الجامعة التقنية الوسطى
كلية التقنيات الصحية والطبية \بغداد
قسم تقنيات المختبرات الطبية

المادة :الأحياء المجهرية

المرحلة :الثانية

Training package in theory lecture

Medical Microbiology

For

The students of second class in Medical laboratory department

By

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Title: Introduction and Classification of bacteria **Lec 1** **العنوان**

Name of the instructor: **اسم المحاضر:**

Professor Dr. Mohammed Flaih

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: المقدمة:

Microbiology is a specialized area of **biology*** that deals with living things, ordinarily too small to be seen without magnification. Such **microscopic*** also study of microorganism (M.O) which are unicellular or cell-cluster microscopic organisms, microbiology involves study in numerous areas involving cell structure, function, genetics, immunology, biochemistry, epidemiology, and ecology.

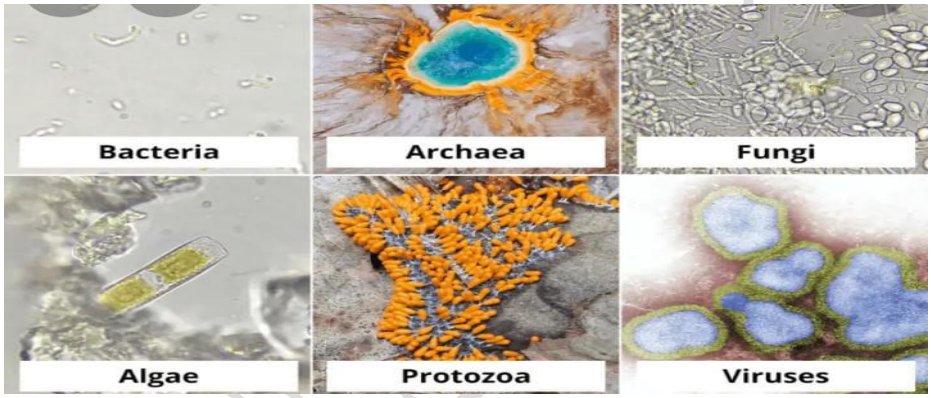
Microorganisms, also called microbes, are organisms that require a microscope to be readily observed. It be found in every ecosystem and in class association with every type of multicellular organisms.

M.O are the oldest organisms, having evolved over the 4 billion years of earth's history to the modern varieties we now observe.

Microbes are classified into groups according to evolutionary relationships, provided with standard scientific names, and identified by specific characteristics.

They populate the healthy human and animals body by billions as **Normal flora** and even participants in body functions, for ex. Bacteria play a role in the degradation in intestinal contents.

These M.O consist of archaea, bacteria, viruses, fungi, protozoa, algae, and **helminthes**, which caused infection and spread of human diseases.
Few species of M.O that harmful to human either by production toxic compounds and enzymes **or** direct infection by their virulence factors are characterized as **pathogens .**



Pre test: **الاختبار القبلي:**

Q\ What is meaning of microbiology and microorganism

Q\ Microorganism consist of …….,…….,……..,…….,…….,……..,

Scientific content: المحتوى العلمي:

Microbiology is a specialized area of **biology*** that deals with living things, ordinarily too small to be seen without magnification. Such **microscopic*** also study of microorganism (M.O) which are unicellular or cell-cluster microscopic organisms, microbiology involves study in numerous areas involving cell structure, function, genetics, immunology, biochemistry, epidemiology, and ecology.

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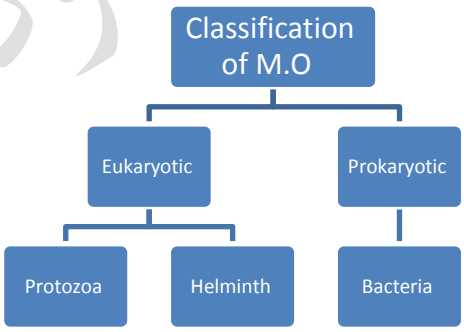
Classification of M.O



Bacteria is an unicellular m.o which have a rigid cell wall surrounding the cell membrane that determine the shape of bacteria.

