

Shatt Al -Arab University

Faculty of science

Pathological analysis science.



Pathogenic Bacteriology

Lect-3-

Bacterial growth and reproduction

Prof. Dr. Mohammed A. fayyadh

LEARNING OBJECTIVES

Define the generation time for bacterial growth based on binary fission

Describe and draw bacterial growth curve

Explain several laboratory methods used to determine viable and total cell counts in bacterial populations undergoing exponential growth

What is Bacterial Growth?

Bacterial growth is an increase in all cell components, which ends in multiplication of the cell leading to an increase in population.

What do we mean by Bacterial Growth?

1-an increase in the size of the cell

2-an increase in the number of individual cells.

Bacterial Growth and Survival

The survival of any microbial group within an environment depends on 2 factors:

1-The availability of Nutrients in the media.

2-The ability of the microbe to consume these nutrients.

The survival and growth of bacteria is evaluated by studying isolated bacteria under optimal condition in laboratories (thus providing the bacteria with excess nutrients) and studying how bacteria consume these nutrients.

Reproduction In Bacteria

#-Bacteria are prokaryotic organisms that reproduce asexually.

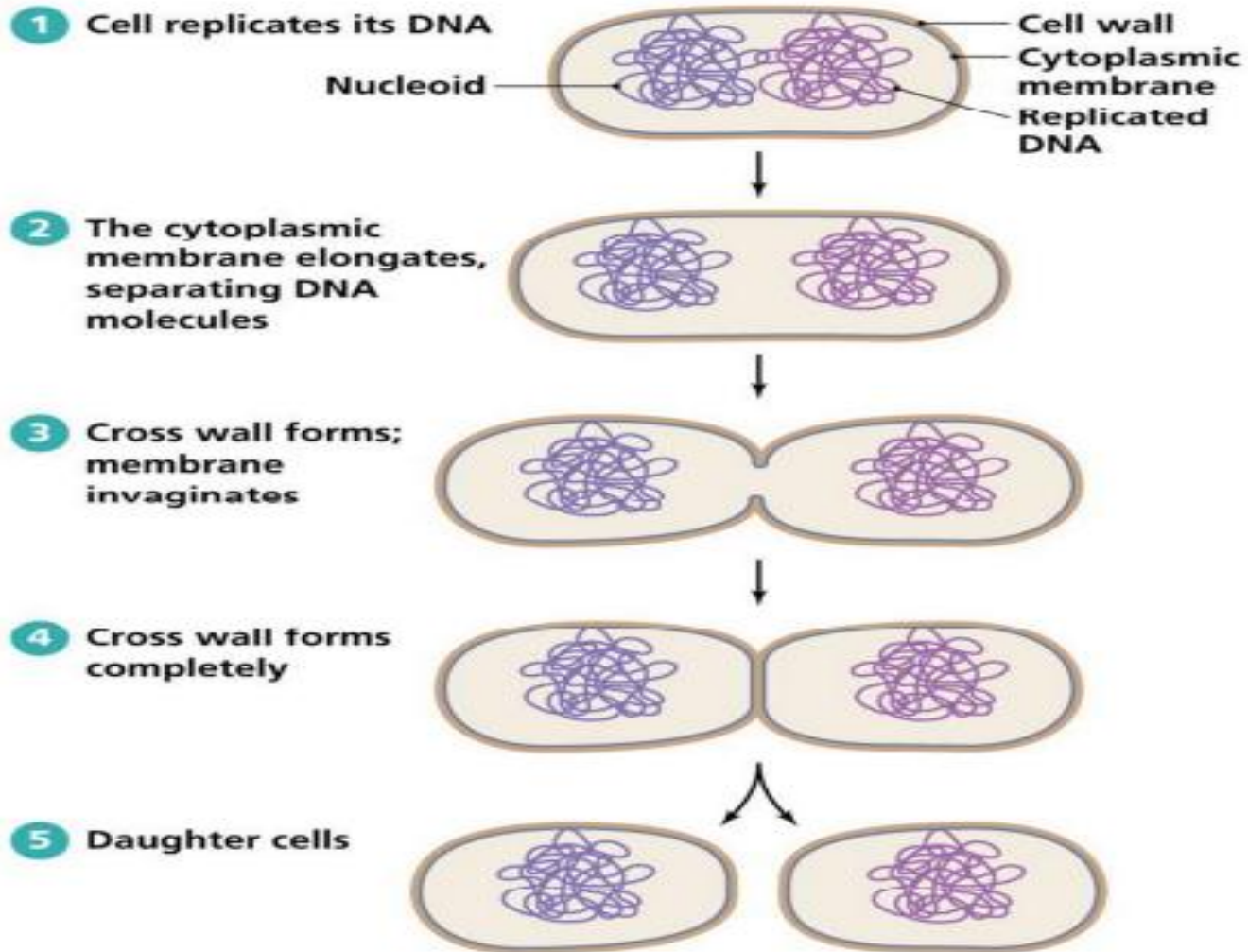
#-Most prokaryotes reproduce by binary fission.

#- Binary fission involves the division of a single cell, which results in the formation of two cells that are genetically identical.

#-In binary fission, the cell elongates, replicates its chromosome, and separates the newly formed DNA molecules so there is one chromosome in each half of the cell.

#-Finally, a septum (or cross wall) is formed at mid cell, dividing the parent cell into two progeny cells, each having its own chromosome and a complement of other cellular constituents.

Binary fission



Doubling time (Generation time)

It is the time required for **binary fission** to occur or the time required for a bacterium to give rise to two daughter cells under optimum conditions.

Doubling time varies from one bacterial species to another and also depends on the growth conditions.

Doubling time (DT) ranges from minutes to hours: For example,

-*E.coli* DT is 20 minutes .

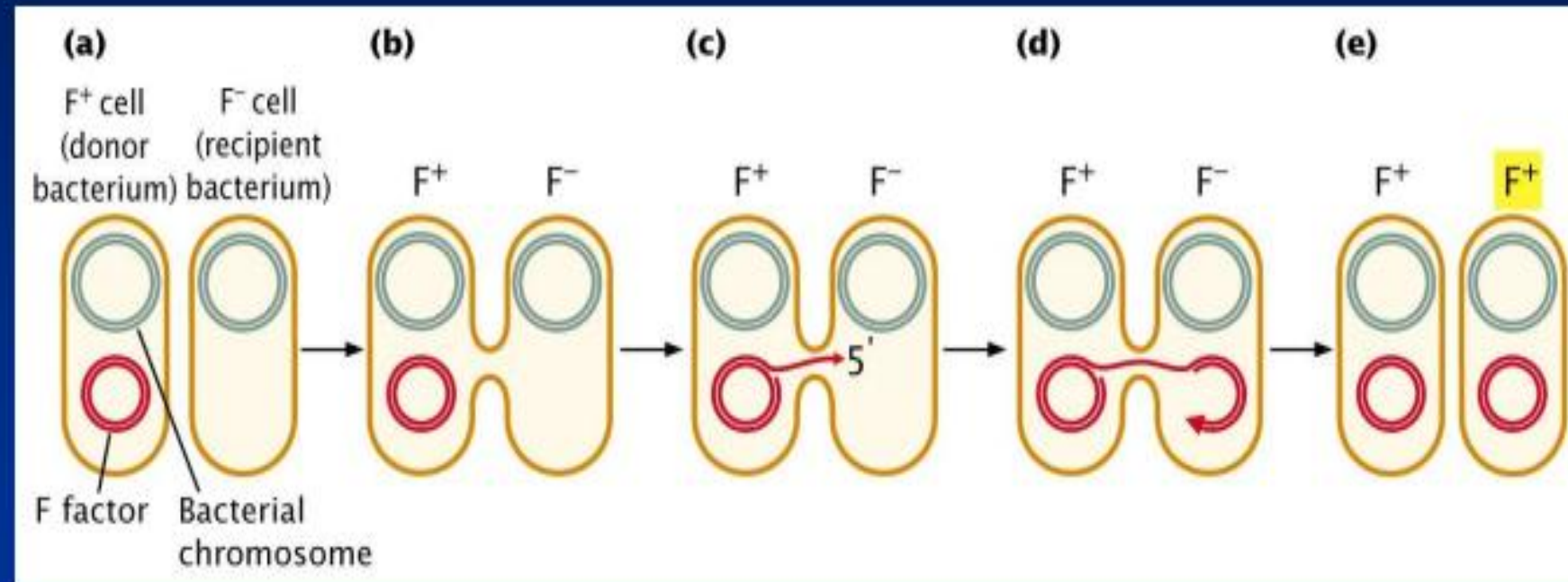
-*M. tuberculosis* has a doubling time of at least 24 h.

BACTERIAL RECOMBINATION

Binary fission is an effective way for bacteria to reproduce, however it is not without problems. Since the cells produced through this type of reproduction are identical, they are all susceptible to the same types of threats, such as environmental changes and antibiotics. These hazards could destroy an entire colony. In order to avoid such perils(مخاطر), bacteria can become more genetically varied through recombination. Recombination involves the transfer of genes between cells. Bacterial recombination is accomplished through conjugation, transformation, or transduction.

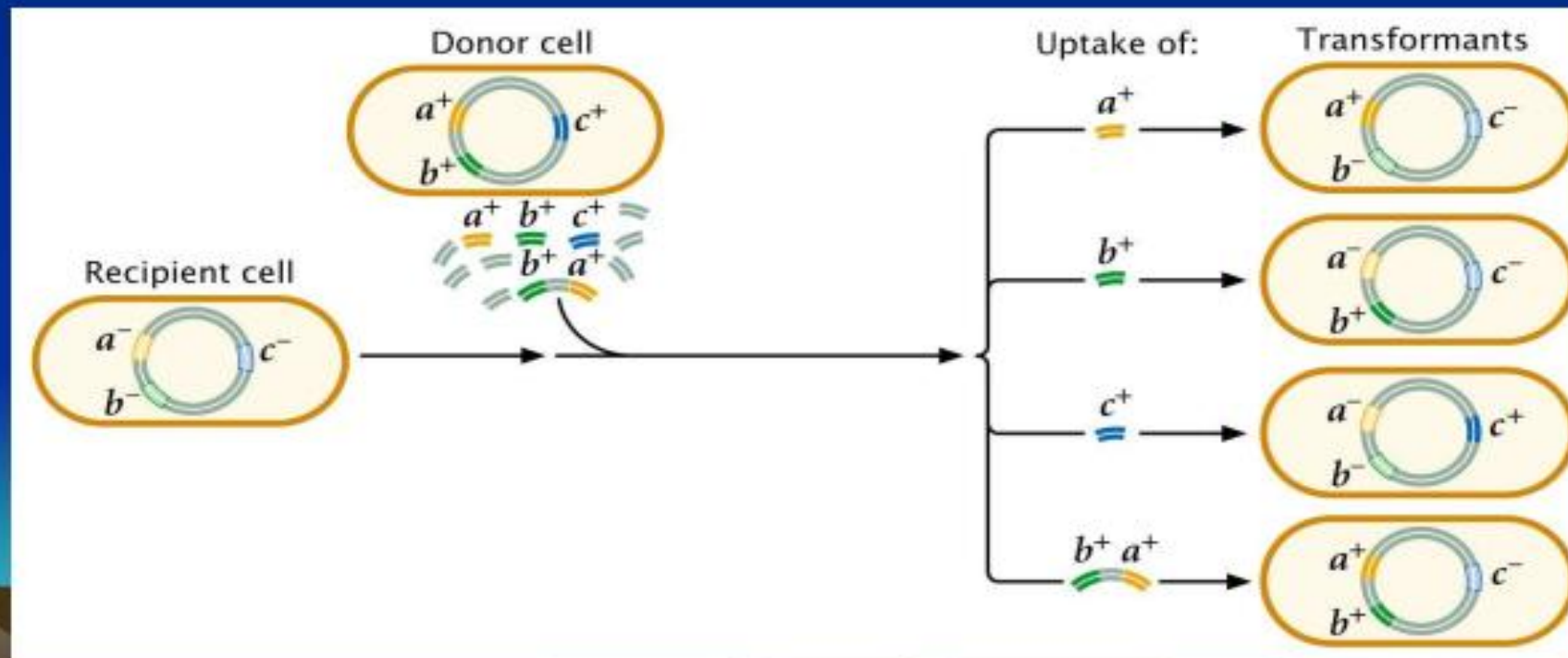
Conjugation

In this two compatible bacteria come in contact and exchange the portion of plasmid or chromosome through conjugation bridge or pilus



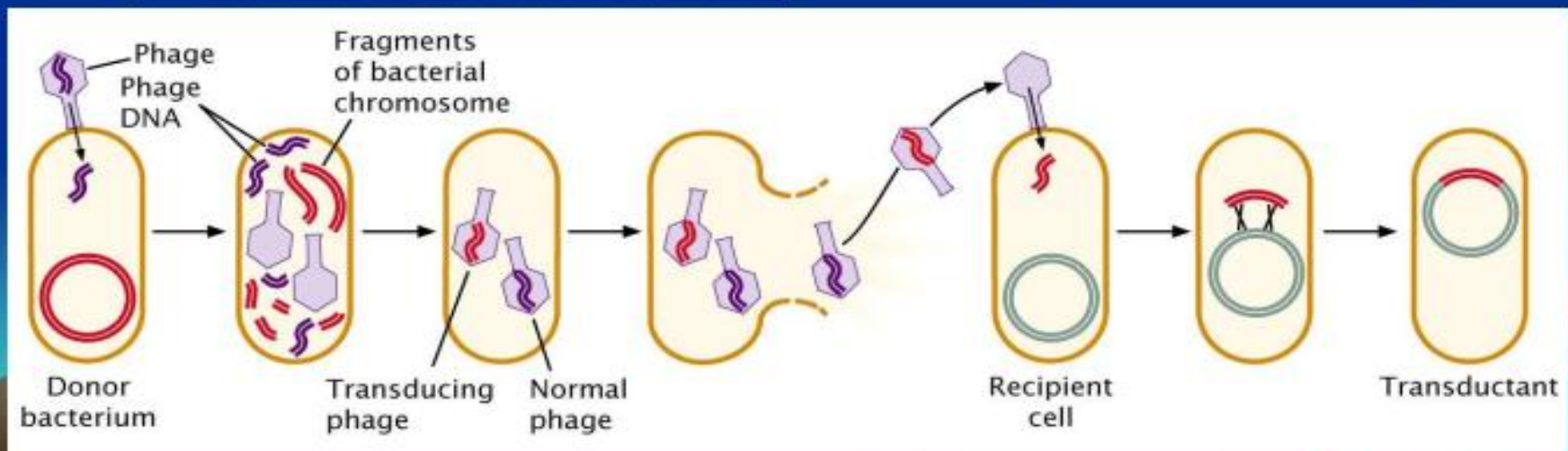
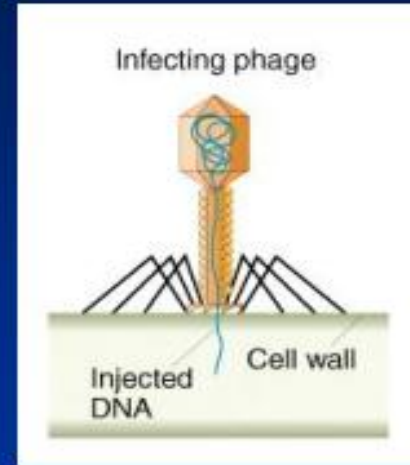
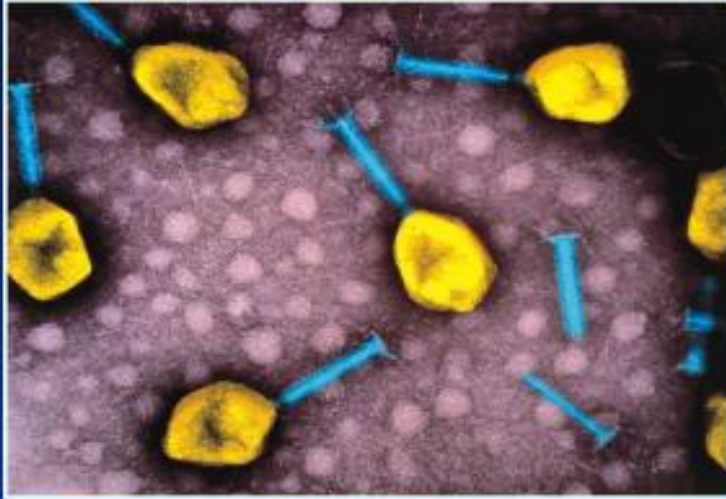
2-Transformation

DNA taken up from external environment by absorption



3-Transduction

Transfer of bacterial genes with a bacteriophage



Requirements for Bacterial growth

The requirements for Bacterial growth can be divided into two main categories:

A- Chemical Factors

B-Physical Factors

A- Chemical Requirements :include

Major Elements (Macro Elements (C-N-P-S)

Trace Elements (Iron-Copper-Molybdenum—Zinc)

Growth Factors (Vitamins-amino acid-purines-pyrimidines)

B- Physical Factors Influencing Bacterial Growth

Temperature

Moisture

pH

Light

**Osmotic
pressure**

Classification of bacteria according to their need for oxygen

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					

Classification of bacteria according to optimum growth temperatures

Psychrophiles (Cold –loving microbes) 0-20C (*Psychrobacter sp*)

**Mesophiles (moderate-temperature loving microbes) 20-45 C
(*Escherichia coli*)**

Thermophiles (Heat –loving microbes) 40-90C (*Clostridium thermosaccharolyticum*)

Bacterial Growth Curve

The bacterial growth curve shows 4 distinct phases:

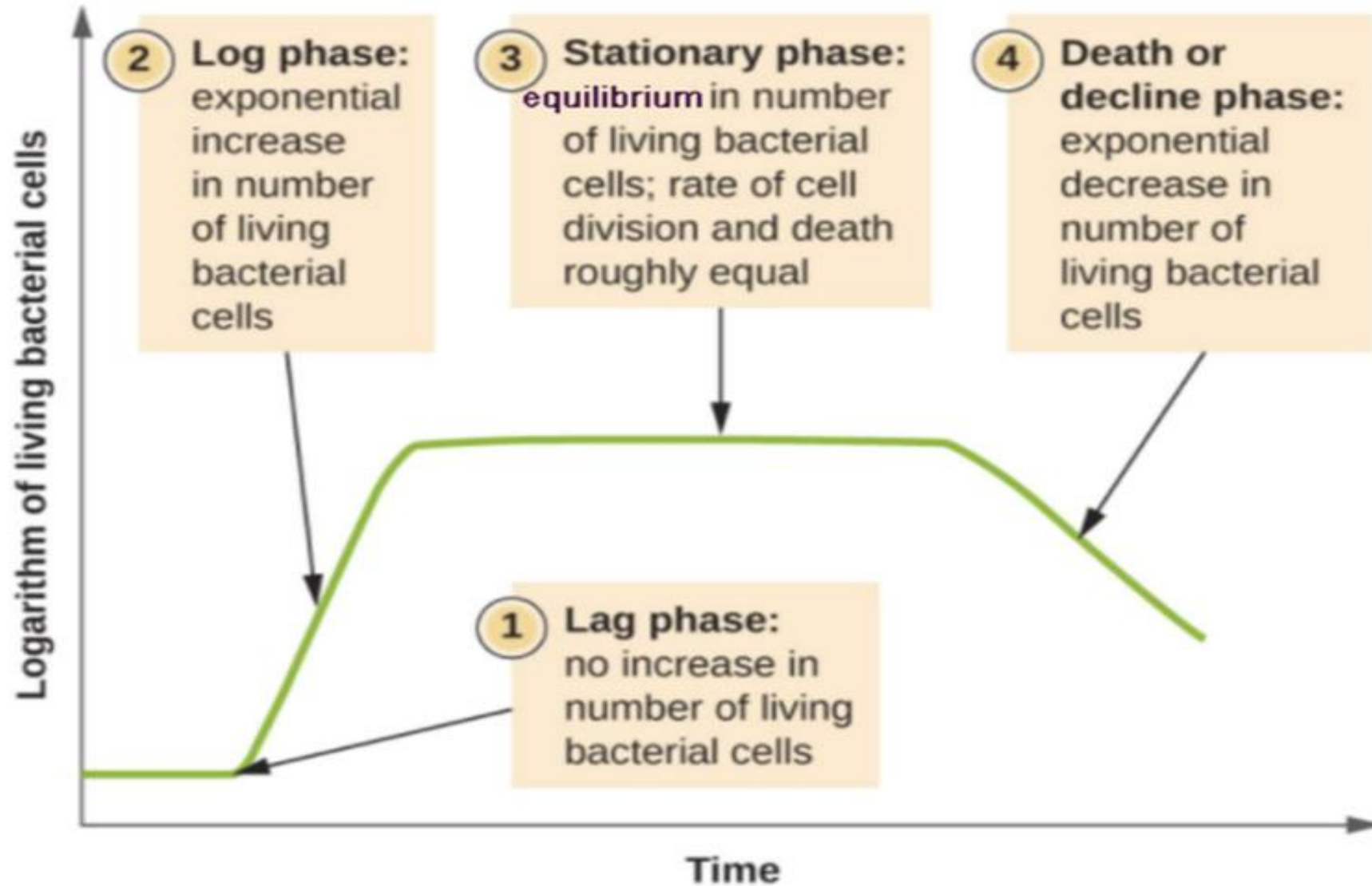
1-Lag phase

2-Log (exponential) phase

3-Stationary phase

4-Dcline (Death) phase

Bacterial Growth Curve



Review Q

Q1) Choose the correct answer .

1-What is the primary method of reproduction in bacteria?

- A) Binary fission
- B) Budding
- C) Mitosis
- D) Meiosis.

2- Which of the following is NOT a method by which bacteria can exchange genetic material?

- A) Conjugation
- B) Transformation
- C) Transduction
- D) Binary fission

3-Which of the following processes contributes to genetic diversity in bacteria?

- A) Binary fission
- B) Conjugation
- C) Transcription
- D) Translation

4-During which phase of bacterial growth does the rate of bacterial death equal the rate of bacterial division?

- A) Lag phase
- B) Log (exponential) phase
- C) Stationary phase
- D) Death phase

5- What term is used to describe bacteria that require oxygen for growth?

- A) Anaerobes
- B) Facultative anaerobes
- C) Aerobes
- D) Microaerophiles.

Q2) Define the following terms:-

1-Transformation 2- conjugation 3- Lag Phase 4- Obligate aerophiles bacteria

Quiz

2- Match the following types of antimicrobials with their actions:

- | | |
|----------------------------|----------------------|
| (a) Kills bacteria | ----- Bacteriostatic |
| (b) Stops bacterial growth | ----- Viricidal |
| (c) inactivate viruses | ----- Sprricidal |
| (d) Kills bacterial spore | ----- fungicidal |
| (c) Kills yeast and mold | ----- Bacteriocidal |

3- Fill in the blanks with the appropriate words.

- a- The type of symbiosis in which both partners benefit is called _____.
- b- The gene transfer mechanism in which bacterial information is carried by a virus is called _____.
- c- The process in which cell-free DNA is taken up by a cell and engages in genetic recombination is called _____.

Thanks for your attention



Shatt Al -Arab University

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Pathogenic Bacteriology

Lect-2-

Structure of Bacterial Cell

- *Prof. Dr. Mohammed A.fayyadh*

Objective

- *State and identify the basic structure of bacterial cells.
- *Clarify the functions of these structure.
- *Explain the differences between Gram-positive and Gram-negative bacterial cells.
- *Recognize the importance of this structure as virulence factors and for different lab diagnostic procedures.

Bacterial Cell Structure

Basic Bacterial Structures

The structure of bacterial cells consists of basic and special structures. **Basic structures** include the cell wall, cell membrane, cytoplasm, nuclear material and ribosome.

Special structures, which are only found in some bacteria, include the flagellum, pilus, capsule, spore, plasmid, etc.

Bacterial Cell Structure

No	Structure	Description	Function
1	Cell Wall	Made mostly of peptidoglycan	Gives shape and protects against osmotic pressure
2	Cell Membrane (Plasma Membrane)	Phospholipid bilayer beneath the cell wall	Controls movement of substances in/out of the cell
3	Cytoplasm	Gel-like substance inside the cell membrane	Site of metabolic reactions; contains enzymes, nutrients
4	Nucleoid	Region containing circular DNA (no membrane)	Carries genetic information (bacterial chromosome)
5	Plasmids	Small, circular extra-chromosomal DNA	Often carry genes for antibiotic resistance or virulence
6	Ribosomes (70S)	Smaller than eukaryotic ribosomes	Protein synthesis
7	Capsule (Glycocalyx)	Slimy or sticky outer layer made of polysaccharides	Protection from phagocytosis, helps in adhesion
8	Flagella	Long, whip-like structures	Motility (movement)
9	Pili (Fimbriae)	Short, hair-like structures	Attachment to surfaces and other cells (e.g., during conjugation)
10	Endospores (in some species)	Dormant structures	Survival in extreme conditions (e.g., <i>Bacillus</i>, <i>Clostridium</i>)

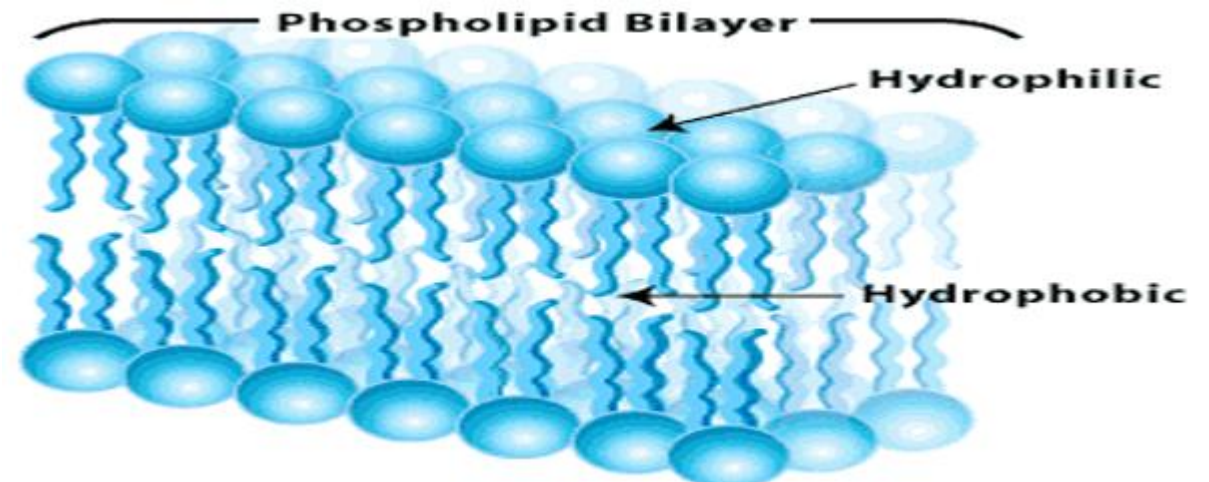
Cell envelope

The cell envelope is all the layers from the cell membrane outward, including the cell wall, the periplasmic space, the outer membrane and the capsule.

Function: To protect the organisms from hostile environments, such as extreme osmolality , harsh chemicals, and even antibiotics.

Cell Membrane

- * Often called the plasma membrane is composed of 2 layers of phospholipids and protein .
- * Phospholipids have polar heads and non-polar tails.
 - * “Polar” implies (تعني) that the heads are hydrophilic.
 - * “Non-polar” means that the tails are hydrophobic.

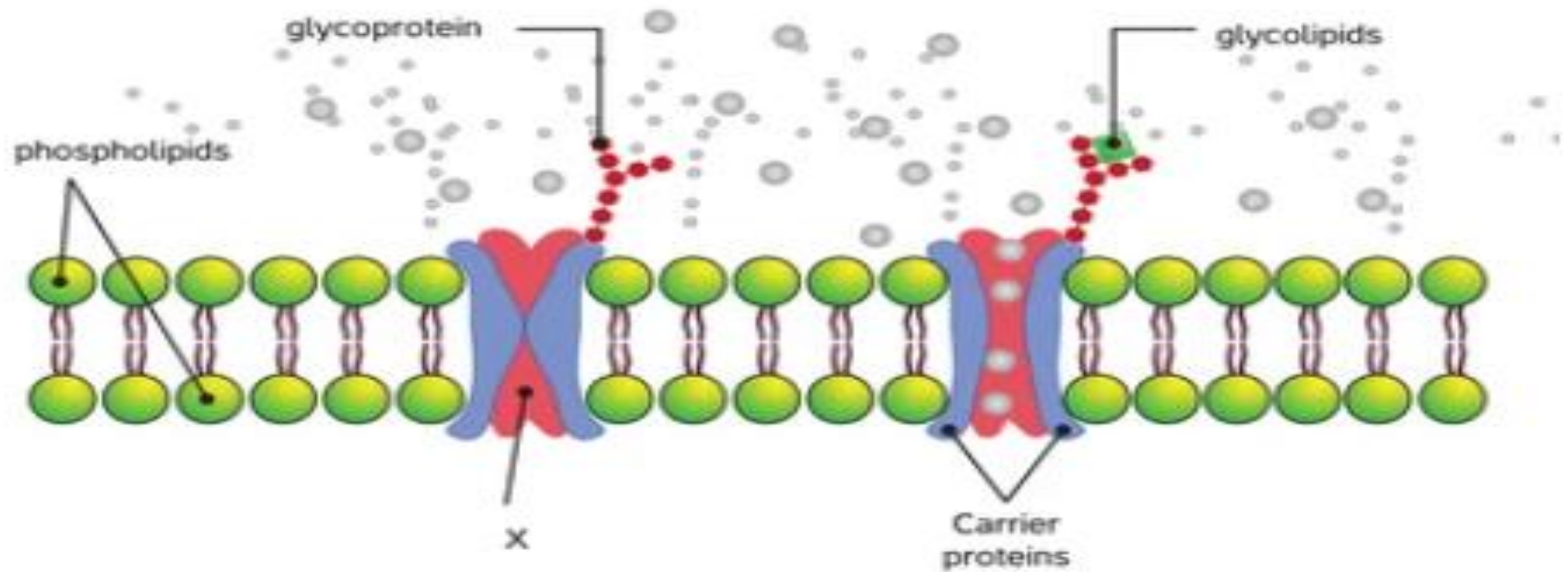


Membrane Proteins

Proteins account 70% of the mass of the membrane, which is a higher proportion than that of mammalian cell membranes.

Most are placed in the membranes so that the hydrophobic amino acids associate with the lipids in the membrane and the hydrophilic amino acids are outside the membrane interacting with the cytoplasm.

