are not effective to protect susceptible persons against vibrios.

3- *V. cholerae* has O–LPS; such that at least 139 O Ag. serogroups are determined .

4- *V. cholerae* strains : O group 1 , and O group 139 cause classic cholera.

5- Occasionally , non O1/ non O139 *V. cholerae* causes cholera–like disease.

6-Ab.s to the O Ag.s tend to protect lab. animals against infections with *V. cholerae*.

7-*V. cholerae* serogroup O1 Ag . has other antigenic determinants ; such that , has further serotyping :

i. serotype Ogawa

ii. serotype Inaba

iii. serotype Hikojima

8- Epidemic *V. cholerae* has 2 biotype :

i. *Classic* biotype

ii. *El Tor* biotype

9-*El Tor* biotype produces a hemolysin.

10 – Molecular techniques can also be used for more *V. cholerae* typing ; that is useful for epidemiological studies.

V . cholerae enterotoxin :

1- A heat –labile enterotoxin with a M.wt. of ≈ 84 kdal. consisting of A and B subunits.

2- Subunit B attached to the receptor on the mucosal cell , and promotes the entry of subunit A into the cell .

3- Subunit A activated and yielding an increased levels of intracellular cAMP, that results in prolonged hypersecretion of water and electrolytes extracellularly . Furthermore , this mechanism exerts an inhibition effect on the absorption of Na^+ and Cl^- ions.

4-Diarrhea occurs , as much as 20-30 l/d , which resulting dehydration , shock , acidosis , and death.

5- The genes of *V. cholerae* enterotoxin are chromosomally encoded .

Pathogenesis and pathology :

1- *V. cholerae* is only a human pathogen .

2- A person with normal gastric acidity , may have to ingest $\approx 10^{10}$ cells to become infected (using water contaminated with *V. cholerae*) .

3- Ingesting food contaminated with *V. cholerae* , $\approx 10^{2} - 10^{4}$ cells would be necessary for infection , due to the buffering capacity of food .

4- Any medications or conditions that decreases stomach acidity , a person would be more susceptible to infection with *V. cholerae* .

5- Cholera disease is not an invasive infection ; the organisms do not reach the bloodstream, but attach to the microvilli of the brush border of intestinal epithelial cells.

6-The bacteria multiply there and liberate cholera toxin ; and perhaps mucinases and endotoxin .

Clinical findings

1- About 60% of classic cholera infections are asymptomatic, 75% of infections are with *El Tor V . cholera*e biotype.

2- The incubation period between the ingestion of contaminated water or food and the development of cholera symptoms is 1- 4 days , depending largely upon the number of organisms taken.

3- A sudden onset of nausea and vomiting , profuse diarrhea with abdominal cramps.

4- Stools resemble **''rice water ''** ,contain mucus , epithelial cells , and large numbers vibrios.

5- Rapid loss of fluids and electrolytes , leading to profound dehydration , circulatory collapse and anuria .

6- The mortality rate without treatment is between 25 – 50 % .

7- El Tor biotype tends to cause milder cholera disease than the classic biotype.

Diagnostic Lab. tests :

1- Specimens : mucus flecks from stools → for culture

2- Smears : made from stool samples ; dark–field or phase-contrast microscopy show the rapidly motile vibrios .

3- Culture :

a- Rapid growth on peptone water , on blood agar (pH ≈ 9.0) , or on TCBS agar

b- Typical colonies can be seen in 18 h.

c- For enrichment , a few drops of stool can be incubated for 6-8 h.s in taurocholate peptone broth (pH 8-9) ;cells from this culture can be stained or subcultured.

d- Specific tests : further identification of *V. cholerae* by slide agglutination tests using anti-O group 1 or group 139 antisera , and biochemical reaction patterns .

Immunity :

1- Gastric acid provides some protection against cholera vibrios.

2- Cholera infection is followed by immunity to reinfection , but with not known duration and degree of immunity

3- In lab. animals , specific lgA antibodies occure in the intestinal lumen . Similar Ab.s appear in serum after infection , but last for few months.

4- Vibriocidal Ab.s in serum with titer ≥ 1: 20 associated with protection against colonization and disease.

5- Antitoxin Ab.s (vaccine) has not been associated with protection.

Treatment

1- The important part of treatment is the replacement of water and electrolytes to compensate the dehydration and salt ions depletion.

2- Administration of effective antibiotics against *V. cholerae* ; e.g. oral doses of tetracycline to reduce bacterial cells in excreted stools.

3- In endemic cholera , *V. cholerae* has developed tetracycline reistance genes carried by transmissible plasmids.

Epidemiology , prevention & control :

1- Six pandemics of cholera occured between 1817 and 1923 , mostly caused by *V. cholerae* O1 of the classic biotype , that originated in Asia (particularly indian subcontinent) .

2-The seventh pandemic began in 1961 in lndonesia , spreaded to Asia, middle east and Africa. This pandemic has been caused by *V . cholerae*

biotype *El Tor* . This wave of cholera appeared in Peru in 1991 and spreaded to south and central America countries . Millions of people have had cholera in that pandemic.

3- Serotype O139 considered to be the causative mo. for cholera eighth pandemic that began in india in 1992 – 1997 and spreaded to Asia.

4- The disease is spread by contact with individuals and by water , food and flies ; as vibrio survive in water for up to 3 weeks . Carriers of cholera has unclear role in transmitting the disease .

5- Control depends on education and on improvement of sanitation of food and water. Patients should be isolated , and their excreta should be disinfected , and following up any personal contacts . Using of chemotherapy may have a place . Repeated injections of cholera vaccine (LPS) extracted from vibrio cells, supply a limited protection to exposed persons (e.g . family members).

Vibrio parahaemolyticus

1- It is a halophilic bacterium that causes acute gastroenteritis after ingestion of contaminated seafood like raw fish or shellfish.

2- After incubation period of 12 – 24 h.s ; nausea , vomiting , abdominal cramps , fever , and watery to bloody diarrhea occure . Fecal leukocytes are often observed . The enteritis tend to subside sponteneuosly in 1 – 4 d.s without treatment .

3- No enterotoxin has yet been isolated from this bacteria .

4- Grow well on blood agar , but not on other differential media for salmonellae and shigellae.

5- Usually identified by its oxidase +ve growth on blood agar .

Other vibrios are :

1- *V . vulnificus*

2- *V . mimicus*

3- *V. fluvialis*

4- *V . alginolyticus*

5- *V .damsela*

causing: gastroenteritis, diarrhea, eye inf.s, ear inf.s, wound inf.s.

6- *V. hollisae*

Tetracycline is the drug of choice ; ciprofloxacin may be effective .