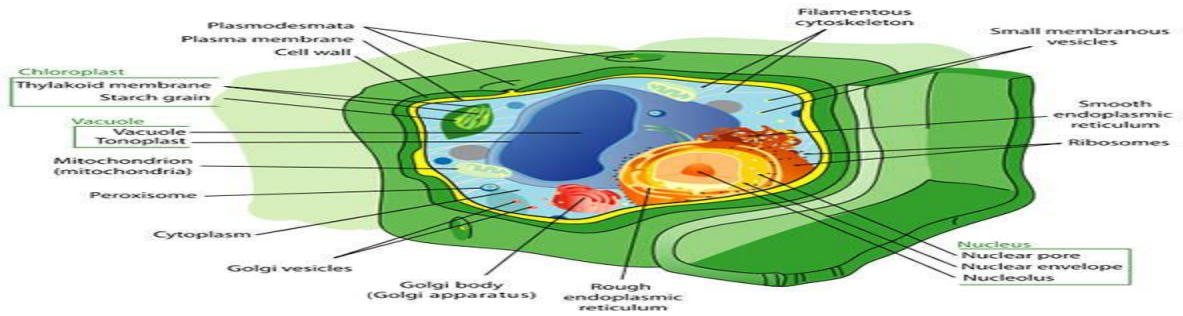
Viruses are obligate intracellular parasite that don't have a cellular structure .

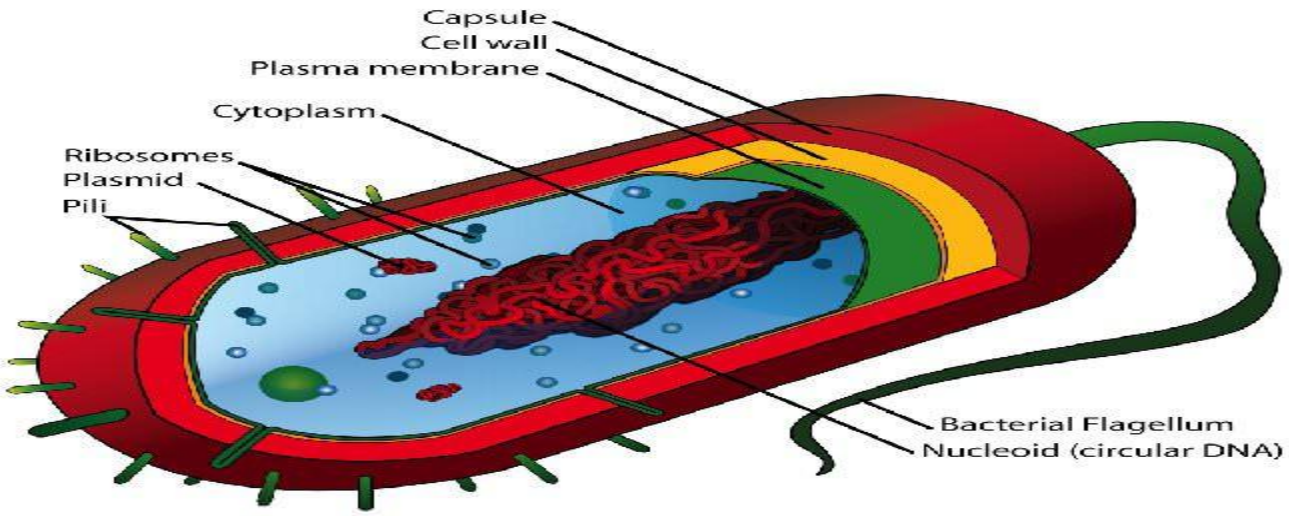
Fungi are non photosynthetic, generally saprophytic eukaryotic m.o some fungi are multicellular filaments called **molds** where as other unicellular called **yeast** .

Protozoa are single called non photosynthetic E. organism that found in different sizes and shapes many protozoa are among the most clinically important parasite of human .

Helminthes are groups of worms that live as parasites they are multicellular E. organisms with complex body organization .

Eukaryotic Cell and Prokaryotic Cell





	Eukaryotic Cell	
Nucleus	Present	Absent
Number of chromosomes	More than one	One--but not true chromosome: Plasmids
Cell Type	Usually multicellular	Usually unicellular (some cyanobacteria may be multicellular)
True Membrane bound Nucleus	Present	Absent
Example	Animals and Plants	Bacteria and Archaea
Genetic Recombination	Meiosis and fusion of gametes	Partial, un directional transfers DNA
Lysosomes and peroxisomes	Present	Absent
Microtubules	Present	Absent or rare
Endoplasmic reticulum	Present	Absent
Mitochondria	Present	Absent
Cytoskeleton	Present	May be absent
DNA wrapping on proteins.	Eukaryotes wrap their DNA around proteins called histones.	Multiple proteins act together to fold and condense prokaryotic DNA. Folded DNA is then organized into a