Cell Membrane



Function of plasma membrane

- (1) Selective permeability and transport of solutes.**
- (2) Electron transport and oxidative phosphorylation**
- (3) Excretion of hydrolytic exo enzymes and pathogenicity proteins.**
- (4) Synthesis of precursors of DNA, cell wall polymers and membrane lipids.**

Permeability and transport

Several mechanisms (transport systems) exist that enable the cell to transport nutrients into and waste products out of the cell.

*These transport systems work against a concentration gradient to increase the nutrient concentrations inside the cell, a function that requires energy.

*There are three general transport mechanisms involved in membrane transport:

1- Passive transport.

2- Active transport.

3- Group translocation.

Bacterial Cell wall

The bacteria cell wall is an important structure which is rigid, present just above the cell membrane and gives a **specific shaped to the cell.**

Because of this cell wall , **bacteria can survive harshest environmental conditions like the drought, heat, chemical exposure, pressure, etc.**

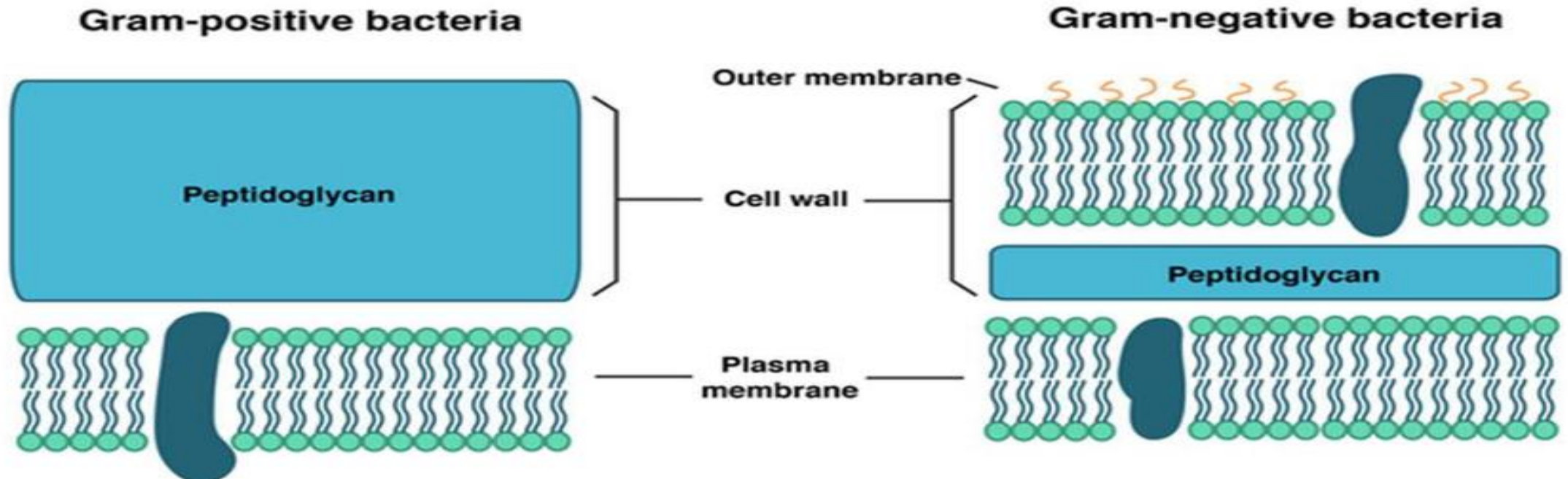
On the basis of cell wall composition, bacteria are classified into two major group ie. **Gram Positive and Gram negative.**

1. Gram positive cell wall

- Peptidoglycan
- Lipid
- Teichoic acid

2. Gram negative cell wall

- Peptidoglycan
- Outermembrane:
 - 1- Lipid
 - 2- Protein
 - 3- Lipopolysaccharide (LPS)



Gram Stain

It is the most important diagnostic procedure in microbiology . It separates most bacteria into two major groups.



Cell Wall: Peptidoglycan Layer

- *Peptidoglycan Polymer (**amino acids + sugars**)

- *Long chains of polysaccharide cross linked by short peptides (amino acid chains).

- *Unique to bacteria.

- *Provides **structural support** and maintains the **shape of the cell**.

Cell Wall : Diaminopimelic acid

#- **It is a unique element** of bacterial cell walls.

#- It is never found in the cell walls of **Archaea or eukaryotes**.

#- Diaminopimelic acid is the immediate precursor of **lysine biosynthesis in the bacterial**.

Cell Wall: Teichoic acids

- Located in the outer layer of** Gram +ve cell wall only
,
such as in the genera Staphylococcus ,
Streptococcus , Bacillus & Clostridium.

Functions of Teichoic acid

1. Provide **rigidity** to the cell-wall.
2. Mediate the **attachment** of some bacterial species such as **Staphylococci and Streptococci** .
3. It is an **acidic polymer** and contributes **negative charge** to the cell wall.

Cell Wall : **Lipopolysaccharide (LPS)**

- *Found in the outer membrane of **Gram-negative bacteria**.
- * Also known as **endotoxins, (Fever causing)**
- *LPS, which is extremely toxic to animals, has been called the **endotoxin of Gram-negative bacteria** because it is firmly bound to the cell surface and is released only when the cells is lysed.
- * Elicit strong **immune responses in animals**.(Cytokine storm)

Outer membrane

- *It is an additional layer present in **gram negative bacteria**.
- *It is composed of **lipid bilayer** , **protein** and **lipopolysaccharide (LPS) layer** .

- **Function:**

- 1-Structure component of gram-ve cell wall
- 2-LPS is an endotoxin produced by gram –ve bacteria
- 3- Lipid-A is antigenic.

Capsule

Capsule is a **thick, gelatinous, and often sticky outer layer** that surrounds the cell wall of some bacterial cells. It is made primarily of **polysaccharides** (sugar molecules), although in some bacteria, it may contain **polypeptides** (proteins).

Functions / Roles of the Bacterial Capsule.

- 1-Protection Against Phagocytosis.**
- 2-Prevents Desiccation (Drying Out).**
- 3-Attachment to Surfaces of Host cells.**
- 4-Protection from Antibiotics and Toxic Compounds.**
- 5-Virulence Factor.**

Example:-

Streptococcus pneumoniae
Klebsiella pneumoniae

Genetic material

Genetic material: The nucleoid or bacterial chromosome comprises a closed circle of double stranded DNA, many times the length of the cell and highly folded and compacted. (The bacterium *Escherichia coli* is around 3–4 μm in length, but contains a DNA molecule some 1400 μm in length.

Plasmid: Its additional DNA in the form of small, self-replicating extra chromosome called plasmids. These do not carry any genes essential for growth and reproduction. and thus the cell may survive without them. They can be very important however, as they may include genes encoding **toxins or resistance to antibiotics**, and can be passed from cell to cell .

Ribosomes: Ribosomes (70s) are composed of a complex of **protein and RNA**, and are the site of **protein synthesis** in the cell.

Inclusion bodies: granular structures. Act as food reserves, and may contain organic compounds such as starch, glycogen or lipid.

Endospores : They are dormant forms of the cell that are highly resistant to extremes of temperature, pH and other environmental factors . Produced by certain bacteria such as *Bacillus and Clostridium* .

Cell Wall appendages

The most important are:-

1-Flagella (Flagellum) –used for bacterial locomotion .

2-Pili (Pilus) , short flagella –They differ from flagella in many features

- they do not penetrate to the plasma membrane
- they are not associated with motility .

Pili function :

* Attachment(adhesion) to host cell . * Transfer of genetic information by conjugation

Types of Flagella



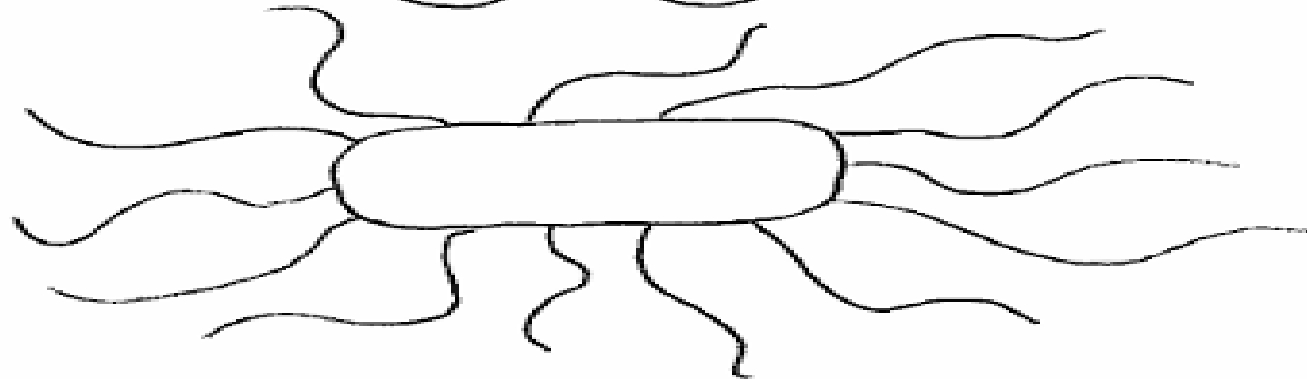
(a) Polar, monotrichous



(b) Polar, amphitrichous



(c) Bipolar, monotrichous



(d) Peritrichous

Comparison between gram positive and gram negative bacteria

Item	Gram positive	Gram negative
Peptidoglycan layer	Thick (multilayered)	Thin (single-layered)
Teichoic acids	Present	Absent
Periplasmic space	Absent	present
Lipopolysaccharide (LPS) content	Virtually none	High
Lipid and lipoprotein content	Low	High
Gram reaction	Retain crystal violet dye and stain dark violet	Can be decolorized to accept counter stain

Review Q

Q/ Compare between Gram +ve and Gram –ve cell wall?

Q/ illustrate the steps of Gram stain.

MCQ:-

1-Which of the following is not typically found in the cytoplasm of bacterial cell:

a)Ribosomes b)Nucleoid c) Endoplasmic reticulum d)Plasmid

2-The rigid outer structure of a bacterial cell that provides shape and protection is called the :

a)Capsule : b)Cell wall : c) Plasma membrane : d)Cytoplasm

3-bacterial flagella are responsible for:

a)Locomotion : b)protein synthesis : c)Energy production :d)DNA replication

4-Which of the following structure is responsible for the attachment of bacteria to host cell surface?

a)Flagella : b)Plasmid : c)Ribosome : d)Pilli

5-which of the following is found in gram-negative but not in gram-positive bacteria?

a)Pilli : b)Ribosome : c)Peptidoglycan : d)Lipopolysaccharide

6. What is the shape of *Bacillus* bacteria?

A) Spherical B) Spiral C) Rod-shaped D) Comma-shaped

7. Which structure helps bacteria in movement?

A) Capsule B) Pili C) Ribosomes D) Flagella

8-The rigid outer covering of a bacterial cell is called the:

A) Plasma membrane B) Cell wall C) Capsule D) Cytoplasm

9-The bacterial cell wall is primarily composed of:

A) Cellulose B) Peptidoglycan C) Chitin D) Starch

10-Which of the following is NOT found in bacterial cells?

A) Ribosomes B) Mitochondria C) Plasmids D) Cell wall

I WISH YOU ALL THE BEST

Prof.Dr. *Mohammed A. Fayyadh*



جامعة شط العرب الاهلية

كلية العلوم - قسم التحليلات المرضية

Pathogenic Bacteriology

Lect-1-

Bacteria Compared with Other Microorganisms

Prof. Dr. Mohammed A. fayyadh

Aims of the lecture

- 1- Differentiate between major groups of microorganisms
- 2- Describe the general characteristics of bacteria
- 3- Differentiate between Prokaryote and Eukaryote cells

Important references

1-Patrick R. Murray, Ken S. Rosenthal, Michael A. Pfaller (2022) Medical Microbiology.

2-Jawetz, Melnick, & Adelberg's Medical Microbiology Twenty-Sixth Edition

3-[Sahra Kırmusaoğlu](#) and [Sonia Bhonchal Bhardwaj](#) (2020) Pathogenic Bacteria

4-Todar's Online Textbook of Bacteriology.

Prokaryotes organisms

A-Domain Eubacteria (True bacteria)

***Bacteria** :are one-celled microscopic living organisms (0.5-2.0 micron in diameter)Belong to **Prokaryotic organisms** and Bacteria **Domain** . These organisms reproduce asexually by binary transverse fission. **They do not possess the nucleus typical of Eukarya**. The cell walls of these organisms are rigid (**with some exceptions, e.g., the mycoplasma**). **It can be grown on culture media**

Bacteria could be found everywhere. They can be either:-

- **Beneficial bacteria**, as in the process of fermentation (such as in vinegar and dairy production), also many bacteria play important role in decomposition. **OR**
- **Pathogenic bacteria** that could cause a disease when enters any living body (human , animal and plants) and it can spread through water, air, soil and through physical contact.

***Chlamydiae.** These organisms are **obligate intracellular parasites** that are able to reproduce in certain human cells only and are found in two stages: the infectious, non reproductive particles called **elementary bodies** (0.3 μm) and the noninfectious, intracytoplasmic, reproductive forms known as **initial (or reticulate) bodies** (1 μm). Gram-negative .They *Chlamydia* cause human respiratory and genitourinary tract disease, and in birds they cause respiratory disease. *Chlamydia trachomatis*

***Rickettsia.** These organisms are obligate intracellular parasites, rod shaped to coccoid, that reproduce by binary transverse fission. The diameter of the individual cell is from 0.3–1 μm .The majority of them are Gram-negative and multiply only within host cells. They lack the enzymatic capability to produce sufficient amounts of ATP to support their reproduction. They obtain the ATP from host cells.

***Mycoplasmas.** Mycoplasmas are bacteria without rigid cell walls. They are found in a wide variety of forms, the most common being the coccoid cell (0.3–0.8 μm). Threadlike forms also occur in various lengths . The colony is “fried egg” appearance . Several of them cause diseases in humans. (pneumonia, respiratory tract disease). *Mycoplasma pneumoniae*: walking pneumonia, atypical pneumonia.

B-Domain :Archaea(Ancient Bacteria)

include three groups:

1.The methanogens:(Strict anaerobes that produce methane (CH₄)from carbondioxide and hydrogen.)

•**ex:***Methanococcus*

2.Extreme halophiles:(Which require high concentrations of salt for survival.)

•**ex:** *Halococcus & Halobacterium*

3.Thermoacidophiles: (Which normally grow in hot , acidic environments.)

•**ex:** *Thremus aquatiquus*

Comparison(Differences) between Eubacteria and Archaea

Feature	Archaea	Bacteria
Cell Wall	No peptidoglycan	Contains peptidoglycan (murein)
Cell membrane	Ether-linked lipids (branched isoprene chains)	Ester-linked lipids (fatty acids)
Habitat	Often found in extreme environments (high salt, temperature, acidity, etc.)	Found in diverse environments, including soil, water, and living hosts
Pathogenicity	No known pathogens	Many are pathogenic (e.g., E. coli, Streptococcus)
Antibiotic Sensitivity	Not sensitive to most antibiotics that affect bacteria	Often sensitive to common antibiotics

Domain :Eukarya

Fungi and Protozoa (Eukaryotes)

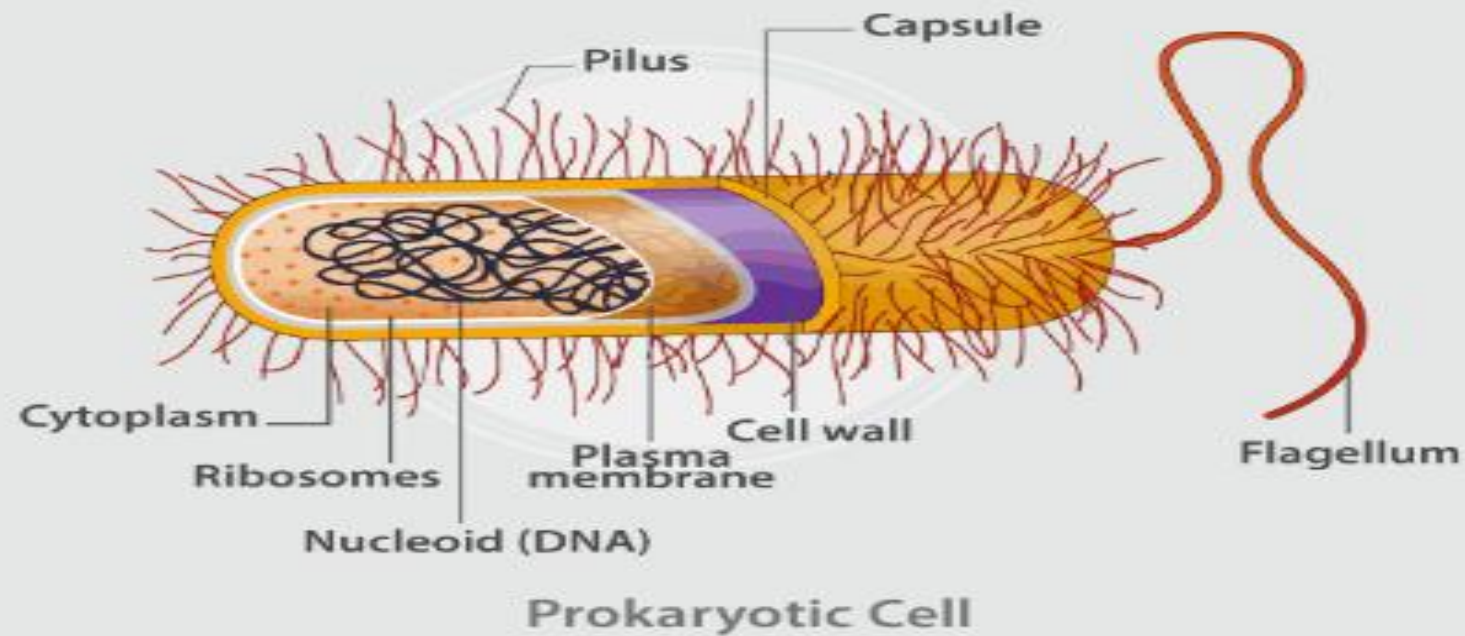
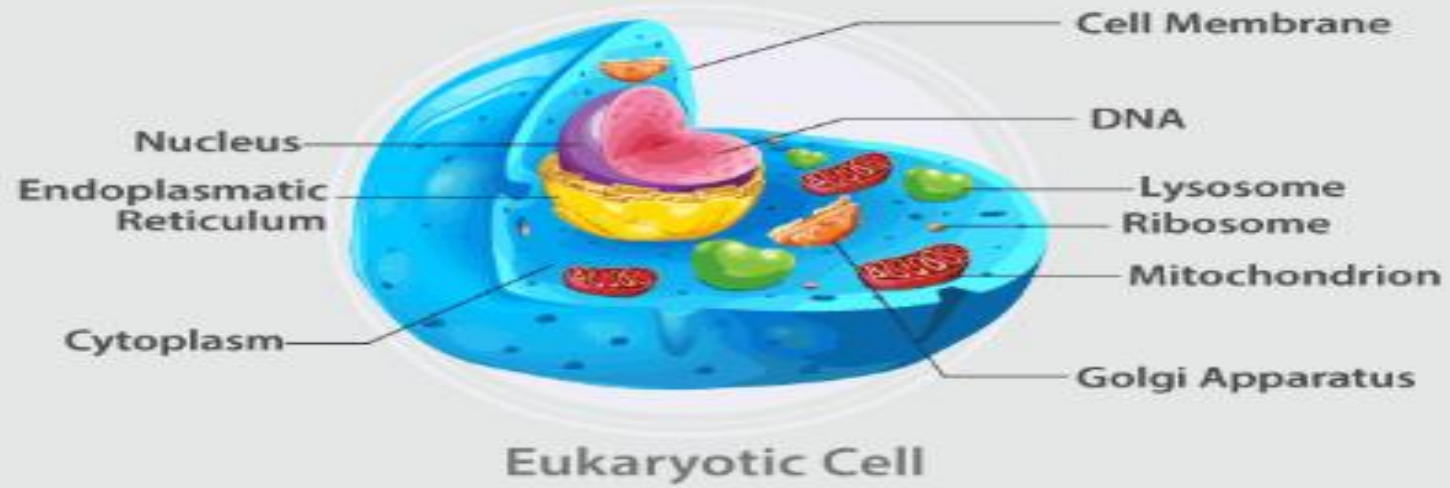
* **Fungi.** Fungi (Mycophyta) are nonmotile eukaryotes with rigid cell walls and a classic cell nucleus. They contain no photosynthetic pigments and are carbon heterotrophic, that is, they utilize various organic nutrient substrates (in contrast to carbon autotrophic plants). of more than 150 000 fungal species(The number of fungal species in nature is estimated at between 1-2 million), **only about 300 are known to be human pathogens.** Most fungal infections occur as a result of **weakened host immune defense.**

***Protozoa.** Protozoa are microorganisms in various sizes and forms that may be free-living or parasitic. They possess a nucleus containing chromosome and organelles such as **mitochondria** (lacking in some cases),**an endoplasmic reticulum** , **pseudopods**, **flagella**, **cilia**, kinetoplasts , etc. Many parasitic protozoa are transmitted by arthropods, whereby multiplication and transformation into the infectious stage take place in the vector.

Animals

Helminths. Parasitic worms belong to the animal kingdom. These are metazoan organisms with highly differentiated structures. Medically significant groups include the trematodes (flukes or flatworms), Cestodes (tapeworms), and nematodes (roundworms). المسطحة والشريطية والخييطية

Arthropods. These animals are characterized by an external chitin skeleton, segmented bodies, jointed legs, special mouthparts, and other specific features. Their role as direct causative agents of diseases is a minor one (mites, for instance, cause scabies) as compared to their role as vectors transmitting viruses, bacteria, protozoa, and helminths



Subcellular Infectious Entities

Viruses. Ultramicroscopic, obligate intracellular parasites that:

contain only one type of nucleic acid, either DNA or RNA,

possess no enzymatic energy-producing system and no protein-synthesizing apparatus.

force infected host cells to synthesize virus particles

Prions (proteinaceous infectious particles). The evidence indicates that prions are protein molecules that cause degenerative (أعتلال) central nervous system (CNS) diseases such as Creutzfeldt-Jakob disease, Kuru, scrapie in sheep, and bovine spongiform encephalopathy (BSE) (general term: transmissible spongiform encephalopathies [TSE]).

Comparison between Prokaryotes and Eukaryotes

	Prokaryotes	Eukaryotes
Type of Cell	Unicellular	Multi-cellular and Unicellular
Nucleus	Absent (There is no membrane surrounding the genetic material.)	Present
Mitochondria	Absent	Present
DNA arrangement	Circular	Linear
Ribosomes	70s (spherical in shape)	80s (linear in shape)
Endoplasmic reticulum	Absent	Present
Cell division	Binary fission (Mitosis)	Mitosis and Meiosis

MCQ

1. Which of the following microorganisms is acellular?

A) Bacteria B) Archaea C) Fungi D) Virus

2. What distinguishes protozoa from bacteria?

A) Protozoa are unicellular; bacteria are multicellular
B) Protozoa are eukaryotic; bacteria are prokaryotic
C) Protozoa have peptidoglycan cell walls
D) Bacteria have a nucleus

4- Which of the following organisms requires a host cell to reproduce?

A) Bacteria B) Fungi C) Archaea D) Virus

5- Which of the following is incorrect about viruses?

A) They are acellular B) They contain both DNA and RNA C) They rely on host cells for replication
D) They are smaller than bacteria

Q2) Define the following terms:- 1-prion 2-Chlamydiae 3-Extreme halophiles

Thanks for your attention

Pro. Dr. Mohammed A Fayyadh



جامعة شط العرب الاهلية

كلية العلوم - قسم التحليلات المرضية

- Pathogenic Bacteriology
- Lect-4-
- Classification of Medically important Bacteria
- *Prof. Dr. Mohammed A. fayyadh*

Introduction

Taxonomy: [Greek taxis = arrangement or order, and nomos = law, or nemein, to distribute or govern] **is defined as the science of biological classification, consists of three separate but interrelated parts: *Classification, Nomenclature and Identification.***

Classification: is the arrangement of organisms into groups or taxa (s., taxon) based on mutual similarity(التشابه المتبادل) or evolutionary relatedness.

Nomenclature: is the branch of taxonomy concerned with the assignment of names to taxonomic groups in agreement with published rules.

Identification: is the practical side of taxonomy, the process of determining that a particular isolate belongs to a recognized taxon.

Taxonomy is important for several reasons :

- 1- It allows us to organize huge amounts of knowledge about organisms because all members of a particular group share many characteristics.
- 2- Taxonomy allows us to make predictions and frame hypotheses for further research based on knowledge of similar organisms.
- 3- Taxonomy places microorganisms in meaningful, useful groups with precise names so that microbiologists can work with them and communicate efficiently.
- 4- Taxonomy is essential for accurate identification of microorganisms. For example, it is essential to clinical microbiology treatment

Classification of Bacteria

Bacteria are classified based on a combination of **morphological**, **physiological**, **biochemical**, **genetic**, and **ecological** characteristics. Below is a summary of the **main criteria** used in bacterial classification:

A-Cell Morphology (Shape and Arrangement)

1-Cocci (Spherical) مكورة

It can be arranged in three forms:-

- Diplococci e.g.: *Streptococcus pneumoniae*



- Chain (Cocci) e.g.: *Streptococcus pyogenes*

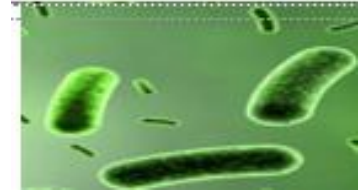


- Cluster or Grape like shape e.g.: *Staphylococcus aureus*

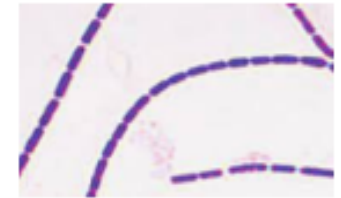


2- Bacilli

Short Bacilli e.g.: *Bacillus subtilis*.



Long Bacilli e.g.: *Lactobacillus*. spp.

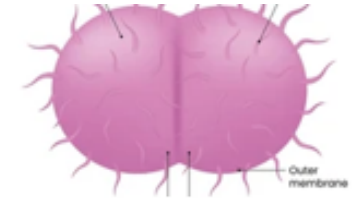


Coccobacilli e.g.: like members of Enterobacteriaceae family

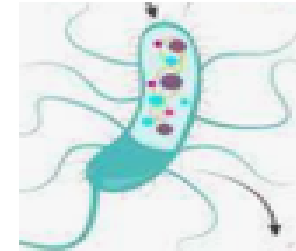


(*Escherichia coli*, *Shigella*, *Salmonella*).

3-Kidney shape: e.g.: *Neisseria gonorrhoeae*.



4-comma shape: e.g.: *Vibrio cholera*.



5- Spiral shape: e.g.: *Helicobacter pylori*



B-Ability to form spores: The bacteria are divided to two groups according to their ability to form spores.

1-Non-spore - former Bacteria: e.g. - *Staphylococcus* spp., *Escherichia coli*,

Streptococcus spp.

2-spore - former Bacteria: e.g.: *Bacillus*, *Clostridium* and *Sporolactobacillus*.

C- Oxygen requirements.

1-Obligates (strict) aerobes bacteria: e.g.: *Bacillus*, *Pseudomonas*.

2-Obligates (strict) anaerobes bacteria: e.g.: *Clostridium*.

3-Facultative anaerobes e.g.: Enterobacteriaceae e.g.: *Escherichia coli*,
Shigella, Salmonella and *Staphylococcus* spp.

4-Microaerophiles e.g. *Helicobacter pylori*. (lower than 21% oxygen)

5-Aerotolerant e.g. *Streptococcus*

D-Reaction to the Gram stain: The Bacteria are divided in two groups according to the reaction with Gram stain.

1- Gram positive bacteria. e.g.: *Streptococcus*, *Staphylococcus*,

Bacillus and *Clostridium*. (purple color)

2- Gram Negative Bacteria. e.g. All the members of Enterobacteriaceae

(*Escherichia coli*, *Shigella*, *Salmonella*,.....) (Pink or Red color)

E- Bacterial Nutrition

1-Autotrophic bacteria: These bacteria synthesize all their food from inorganic substances (CO_2 and hydrogen donor). (i) Photosynthetic bacteria. (ii) Chemosynthetic bacteria.

2-Heterotrophic bacteria: The heterotrophic bacteria obtain their-ready made food from organic substances, living or dead. These bacteria including three types:

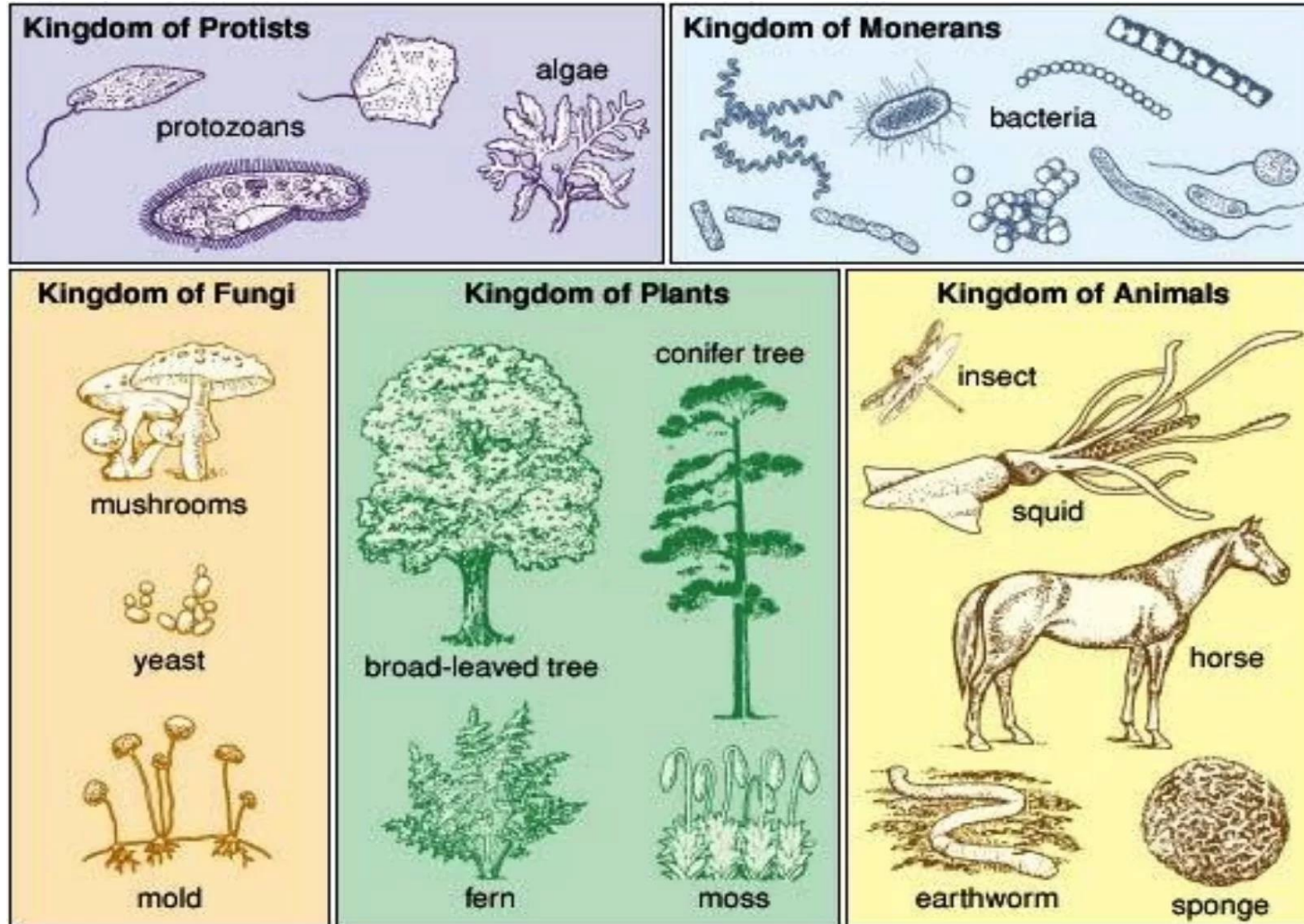
- (i) Saprophytic bacteria.
- (ii) Parasitic bacteria.
- (iii) Symbiotic bacteria.