Eukaryotic Cell		
		variety of conformations that are supercoiled and wound around tetramers of the HU protein.
Ribosomes	larger	smaller
Vesicles	Present	Present
Golgi apparatus	Present	Absent
Chloroplasts	Present (in plants)	Absent; chlorophyll scattered in the cytoplasm
Flagella	Microscopic in size; membrane bound; usually arranged as nine double surrounding two single	Submicroscopic in size, composed of only one fiber
rmeability of Nuclear Membrane	Selective	not present
Plasma membrane with steroid	Yes	Usually no
Cell wall	Only in plant cells and fungi (chemically simpler)	Usually chemically complex

Eukaryotic Cell		
Vacuoles	Present	Present
Cell size	10-100 um	1-10 um

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 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)

Historically, bacteria or **prokaryotes** (prenucleus) were included in a single domain. However, with the more detailed analysis using modern techniques, this domain has now been separated into the Bacteria and the Archaea (ancient bacteria).

The Bacteria contain the **environmental prokaryotes** (blue green or cyanobacteria) and the **heterotrophic medically relevant bacteria**. The Archaea are environmental isolates that live in extreme environments such as **high salt concentrations, jet fuel, or extreme temperatures.** The third domain, Eukarya, **eukaryotes** (true nucleus), also contains medically relevant organisms, including fungi and parasites.

There are several other taxonomic sublevels below the domains, as noted previously; however the typical application of organism classification in the diagnostic microbiology laboratory primarily uses the taxa beginning at the family designation.

Family

A **family** encompasses a group of organisms that may contain multiple genera and consists of organisms with a common attribute. The name of a family is formed by adding the suffix ***-aceae*** to the root name of one of the group's genera, called the **type genus;** for example, the ***Streptococcaceae*** family type genus is ***Streptococcus.*** One exception to the rule in microbiology is the family *Enterobacteriaceae*; it is named after the "enteric" group of bacteria.

Bacterial (prokaryotic)-type species or strains are determined according to guidelines published by the International Committee for the Systematics of Prokaryotes. Species definitions are distinguished using **DNA** profiling, including a nearly complete **16S rRNA sequence** with less than 0% to 5% ambiguity in combination with phenotypic traits.

Genus

Genus (plural, genera), the next taxon, **contains different species that have several important features in common**. Each species within a genus differs sufficiently to maintain its status as an individual species. Placement of a species within a particular genus is based on various genetic and phenotypic characteristics shared among the species. Microorganisms do not possess the multitude of physical features exhibited by higher organisms such as plants and animals.

Species

Species (abbreviated as **sp.**, singular, or **spp.**, plural) is the most basic of the taxonomic groups and can be defined as a collection of bacterial strains that share **common physiologic and genetic features and differ notably from other microbial species.**

Occasionally, taxonomic subgroups within a species, called **subspecies,** are recognized.

Nomenclature

Nomenclature is the naming of microorganisms according to established rules and guidelines set forth in the International Code of Nomenclature of Bacteria (ICNB) or the **Bacteriological Code** (BC). It provides the accepted labels by which organisms are universally recognized. Because genus and species are the groups commonly used by microbiologists, the discussion of rules governing microbial nomenclature is limited to these two taxa. In this **binomial** (two name) system of nomenclature, every organism is assigned a **genus and a species** of Latin or Greek

derivation. Each organism has a scientific "label" consisting of two parts: the genus designation, in which the first letter is always capitalized, and the species designation, in which the first letter is always lowercase. The two components are used simultaneously and are printed in italics or underlined in script. For example, the streptococci include *Streptococcus pneumonia.*

Post test: **الاختبار البعدي:**

Q\ Enumerate classification of microorganism

Q\ Compare between Eukaryotic cell and prokaryotic cell

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المادة :الأحياء المجهرية

المرحلة :الثانية

Title: The Structure of a Generalized Procaryotic cell Lec2,3 العنوان: Cell

Name of the instructor: **اسم المحاضر:**

Estabraq Ali AL-Sodani د.محمد فليح د. استبرق علي السوداني

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

• Procaryotic cells are the smallest, simplest, and most abundant cells on earth.
• Representative procaryotes include bacteria and archaea, both of which lack a nucleus and organelles but are functionally complex.
• The structure of bacterial cells is compact and capable of adaptations to a myriad of habitats.
• The cell is encased in an envelope that protects, supports, and regulates transport.
• Bacteria have special structures for motility and adhesion to the environment.
• Bacterial cells contain genetic material in a single chromosome, and ribosomes for synthesizing proteins.
• Bacteria have the capacity for reproduction, nutrient storage, dormancy, and resistance to adverse conditions.
• Shape, size, and arrangement of bacterial cells are extremely varied.
• Bacterial taxonomy and classification is based on their structure, metabolism, and genetics.

Bacterial cells appear featureless and two-dimensional when viewed with an ordinary microscope. Not until they are subjected to the scrutiny of the electron microscope and biochemical studies does their intricate and functionally complex nature become evident. The descriptions of bacterial structure, except where otherwise noted, refer to the **bacteria,*** a category of procaryotes with

peptidoglycan in their cell walls. presents a three dimensional anatomical view of a generalized (rod-shaped) bacterial cell. As we survey the principal anatomical features of this cell, we will perform a microscopic dissection of sorts, following a course that begins with the outer cell structures and proceeds to the internal contents.

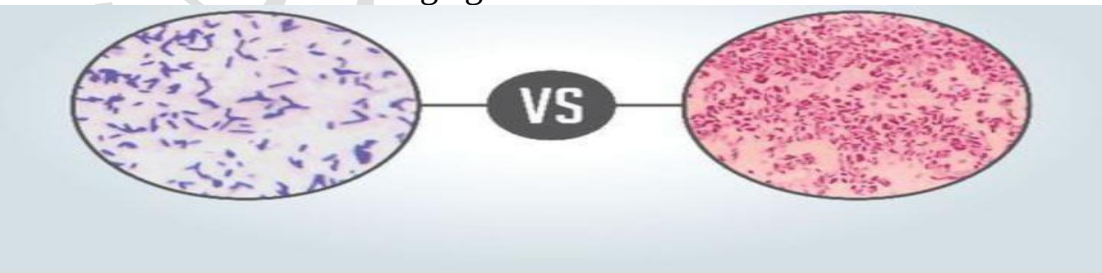
العنوان: **Pre test:**

Q\ Enumerate shapes of bacteria?

المحتوى العلمي: **Scientific content:**

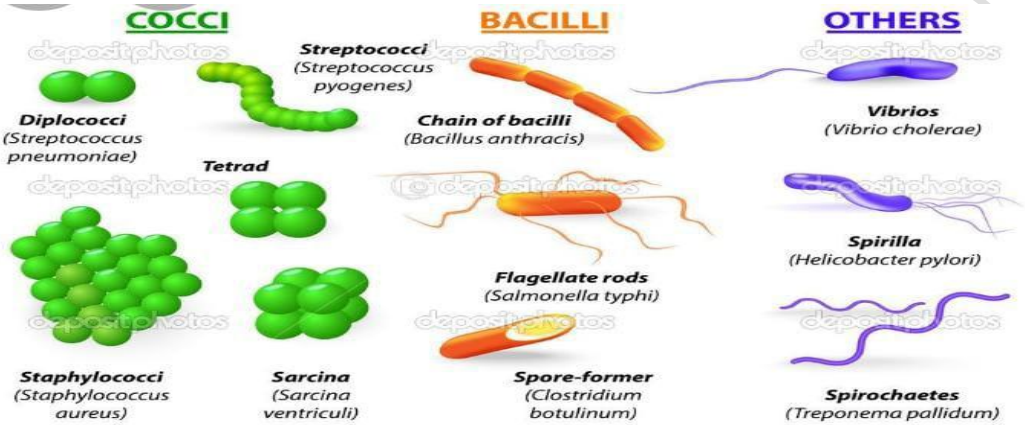
Bacterial Morphology

Most clinically relevant bacterial species range in size from 0.25 to 1 μm in width and 1 to 3 μm in length, thus requiring microscopy for visualization. Just as bacterial species and genera vary in their metabolic processes, their cells also vary in size, morphology, and cell-to-cell arrangements and in the chemical composition and structure of the cell wall. The bacterial cell wall differences provide the basis for the **Gram stain,** a fundamental staining technique used in bacterial identification schemes. This staining procedure separates almost all medically relevant bacteria into two general types: **gram-positive** bacteria, which stain a deep blue or purple, and **gram-negative** bacteria, which stain a pink to red.This simple but important color distinction is the result of differences in the constituents of bacterial cell walls that influence the cell's ability to retain differential dyes after treatment with a decolorizing agent.