Scientific classification of bacteria

Classification is a method for organizing microorganisms into groups or taxa based on similar morphologic, physiologic, and genetic traits. The hierarchical classification (التصنيف الهرمي) system consists of the following taxa designations:

- **Domains** (Bacteria, Archaea, and Eukarya)
- **Kingdom** (contains similar divisions or phyla; most inclusive taxa)
- **Phylum** (contains similar classes; equivalent to the Division taxa in botany)
- **Class** (contains similar orders)
- **Order** (contains similar families)
- **Family** (contains similar genera)
- **Genus** (contains similar species)
- **Species** (specific epithet; lowercase Latin adjective or noun; most exclusive taxa)

In 1969 **Robert Whittaker** proposed a **five kingdom** for all living organisms:



Whittaker's **Five** Kingdom Classification

In 1978 **Carl Woese** proposed **Domain** as level of classification above **kingdom**.

There are three domains based on the following distinguishing criteria:

1-Cell wall composition 2-Membrane lipids 3-RNA sequence
4-Protein synthesis 5-Antibiotic sensitivity

The Domains are:-

- **A- Domain Eubacteria** (True bacteria)
- **B- Domain Archaeabacteria** (Ancient bacteria)
- **C- Domain Eukarya:** All eukaryotes(**Protista, Fungi, Plantae , and Animalia**).

Taxonomy of Medically Important Bacteria by Phylum

- **Phylum: Proteobacteria** (Gram-negative rods and cocci

Includes many human pathogens)

- **Class: Gammaproteobacteria**

- **genus:** *Escherichia* *E. coli*

- *Salmonella* *S. typhi*

- *Shigella* *S. dysenteriae*

- **Class: Betaproteobacteria**

- **genus:** *Neisseria* *N. meningitides* , *N. gonorrhoeae*

-Class: Epsilonproteobacteria

-genus : *Helicobacter* *H. pylori*

Phylum: Firmicutes (Gram-positive cocci and rods)

-Class: Bacilli

-genus : Bacillus *B. anthracis*

- :Staphylococcus *S. aureus*

-Class: Clostridia

-genus : Clostridium *C. tetani*

Phylum: Actinobacteria (High G+C ; Gram-positive rods)

-genus : Mycobacterium *M. tuberculosis*

-genus : Corynebacterium *C. diphtheriae*

Example: Taxonomic Ranks of the bacterium *Escherichia coli*

Example: Taxonomic Ranks of the bacterium *Escherichia coli*

Formal Rank	Example
Domain	Bacteria
Phylum	Proteobacteria
Class	<i>Gammaproteobacteria</i>
Order	<i>Enterobacteriales</i>
Family	<i>Enterobacteriaceae</i>
Genus	<i>Escherichia</i>
Species	<i>coli</i>

Prokaryote identification

- Various techniques are employed to characterize and identify microorganisms

A-Phenotypic characteristics:

-Microscopic morphology:

arrangement , staining , culturing ,other structures

B-Metabolic differences:

fermentation and oxidation test , other biochemical tests

C-Serology: antibody antigen reaction

D-Fatty acid analysis

E-Genotypic characteristics

- Nucleic acid probes : DNA amplification :rRNA sequencing

Bergey's Manual of Systematic Bacteriology

Bergey's Manual of Systematic Bacteriology is a **comprehensive reference work** that provides detailed descriptions of the **classification, identification, and taxonomy of bacteria**. It is considered the **gold standard** in bacterial taxonomy.

- Named after **David Hendricks Bergey**, an American bacteriologist.
- First published in **1923** as “**Bergey's Manual of Determinative Bacteriology**”.
- Later evolved into two main works:

- 1. Bergey's Manual of Systematic Bacteriology** – For scientific classification (taxonomy)
- 2. Bergey's Manual of Determinative Bacteriology** – For laboratory identification

MCQ

1-Bacteria are primarily classified based on:

- A) Their DNA sequence only B) Color of the colony C) Shape, cell wall structure, and metabolism D) Their habitat only

2- Which of the following is a coccus-shaped bacterium?

- A) Escherichia coli B) Streptococcus pneumonia C) Spirillum minus D) Vibrio cholera

3-In Gram staining, Gram-positive bacteria appear:

- A) Red or pink B) Green C) Blue or purple D) Colorless

4- Bacteria that can live without oxygen are called:

- A) Aerobic B) Anaerobic C) Facultative D) Obligate aerobes

5-Which of the following is a Gram-negative rod?

- A) Staphylococcus aureus B) Bacillus subtilis C) Escherichia coli D) Streptococcus pyogenes

6-The binomial nomenclature system gives each bacterium a:

- A. Class and species name B. Family and genus name
- C. Genus and species name D. Domain and species name

7-Which of the following bacteria belongs to the phylum Firmicutes?

- A. Escherichia coli B. Bacillus subtilis C. Pseudomonas aeruginosa D. Neisseria gonorrhoeae

8- What is the lowest (most specific) level of classification for bacteria?

- A. Genus B. Family C. Order D. Species



جامعة شط العرب الاهلية

كلية العلوم - قسم التحليلات المرضية

Pathogenic Bacteriology

Lect-5-

Normal Flora

- *Prof. Dr. Mohammed A. fayyadh*

Normal Microflora (Flora) or Human Microbiota

Some terms should be know:-

Saprophytes:-These microorganisms are **nonpathogenic**; their natural habitat is dead organic matter.

Parasites:-Unicellular or metazoan organism living in or on an organism of another species (host) on the expense of the host.

Pathogen:- any organism can cause disease to human, animals and plants .

Opportunistic pathogen : An agent capable of causing disease only when the hosts resistance is impaired(when the patient is **immunocompromised**). these are frequently germs of the normal flora or occasionally from the surrounding environment, animals, or other germ carriers.

- **Symbiosis**: it is the relationship between two dissimilar organisms that are adapted to live together.

- **There are three types of symbiosis:**

1. **Mutualism**: close relationship in which the parasite and the host depend on each other to survive. It's beneficial for both of them .E.g.: the **protozoa** in the digestive tract of **termites**.

2. **Commensalism**: It's beneficial for the parasite, at the same time it's harmless for the host . E.g.: *E. coli* .

3. **Parasitism**: The parasite is harmful to the host . E.g.: *Entamoeba histolytica* , *Giardia lamblia* .

What are Normal Flora?

- Normal flora are microorganisms (bacteria, fungi, protozoa, and viruses), mostly bacteria that continuously inhabited the human body (**Resident Normal Flora**).
- Under normal conditions in a healthy human they are harmless and may even be beneficial.
- Also called **Commensals organisms that dine together** or **Microflora. Or Microbiota**

Note about Normal Flora

- *A fetus is sterile when born (No Normal Flora), then newborn start having the normal flora from its mother, air, food and the environment.
- *Our internal organs are sterile like the spleen(الطحال), liver, pancreas, bladder(المثانة), CSF (Cerebrospinal fluid), and blood unless during infection.
- *Normal flora differ from one human to another depending on age, diet, and geographic habitat.
- *When the number of resident normal flora is greatly reduced, opportunistic microbes can easily cause infections in these areas e.g. *Candida albicans* that cause candidiasis. (داء المبيضات)

Location of normal flora in the body

Normal flora is found on or in most parts of the body, especially those exposed directly or indirectly to the external environment. Below are the most important locations where it is found.

- 1. Skin**
- 2. Eyes and Ears**
- 3. Respiratory Tract**
- 4. Oral Cavity (Mouth)**
- 5. Gastrointestinal Tract**
- 6. Urogenital Tract**

Normal Flora in the Skin

- Resident bacteria of the skin are mainly bacteria and fungi and can be in any layer of the skin.

1-Aerobic Bacteria

- Present in the outer layer of skin.
- Staphylococcus epidermidis* (accounts 90%) +
Staphylococcus aureus.

2-Anaerobic Bacteria: (More than Aerobic bacteria)

- Present in the deeper skin layers, hair follicles, and sweat & sebaceous glands.
- Propionibacterium acnes*. (بكتريا حب الشباب)

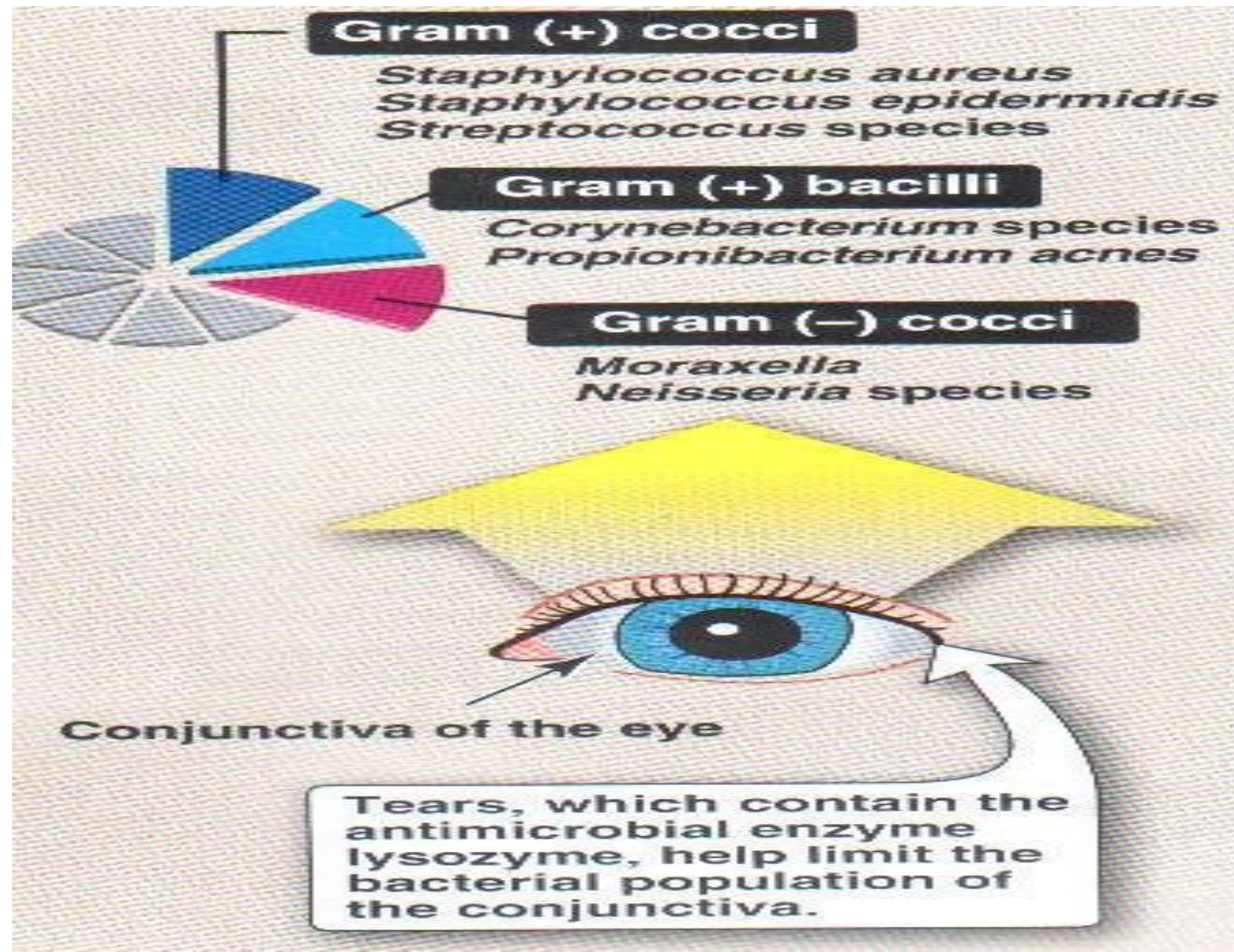
- The number and variety of normal flora depends on :
 1. Moisture.
 2. pH
 3. Temperature.
 4. Salinity.
 5. Chemical waste (urea , fatty acids ,..)
 6. Other microbes.
- Skin normal flora are generally harmless but it might cause **bloodstream infections** if skin was penetrated.
- Healthcare workers should maintain proper hygiene (skin and clothing) to prevent self infection or transferring infection to patients.

Normal Flora in the EYES

- The external surface of the eye is cleaned and protected by tears, mucus, and oil.
- Tears, that contain **Lysozyme enzyme**, will reduce the number of normal flora of the eye.
- **The conjunctiva** (الملتحة) of the eye has primarily **Staphylococcus, Streptococcus and Corynebacterium**.

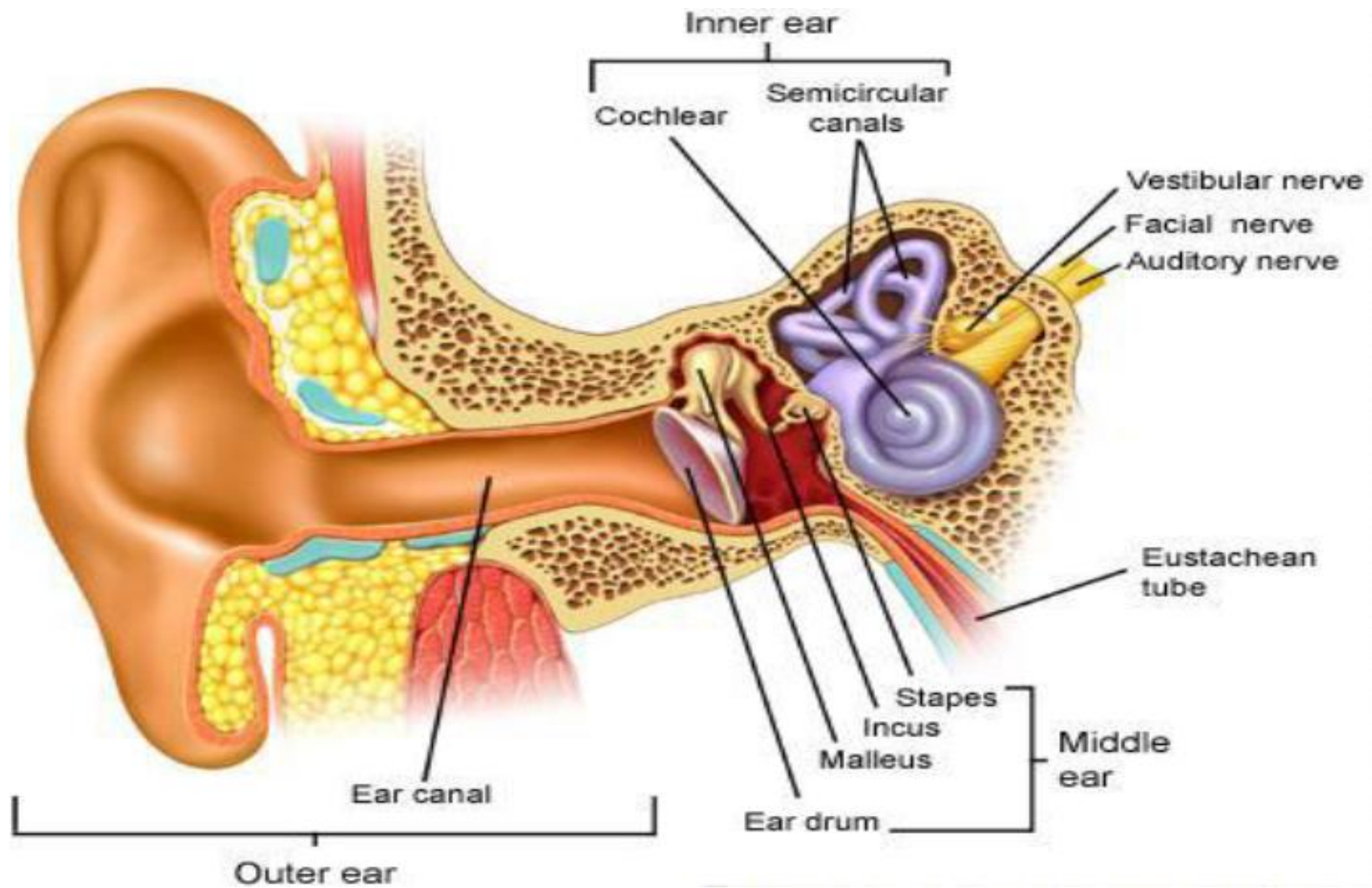
The conjunctiva covers the white part of the eye and lines the inside of the eyelids.

Normal Flora in the Eye



Normal Flora in the EARS

- The middle ear and inner ear: **are usually sterile.**
- **The outer ear and the auditory canal:** contain the same normal flora of the moist areas like nose and mouth.
- When the person coughs, sneezes, or blows his nose, these microbes may move into the middle ear where they cause infection.
- Also infection of the middle ear develops If the **Eustachian tube** does not open and close properly to maintain air pressure.



Normal Flora in Respiratory Tract

- Upper Respiratory Tract:

- Nose and throat have many microorganisms: -Normal flora: *Corynebacterium*(diphtheroids).

- Opportunistic: Staphylococcus, Streptococcus

- Carriers: only in some people have virulent bacteria like *C. diphtheria* and *S. pneumoniae*.

- Lower Respiratory Tract:

- Is usually sterile because the mucous membranes and lungs remove any microbes.

Normal Flora in the Oral Cavity (Mouth)

- They have both aerobic and anaerobic bacteria. The most common ones are: *C. diphtheroides*, *S. aureus*, *S. epidermidis*.
- Also yeasts, molds, protozoa, and viruses can be living in the mouth.
- **Teeth and Gingiva: Streptococcus mutants**
- Poor dental hygiene help bacteria to grow and cause **dental caries**(تسوس الاسنان), **gingivitis**,...
- After dental surgeries, there might be a risk of bloodstream infection that might cause **endocarditis**.(التهاب الشغاف)

Normal Flora in the Gastrointestinal Tract

- **Stomach:** Only few bacteria are present in the stomach due to gastric enzymes and acidic pH.
- **Small intestine:** Only few normal flora are present in the upper part of small intestine because **bile** kills them. Lower parts have more no. of normal flora.
- **Large intestine:**
 - Has more bacteria than any other part of the body. 99% of normal flora in the large intestine are anaerobic *Bacteroides spp.*
 - Also many fungi, protozoa, and viruses can live there.
 - Many of the normal flora are opportunistic **i.e. if they move to other areas e.g. *E.coli* cause urinary infection.**

Normal Flora in the Urogenital Tract

-Urinary Tract

- Kidneys, Ureters and Urinary Bladder (المثانة): are sterile.
- Lower Urethra and external opening: bacteria, yeast, and viruses. has the same bacteria present on the skin.

-Genital Organs

- Male and female genitals: are sterile except vagina.
- Vagina: *Lactobacillus spp.* *Candida albicans*

Beneficial Functions of Normal Flora

1-**Protect** - Normal flora protect our organs and systems that are in direct contact with the external environment from invading pathogens. Some normal flora **produce substances** that kills pathogens while others **compete** with them for nutrients.

2-In newborns, normal flora **stimulates** the development of immune system.

3-Normal flora of the gut **provides important nutrients** such as Vitamin K which aid in digestion and absorption of nutrients.

Harmful Effect of Normal Flora

1. When the normal flora are displaced from their normal site of the body e.g. **bloodstream infections by *Staphylococcus epidermidis***.
2. When potential pathogens (المسببات المحتملة) gain a competitive advantage due to diminished populations of harmless competitors e.g. ***Clostridium difficile*** growing in the gut after antibiotic therapy.
3. When harmless, commonly ingested food substances are converted into carcinogenic derivatives by bacteria in the colon, for example, **sodium cyclamate (a sweetener)**.
4. When individuals are immunocompromised, normal flora can overgrow and become pathogenic.

MCQ

1. Which of the following best describes normal flora?

- A. Pathogenic microorganisms that cause disease B. Microorganisms that normally live on or in the human body without causing harm C. Microorganisms that only live in the environment D. Microorganisms found only in laboratory cultures

2. Which of the following is a beneficial function of normal flora?

- A. Producing toxins harmful to the host B. Competing with pathogens for nutrients and space
C. Causing inflammation in tissues D. Destroying body cells

3-Where is the largest population of normal flora found in the human body?

- A. Skin B. Mouth C. Large intestine D. Lungs

4. Normal flora help in protecting the host by:

- A. Producing antibiotics B. Competing with pathogens C. Stimulating the immune system
D. All of the above

5-Which of the following can result from disruption of normal flora (e.g., by antibiotics)?

- A. Enhanced immunity B. Opportunistic infections C. Increased nutrient absorption
D. Decreased pathogenic growth

See you next week



جامعة شط العرب الاهلية

كلية العلوم - قسم التحليلات المرضية

Pathogenic Bacteriology

Lect-6-

Pathogenesis of Bacteria

- *Prof. Dr. Mohammed A. fayyadh*

Basic terms

Basic terms frequently used in describing aspects of pathogenesis:

- * **Pathogenicity:** The ability of an infectious agent to cause disease.
- * **Virulence:** The quantitative ability of an agent to cause disease.
- * Virulent agents cause disease when introduced into the host in small numbers.
- * Virulence involves invasiveness and Toxigenicity.

*** Toxigenicity:** The ability of a microorganism to produce a toxin that contributes to the development of disease.

*** Invasion:** The process whereby bacteria, parasites, fungi and viruses enter the host cells or tissues and spread in the body.

- **Pathogen:** A microorganism capable of causing disease.
- **Non-pathogen:** A microorganism that does not cause disease. It may be part of the normal flora.
- **Opportunistic pathogen:** An agent capable of causing disease only when the host's resistance is impaired (e.g. the patient is immunocompromised).

Characters of pathogen

A-Pathogenic organism should be able to enter the host body.

B-Pathogenic organism should be able to multiply in tissue.

C-Pathogenic organism should be able to damage the tissue.

D-They must be capable to resist the host defense.

Robert Koch postulates

In 1884, Robert Koch proposed a series of postulates in his treatise on *Mycobacterium tuberculosis* and tuberculosis.

These postulates have been applied more broadly to link many specific bacterial species with particular diseases.

Koch's postulates (GERM THEORY of disease)

1-The microorganism should be found in all cases of the disease in question, and its distribution in the body should be in accordance with the lesions observed.

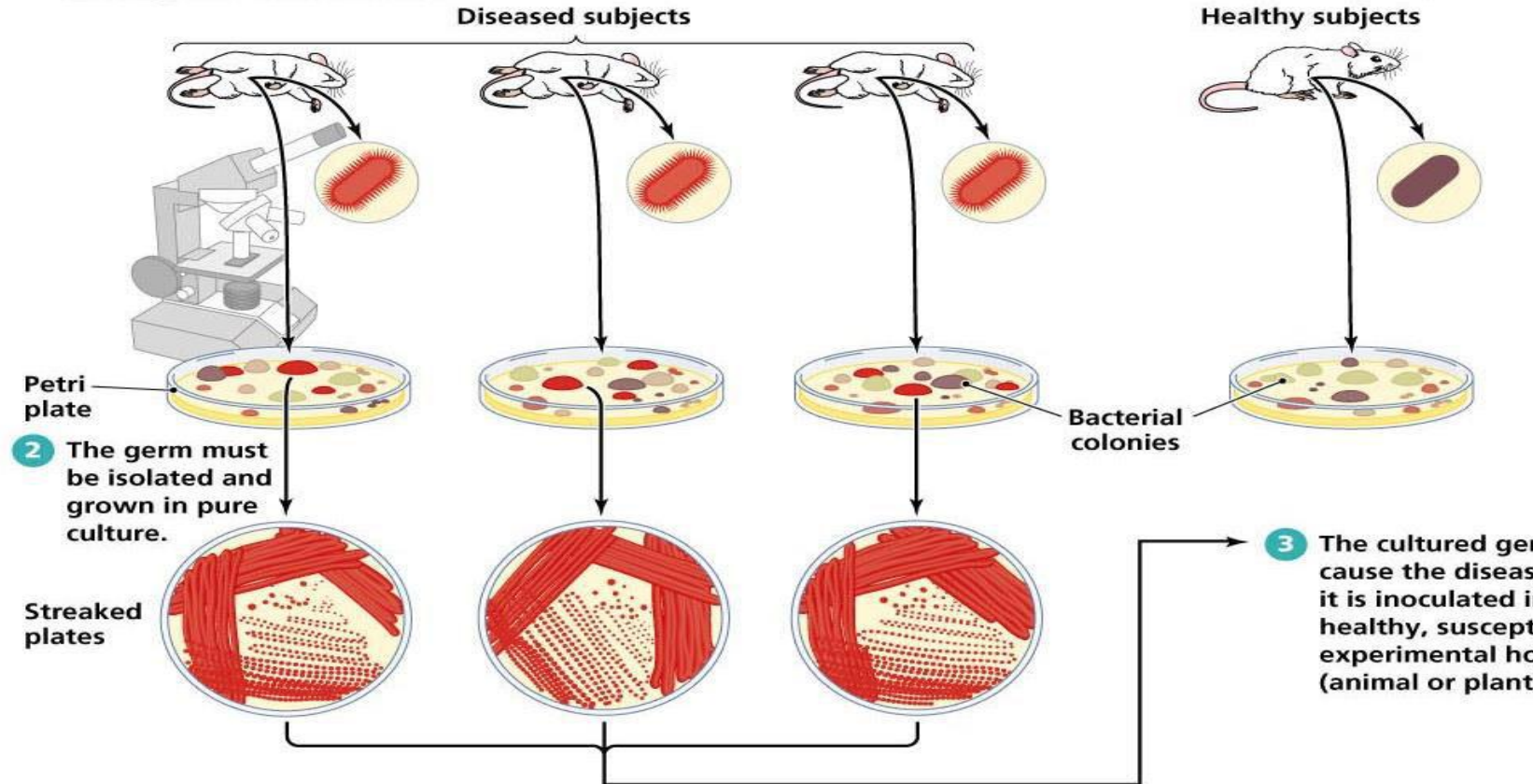
2-The microorganism should be grown in pure culture in vitro for several generations.

3-When such a pure culture is inoculated in to susceptible animal species, the typical disease must result.

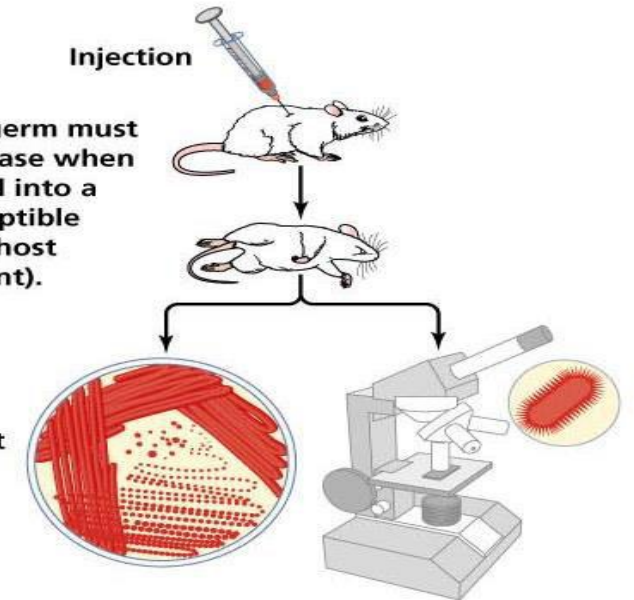
4-The microorganism must again be isolated from the lesions of such experimentally produced disease.

Koch's postulates

- 1 The suspected germ must be present in every case of the disease.



- 4 The same germ must be reisolated from the diseased experimental host.



Exceptions to Koch's Postulates

*Using Koch's postulates is not feasible in all cases

1- Some pathogens can't be cultured in the laboratory

2- Some diseases are caused by a combination of pathogens and other cofactors

3- Ethical considerations prevent applying Koch's postulates to pathogens that require a human host.

Virulence Factors of Pathogenic Bacteria

- Virulence factors contribute to an organisms virulence are:-

A-Adhesion factors

B-Biofilms

C-Extracellular enzymes

D-Toxins

E-Antiphagocytic factors

A-Adhesion factors

Certain bacteria have specialized structures which allow them to adhere to specific sites on surface of human cell, thereby enhancing their ability to cause diseases. The most important are:-

1. Capsule:

- Which mediate adherence of pathogen to specific receptors on host cell surface. prevent the phagocytes from adhering to bacteria, therefore act as Antiphagocytic factor.

2. Pili:

- Which mediate adherence to specific sites on surface of host cell.

B-Biofilms

A biofilm is a group of bacterial cells that stick to each other and often to a surface, enclosed within a protective, sticky, gel-like material they secrete, called the **extracellular polymeric substance (EPS)**.

Biofilms play a crucial role in bacterial pathogenesis (the ability of bacteria to cause disease) by helping pathogens survive, evade host defenses, and resist treatment.

C-Extracellular enzymes

- Enzymes secreted by the pathogen Dissolve structural chemicals in the body Help pathogen maintain infection, invade further, and avoid body defenses.