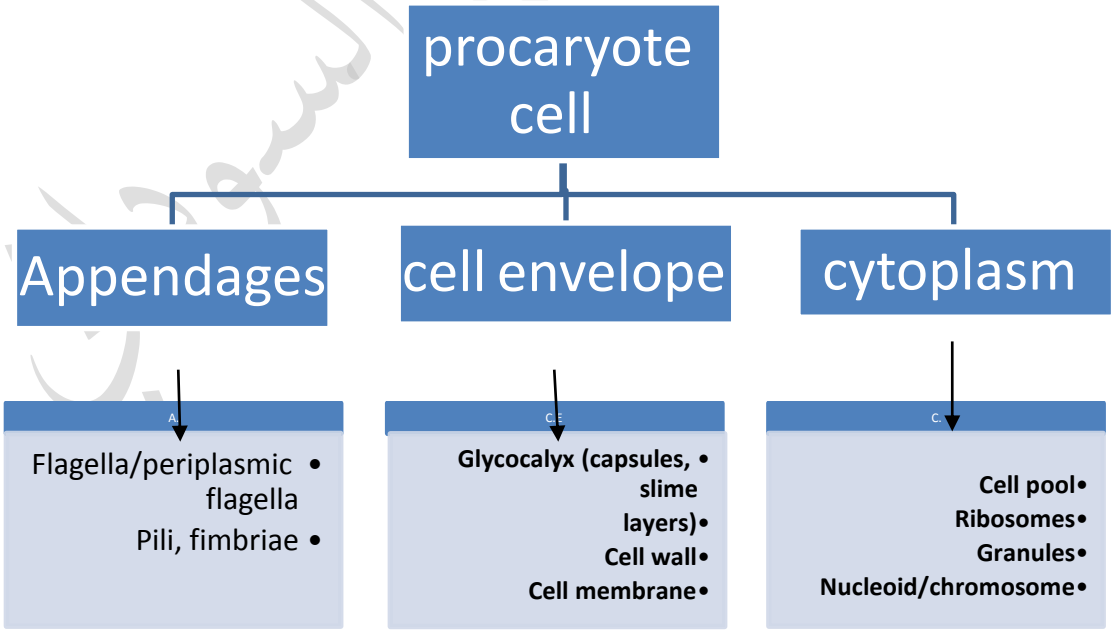


Common bacterial cellular morphologies include **cocci** (circular), **coccobacilli** (ovoid), and **bacilli** (rod shaped), as well as **fusiform** (pointed end), curved, or

spiral shapes. Cellular arrangements are also noteworthy. Cells may characteristically occur singly, in pairs, or grouped as tetrads, clusters, or in chains. The determination of the Gram stain reaction and the cell size, morphology, and arrangement are essential aspects of bacterial identification.