1- Collagenase and hyaluronidase ; which degrade collagen and hyaluronic acid ,thereby allowing the bacteria to spread through host tissues.

2- Coagulase; which accelerates the formation of fibrin clot from fibrinogen around bacteria to protect the bacteria from phagocytosis action. fibrinogen--**Coagulase** --fibrin

3- IgA protease; which degrades this immunoglobulin; allowing the bacteria to adhere to mucous membrane.

4- **leucocidin** = leukocidin (Toxin); which can destroy both neutrophils and macrophages.(WBC) .

5- **Hemolysin (Toxin)** which can destroy RBC .

D-Toxin

Toxin are Chemicals that harm tissues or trigger host immune responses that cause damage

Toxemia refers to toxins in the bloodstream that are carried beyond the site of infection.

- The bacteria produce two types of toxins the are :-.

- **Exotoxins**

- **Endotoxins.**

Exotoxin is produce by G+ ve and G- ve bacteria. In contrast, **endotoxin** which is produce by only G- ve bacteria. **Exotoxin** is protein, whereas **endotoxin** is lipid and carbohydrate.

Exotoxins

Endotoxin

Source	Mainly Gram-positive and Gram-negative bacteria	Gram-negative bacteria
Relation to bacteria	Metabolic product secreted from living cell	Portion of outer (cell wall) membrane released upon cell death
Chemical nature	Protein or short peptide	Lipid portion of lipopolysaccharide (lipid A) of outer (cell wall) membrane
Toxicity	High	Low, but may be fatal in high doses
Heat stability	Typically unstable at temperatures above 60°C	Stable for up to 1 hour at autoclave temperatures (121°C)
Effect on host	Variable depending on source; may be cytotoxin, neurotoxin, enterotoxin	Fever, lethargy, malaise, shock, blood coagulation
Fever producing?	No	Yes
Antigenicity ^a	Strong: stimulates antitoxin (antibody) production	Weak

Quorum sensing (QS)

Quorum sensing (QS) is a cell-to-cell communication mechanism used by bacteria to detect and respond to changes in cell population density through the production and detection of **autoinducers** (signaling molecules). It plays a **critical role** in regulating genes involved in **virulence, biofilm formation, motility, and antibiotic resistance**

Stages of bacterial pathogenesis

A-Transmission and portal of entry

1-Mode of transmission :-

a-Inhalation b-Ingestion c-Skin contact d-Blood transfusion e-Sexual intercourse.

2- Portals of entry :-

a- Respiratory tract. b-gastrointestinal tract. c-Skin d- Genital tract.

B-Adherence

C-Invasion and damage of host tissues:

MCQ

1. Which of the following is NOT considered a bacterial virulence factor?

A. Capsule B. Pili (fimbriae) C. Ribosome D. Exotoxin

2. The primary function of bacterial capsules in pathogenesis is to:

A. Aid in nutrient uptake B. Facilitate motility C. Evade phagocytosis D. Promote conjugation

3-Which virulence factor is responsible for tissue degradation and spreading of bacteria in host tissues?

A. Hemolysin B. Hyaluronidase C. Catalase D. Beta-lactamase

4- Endotoxins are part of which bacterial structure?

A. Flagella B. Outer membrane of Gram-negative bacteria
C. Peptidoglycan layer D. Cytoplasmic membrane

5. Which enzyme produced by bacteria breaks down immunoglobulin A (IgA)?

A. Coagulase B. IgA protease C. Urease D. Lecithinase

Fill in the blank with suitable terms

- 1-The ability of a bacterium to cause disease is known as its _____.
- 2-Bacterial structures such as _____ and _____ help in adherence to host tissues during the initial stages of infection.
- 3_____ are toxic proteins secreted by bacteria that target specific host functions or cells.
- 4-_____ are structural components of the bacterial cell wall in Gram-negative bacteria that can trigger strong immune responses.
- 5-The secretion system used by many Gram-negative bacteria to inject virulence factors directly into host cells is called the _____ **secretion system**.
- 6-The capsule in bacteria primarily aids in evading the host's _____ response.
- 7-Bacteria that live inside host cells to avoid immune detection are called _____ **pathogens**.
- 8-A common bacterial strategy to acquire iron from the host is the secretion of _____.
- 6-The process by which non-pathogenic bacteria acquire virulence genes from other organisms is known as _____ **gene transfer**.
- 7-_____ is a phase in bacterial pathogenesis where the bacteria increase in number and begin to produce toxins.

Q2) Explain why Koch's postulates is not feasible in all cases.

Q4)list the steps of Koch's postulates .

Thanks for your attention



جامعة شط العرب الاهلية

كلية العلوم - قسم التحليلات المرضية

Pathogenic Bacteriology

Lect-7-

Anerobic Bacteria

- *Prof. Dr. Mohammed A. fayyadh*

Introduction to Anaerobic Bacteria

Anaerobic bacteria are organisms that thrive in environments **devoid of oxygen**. They may die or fail to grow in the presence of atmospheric levels of oxygen (21%).

Types of Anaerobes:

Obligate Anaerobes: Cannot tolerate oxygen (e.g., *Clostridium perfringens*).

Facultative Anaerobes: Can grow with or without oxygen (e.g., *Escherichia coli*).

Aerotolerant Anaerobes: Do not use oxygen but can survive its presence (e.g., *Lactobacillus* spp.).

Microaerophiles: Require low levels of oxygen (not strictly anaerobic but relevant in low-oxygen environments). (e.g., *Helicobacter pylori*)

Where Anaerobes are Found

*Normal flora of:

- Gastrointestinal tract
- Oral cavity
- Skin (especially sebaceous glands) الغدد الدهنية
- Female genital tract

*Environmental sources:

- Soil
- Sediments
- Deep wounds with compromised blood supply

Characteristics of Anaerobic Infections

- Often **polymicrobial** (mixed with aerobic bacteria).
- Associated with **foul odor, gas production, and tissue necrosis.**
- Frequently occur in **low oxygen** environments such as:
 - Abscesses
 - Deep tissue injuries
 - Post-surgical wounds
 - Necrotic tissue

Common Anaerobic Pathogens

Genus	Notable Species
Clostridium	C. perfringens, C. tetani, C. botulinum, C. difficile
Bacteroides	B. fragilis
Fusobacterium	F. necrophorum
Peptostreptococcus	Multiple species
Propionibacterium (Cutibacterium)	C. acnes
Actinomyces	

Genus : Clostridium

4 important species:

- *C. tetani*
- *C. botulinum*
- *C. perfringens*
- *C. difficile*

Characteristic of Clostridium

Clostridium is:- G+ve: obligate anaerobic: spore-forming bacilli.

- Four main species responsible for disease in humans:

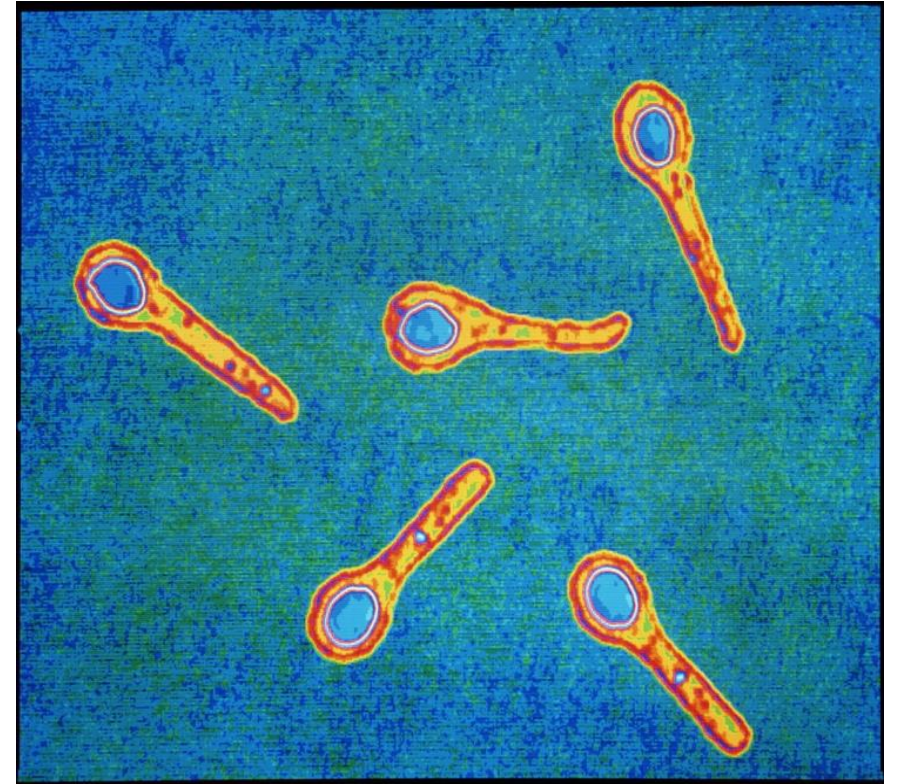
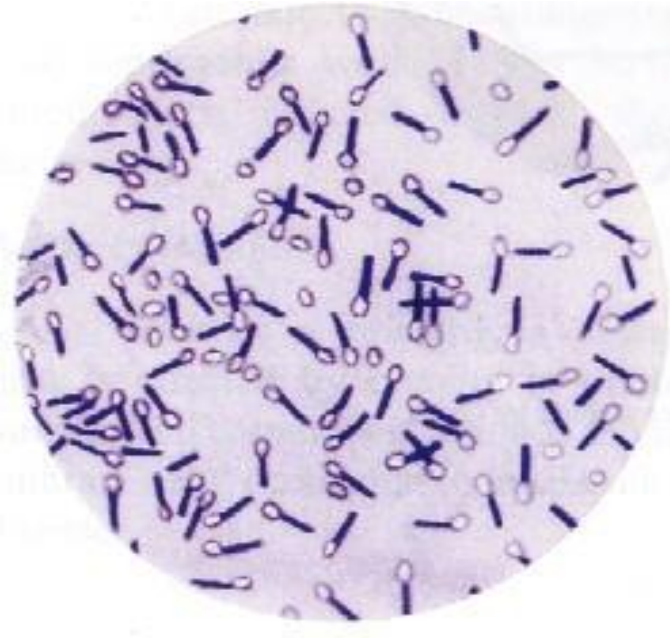
1-*Clostridium tetani* (تسبب مرض الكزاز)

- Rod-shaped, rounded ends.
- Motile with Peritrichous flagella
- Spores are rounded terminal, form drum-stick appearance.(tennis racket)
- Spores are highly resistant, may remain alive for years, and resist boiling for 15-90 min., some may resist dry heat at 150 C° for 1 hour.

Clostridium tetani causes **tetanus**, a serious nervous system disease triggered by a powerful neurotoxin called **Tetanospasmin**.

Q) What is the main symptoms of Tetani :(Home work).

Clostridium tetani



C. tetani their spores are terminally location

2-Clostridium botulinum

- Rod-shaped, single or in pairs or in chains
- **Spores are oval, subterminal , bulging** (منتفخة)
- Motile with 4-8 Peritrichous flagella
- Form large irregular, glistening (لامعة) colonies with fimbriated border. (حواف مزخرفة)
- Meat is digested in cooked meat medium with putrid odor .
- Spores are destroyed at 180 C° in dry heat for 5-15 min., and in boiling for 5 hours.

#-Infants — especially those under **12 months old** — have an **underdeveloped intestinal microbiota**. This means their gut lacks enough “good” bacteria to compete with or inhibit *C. botulinum* spores.

#-Once the spores are ingested (from contaminated honey), they can **germinate inside the infant’s intestine**. The bacteria then produce **botulinum toxin**, (neurotoxins).

#-The **botulinum toxin** blocks nerve function, preventing muscles from contracting. This leads to **flaccid paralysis**(شلل رخو) — symptoms include:-

#-Constipation (often the first sign) ; Weak cry; Poor feeding ; Muscle weakness or “**floppiness**”ترهل ; Breathing difficulties in severe cases.

3-Clostridium perfringens

***Gram+ve , obligate anaerobe.**

***Non-motile.**

***Spore forming, Spores are oval, central or subterminal ,not bulging.**

***Capsulated**

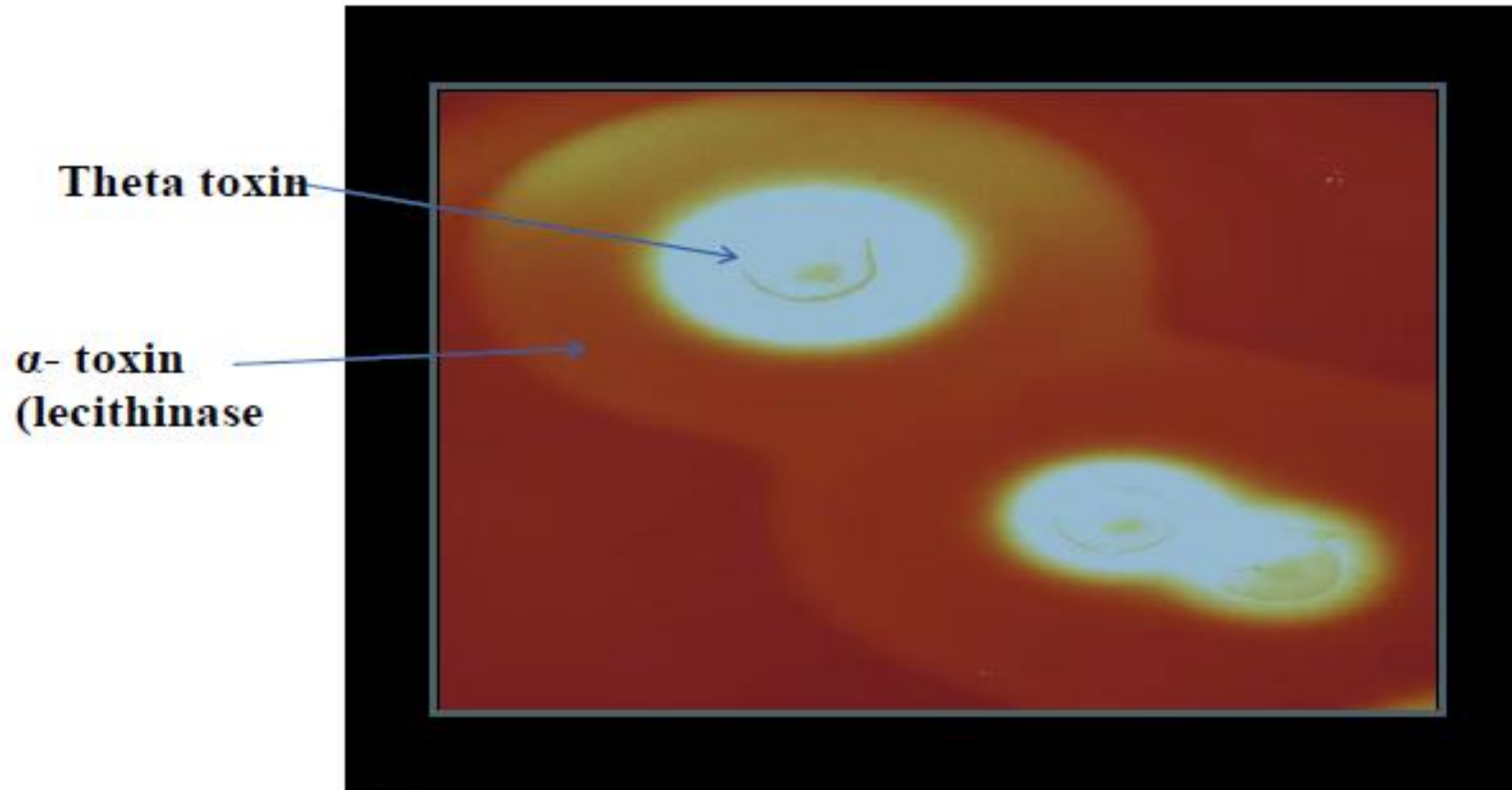
***Grow best on media containing carbohydrates such as ;glucose agar, or glucose blood agar.**

*Spores of strains of food poisoning resist boiling for 1-3 hours . Other strains can resist boiling for several hours.

*Produce double zone inhibition {narrow zone of complete hemolysis(**theta toxin**),while α -toxin (**lecithinase**) produce a wide zone of hemolysis}.**Theta and α -toxin** are Virulence factors

- ***C. perfringens* cause gas gangrene and food poisoning**

C. Perfringens produce double zone of hemolysis



Types of spores



C.tetani



C.perfringens



C.botulinum

4- *Clostridium difficile*

- *which overgrows during antibiotic therapy, may lead to **pseudomembranous colitis**. (التهاب القولون الغشائي الكاذب)
- *Gram-positive, rod-shaped (bacillus) ; Forms **subterminal endospores**; Motile with peritrichous flagella; Normal flora of the **human intestine** (small numbers in healthy individuals) and in the **environment** (hospitals, soil, feces).
- * Produces a characteristic "**horse stable**" **odor** due to short-chain fatty acids- **Ferments carbohydrates**- **Catalase and oxidase negative**.

Laboratory diagnostic tests

Clostridium spp. could be divided into:

1. **Saccharolytic organisms;** *C. perfringens*, ferments carbohydrates, produces acid and gas
2. **Proteolytic organisms;** *C. tetani*, digest proteins with blackening and bad smell production.
3. **Mixed saccharolytic & proteolytic;** certain groups of *C. botulinum*.

Specimen: Wound, food, stool.

1.Gram stain: G+ve rods with spore site according to spp

2.Culture: Cultures are incubated under anaerobic conditions.

a.Blood agar:

*Most clostridia produce flat , gray colonies.

*They are also usually hemolytic.

- *Clostridium perfringens* produces raised, entire colonies with double zone hemolysis, the complete zone round the colony is due to theta toxin, and the wider zone around it is due to alpha toxin (lecithinase).

Micro & Macroscopic C. perfringens



Gram stain: G+ve rods



***C. perfringens* on blood agar
with double zone of hemolysis**

b. Robertson's medium (cooked meat medium):

- *-Cooked Meat Medium provides a favorable environment for the growth of anaerobes, since the muscle protein in the heart tissue is a source of amino acids and other nutrients.
- *-The **sulfhydryl groups**, which exert the reducing effect, are more available in denatured protein; therefore, the meat particles are cooked for use in the medium.
- *-Cooked Meat Medium is still widely used for the cultivation and maintenance of clostridia and for determining proteolytic activity of anaerobes.
- *-On **Robertson's cooked meat medium** → blackening of meat will be observed

The anaerobic conditions can be provided by:

(Growth & gas produced are evident).

Gas produced lifted the cooked meat particles to the surface.



Cooked Meat Medium Tubes

Reaction on Cooked Meat Medium

- Saccharolytic reaction

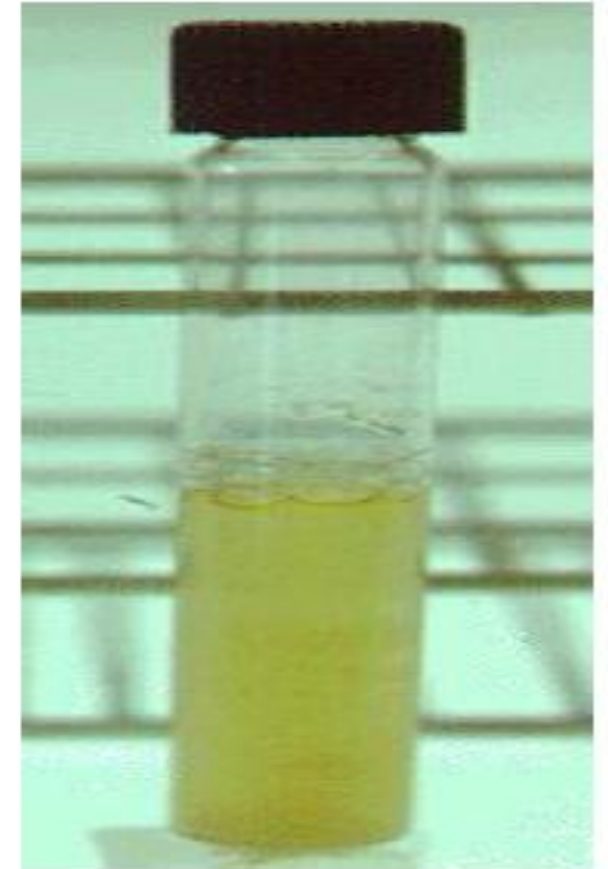
- It causes fermentation of glycogen of muscles
- Production of acid and gas
- Meat particles remain intact
- e.g *C. Perfergines*

- Proteolytic Reaction

- It causes digestion of meat particles with the production of H_2S and NH_3
- Formation of black, foul smelling due to sulfur compounds
- e.g *C. tetani*.

C. Brewer Thioglycollate Medium:

- Contains highly nutritious proteose peptone and beef infusion which support the growth of fastidious bacteria.
- Sodium thioglycollate helps to create anaerobic conditions.
- **Methylene blue** indicates oxygen content of the medium by exhibiting a bluish-green color of the medium in the presence of oxygen.
- Brewer's anaerobic jar is used for growing bacteria under anaerobic condition.



Reducing agents in the medium absorb oxygen and allow obligate anaerobes to grow

The anaerobic conditions can be provided by:



Anaerobic Jar

d. Egg yolk agar medium

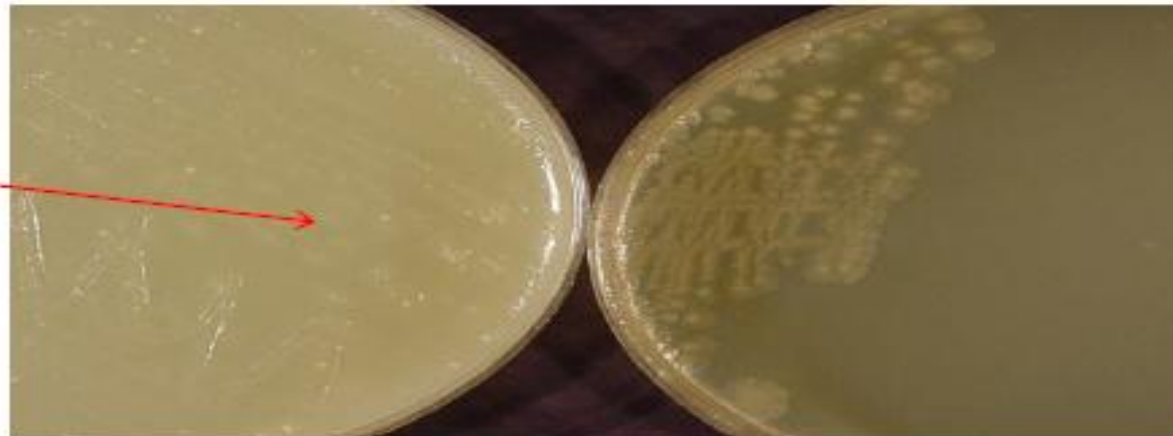
Definitive **diagnostic** test for *C. perfringens*

with *C. perfringens* growth



White opaque insoluble precipitation around colonies due to lecithinase activity

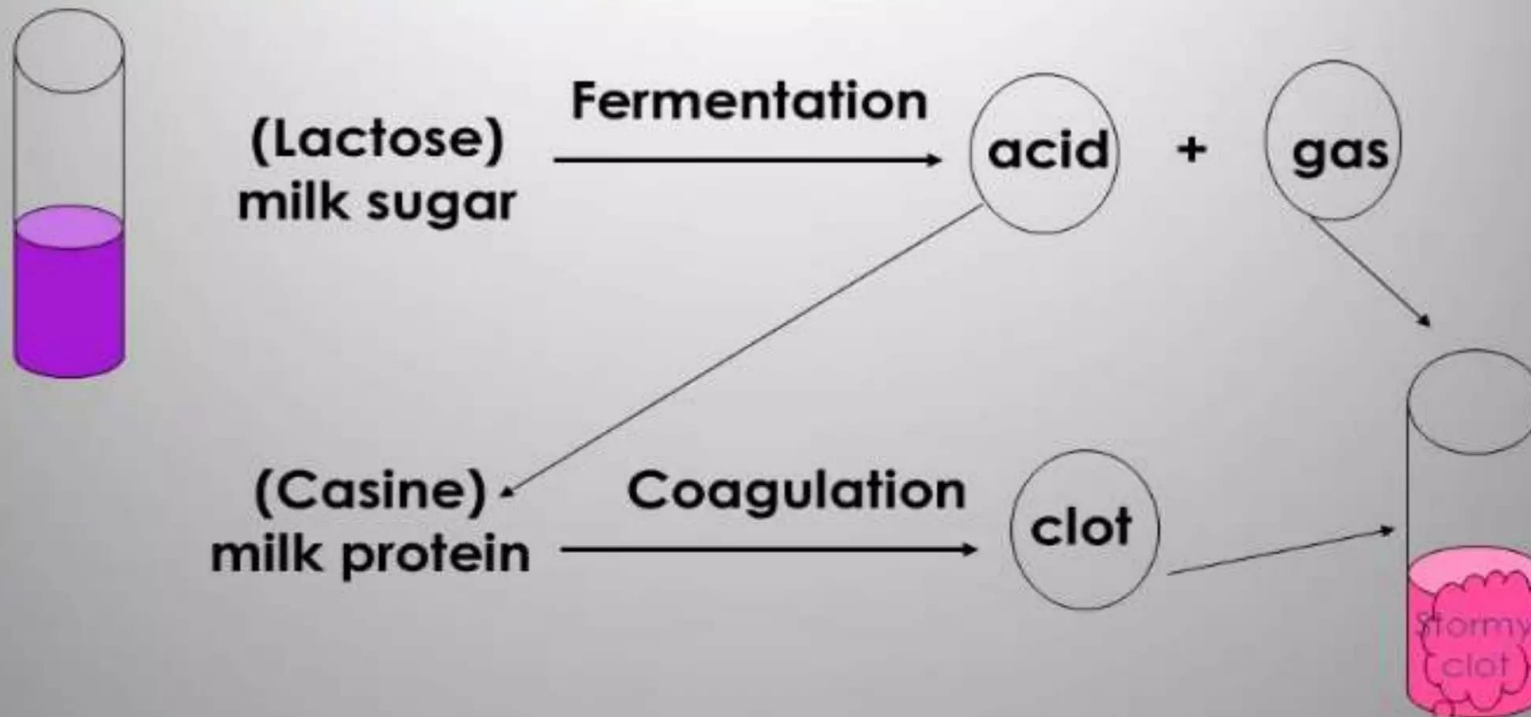
C. perfringens



3. Biochemical tests

a. Stormy fermentation

Stormy Clot Formation:



The *C. Perfringens* cause rapid fermentation of lactose in litmus milk and the gas produced split the clot (**Stormy Clot Formation**).

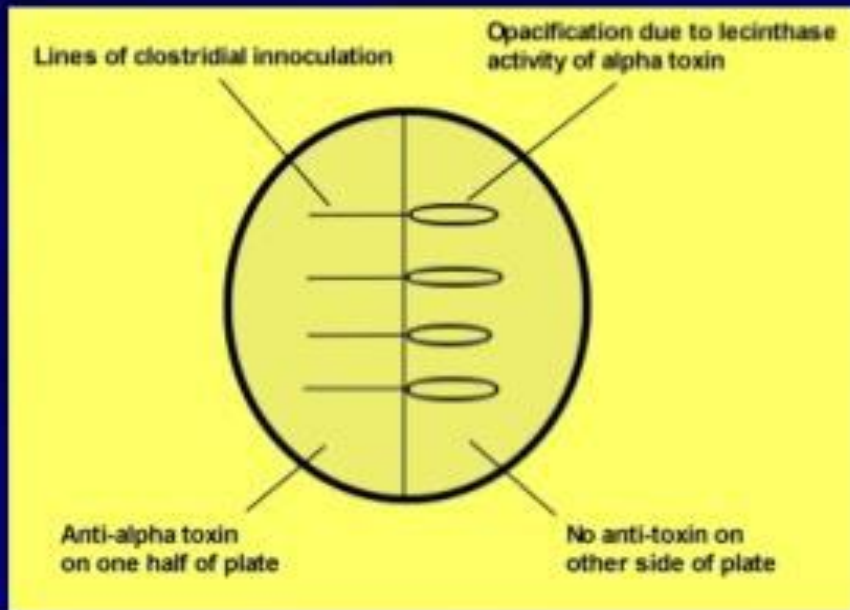


Stormy fermentation

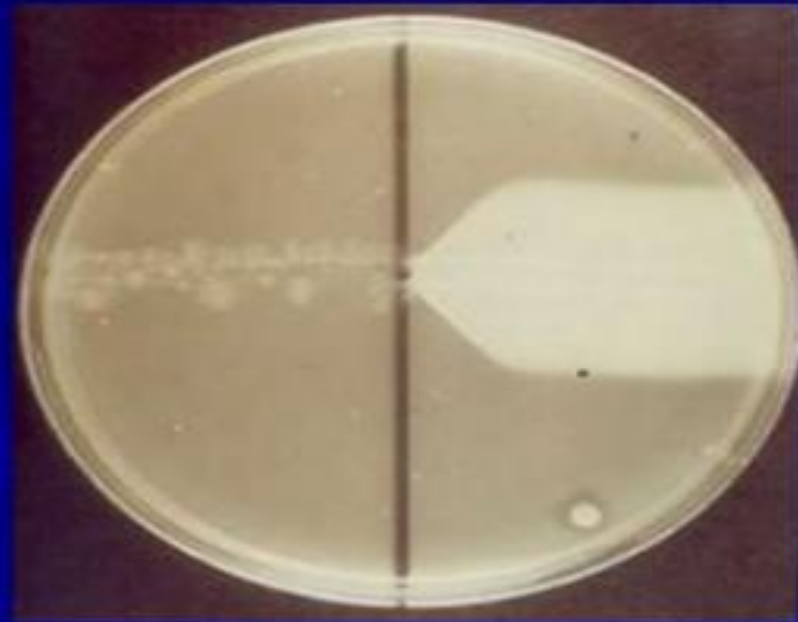
b. Nagler's reaction for the detection of lecithinase activity

- Nagler's reaction is related to the detection of lecithinase activity (alpha toxin of *Clostridium perfringens*). Nagler's reaction is a test used to identify bacteria that produce the enzyme *lecithinase*, which hydrolyzes lecithin, a phospholipid found in egg yolk.
- An egg-yolk plate is divided into two halves. Over one half is spread with a specific antitoxin.
- The culture to be tested, together with a positive control is streaked across the plate, going from the untreated area of the plate to the area that is covered with antitoxin.
- The culture is then incubated anaerobically.
- Following incubation, *lecithinase activity*, caused by the action of the *Clostridium perfringens*-toxin is seen as a precipitate in the medium on the side of the plate that did not receive the antitoxin, but not on the side that was treated.

Nagler Reaction



Procedure of Nagler Reaction



Positive Nagler Reaction

6. Serological test

- C. difficil Toxin in stool by ELISA
- C. tetani Toxin in serum by ELISA
- C. botulinum Toxin in serum by ELISA

Fill in the blank with suitable terms

- 1-*Clostridium* species are typically Gram-_____ and are known for being _____-forming bacteria.
- 2-*Clostridium tetani* causes the disease known as _____.
- 3-The neurotoxin produced by *Clostridium botulinum* is called _____.
- 4-*Clostridium difficile* is a major cause of _____-associated diarrhea, especially after antibiotic treatment.
- 5-The main virulence factors of *Clostridium perfringens* include _____ toxins that cause tissue necrosis.
- 6-The disease **gas gangrene** is most commonly associated with *Clostridium* _____.
- 7-*Clostridium botulinum* causes flaccid paralysis by blocking the release of the neurotransmitter _____.
- 8-*Clostridium difficile* produces two major toxins: toxin A (an _____ toxin) and toxin B (a _____ toxin).
- 9-*Clostridium* species are anaerobic, meaning they thrive in environments with little or no _____.
- 10-Infant botulism is often associated with ingestion of *Clostridium botulinum* spores found in _____.

MCQ

1. What type of bacteria are most *Clostridium* species?

A. Gram-positive, aerobic B. Gram-negative, anaerobic C. Gram-positive, anaerobic D. Gram-negative, aerobic

2. *Clostridium botulinum* produces a toxin that causes which type of paralysis?

A. Spastic paralysis B. Flaccid paralysis C. Localized muscle stiffness D. Respiratory stimulation

3-Which of the following is a major risk factor for *Clostridium difficile* infection (CDI)?

A. Iron deficiency B. Antibiotic use C. Vaccination D. Viral infection

4-*Clostridium perfringens* is commonly associated with which condition?

A. Botulism B. Diarrhea C. Tetanus D. Gas gangrene

5-Which of the following foods is most commonly associated with infant botulism?

A. Peanut butter B. Cow's milk C. Honey D. Yogurt

Thank You



جامعة شط العرب الاهلية

كلية العلوم - قسم التحليلات المرضية

Pathogenic Bacteriology

Lect-8-

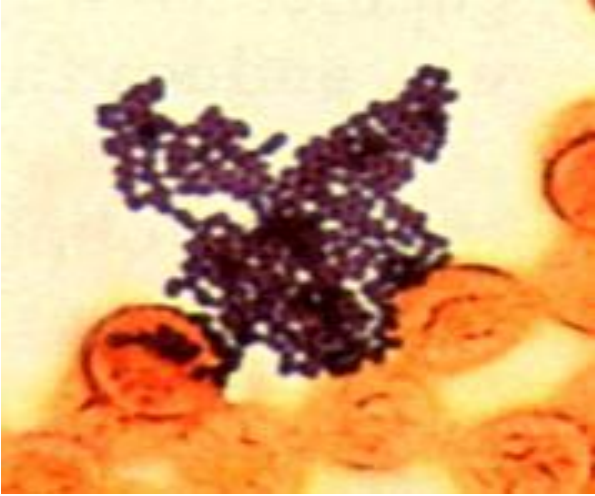
Gram + Cocci

Prof. Dr. Mohammed A. fayyadh

Questions should be answer through lecture

- what is staphylococci ??
- what are diseases that causing? And how?
- How can diagnose it in laboratory?
- How can differentiate from closely related species?

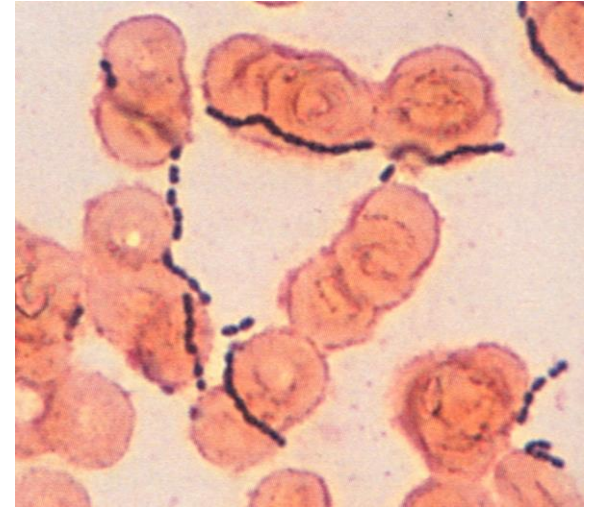
Pyogenic Cocci



Staphylococcus
Gram-positive
Catalase +



Neisseria
Gram-negative
Diplococci



Streptococcus
Gram-positive
Catalase -ve

Gram positive cocci - *Staphylococci*

(Nosocomial Infection - Hospital Acquired Infection)

General Features:-

1- is a genus of **Gram-positive** bacteria. Under the microscope, they appear spherical (**cocci**), and form in grape-like **clusters**.

2-**facultative anaerobic** organisms (capable of growth both aerobically and anaerobically).

3-Are **non-motile, non-capsulated** (some strain have capsule), **non-spore** forming.

4-*Staphylococci* are **oxidase** negative & **catalase** positive which one feature that distinguishes from *Streptococci*.

5-*Staphylococci* are part of normal flora of human skin, nose, respiratory and gastrointestinal tracts.

***Staphylococcus* has at least 30 spp., three spp. of clinical importance are :-**

-Coagulase – positive *Staphylococci*:-

□ ***Staphylococcus aureus*.**

-Coagulase – Negative *Staphylococci*:-

□ ***Staphylococcus epidermidis* .**

□ ***Staphylococcus saprophyticus*.**

Staphylococcus aureus

Transmission: *S. aureus* is major pathogenic spp. for human. Transmission of bacteria from human to human by skin , mucous membranes , inhalation of respiratory secretion or consumption of contaminated food which can then result in food poisoning.



Virulence Factors of *S. aureus*

A- Cell wall virulence factors (capsules , peptidoglycan , protein A , teichoic acid) .

B- Enzymes:-

1- Coagulase: Converts **fibrinogen** into **fibrin** to form blood clots around bacteria (Fibrin clots **hide** the bacteria from phagocytic cells) to protect the bacteria from phagocytosis action.

2- Staphylokinase :- Dissolves fibrin threads in blood clots, allowing *S. aureus* to **free** itself from **clots**.

3-Leukocidins:- enzymes that attack certain types of WBCs which can destroy both neutrophils and macrophages.

4-Hyaluronidase:- break down hyaluronic acid, thereby allowing the bacteria to spread through host tissues.

5- β -lactamase (Penicillinase):- Breaks down penicillin (Allows the bacteria to survive treatment with β -lactam antimicrobial drugs).
Methicillin resistant *Staphylococcus aureus* (MRSA).

6-Lipases :- Digest lipids, allowing staphylococcus to grow on the skin's surface and in oil glands.

C- Cytolytic exotoxin:- α , β , γ , and δ toxin attack red blood cell, macrophage , lymphocyte , neutrophils or platelets and often referred to as hemolysis. (Alpha, Beta ,Gamma , Delta)

D- Superantigen exotoxins:

□ **Enterotoxins:-** (six major antigenic types: A, B, C, D, E, and G): when these bacteria contaminate food and allowed to grow they secrete enterotoxin , ingestion of which can cause food poisoning.

□ **Toxin shock syndrome toxin.** متلازمة صدمة السموم

□ **Exfoliative toxin :-** it cause scalded skin syndrome in children.

A comparison of the Virulence factors of Staphylococcus species

19-t01_staphvirulence_t	<i>S. aureus</i>	<i>S. epidermidis</i>
Protein A	+	—
Coagulase	+	—
Catalase	+	+
Hyaluronidase	+	—
Staphylokinase	+	—
Lipase	+	+
β -lactamase (penicillinase)	+	—

Clinical Significance:-

Staphylococcal Infections are Classified as:

1-Cutaneous Diseases (Localized Skin Infection)

- Folliculitis** – hair follicle infection. التهاب بصيلات الشعر
- Furuncle** – boil. الدمل
- Impetigo (children)**- red patches; pus-filled crust. القوباء



Impetigo



Furuncle

2- Systemic disease:-

□- **Bacteremia** :- presence of bacteria in the blood from wound infections.(50% are nosocomial infections).

□-**Acute endocarditis**:- occurs when bacteria attack the lining of the heart; 50% mortality rate.

generally associated with intravenous drug abuse , acute endocarditis is caused by injection of contaminated preparation or by needles contaminated with *S. aureus*.

□-**Pneumonia** :- inflammation of the lungs in which the alveoli(حويصلات) and bronchioles (قصيبات) become filled with fluid.

□-**Toxin shock syndrome**:- TSS toxin produced by *S. aureus* is absorbed into the blood and causes shock. results in high fever , rash skin(طفح جلدي), vomiting , diarrhea and hypotension .

□-**Staphylococcal Gastroenteritis** : this is caused by ingestion of food contaminated with enterotoxin producing *S. aureus*. The incubation period (8-30 h). The symptoms severe nausea (غثيان), vomiting and diarrhea.

Common sources: foods that are improperly stored, especially those rich in protein (e.g., custards, meats, mayonnaise-based salads).

□- **Scalded Skin Syndrome** : this involves the appearance of superficial bullae (فقاعات) resulting from action of an exfoliative toxin. severe exfoliation affecting almost the entire body.



3-Oral Staph Infection:- is rare, it's possible to have a staph infection in your mouth. Staph bacteria are also capable of colonizing the mouth. One study found that 94 percent of healthy adults carried some form of Staphylococcus bacteria in their mouth and 24 percent carried *S. aureus*.

S. aureus bacteria have also been found in 0.7 to 15 percent of dental abscesses. A **dental abscess** is a pocket of pus that develops around a tooth due to a bacterial infection. Symptoms can include:

- =Pain, redness, and swelling around the affected tooth.
- =Swelling in your cheeks or face.
- =fever.
- =Bad taste or bad smell in your mouth.
- = Sensitivity to hot or cold food and drink in the affected area.
- =Difficulty opening your mouth and chewing food.



Gingival abscess



Coagulase – Negative *Staphylococci*

Staphylococcus epidermidis & *Staphylococcus saprophyticus* :-

S. epidermis is part of the normal flora on the skin and mucous membrane but can enter the bloodstream (**bacteremia**) and cause infection , especially at the site of **implants** .it commonly infects **intravenous catheters** and **prosthetic implants, prosthetic heart valves** (endocarditis's) and **prosthetic joints** (arthritis or osteomyelitis). Strains of *S. epidermidis* that produce a **glycocalyx** are more likely to adhere to prosthetic implant material.

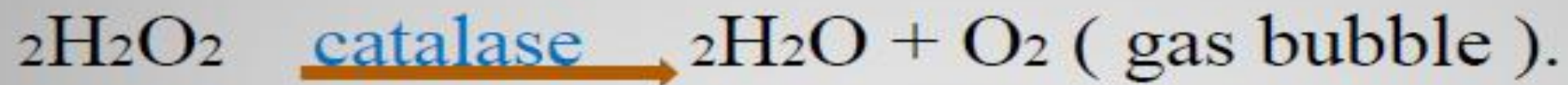
S. saprophyticus causes urinary tract infections , this organism is a frequent causes of **cystitis** in women , probably related to it occurrence as part of normal vaginal flora.

Laboratory Diagnosis

1- Microscopic smear (stain smear show G⁺ cocci , cluster shaped).

2- Lab isolation (*S. aureus* usually forms gray to deep **golden** yellow colonies, *S. aureus* produced alpha-toxin that causes wide zone of clear (beta – type) hemolysis on blood agar, while *S. epidermidis* colonies usually are gray to **white** and is not hemolytic on blood agar).

3-Catalase test (positive test):- the enzyme catalase mediates the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water. The presence of the enzymes in bacterial isolated is evident when a small inoculum is introduced into hydrogen peroxide and rapid elaboration oxygen (bubbles) and water.

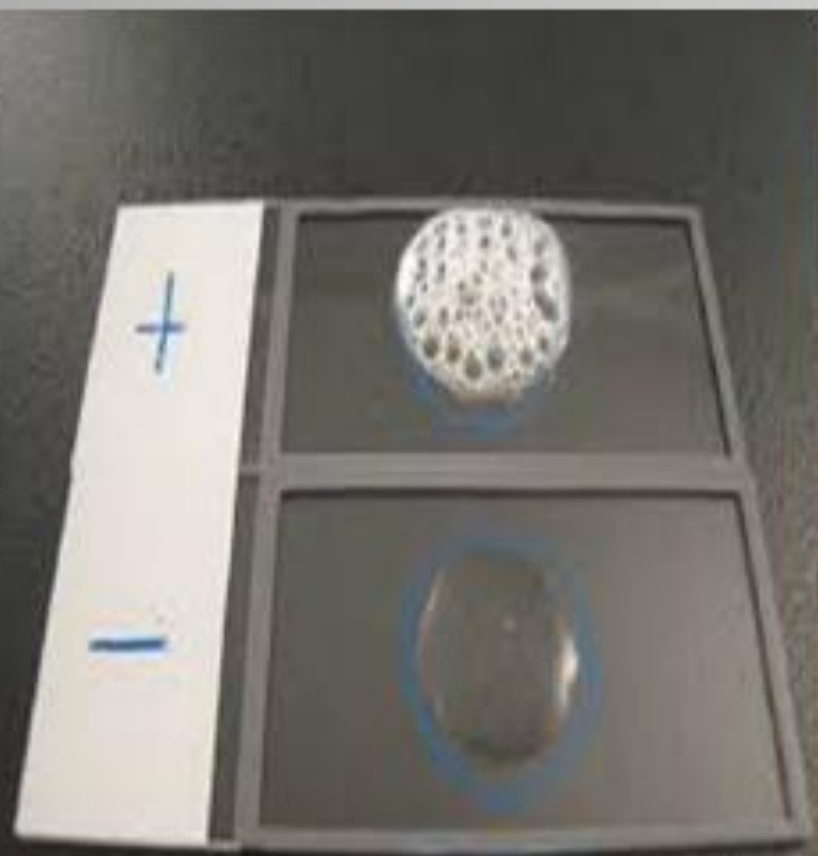


The result $\longrightarrow$ positive test.

No bubbles result $\longrightarrow$ negative test.

Note1 / blood agar will give a false positive result
Because blood contain the **catalase enzyme** .

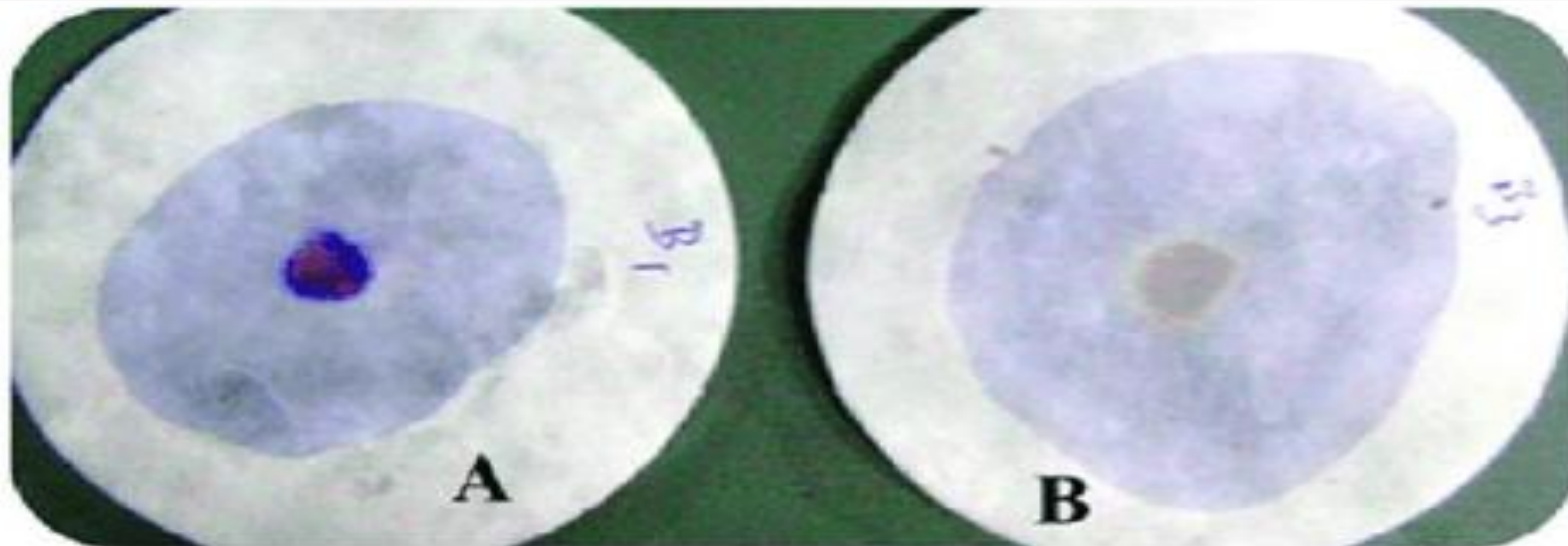
Note 2 / Platinum wires loop reaction H_2O_2 give false positive result. Preferably use plastic loop or applicator stick or glass rod.



ASM Worded, Image © Peter



4-Oxidase Test :- Used to determine if a bacterium produce of **cytochrome c oxidase**. small area of filter paper is soaked with a freshly prepared 1% oxidase reagent , bacterial colony to be tested is picked from agar by stick or glass rod and put it on the soaked area. (A) **positive result** is indicated by formation of deep **purple color** (dark blue) while the reagent is colorless when the (B) **negative result**.



5- Plasma Coagulase :- is a biochemical test used that differentiate *s. aureus* from other *staphylococcus* species like *s. epidermidis* & *s. saprophyticus*. Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of **fibrinogen** to **fibrin**.

s. aureus  coagulase positive.

s. epidermidis & *s. saprophyticus*  coagulase negative

S. aureus produces two forms of coagulase (i.e., bound coagulase and free coagulase). Bound coagulase, otherwise known as "**clumping factor**", can be detected by carrying out a **slide coagulase** test, and free coagulase can be detected using a **tube coagulase test**.

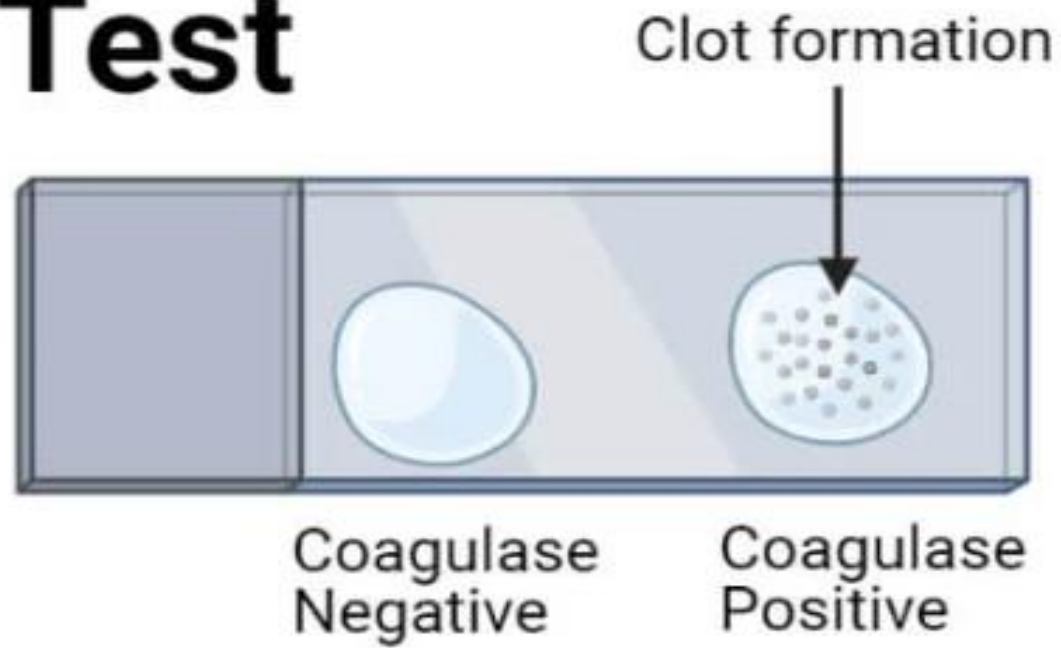
Slide Test

- 1-About 10 μ l of deionized water or physiological saline is added to a slide.
- 2-Several colonies from a fresh culture are collected with an inoculating loop and are emulsified into the water to obtain a smooth milk-colored suspension.
- 3-A drop of a rabbit or human plasma is added to the slide, and the clumping is observed immediately, not to exceed 10 seconds.

Tube Test

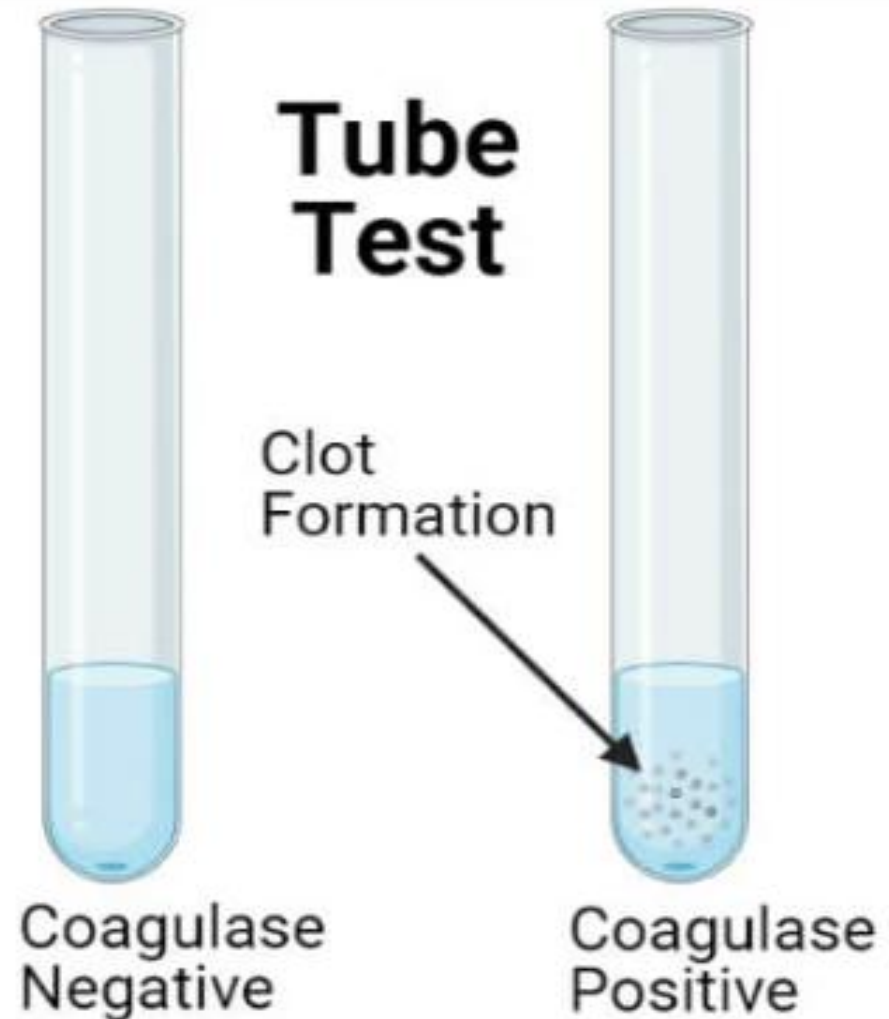
- 1-The rabbit plasma is diluted with physiological saline. (Add 0.2 ml plasma in 1.8 ml saline)
- 2-5 ml of the diluted plasma is then added to a test tube. About 5 drops of the test organism culture are added to the test tube.
- 4-The test tube is mixed and incubated at 37°C for an hour.
- 5-The tube is finally observed for clot formation. If no clotting is observed, the tube should be examined **at 30 minutes intervals of up to 6 hours.**

Coagulase Test

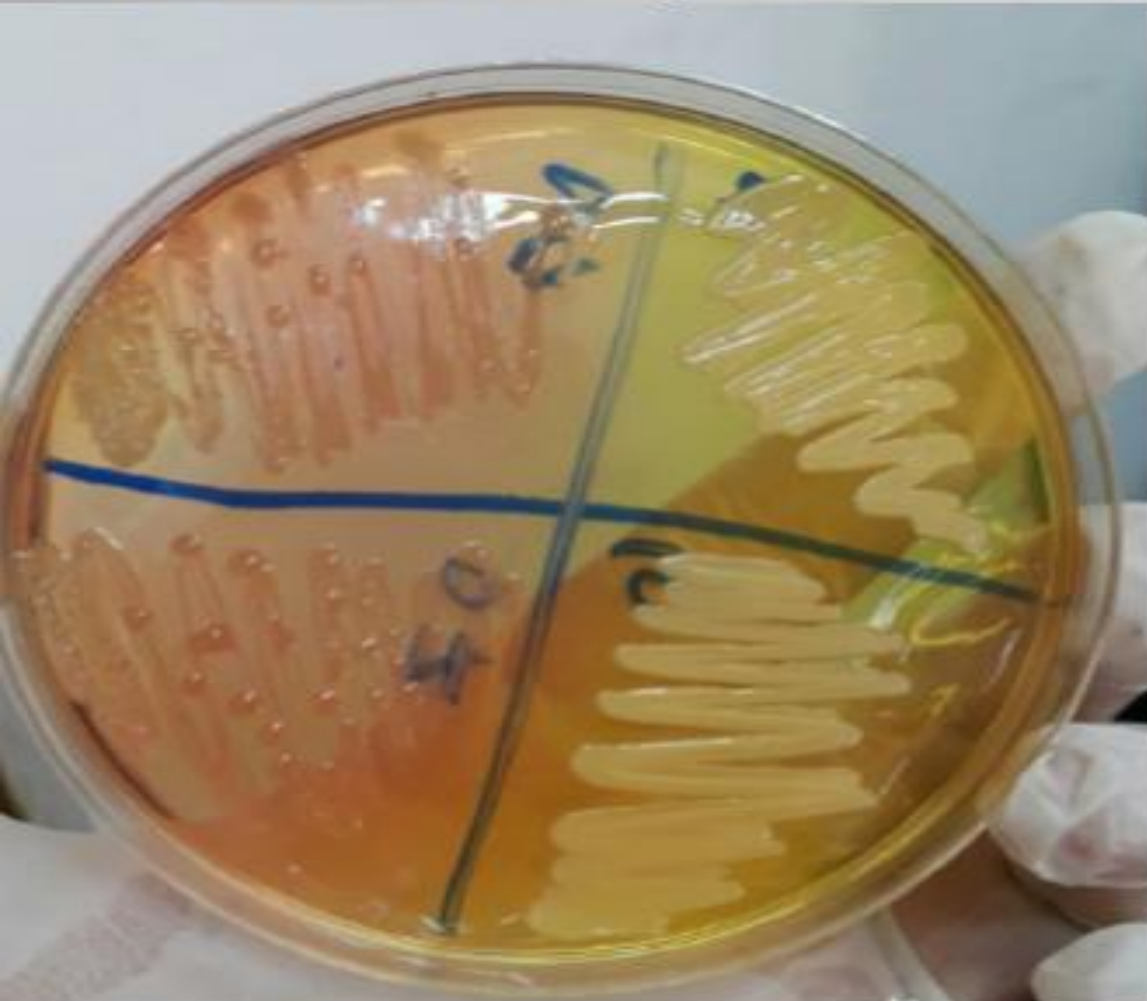


Slide Test

Tube Test



6- Mannitol salt agar (differential media) *S. aureus* produces yellow colonies with yellow zones, whereas *S. epidermidis* produce small pink or red colonies with no color change to the medium.