Parts of prokaryotic cell



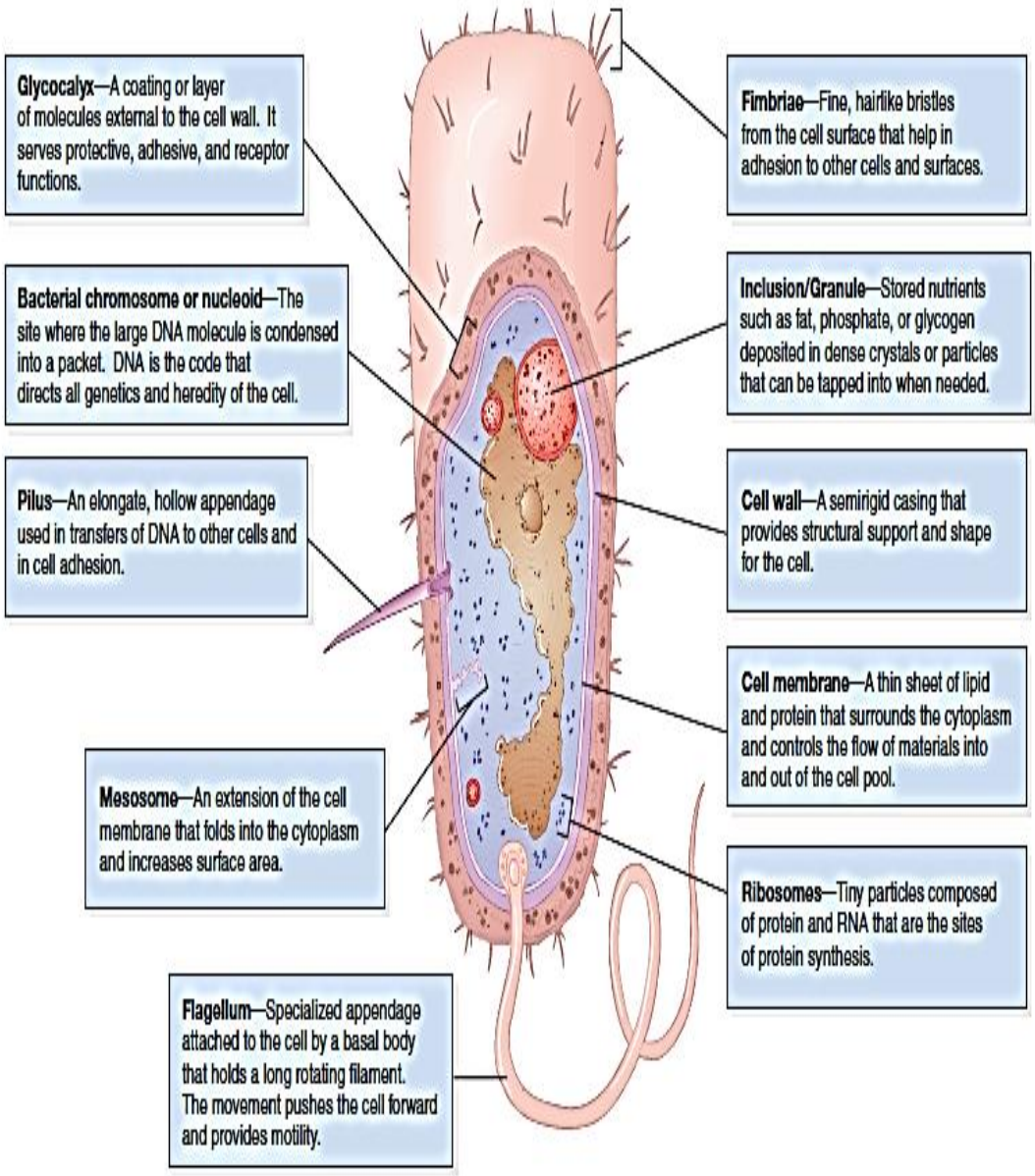


FIGURE 4.1

Structure of a prokaryotic cell. Cutaway view of a typical rod-shaped bacterium, showing major structural features. Note that not all components are found in all cells.

1-THE CELL ENVELOPE: THE OUTER WRAPPING OF BACTERIA

The majority of bacteria have a chemically complex external covering, termed the **cell envelope,** that lies outside of the cytoplasm.
It is composed of three basic layers known as the glycocalyx, the cell wall, and the cell membrane . The layers of the envelope are stacked one upon another and are often tightly bonded together like. Although each envelope layer performs a distinct function, together they act as a single protective unit. The envelope is extensive and can account for one-tenth to one-half of a cell's volume.

A-The Bacterial Surface Coating, or Glycocalyx

The bacterial cell surface is frequently exposed to severe environmental conditions. The **glycocalyx** develops as a coating of macromolecules to protect the cell and, in some cases, help it adhere to its environment. Glycocalyces differ among bacteria in thickness, organization, and chemical composition. Some bacteria are covered with a loose, soluble shield called a **slime layer** that evidently protects them from loss of water and nutrients. Other bacteria produce **capsules** of repeating polysaccharide units, of protein, or of both. Acapsule is bound more tightly to the cell than a slime layer is, and it has a thicker, gummy consistency that gives a prominently sticky (mucoid) character to the colonies of most encapsulated bacteria .

Specialized Functions of the Glycocalyx

Capsules are formed by a few pathogenic bacteria, such as *Streptococcus pneumonia* (a cause of pneumonia, an infection of the lung),*Haemophilus influenzae* (one cause of meningitis), and *Bacillus anthracis* (the cause of anthrax). Encapsulated bacterial cells generally have great pathogenicity because capsules protect the bacteria against white blood cells called phagocytes.

B-Peptidoglycan (PG) Cell wall

Immediately below the glycocalyx lies a second layer, the **cell wall.** This structure accounts for a number of important bacterial characteristics.
In general, it determines the *shape of a bacterium, and it also provides the *kind of strong structural support necessary to keep a bacterium from bursting or collapsing because of changes in osmotic pressure.