Staphylococcus epidermidis

Staphylococcus aureus

Nutrient Agar
(NA)



Mannitol Salt
Agar (MSA)



7-Novobiocin test

Staphylococcus saprophyticus (A) are **resistant** while *S. aureus* and *S. epidermidis* are sensitive .(B)

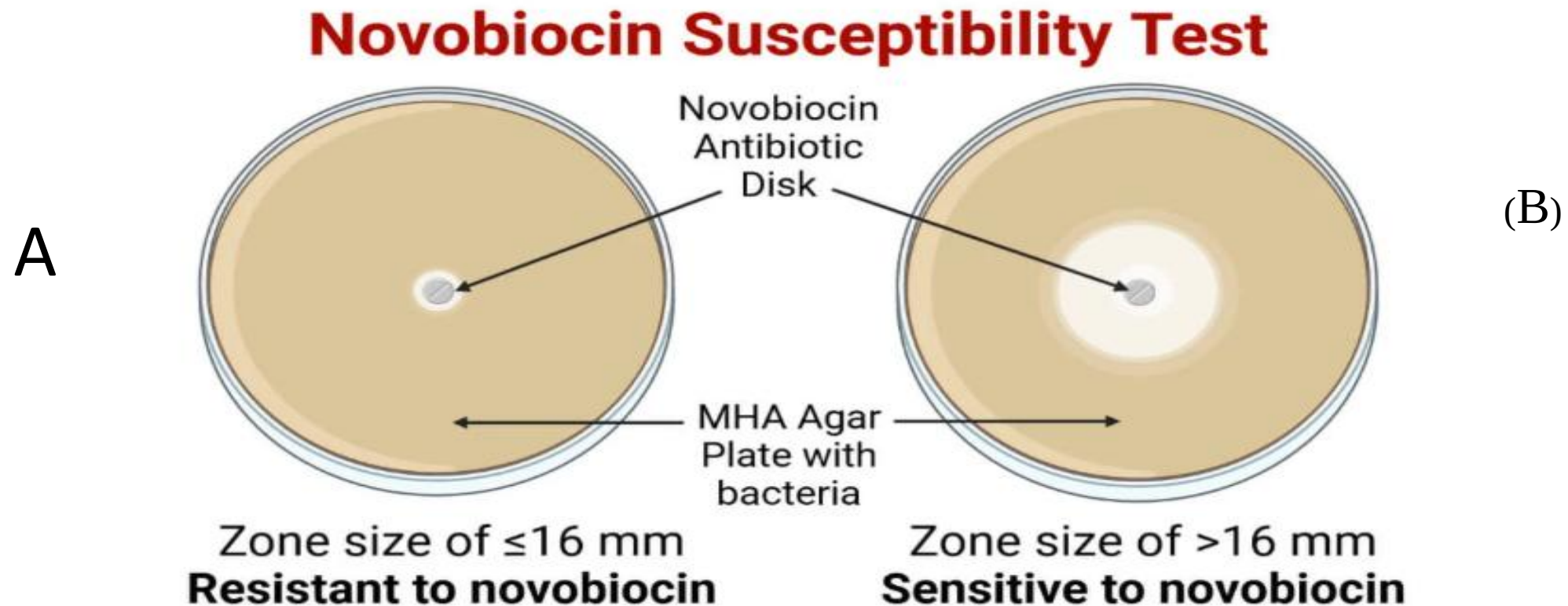
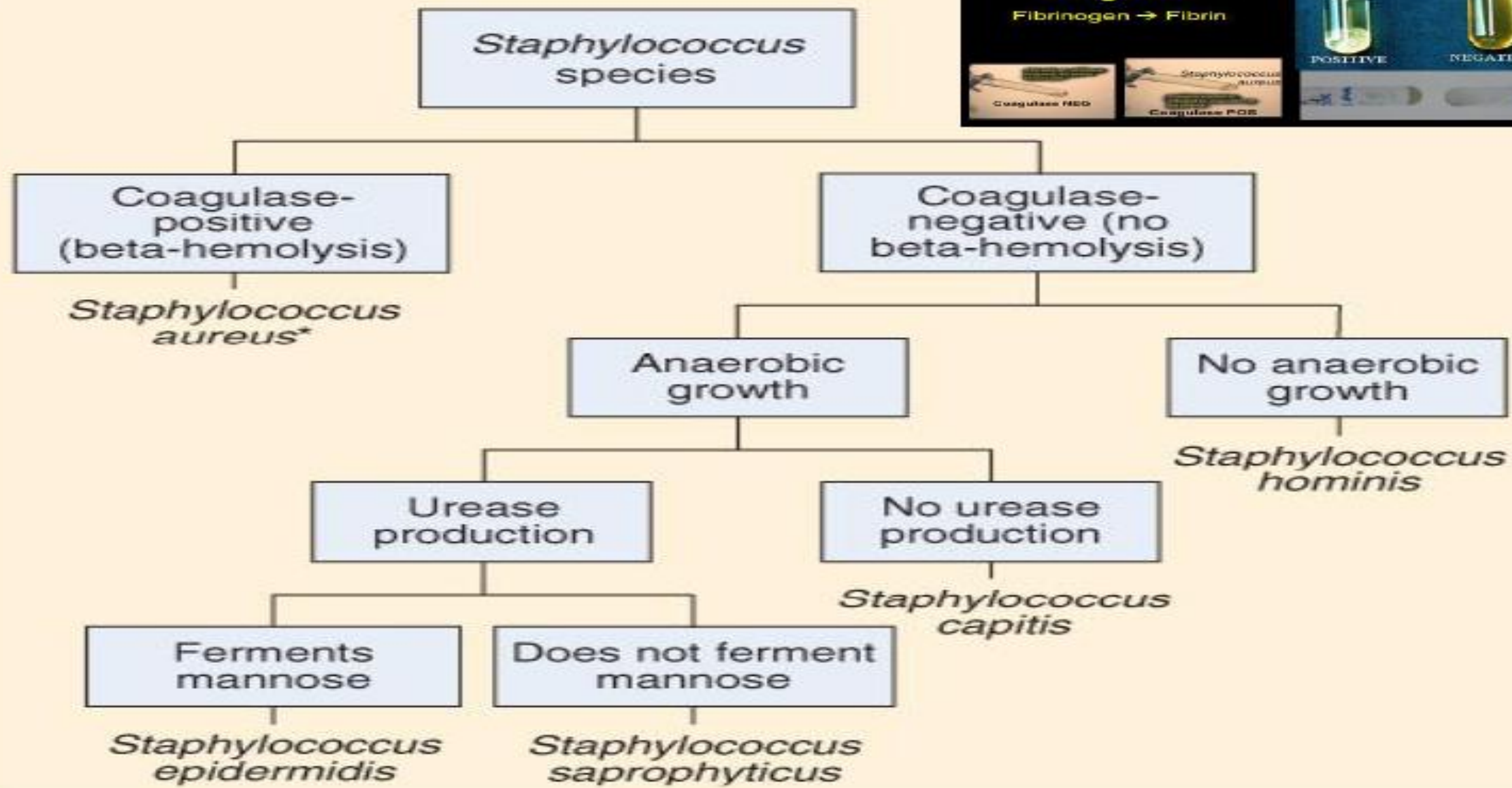


TABLE 18.2

Separation of Clinical
Species of *Staphylococcus*Differential
Characteristics**Coagulase**
Fibrinogen → Fibrin

1. Which of the following is a distinguishing feature of *Staphylococcus aureus*?

A. Catalase-negative B. Coagulase-positive C. Oxidase-positive D. Gram-negative

2-*Staphylococcus aureus* is best described as:

A. Gram-negative rod B. Gram-positive cocci in clusters C. Gram-positive cocci in chains D. Gram-negative diplococci

3-The enzyme that differentiates *Staphylococcus* from *Streptococcus* species is:

A. Coagulase B. Catalase C. Urease D. Lipase

4-Which type of infections are commonly caused by *Staphylococcus epidermidis*?

A. Food poisoning B. Skin abscesses C. Prosthetic device and catheter-related infections D. Toxic shock syndrome

5-The rapid test to differentiate *S. aureus* from coagulase-negative staphylococci is:

A. Catalase test B. Coagulase test C. Oxidase test D. Indole test

Q1: What is the shape and Gram reaction of Staphylococci?

Q2: Which test differentiates *Staphylococcus aureus* from other Staphylococci?

Q3: Name two coagulase-negative Staphylococci (CoNS).

Q4: What diseases are commonly caused by *S. aureus*?

Q5: What is the main infection caused by *S. epidermidis*?

Thank you



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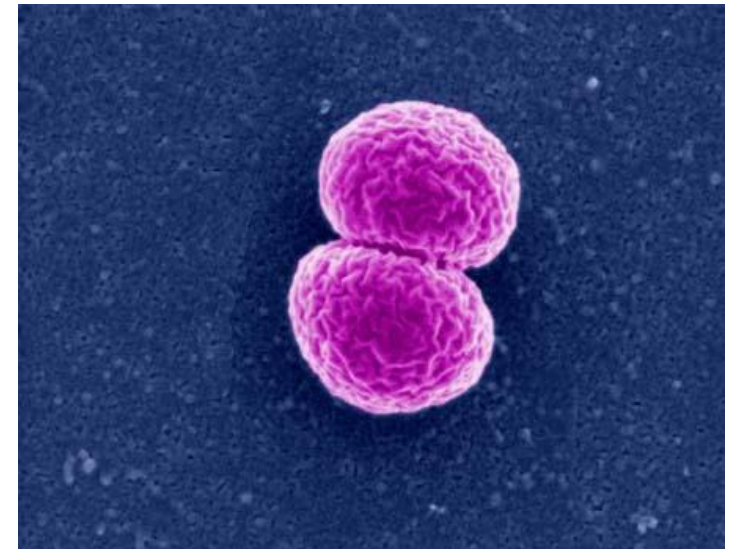
Pathogenic Bacteriology Lect-9-

Gram Negative cocci Neisseria

Prof. Dr. Mohammed A. fayyadh

Gram Negative cocci *Neisseriae*

The *Neisseriae* are gram negative cocci that usually arranged in pairs. *Neisseria gonorrhoeae* (gonococci) and *Neisseria meningitides* (meningococci) are pathogenic for human and typically are found inside **Polymorphonuclear cells** (PMNs). Some *Neisseriae* are normal inhabitants of human respiratory tract rarely causing disease and occur extracellularly.



Gonococci and meningococci are closely related with 70% DNA homology. Meningococci have polysaccharide capsule, whereas gonococci does not. Meningococci rarely have plasmids whereas most gonococci do. They can differentiate by usual clinical presentation of the disease, meningococci are typically found on respiratory tract and cause meningitis , while gonococci cause genital infection (Gonorrhoea).

Morphology:

-The typical Neisseria is a gram negative non-motile diplococci kidney shape(the flat or concave sides are adjacent).

Culture:

On special enrichment media e.g.Mullar-Hinton , Modified thayer - Martin or Heated blood agar (Chocolate agar). They produce convex glistening, non-pigmented, non-hemolytic colonies. They required 5% CO₂ (Candle jar). The Neisseria gives **oxidase positive** that **differentiate them from streptococci.**



They caused gonorrhea , which is globally distributes. It is exclusively transmitted by sexual contact usually by men or women with asymptomatic infection. The infectivity rate after a single contact with infected partners 20-30% for men and greater for women. *N.gonorrhea* is antigenically heterogeneous and capable of changing its surface structure in vivo &in vitro. These bacteria can avoid host defense by several types of surface structures.

Types of surface structures.

1.Pili:hair like projections extend from the surface. They enhance attachment to host cell and resist phagocytosis.

2.Por:they extend through the bacterial cell membrane in trimers forming pores through which nutrient are passed.

3.Opa: enhance adherence of gonococci within colonies and helps attachment of bacteria to the host cell.

Opa refers to a family of **outer membrane proteins** known as **Opacity-associated proteins**. (بروتين يسبب العتمة)

4.Lipopolysaccharide capsule: Gonococci can express antigenically different types of LPS at the same time.

Pathogenesis:

Gonococci attach the mucous membrane of the genitourinary tract, eye, rectum and throat producing acute suppuration that may lead to tissue invasion followed by chronic inflammation and fibrosis . In males usually causing urethritis with yellow creamy pus and painful urination. This process may extent to epididymis (البربخ). In untreated cases, fibrosis occurs that may lead to urethral stricture. Urethral infection in man may be asymptomatic. In females the primary infection is in the endocervix (عنق الرحم) and extends to urethra and vagina. Resulting in mucopurulent discharge that may extent to uterine tubes causing salpingitis (التهاب قناة فالوب), fibrosis & obliteration of tubes (**Infertility develop in 20% of women with salpingitis**). Chronic gonococcal cervicitis or proctitis (التهاب عنق الرحم والمستقيم) is often asymptomatic. Gonococcal bacteremia leads to skin papules, & it may also cause suppurative arthritis usually of knee, ankles & wrists (الركبة والكاحلين) (السائل). Gonococci can be cultured from blood and synovial fluid (والمعصمين). Gonococci in 30% of patients with gonococcal urethritis. (الزليلي)

Post-gonococcal urithritis (PGU): is common chronic sequelae of untreated or badly managed gonorrhoea, usually caused by different pathogens.

Penicillinas producing *N. gonorrhoea* (PPNG): are a totally penicillin resistant gonococci appeared in different parts of the world & highly prevalent among certain population (e.g. prostitutes).

Gonococcal ophthalmia neonatorum: An infection of the eye of newborn acquired during passage through infected birth canal. Untreated cases may lead to blindness. To prevent GON, instillation of 1% tetracycline ointment or 0.5% erythromycin ointment into the conjunctival sac of newly born is compulsory in certain developed countries. Silver nitrate is also effective & is the classical method, but it is irritant to conjunctiva.



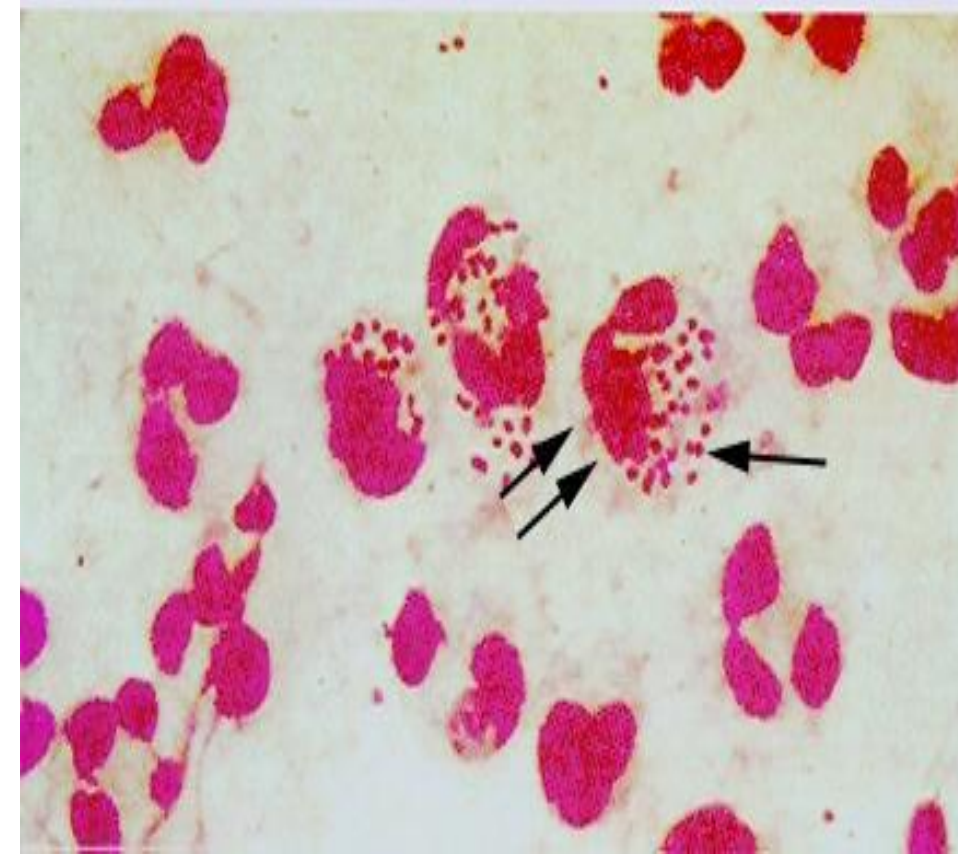
Laboratory diagnosis:

1.Specimens: Pus discharge from urethra, conjunctiva, synovial fluid, blood.

2.Smears :To detect intracellular (within pus cells) gram negative diplococci (Diagnostic). In men the sensitivity is 90% & specificity is 99%. In women (Endocervical exudate), the sensitivity is 50% & the specificity is 95%.

3. Culture: on special enrichment media (e.g. Modified Thayer-Martin agar, chocolate agar) under 5% CO₂.

4.Serology: Detection of specific antibodies against pili, outer membrane protein & LPS by ELISA.



Sensitivity and specificity

- **Sensitivity**
- **Definition:** The ability of a test to correctly identify people **who have the disease**.
- **In other words:** “If someone *has* the disease, how often will the test be positive?”
- **Formula:**

$$\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}}$$

- **High sensitivity** means very few cases of the disease are missed (few false negatives).

✓ Example:

If a COVID test has **95% sensitivity**, it correctly detects 5 out of 100 infected people — 5 will be missed.

-Specificity

- **Definition:** The ability of a test to correctly identify people **who do not have the disease**.
- **In other words:** “If someone *doesn't* have the disease, how often will the test be negative ?

- **Formula:**

$$\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}}$$

- **High specificity** means very few healthy people are wrongly labeled as sick (few false positives).

✓ Example:

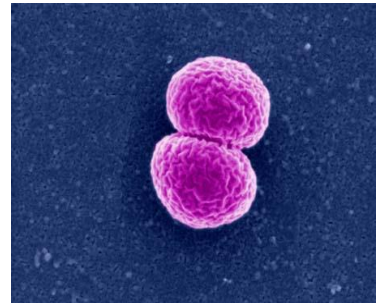
If a test has **98% specificity**, it correctly gives a negative result to 98 out of 100 healthy people — 2 will get a false positive.

Neisseria meningitidis

The most important serogroups associated in disease in human are A, B, C, Y and W-135. 5-30% of the normal population carry meningococci in nasopharynx.

Pathogenesis:

Human is the only natural host for meningococci. The **nasopharynx** is the portal of entry. The bacteria attached to the epithelial cells by pili. Then reaching the bloodstream (Bacteremia). Meningitis is the most common complication of meningococemia. Usually begins suddenly with intense headache , vomiting & stiffness of the neck that may progress to coma.



Laboratory diagnosis:

1.Specimens: blood (for culture), CSF(for culture ,smear & biochemical investigations).
Nasopharyngeal swab for detection of carriers.

2. Smears: Intracellular G-diplococci (with flat adjacent sides).

3.Culture: on special enrichment media under 5% CO₂ and further colony identification through carbohydrate fermentation & agglutination with specific antisera..

4. Serology: Latex agglutination test or hemagglutination test with specific antibodies to meningococcal polysaccharide.

Differences between *Neisseria gonorrhoeae* and *N. meningitidis*

- Both *Neisseria gonorrhoeae* and *Neisseria meningitidis* are:
 - Gram-negative diplococci
 - **Oxidase-positive**
 - Catalase-positive
 - **Non-motile**
- +carbohydrate fermentation test can distinguish between them

Carbohydrate Fermentation Results

Organism	Glucose	Maltose	Lactose	Sucrose	Key Difference
<i>Neisseria gonorrhoeae</i>	+	-	-	-	Ferments only glucose
<i>Neisseria meningitidis</i>	+	+	-	-	Ferments glucose and maltose

CSF= **Cerebrospinal Fluid** — it's a **clear, colorless body fluid** that surrounds and protects your brain and spinal cord.

Main Functions:-

Protection (Cushioning): Buoyancy:

Waste removal: Nutrient transport:

Pressure regulation.

MCQ

1-Which of the following statements about *Neisseria gonorrhoeae* is TRUE?

- A. It is an encapsulated organism
- B. It ferments both glucose and maltose
- C. It is oxidase positive and ferments only glucose
- D. It is a motile organism

2-*Neisseria meningitidis* differs from *N. gonorrhoeae* in that it:

- A. Is oxidase negative
- B. Ferments only glucose
- C. Possesses a polysaccharide capsule
- D. Is resistant to penicillin

3- The principal virulence factor of *Neisseria meningitidis* that allows it to evade phagocytosis is:

- A. Lipooligosaccharide (LOS)
- B. Pili
- C. Capsule
- D. IgA protease

4-The mode of transmission of *Neisseria meningitidis* is:-

- A. Sexual contact
- B. Airborne droplets
- C. Fecal-oral route
- D. Vector-borne

5-Which laboratory test can distinguish *N. gonorrhoeae* from *N. meningitidis*?

- A. Oxidase test
- B. Gram staining
- C. Sugar fermentation test
- D. Catalase test

Q1-Name the two pathogenic species of *Neisseria* in humans

Q2-Which *Neisseria* species is **capsulated**

Q3-Which carbohydrate(s) are fermented by *N. gonorrhoeae*?

Q4-Name one important virulence factor common to both *N. gonorrhoeae* and *N. meningitidis*.

Q5-What enzyme produced by *Neisseria* helps it evade mucosal immunity?

Thank you all



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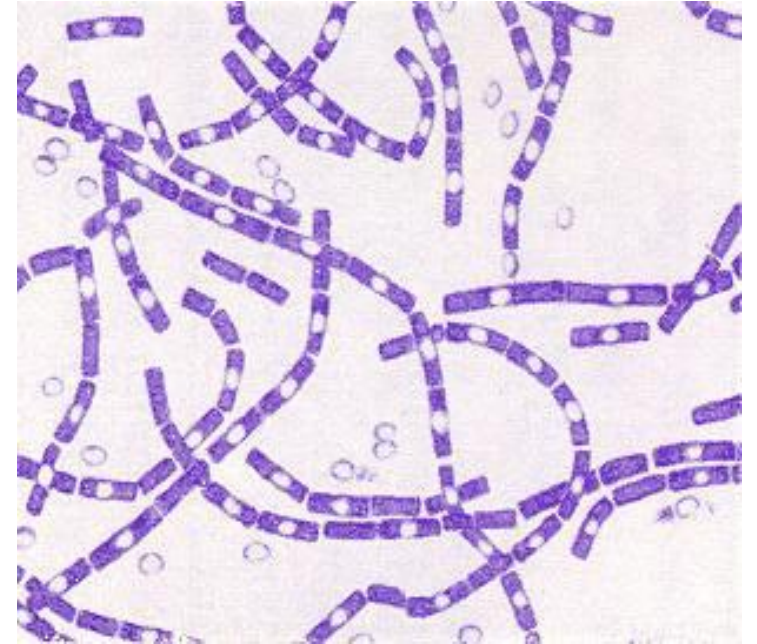
كلية العلوم - قسم التحليلات المرضية

Pathogenic Bacteriology

Lect-10-

Gram Positive Rod (*Bacillus anthracis*)

Prof. Dr. Mohammed A. fayyadh



Gram Positive Rod (Introduction)

Medically important **gram-positive rods (bacilli)** include several genera that are significant due to their ability to cause disease in humans. They can be categorized into **spore-forming** and **non-spore-forming** rods.

1. Spore-Forming Rods.

- *Bacillus anthracis* – causes **anthrax**
- *Bacillus cereus* – causes **food poisoning**, opportunistic infections
- *Clostridium tetani* – causes **tetanus**
- *Clostridium botulinum* – causes **botulism**
- *Clostridium perfringens* – causes **gas gangrene**, food poisoning

2- Non-spore-forming

- *Listeria monocytogenes* (aerobic, non-spore-forming)
 - Causes **listeriosis** (meningitis in neonates, pregnant women, immunocompromised)
- *Corynebacterium diphtheriae* (aerobic, non-spore-forming)
 - Causes **diphtheria**

Bacillus anthracis (Anthrax)

- **Anthrax** is primarily a disease of herbivorous animals (zoonosis). It is an acute disease caused by *B. anthracis*. Most forms of the disease are lethal, and it affects both humans and other animals.
- *B. anthracis* is a large G+ve bacilli found in chains, 1 - 1.2µm in width & (3 - 5µm) in length . Forms oval, centrally located endospores (**Spores: Dipicolinic acid**) with Glutamyl-polypeptide capsule and it is Non motile.
- **Anthrax toxin** is encoded on one plasmid, and the polyglutamate capsule is encoded on a different plasmid.

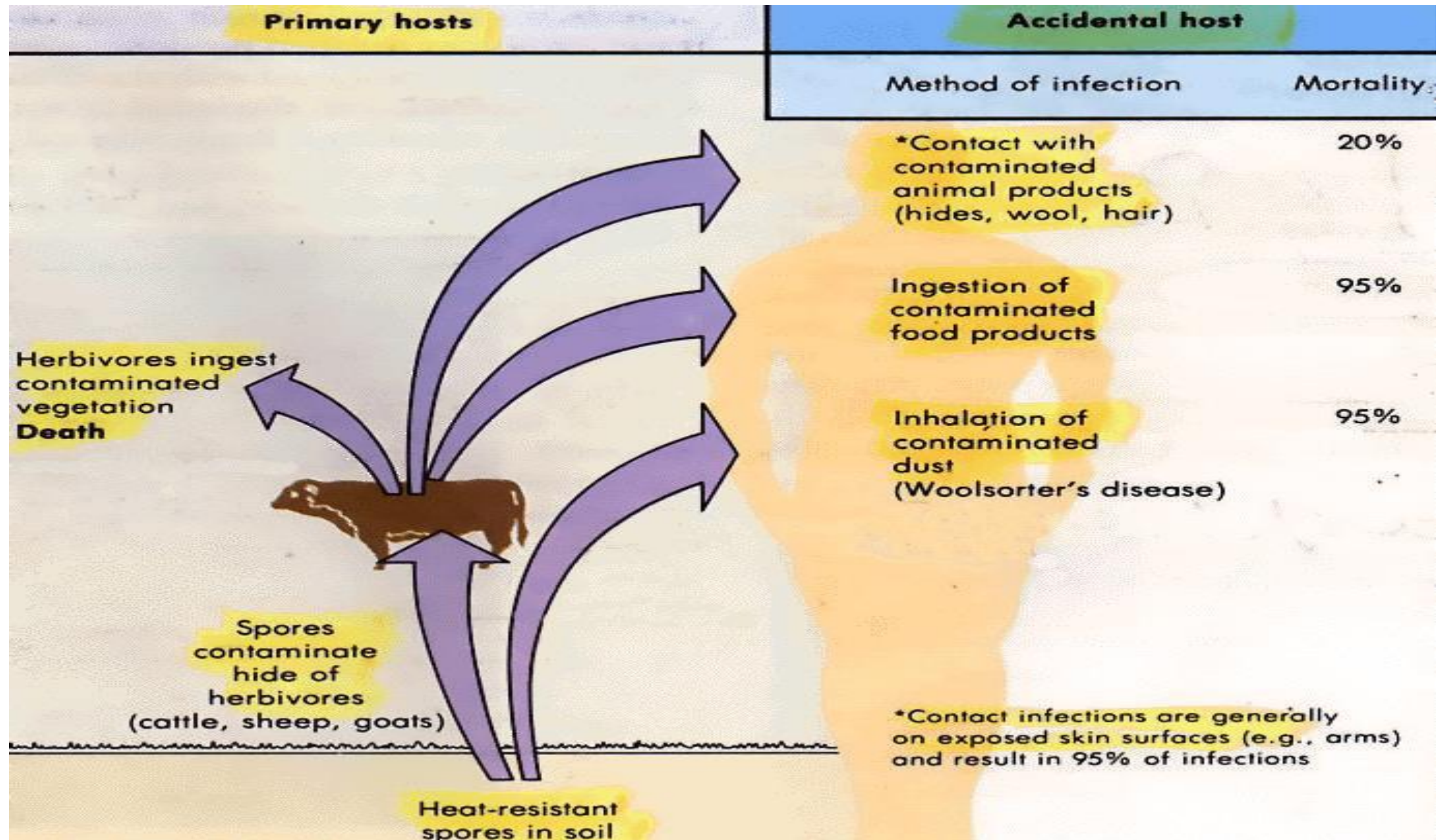
Not: Dipicolinic acid (DPA), is a chemical compound found in high concentrations in bacterial endospores that provides resistance to heat and other harsh conditions.

Route of bacterial entry to human body

1. **Injured skin (cutaneous anthrax)** (most common).
2. **Inhalation of spores (pulmonary anthrax "wool sorter's disease")**
(deadly). is not communicable from person to person
3. **Mucous membranes (gastrointestinal anthrax)** (rarely)

- Anthrax is an occupational disease (مرض مهني) (animal's handlers, veterinarians, agricultural workers).
- *Bacillus anthracis* is a potential biological warfare agent

Q) What is bioterrorism ?



Virulence factors

1- Capsule

Protects against phagocytosis & lysis during vegetative state

2-Exo toxin

a-Protective antigen (PA)

b- edema factor (EF)

c- lethal factor (LF)

The pathogenicity results from its antiphagocytic capsule as well as from a toxin that causes edemas and tissue necrosis. ***B anthracis*** invades host and produce exotoxin.

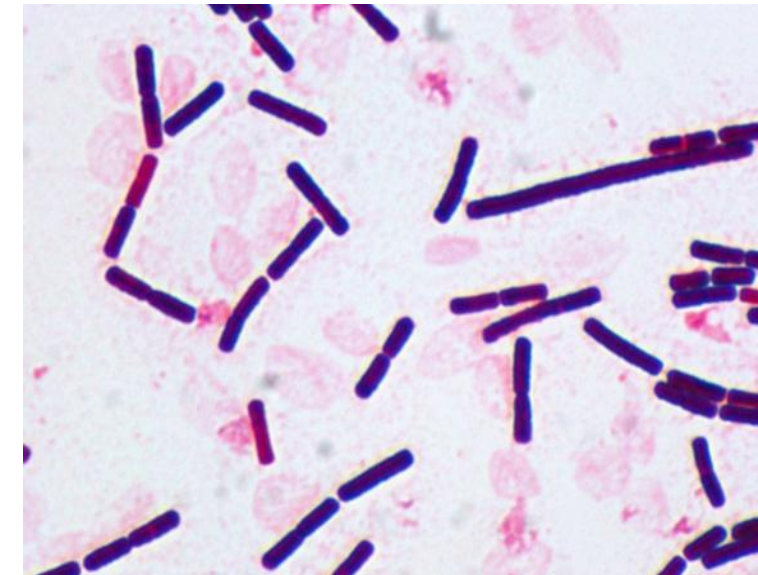
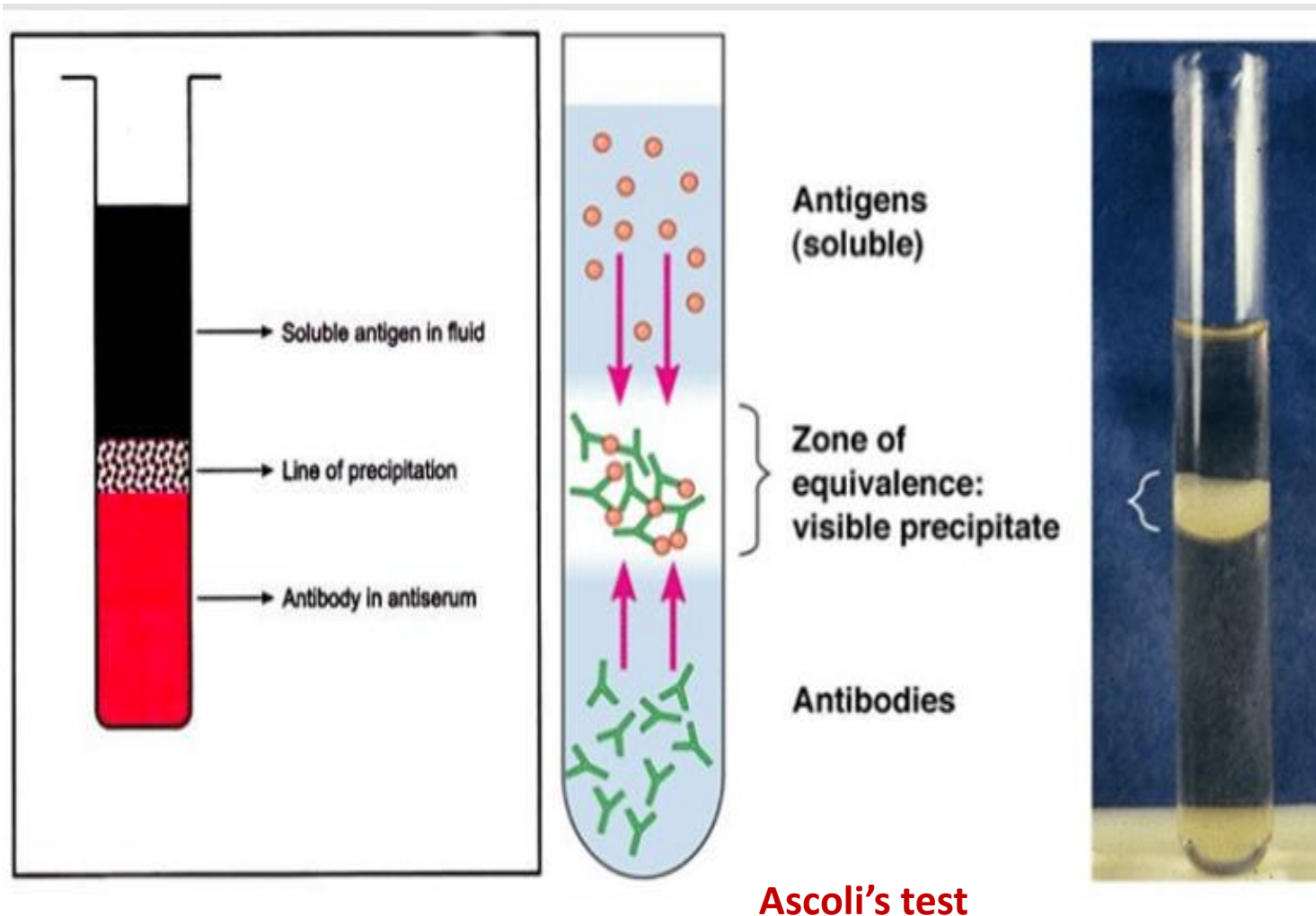
Diagnostic Laboratory Tests

- 1- **Specimens:** Fluid, pus from local lesion, blood and sputum.
- 2- **Staining methods:** Gram Stain
- 3- **Cultivation:** On Blood agar
- 4- **Demonstration of capsule:** (McFadyean's stain).
- 5- **Polymerase chain reaction (PCR).**
- 7- **Ascoli's test.**



-McFadyean's stain is a polychrome methylene blue stain that demonstrates the capsule of *Bacillus anthracis* as a distinctive purple or pinkish capsule around blue-stained bacilli under the microscope.

-Ascoli's test is a precipitation (immunodiffusion) test that detects anthrax antigen in suspected contaminated materials using known anthrax antiserum.



McFadyean's stain

Bacillus cereus

B. cereus causes food poisoning (food born gastroenteritis)

Transmission: (Food –born) *Bacillus cereus*

B. cereus Cause Two types food poisoning

1- Emetic type (النوع القيئي) associated with fried rice.

2- Diarrheal type associated with meat dishes and sauces

Spores on grains such as rice survive steaming and rapid frying. The spores germinate when rice is kept warm for many hours (e.g., **reheated fried rice**). The portal of entry is the gastrointestinal tract.

Differences between *Bacillus anthracis* from *Bacillus cereus* in the lab?

B. anthracis is **non-motile and non-hemolytic**, while *B. cereus* is **motile and β -hemolytic**.

MCQ

1. Which of the following characteristics best distinguishes *Bacillus anthracis* from *Bacillus cereus*?

- A. Gram-positive rod shape B. Spore formation C. Non-motility
D. Aerobic growth

2-The capsule of *Bacillus anthracis* is composed of:

- A. Polysaccharide B. Polypeptide (D-glutamic acid)
C. Lipopolysaccharide D. Teichoic acid

3-The toxin of *Bacillus anthracis* consists of all the following components EXCEPT:

- A. Protective antigen B. Edema factor C. Lethal factor D. Neurotoxin

4-Which disease is caused by *Bacillus cereus*?

- A. Gas gangrene B. Food poisoning C. Tetanus D. Diphtheria

5-The most common form of anthrax in humans is:

- A. Inhalational anthrax B. Gastrointestinal anthrax C. Cutaneous anthrax D. Injectional anthrax

Q1) List the three components of the anthrax toxin.

Q2) How can *Bacillus anthracis* be differentiated from *Bacillus cereus* in the lab

Q3) What is the main virulence factor of *Bacillus anthracis* that prevents phagocytosis?

NEVER STOP LEARNING



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كلية العلوم - قسم التحليلات المرضية

Pathogenic Bacteriology

Lect-11-

Gram Negative Rod(Enterobacteriaceae)

Prof. Dr. Mohammed A. fayyadh

Gram-negative Rods Enterobacteriaceae and Pseudomonas

Learning objectives:

After this lab. You must be able to:

A-Describe microscopic morphology and cultural biochemical characteristics of each member in this family.

B-List infections caused by each of these members.

C- Differentiate each member of this family from each other.

D-Discuss principles of biochemical tests of each member in this family.

Gram-Negative bacilli

Large group of diverse organisms, they are divided to:

- *Gram-Negative Rods related to **gastrointestinal tract**.
- *Gram-Negative Rods related to **respiratory tract**.
- *Gram-Negative Rods related to **animal source**.

General characters of *Enterobacteriaceae*

This is a large group of microorganisms that most of them live as a normal microbiota in the intestine (colon) of human and may be other parts of the body, and cause several enteric diseases as well as other diseases that involve the respiratory, genitourinary, the meninges and the skin. The general characters of this family are:-

- 1- Gram negative 2- Non spore-forming rods.**
- 3- Some are motile, others are capsule producing. 4- Facultative anaerobes**
- 5- Ferment glucose with or without gas 6- Grow on ordinary media**
- 7- Oxidase negative**

Lactose fermenting

Escherichia

Klebsiella

Enterobacter

Citrobacter

Enterobacteriaceae

Lactose fermenting

Non-Lactose fermenting

NON Lactose fermenting

Proteus

Salmonella,

Shigella,

Yersinia
spp

Clinical Significance of Enteric

Enteric are ubiquitous in nature, some live in water, soil and sewage and most are present in the **intestinal tract of animals and humans as commensal flora**; therefore, they are sometimes call **“fecal coliforms”** Based on clinical infections produced, enteric are divided into two categories: –

- ***True pathogen** - Salmonella, Shigella, Yersinia spp and some strains of E. coli

- * **Opportunistic pathogens** – normally part of the intestinal flora that may produce infection outside the intestine

Colonial Morphology

& Ability to ferment lactose:

□ lactose-fermenting strains *pink-purple colonies (e.g., Escherichia, Klebsiella, Enterobacter).

□ Non lactose-fermenting strains * colorless colonies (e.g., Proteus, Salmonella, Shigella, and Yersinia spp).

Escherichia coli

*One of the most predominant intestinal flora, causes wide range of infections including: UTI(urogenital tract infections), gastrointestinal infection, meningitis, wound infection and bacteremia.

*Ferments lactose. – Have characteristic metallic green sheen on EMB.(Eosin Methylene Blue)

- * Positive in indole and methyl red tests

- * Negative in Voges-Proskauer and Citrate tests

- * Does NOT produce H₂S

- * Usually motile.

- * IMViC test: + + - -

- *TSI test: A/A/ -

Home Work

What is the following test mean .

* Voges-Proskauer and Citrate tests

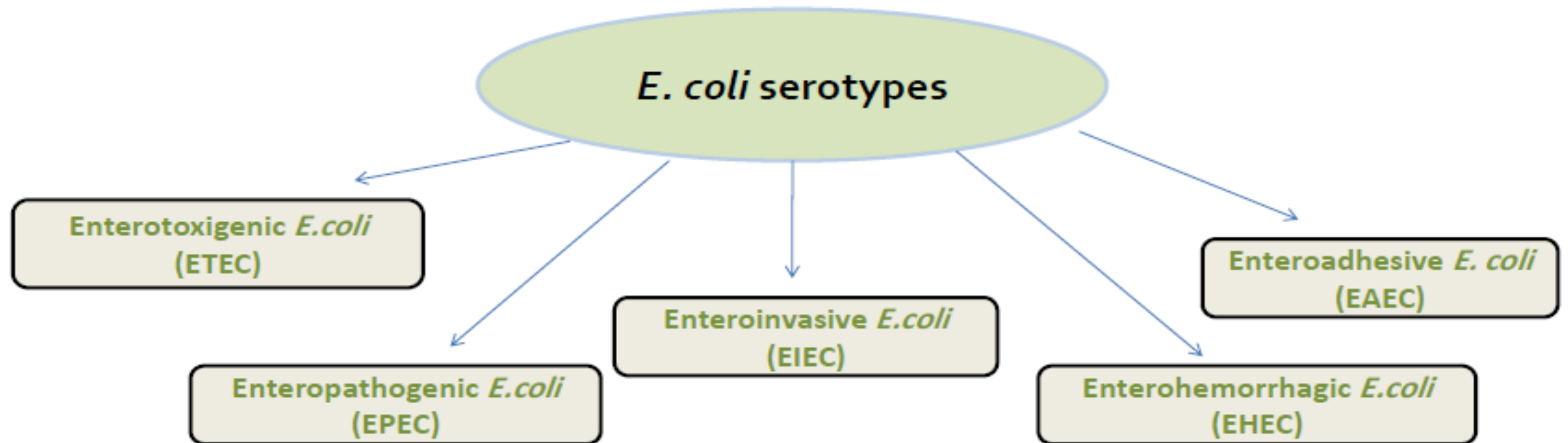
* * IMViC test: + + - -

*TSI test: A/A/ -

E. coli (*Escherichia coli*) infections

1) **INTESTINAL** infections:(caused by some strains)

(e.g. Traveler's diarrhea, dysentery, hemorrhagic infection).



2) EXTRAINTESTINAL infections

***UTI(urogenital tract infections) , Wound infections, Pneumonia, Bacteremia, Septicemia.**

***It is the common cause of G-ve nosocomial infection.**

Klebsiella spp

The genus was originally divided into 3 main species based on biochemical reactions. These are (1) *Klebsiella pneumoniae*, (2) *Klebsiella ozaenae*, (3) *Klebsiella oxytoca*. **General features are:-**

- ❖ **Normal flora of GIT (Gastrointestinal tract) .**
- ❖ **Non - motile.**
- ❖ **Capsulated by heavy capsule**
- ❖ **Mucoid colonies.**
- **Diseases:**
 - **Necrotizing Pneumonia , Nosocomial infections like UTI (urogenital tract infections) mainly in debilitated patients.**

Klebsiella pneumoniae

1-*K. pneumoniae* is typically colonizes **human mucosal** surfaces of the **oropharynx** and **gastrointestinal (GI)** tract. It is recorded to be associated with **pneumonia** in the alcoholic and diabetic patient population. *K. pneumoniae* is also a well-known cause of **community-acquired** pneumonia.

2- It is mostly commonly isolated **Gram-negative, non-motile** bacteria possesses a **polysaccharide capsule**, which **protects against phagocytosis** and antibiotics and makes the colonies **moist** and **muroid**. has a distinctive “**yeasty**” odor.

Antigenic Structure

Members of the genus *Klebsiella* form large capsules consisting of **polysaccharides (K antigens)** covering the **somatic (O or H)** antigens and can be identified by capsular swelling tests with specific antisera.

Note:- The somatic (O) and flagellar (H) antigens are surface antigens used in the classification and identification of Gram-negative bacteria like *Klebsiella*.

Klebsiella has **O antigens**, which are part of the **lipopolysaccharide (LPS)** layer on the outer membrane.

Klebsiella species are **non-motile**, meaning they **do not have flagella**, and therefore **do not possess H antigens**.

***Klebsiella pneumoniae* Growing on MacConkey, Nutrient, Blood and Eosin Methylene Blue agar plates respectively.**



Thanks for your attention



جامعة شط العرب الاهلية كلية العلوم - قسم التحليلات المرضية

Pathogenic Bacteriology Lect-12-

Gram Negative Rod (Salmonellae and Shigella)

Prof. Dr. Mohammed A. fayyadh

The salmonella

-Pathogenic for humans or animals when acquired by the oral route.

- The bacteria transmitted to human via **contaminated food, contaminated water or from person to person**
- They cause different infections for human such as :-
 - Gastroenteritis
 - Systemic infection (bacteremia)
 - Enteric fever (**Typhoid**).

The morphology and growth characteristics

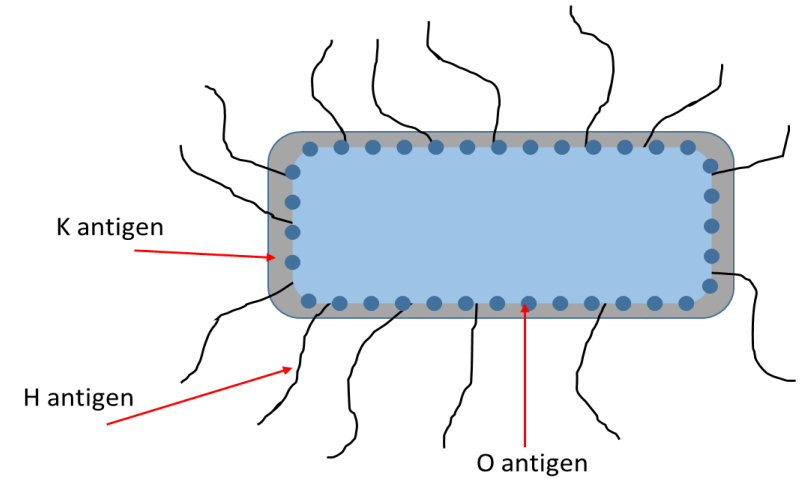
- Gram-negative bacilli .
- Most isolates are motile with Peritrichous flagella.
- Grow readily on simple media
- They almost never ferment lactose or sucrose.**
- They form acid and sometimes gas from glucose and mannose.
- They usually produce H₂S.
- They survive freezing in water for long periods.
- Resistant to some certain chemicals (eg , Brilliant green , sodium Tetrathionate , sodium deoxycholate) that inhibit other enteric bacteria.

(Note)Such compounds are therefore useful for inclusion in media to isolate salmonellae from feces.

Brilliant Green:- is a synthetic triphenylmethane dye(Used as a selective dye in microbiological media)

Surface antigens and variation

- As a member of the enteric family, Salmonella possess the three antigens :
- **Somatic (O)** antigen, **Flagellar (H)** antigen , and **capsular K** antigen (called Vi in salmonella)
- Flagellar (H) antigen can be found in two forms called phase 1 and phase 2 and the organisms can switch from one phase to another
- **This is called phase variation.**
- Salmonella can loss H antigens and become non motile
- It can also loss O antigen, and switch from smooth to rough colony
- Vi (K)Antigen can be lost partially or completely



Classification

- The genus *Salmonella* is divided into two species.
- Salmonella enterica* and *Salmonella bongori*
- Based on phenotypic profiles , *S. enterica* were divided into 6 subspecies
 - enterica(subspecies I).
 - salamae(subspecies II)
 - arizonae(subspecies IIIa)
 - diarizonae(subspecies IIIb)
 - houtenae(subspecies IV)
 - indica(subspecies VI)
- The subspecies I strains (written as *S enterica subspecies enterica*) is responsible for the majority of human illness

- ***Salmonella enterica*** can also be divided into “Typhoidal” and “non Typhoidal” depending on their serotypes

- Typhoidal salmonella refers to the serotypes that responsible for **Typhoid** (enteric) fever which include :

- *Salmonella Paratyphi* A (serogroupA), ((***Salmonella enterica*** serogroup))***Paratyphi***

- *Salmonella Paratyphi* B (serogroupB),

- *Salmonella Choleraesuis* (serogroupC1), and

- *S. Typhi*(serogroupD).

- **Non typhoidal** refers to other serotypes that do not cause typhoid fever such as **Enteritidis** and **Typhimurium**

Pathogenesis and disease

&-Infection with **Typhoidal salmonellae** (S. Typhi and S. Paratyphi) believed to be acquired from a human source (as these serotypes are highly adapted and restricted to human host).

&-**Non-typhoidal** serotypes , which responsible for gastroenteritis, can be acquired from different animal sources as well as from environment.

&-Different animal can be carriers for salmonella and act as a the reservoir for human infection; these include poultry, pigs, rodents, cattle, pets and many others.

&-Transmission: By oral route, via ingestion of contaminated food or drink.

&-The mean infective dose to produce clinical or subclinical infection in

humans is 10^5 --- 10^8 salmonellae

The main Diseases produced by Salmonellae :-

- I. Enteric fever (Typhoid)** -Sever systemic infection
- Caused by *Salmonella* typhi (most common), and *Salmonella* Paratyphi
 - Transmission : ingestion of contaminated food or drinks
 - Incubation period : 10 -15 days
 - Clinical manifestation : fever, malaise, headache, constipation, bradycardia, and myalgia occur after the incubation period
 - Fever reach a plateau (39–40 °C)
 - The spleen and liver are enlarged
 - Rose spots (1-4 mm) blanching pink muscle continuously observed on the chest and abdomen in less than 5% of the cases
 - Abdominal symptoms include : diarrhea, constipation , and general abdominal pain

II . Enterocolitis

- *S. Typhimurium* and *S. Enteritidis* are the dominant causes , but enterocolitis can also be resulted from any other serotypes (around 1400 serotype) of serogroup I salmonellae.
- Eight to 48 hours after ingestion of salmonellae, there is nausea, headache, vomiting, and profuse diarrhea, with few leukocytes in the stool
- Low-grade fever and abdominal cramping are very common
- Diarrhea is self limited typically last for 3 to 7 days
- Bacteremia is rare (2–4%) except in immunodeficient persons.

Laboratory diagnosis

A. Specimens :

- Non-typhoidal salmonella : fresh stool
- Typhoidal salmonella : blood , bone marrow (less practical) , other sterile sites, urine or intestinal secretion

B. Culture : specimens can be plated on different media including:

1-Differential media :

- **MacConkey, or deoxycholate medium** permits rapid detection of lactose non fermenters(salmonella and other genera) :
- **Bismuth sulfite medium** : rapid detection as many salmonellae form black colonies due to H₂S production.

2-Selective media: such as :

- **Salmonella-Shigella agar** (S-S agar), Hektoen enteric agar.



Salmonella on SS Agar

C. Serological test

1. Agglutination test : using known sera against unknown culture

- It is useful for rapid preliminary identification culture
- commercial kits available to agglutinate and serogroup salmonellae by their O antigens.

2. Tube dilution agglutination test .

- Used to detect the antibodies raised against the O and H antigens.

D. Nucleic acid amplification tests

- There are several commercial kits for direct detection of salmonella in fecal samples with acute diarrhea.

The Shigella

Shigella is the causative agents of **Shigellosis** (Bacillary dysentery)

- The intestinal tracts of humans and other mammalian is the natural habitats of this bacteria
- The bacteria transmit from person to another by fecal-oral route

Morphology and identification

A. Typical Organisms

- gram-negative rods.
- facultative anaerobes but grow best aerobically.
- produce Convex, circular, transparent colonies with intact edges.

B. Growth Characteristics

- All shigellae ferment glucose except *Shigella sonnei* ; they do not ferment lactose.
- Ferment carbohydrates and produce acid, but rarely produce gas.
- They may also be divided into those that ferment mannitol and those that do not ferment mannitol.

Antigenic Structure

1.Somatic O antigens : The shigellae are divided into four ‘major’ O antigenic groups, designated A, B, C, and D.

2.K antigen: presence in some strains , and it is not important for serologic typing

3.H antigen: shigellae is non-motile and does not have H antigen.

- The somatic O antigens is overlapping with other enteric bacteria such as *E. coli*

- Therefore identification of shigellae should be made by a combination of antigenic and biochemical properties .

Classification

- Depending on the combination of biochemical and serological characteristics, Shigellae are classified into four species or groups (A, B, C, D)
- They are also divided into mannitol fermenting species and mannitol non fermenting species
- There are more than 40 serotypes according the their serologic specificity depends on the polysaccharide .

Shigella species	Group	Manitol fermentation
<i>S. dysenteriae</i>	A	-
<i>S. flexneri</i>	B	+
<i>S. boydii</i>	C	+
<i>S. sonnei</i>	D	+

Pathogenesis

&-The infections is limited to the gastrointestinal tract

&-systemic invasion (bloodstream) is very rare.

&-The infective dose is around (10-100) organisms (which is low in comparison to salmonellae and vibrios).

&-The essential pathologic process involve :

+invasion of the mucosal epithelial cells .

+Multiplication and spread within the epithelial cell cytoplasm.

+ Microabscesses in the wall of the large intestine and terminal ileum lead to necrosis of the mucous membrane, superficial ulceration, bleeding, and formation of a “pseudomembrane” on the ulcerated area.

Toxins

A. Endotoxin

- As any **Gram negative bacteria** , all Shigellae release their toxic lipopolysaccharide after their hydrolysis .
- Their endotoxin may responsible for irritation of the bowel wall.

B. Exotoxin

- *S. dysenteriae* type 1 (Shiga bacillus) produces a heat-labile exotoxin
- It acts as enterotoxin and neurotoxin
- **The enterotoxinic activity:** it produces diarrhea as does the E coli Shiga-like toxin, perhaps by the same mechanism. In humans, the exotoxin also inhibits sugar and amino acid absorption in the small intestine.

Clinical Findings of bacillus dysentery

&-After (1–2 days) of incubation, there will be a sudden onset of abdominal pain, fever, and watery diarrhea.

&-The diarrhea is a consequence of the **exotoxin** activity in the intestine

&-After 1-2 days , the stools increases (but with less liquid) and often contains mucus and blood (due to invasion of intestinal mucosa).

&-This is will be accompanied with rectal spasms.

&-In adults , the infection is mainly self-limited , and the fever and the diarrhea can disappear spontaneously within 2-5 days in more than 50 % of the cases .

&-In elderly and children, dehydration and acidosis can be developed and it can even lead to death.

&-Some people become chronic intestinal carriers (rare) for the dysentery bacilli and may re-infected many times .

Diagnostic Laboratory Tests

A. Specimens

- Specimens include fresh stool, mucus flecks, and rectal swabs for culture.
- Large numbers of fecal leukocytes and some red blood cells often are seen microscopically.

B. Culture on :

- differential media (e g, MacConkey)
- selective media (Hektoen enteric agar or Salmonella–Shigella agar)
- The growing colorless (non-lactose fermenting) colonies.
- **TSI agar: Shigella can be diagnostic as it is H₂S –ve, produce acid but not gas. (Triple Sugar Iron agar- glucose, lactose, sucrose)**

C. Motility test can be done : the bacteria are non motile

D. slide agglutination by specific Shigella antisera (not depended in diagnose Shigella infections)

Epidemiology

- Shigellae are transmitted by fecal-oral route, food and flies
- Most cases of Shigella infection occur in children younger than 10 years of age.
- Shigellosis, caused primarily by *S sonnei*, has become an important problem in daycare centers in some places.
- *S dysenteriae* can spread widely.

Prevention and Control

Some controlling measures can be taken to eliminate the bacteria from infecting human such as :

- 1) sanitary control of water, food, and milk; sewage disposal and fly control**
- 2) isolation of patients and disinfection of excreta**
- 3) detection of subclinical cases and carriers, particularly food handlers**
- 4) antibiotic treatment of infected individuals.**

Review Q

- 1-How are Shigella and Salmonella transmitted?
- 2-Why is Shigella more contagious than Salmonella?
- 3-List the main species of Shigella and Salmonella
- 4-What is the main characters of Enterobacteriaceae .
- 5-Classify Enterobacteriaceae according to their ability to ferment lactose .

Thanks for your attention



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Pathogenic Bacteriology

Lect-13-

Gram -Negative Rod related to Respiratory Tract

Prof. Dr. Mohammed A. fayyadh

Gram -Negative Rod related to Respiratory Tract

- There are three medically important gram-negative rods :
 - *Haemophilus influenzae*,
 - *Bordetella pertussis*,
 - *Legionella pneumophila* .
- *H. influenzae* and *B. pertussis* are found only in humans, whereas *L. pneumophila* is found primarily in environmental water sources.

Haemophilus influenzae

- Diseases

- *H. influenzae* : *Meningitis in young children.

Otitis media, sinusitis, conjunctivitis, epiglottitis, Facial cellulitis and sepsis in children.

***Pneumonia in adults, particularly in those with chronic obstructive lung disease.**

Important Properties

- **H. influenzae* is a small gram-negative rod (coccobacillus) with a polysaccharide capsule.
- *Serologic typing is based on the antigenicity of the capsular polysaccharide.
- * **Six serotypes**, type b causes most of the severe, invasive diseases, such as **meningitis and sepsis**. (Hib)
- ***Unencapsulated** and therefore untypeable strains can also cause disease, especially diseases of the upper respiratory tract such as **sinusitis and otitis media**, but are usually noninvasive.
- **H. influenzae* infects only humans; there is no animal reservoir.



#- It enters the body through the upper respiratory tract, resulting in either asymptomatic colonization or infections.

#-The organism produces an **IgA protease that degrades secretory IgA, thus facilitating attachment to the respiratory mucosa.**

#-The organism can enter the bloodstream (bacteremia) and spread to the **meninges.**

#-Meningitis is caused primarily 95% by of which possess the type b capsule.

#-The non-encapsulated strains are frequently involved in otitis media, sinusitis, and pneumonia.

#-Virulence factor** involves the Antiphagocytic capsule and endotoxin**

#-No exotoxin is produced.

- **IgA (Immunoglobulin A)** is a type of antibody—one of the five major classes your immune system makes (IgA, IgG, IgM, IgD, IgE).
- **What it does**
- IgA is the main antibody that protects **mucosal surfaces**, meaning areas where your body meets the outside world, such as:
 - **Respiratory tract** (nose, lungs)
 - **Gastrointestinal tract** (stomach, intestines)
 - **Urogenital tract**
 - **Saliva, tears, and breast milk**

Clinical Findings

- 1- Most infections occur in children between the ages of 6 months and 6 years, with a peak in the age group from 6 months to 1 year.
- 2- The rapid onset of fever, headache, and stiff neck along with drowsiness.
- 3- Sinusitis and otitis media cause pain in the affected area, opacification of the infected sinus, and redness with bulging of the tympanic membrane.
- 4- Rarely, epiglottitis, which can obstruct the airway. A swollen "cherry-red" epiglottis is seen.
- 5- Pneumonia in elderly adults, especially those with chronic respiratory disease, can be caused by untypeable strains of *H. influenzae*.



Facial cellulitis

**Acutely inflamed epiglottitis associated with
Haemophilus influenza type B**

Laboratory Diagnosis

1. Isolation of *H. influenzae* on chocolate agar enriched with two growth factors required for bacterial respiration, for adequate energy production namely, **factor X (a heme compound) and factor V (NAD)**.
2. Other species such as *Haemophilus parainfluenzae*, do not require both factors. (Blood Agar)
3. Biochemical tests
4. The capsular swelling (quellung) reaction.
5. Fluorescent-antibody staining (encapsulated) and counter immunoelectrophoresis or latex agglutination tests, which detect the capsular polysaccharide.

What the Quellung Reaction Is

“Quellung” means *swelling* in German.

When **specific anticapsular antibodies** are added to bacteria with a polysaccharide capsule, the capsule becomes **more visible and appears to swell** when viewed under a microscope.

- **How It Works**

- A bacterial sample is mixed on a slide with:
 - Specific **antisera** (antibodies) against a bacterial capsule.
 - Often **methylene blue or India ink** as a background contrast.
- Under a microscope:
 - The capsule appears **enlarged, clear, and sharply outlined**.

Prevention

- Vaccine contains the capsular polysaccharide of *H. influenzae* type b conjugated to diphtheria toxoid or other carrier protein ,effective in young children.
- It is given between the ages of 2 and 15 months. This vaccine is reduced the incidence of meningitis by approximately 90% in immunized children.
- Meningitis in close contacts of the patient can be prevented by rifampin.
- Rifampin is used because it is secreted in the saliva to a greater extent than ampicillin.

Bordetella

- *B. pertussis* causes whooping cough (pertussis).
- Important Properties
- *B. pertussis* is a small, coccobacillary, encapsulated gram-negative rod.

Pathogenesis & Epidemiology

- 1.B. pertussis*, a pathogen only for humans
- 2.It is transmitted by airborne droplets produced during the severe coughing episodes.**
- 3.The organisms attach to the ciliated epithelium of the upper respiratory tract but do not invade the underlying tissue.*
- 4.Decreased cilia activity followed by death of the ciliated epithelial cells.**
- 5.Pertussis is a highly contagious disease that occurs primarily in infants and young children and has a worldwide distribution.*

Virulence factors play a role in the pathogenesis:

1. Attachment of the organism to the cilia of the epithelial cells is mediated by a protein on the pili called **filamentous hemagglutinin**

2. Pertussis toxin

***It is an A-B subunit toxin, B mediates its binding to receptors on the surface of respiratory tract epithelial cells..**

Diagnostic test for *Bordetella pertussis*

The **best diagnostic test** for *Bordetella pertussis* depends on the stage of illness, but **the single most accurate and widely recommended test today is:**

★ **PCR (Polymerase Chain Reaction) from a nasopharyngeal swab**

Rivew Q

- 1-Which capsule type is most associated with severe invasive disease cause by *Haemophilus influenzae*?
- 2- Which disease is *H. influenzae* type b known for causing in children
- 3- What disease does *Bordetella pertussis* cause ?
- 4- What laboratory test is most specific for diagnosing pertussis?

MCQ

1. *Haemophilus influenzae* is best described as:
A. Gram-positive rod B. Gram-negative coccobacillus
C. Acid-fast bacillus D. Gram-negative diplococcus
- 2- Which capsule type is most virulent in *H. influenzae*?
A. Type a B. Type b C. Type c D. Type d
- 3- *H. influenzae* requires which factors for growth on chocolate agar?
A. X factor and Y factor B. V factor and W factor C. X factor and V factor
D. A factor and C factor
- 4- *Bordetella pertussis* causes which disease?
A. Diphtheria B. Tetanus C. Whooping cough D. Scarlet fever
- 5- The preferred diagnostic test for pertussis is:
A. Blood culture B. Sputum Gram stain C. PCR from nasopharyngeal swab
D. CT scan

True & False Q

1. *H. influenzae* requires both X and V factors for growth.
2. Non-typeable *H. influenzae* strains are the main cause of invasive meningitis.
3. Chocolate agar supplies X and V factors because red blood cells are lysed by heating.
- 4- whooping cough disease caused by *Haemophilus influenza*
- 5- *Bordetella . pertussis* is an intracellular pathogen that invades epithelial cells

Never forget two people in your life....