The cell walls of most bacteria gain their relatively rigid quality from a unique macromolecule called **peptidoglycan (PG).** Peptidoglycan is only one of several materials found in cell walls, and its amount and exact composition vary among the major bacterial groups.

Because many bacteria live in aqueous habitats with a low solute concentration, they are constantly absorbing excess water by osmosis.

Some factors effect in C.W

1-Several types of **drugs** used to treat infection (penicillin, cephalosporins) are effective because they target the peptide cross-links in the peptidoglycan. **lysis*** .

2-Some **disinfectants** (alcohol, detergents) also kill bacterial cells by damaging the cell wall.

3-**Lysozyme**, an enzyme contained in tears and saliva, provides a natural defense against certain bacteria by hydrolyzing the bonds in the glycan chains and causing the wall to breakdown.

Differences in Cell Wall Structure

The Gram-Positive Cell Wall

The bulk of the gram-positive cell wall is a thick, homogeneous sheath of peptidoglycan ranging from **20-80** nm in thickness. It also contains tightly bound acidic polysaccharides, including teichoic acid and lipoteichoic acid . Teichoic acid is a polymer of ribitol or glycerol and phosphate embedded in the peptidoglycan sheath. Lipoteichoic acid is similar in structure but is attached to the lipids in the plasma membrane. These molecules appear to function in cell wall maintenance and enlargement during cell division, and they also contribute to the acidic charge on the cell surface.

The Gram-Negative Cell Wall

The gram-negative cell wall is more complex in morphology because it contains an **outer membrane OM,** has a thinner shell of peptidoglycan, and has an extensive space surrounding the peptidoglycan. The outer membrane is somewhat similar in construction to the cell membrane, except that it contains specialized types of polysaccharides and proteins. The uppermost layer of the OM contains lipopolysaccharide (LPS). The polysaccharide chains extending off the surface function as antigens and receptors. The innermost layer of the OM is another lipid layer anchored by means of lipoproteins to the peptidoglycan layer below. The outer membrane serves as a partial chemical sieve by allowing only relatively

small molecules to penetrate. Access is provided by special membrane channels formed by ***porin proteins*** that completely span the outer membrane. The size of these porins can be altered so as to block the entrance of harmful chemicals, making them one defense of gram-negative bacteria against certain antibiotics.

The bottom layer of the gram-negative wall is a single, thin (1–3 nm) sheet of peptidoglycan. Although it acts as a somewhat rigid protective structure as previously described, its thinness gives gram-negative bacteria a relatively greater flexibility and sensitivity to lysis. There is a well-developed *periplasmic space* surrounding the peptidoglycan. This space is an important reaction site for a large and varied pool of substances that enter and leave the cell.