1) The person who was with you in every pain .(**YOUR MOTHER**)

2)The person who lost everything just to make you win (**YOUR FATHER**)



جامعة شط العرب الاهلية كلية العلوم - قسم التحليلات المرضية

Pathogenic Bacteriology

Lect-14-

Gram -Negative Rod related to animal source.
(Brucella)

Prof. Dr. Mohammed A. fayyadh

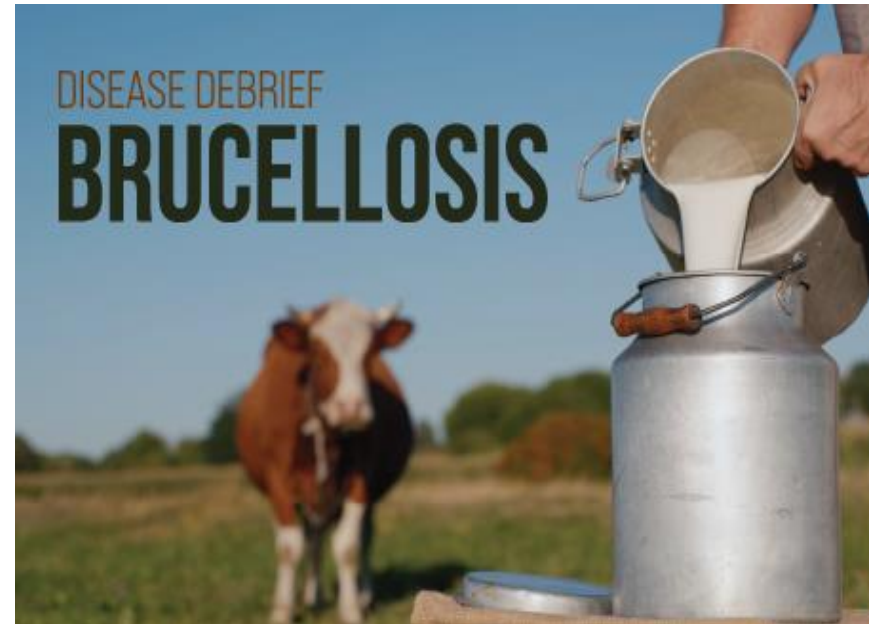
Brucella and Brucellosis

Objective:

- 1-Identify Brucella spp.
- 2-Describe the characteristic features.
- 3-State how Brucellosis could be transmitted to human.
- 4-State how to control and prevent the infection.

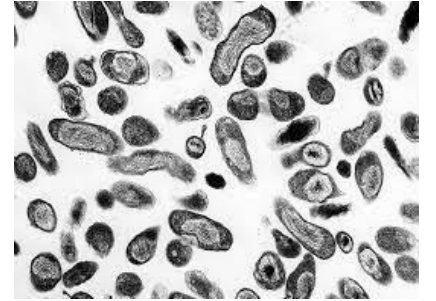
Brucellosis :is an infectious disease caused by contact with animals carrying bacteria called *Brucella*.

- It is called also Undulant fever, Contagious abortion, Malta fever and Mediterranean fever .



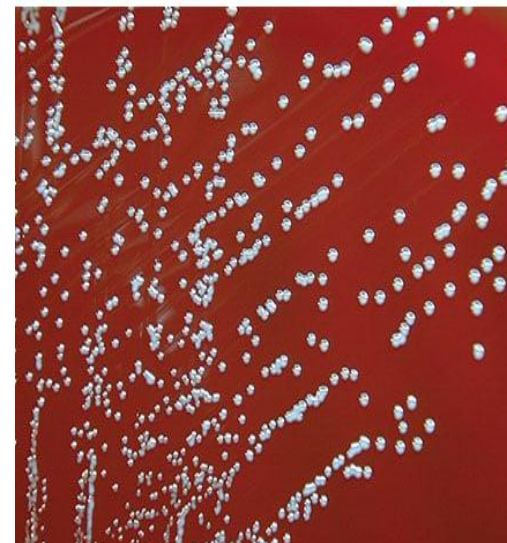
Brucella : Important properties

- 1-Small G⁻ve coccobacilli, aerobic, non-motile.
- 2-Obligate intracellular parasite.
- 3-Sensitive to heat and acidity.
- 4-Killed in milk by pasteurization.
- 5-Catalase +ve, oxidase +ve,
- 6-Utilize carbohydrates and produce neither acid nor gas.



Coccobacilli are bacteria that have a shape between cocci (spherical) and bacilli (rod-shaped). , they appear short, plump, and oval.

Biochemical Test of *Brucella melitensis*



Important species of Brucella

The major human pathogens and their animal reservoirs are:

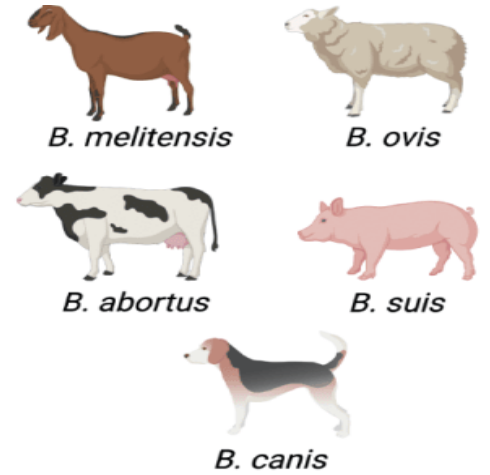
- *B. melitensis*----goats
- *B. abortus*----cattle
- *B. suis*-----pigs
- *B. canis*---dogs.

Higher risky group :

Veterinarians, farmers,
slaughterhouse workers,
(عمال المسالخ)
laboratory technicians
and animal handlers.



Brucella species and natural hosts



Pathogenicity :

The common routes of infection in humans are:

- 1-Intestinal tract (**by ingestion of infected milk**).
- 2-Mucous membranes (**droplets**).
- 3-Skin (**contact with infected tissues of animals**).

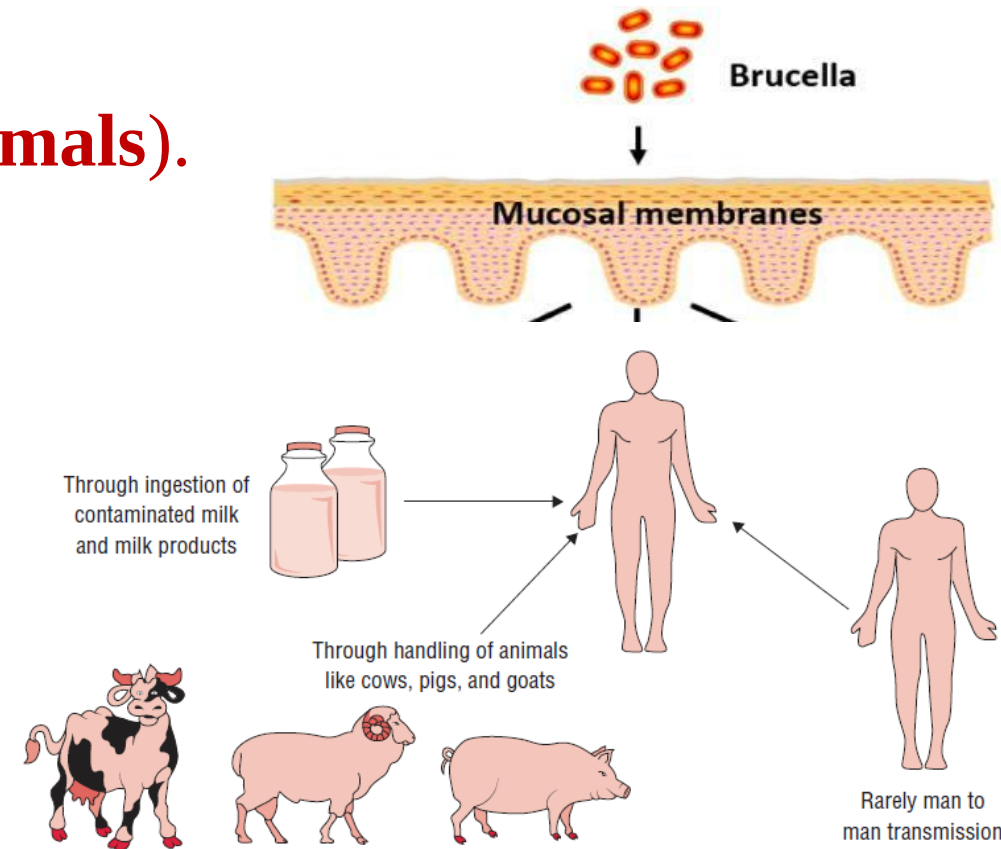
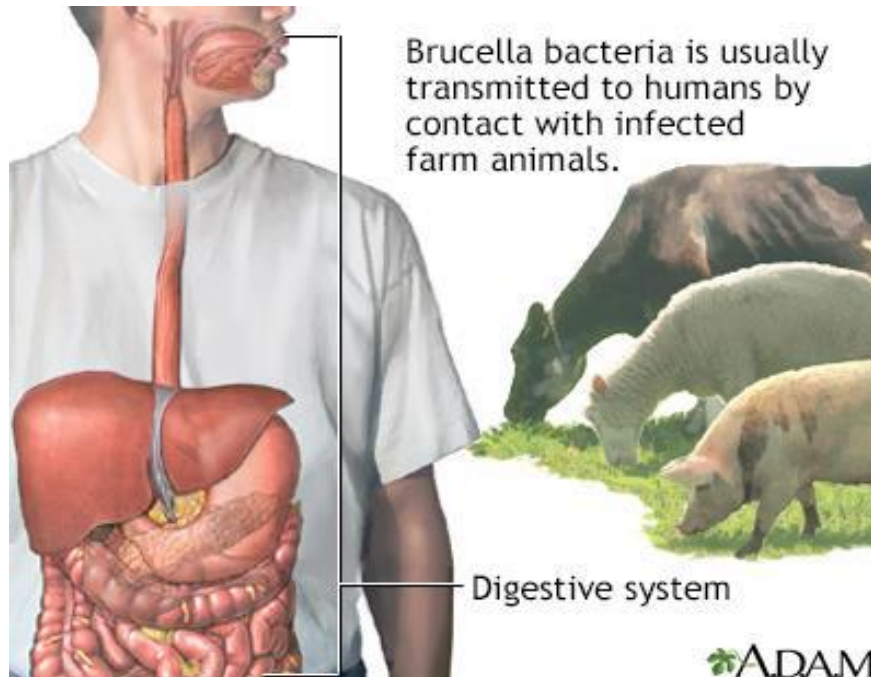


FIG. 40-1. Transmission of brucellosis.

Types of Brucella pathogenicity:

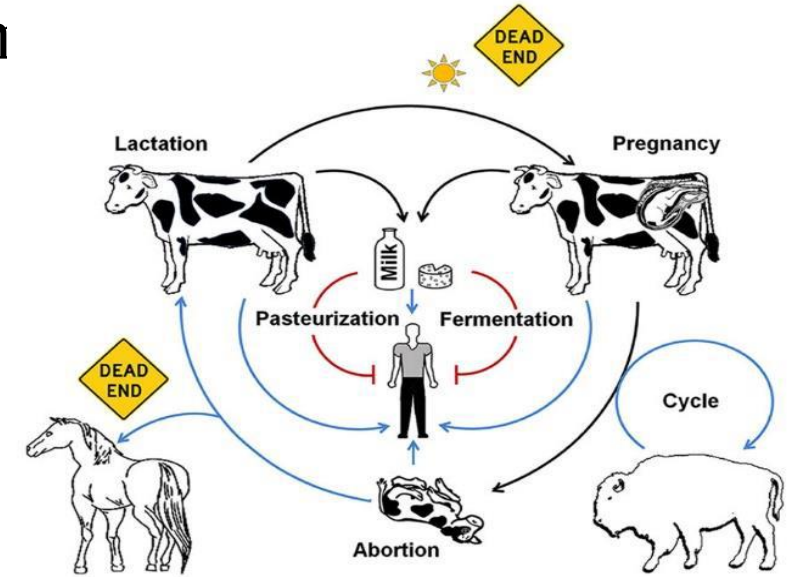
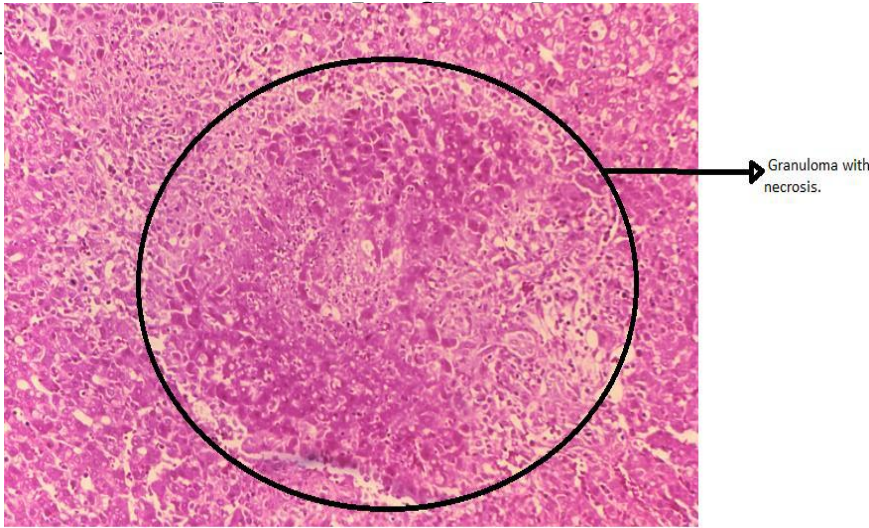
1-*B. melitensis*.... Acute and severe.

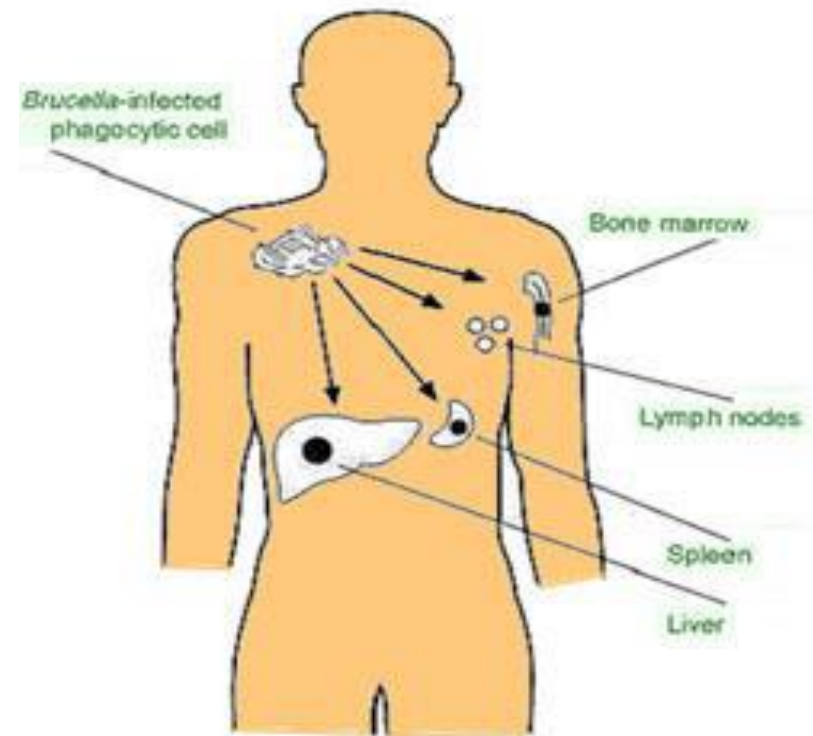
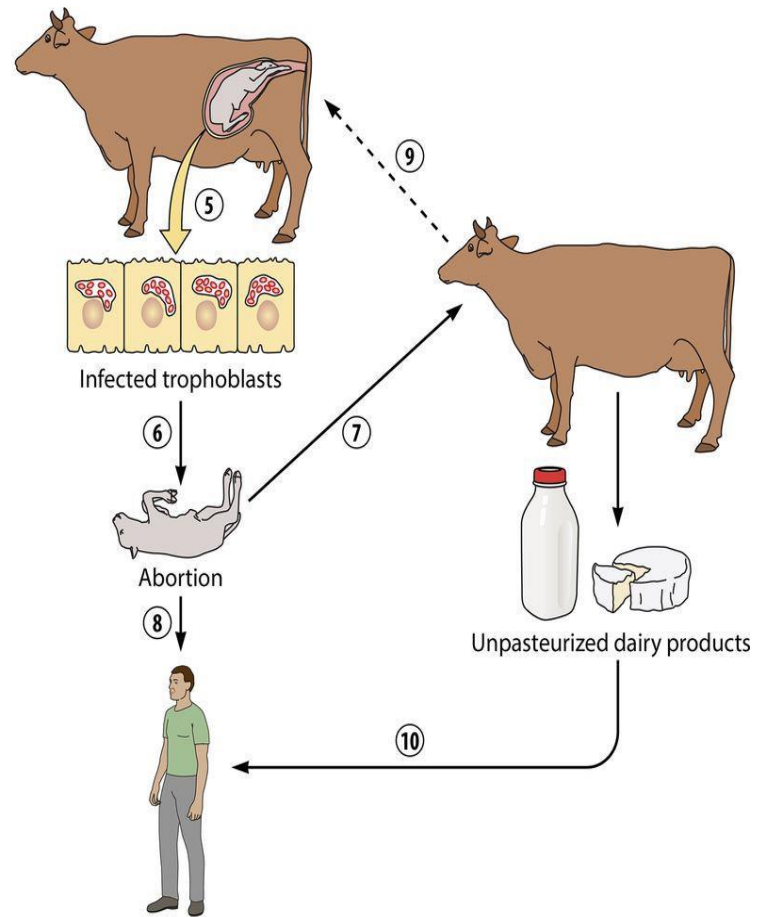
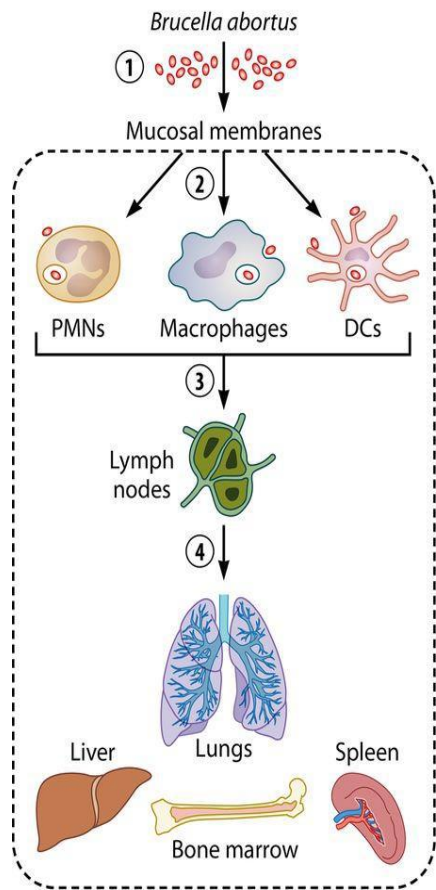
2-*B. abortus*..... Mild disease without suppurative complication.

3-*B. suis*..... Chronic with suppurative lesions.

4-*B. canis*.....Mild disease

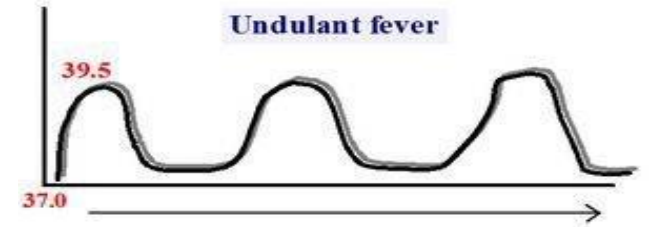
Infected animals may shed high numbers of bacteria in n
placental membra





Clinical findings:

- Incubation period 1-6 weeks.
- The undulating (rising and falling) fever pattern that gives the disease its name.
- Weakness , headache and fatigue commonly occur.
- Swollen lymph nodes and Bacteremia during the early phase
- Osteomyelitis is the most frequent complication.
- After the initial infection, a chronic stage may develop.



- Brucella cannot be isolated from the patient at this stage, but the agglutinin titer may be high.
- The diagnosis of “chronic brucellosis” is difficult to establish with certainty unless local lesions are present.
- In **pregnant animals** leads to **placentitis and abortion** of these species.
- Placenta and fetal membranes of cattle, swine(Pigs), sheep and goats contain erythritol , a growth factor for ***Brucella***.
- There is **no erythritol in human** placentas.

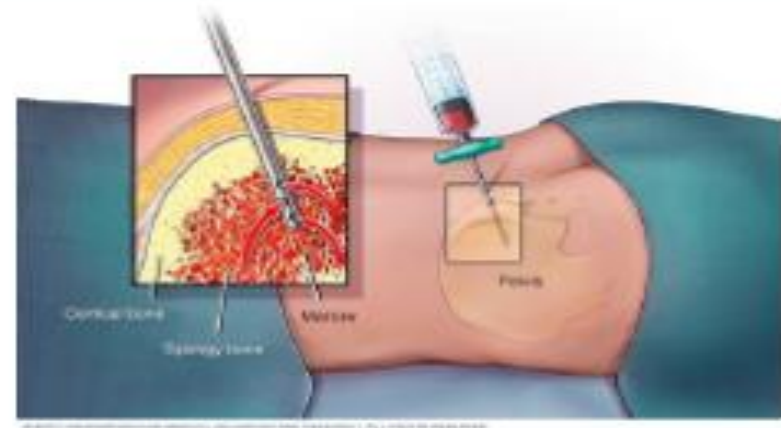
Lab. Diagnosis :

• Specimen:

- 1- Blood, bone marrow and biopsy for culture.
- 2- Serum for serology.

• Culture:

- Brucella agar and Trypticase Soy agar in 8-10% CO₂ at 35-37°C. Observed for 3 weeks before being discarded as negative.
- Slide agglutination test with Brucella antiserum.



(a) *Brucella* spp.



MCQ

1. Brucella species are best described as:

- A. Gram-positive cocci B. Gram-negative coccobacilli
C. Acid-fast bacilli D. Anaerobic Gram-negative rods

2. The primary route of transmission of Brucellosis in humans is:

- A. Mosquito bites B. Consumption of unpasteurized dairy products
C. Person-to-person spread D. Contaminated water

3. The most pathogenic species causing systemic illness in humans is:

- A. Brucella canis B. Brucella melitensis C. Brucella suis D. Brucella abortus

4. Brucella organisms are:

- A. Non-motile and non-spore-forming B. Motile with polar flagella
C. Spore-forming bacilli D. Strict anaerobes

5- Which clinical feature is most characteristic of brucellosis?

- A. Rash with desquamation B. Undulating fever C. Bloody diarrhea
D. Hemorrhagic pneumonia

Review Q

Q: What type of microorganism is *Brucella*?

Q: What disease does *Brucella* commonly cause in humans?

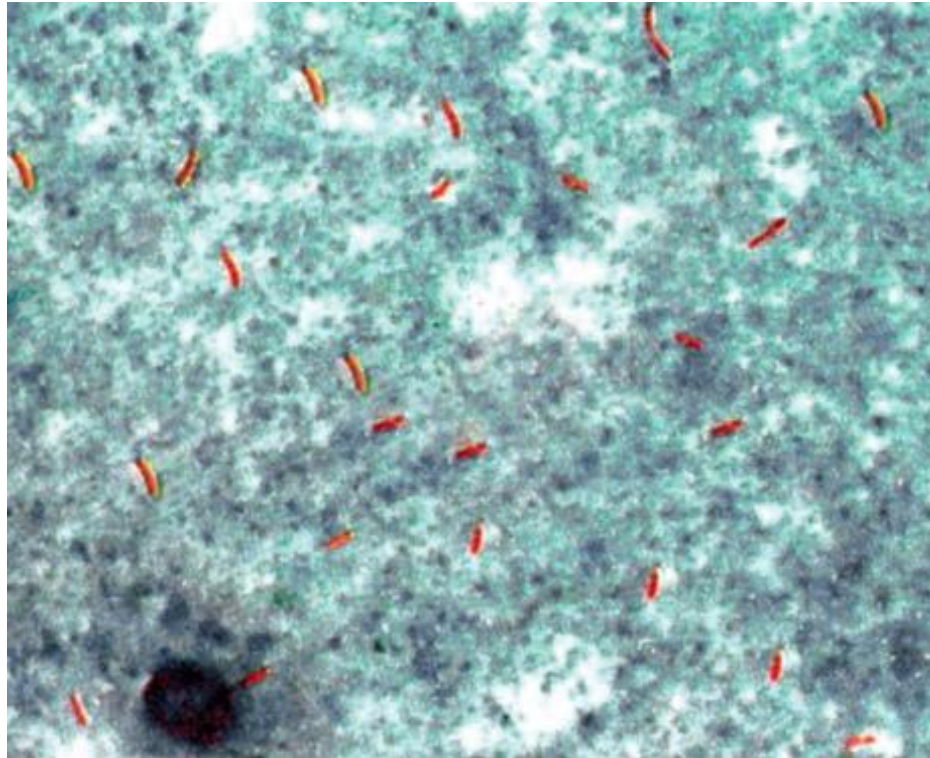
Q: What is the main route of human infection?

Q: Which species of *Brucella* is most commonly associated with human infection?

Q: What is a classic clinical feature of brucellosis?

Mycobacterium tuberculosis

It Cause Tuberculosis



Q-Why *Mycobacterium tuberculosis* Does Not Stain With Gram Stain

A- Because the High Lipid Content in Cell Wall (Mycolic Acids)

- The cell wall of *M. tuberculosis* contains **very long-chain mycolic acids** (lipids).
- These waxy lipids make the cell wall **hydrophobic**.
- As a result, the **crystal violet dye in Gram staining cannot penetrate** effectively

Helicobacter pylori

It cause **Inflammation of the stomach lining**
Gastric ulcers (stomach).



Wishing you success



جامعة شط العرب الاهلية

كلية العلوم - قسم التحليلات المرضية

Lec-15-Mycobacterium tuberculosis

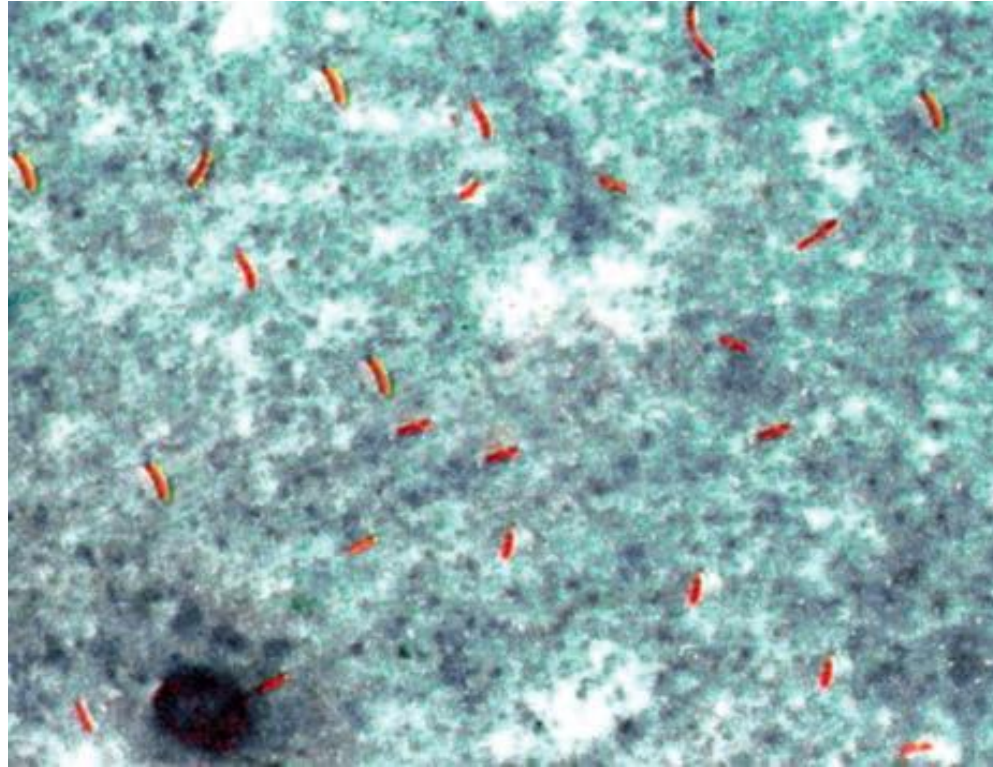
Prof. Dr. Mohammed A. fayyadh

Morphology of Mycobacterium

M. tuberculosis* is a slender, straight or slightly curved rod with rounded ends, about $3\text{ }\mu\text{m} \times 0.3\text{ }\mu\text{m}$, in pairs or as small clumps. The bacilli are non-motile, non-spore forming, non-capsulated and acid-fast. **They are gram-positive but are difficult to stain. When stained with carbol fuchsin by the Ziehl-Neelsen method, they resist de-colorization by 20 percent sulfuric acid and absolute alcohol for 10 minutes **(acid and alcohol fast)**.

With this stain, the **Mycobacterium** stain **bright red**, while the tissue cells and other organisms are stained blue. Organisms in **tissue** and **sputum smears** often stain **irregularly** and **have a beaded or barred** appearance, presumably because of their **vacuoles** and **polyphosphate** content.

Acid fastness has been ascribed to the presence in the bacillus of **mycolic acid**. It is related to the **integrity of the cell** and appears to be a property of the lipid-rich waxy cell wall. Staining may be uniform or granular.



Mycobacterium tuberculosis in Ziehl-Neelsen stained smear

Cultural Characteristics

- *M. tuberculosis* is an **obligate aerobe**. The optimal growth temperature of tubercle bacilli is 35 to 37°C but they fail to grow at 25°C or 41°C. Optimum pH is 6.4 to 7.0. The bacilli grow slowly, the **generation time *in vitro*** being **14 to 15 hours**. Colonies appear in about **two weeks** and may sometimes take up to **eight weeks**.
- The solid medium most widely employed for routine culture is **Lowenstein-Jensen (LJ) medium** without starch.

- **Human tubercle bacilli** produce visible growth on LJ medium in about 2 weeks, although on primary isolation from clinical material colonies may take up to 8 weeks to appear. On solid media, *M. tuberculosis* forms **dry, rough, raised, irregular** colonies with a **wrinkled** surface. They are **creamy white, becoming yellowish or buff colored on further incubation.**

Morphology of *Mycobacterium tuberculosis* on **Lowenstein-Jensen (LJ) medium**



Clinical Findings of *Mycobacterium tuberculosis*

1. General (Systemic) Symptoms

A-Fever (often low-grade, evening rise) **B-Night sweats** **C-Weight loss**
D-Anorexia (loss of appetite) **E-Fatigue and malaise**

2. Pulmonary Tuberculosis (most common)

- **Productive cough** (lasting >3 weeks)
- **Hemoptysis** (coughing blood)
- **Chest pain** (pleuritic or dull ache)
- **Dyspnea** (late stages)

Q-Why *Mycobacterium tuberculosis* Does Not Stain With Gram Stain

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I wish you all the best