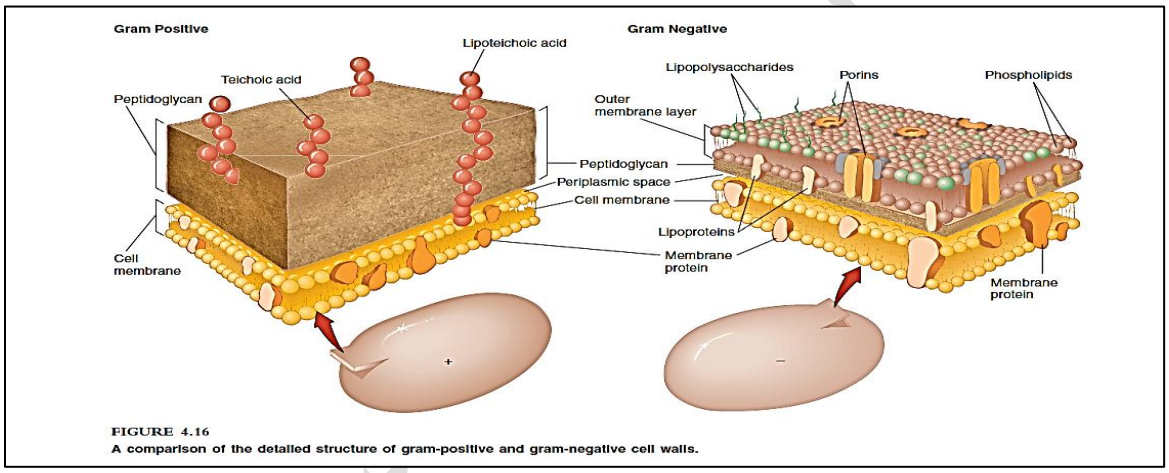


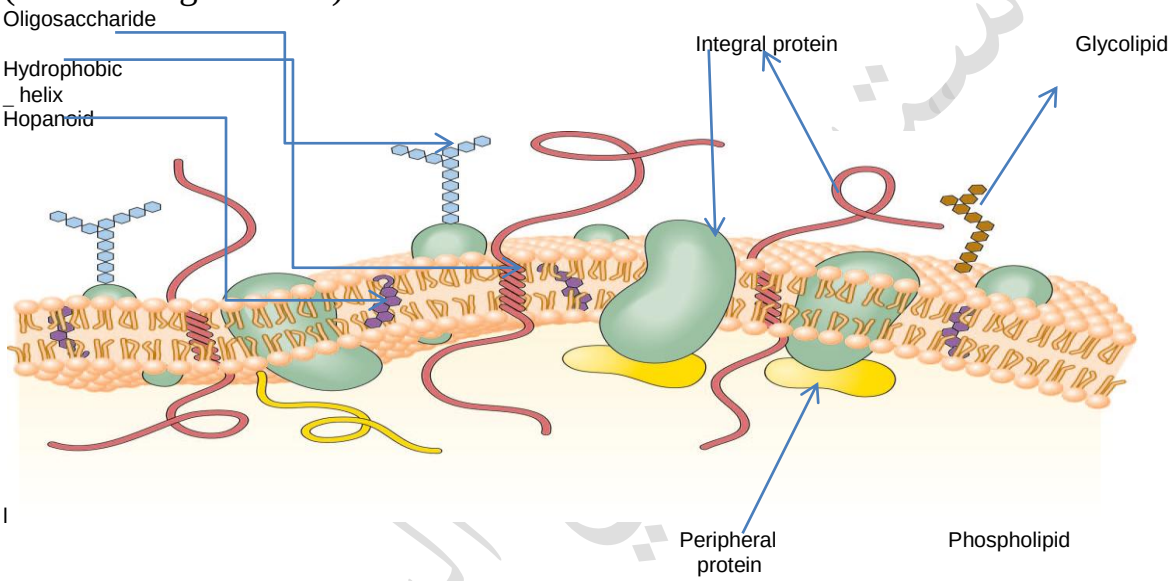
FIGURE 4.16
A comparison of the detailed structure of gram-positive and gram-negative cell walls.

TABLE 4.1
Comparison of Gram-Positive and Gram-Negative Cell Walls

Characteristic	Gram-Positive	Gram-Negative
Number of major layers	1	2
Chemical composition	Peptidoglycan Teichoic acid Lipoteichoic acid	Lipopolysaccharide Lipoprotein Peptidoglycan
Overall thickness	Thicker (20–80 nm)	Thinner (8–11 nm)
Outer membrane	No	Yes
Periplasmic space	Narrow	Extensive
Porin proteins	No	Yes
Permeability to molecules	More penetrable	Less penetrable

C- CELL MEMBRANE STRUCTURE

Appearing just beneath the cell wall is the **cell,** or **cytoplasmic, membrane,** a very thin (5–10 nm), flexible sheet molded completely around the cytoplasm. Its general composition was described as a lipid bilayer with proteins embedded to varying degrees. Bacterial cell membranes have this typical structure containing primarily phospholipids (making up about 30–40% of the membrane mass) and proteins (contributing 60–70%).



In some locations, the cell membrane forms internal pouches in the cytoplasm called **mesosomes*.** These are prominent in gram-positive bacteria but are harder to see in gram negative bacteria because of their relatively small size. Mesosomes presumably increase the internal surface area available for membrane activities.

Functions of the Cell Membrane

Since bacteria have none of the eucaryotic organelles, the cell membrane provides a site for functions such as

1-Energy reactions.

2-Nutrient processing, and synthesis.

3-A major action of the cell membrane is to regulate *transport,* that is, the passage of nutrients into the cell and the discharge of wastes.

4- Although water and small uncharged molecules can diffuse across the membrane unaided.

5-membrane is a *selectively permeable* structure with special carrier mechanisms for passage of most molecules.
6-The cell membrane is also involved in *secretion*, or the discharge of a metabolic product into the extracellular environment.
7-Most enzymes of respiration and ATP synthesis reside in the cell membrane since procaryotes lack mitochondria.
8-Other products (enzymes and toxins) are secreted by the membrane into the extracellular environment.

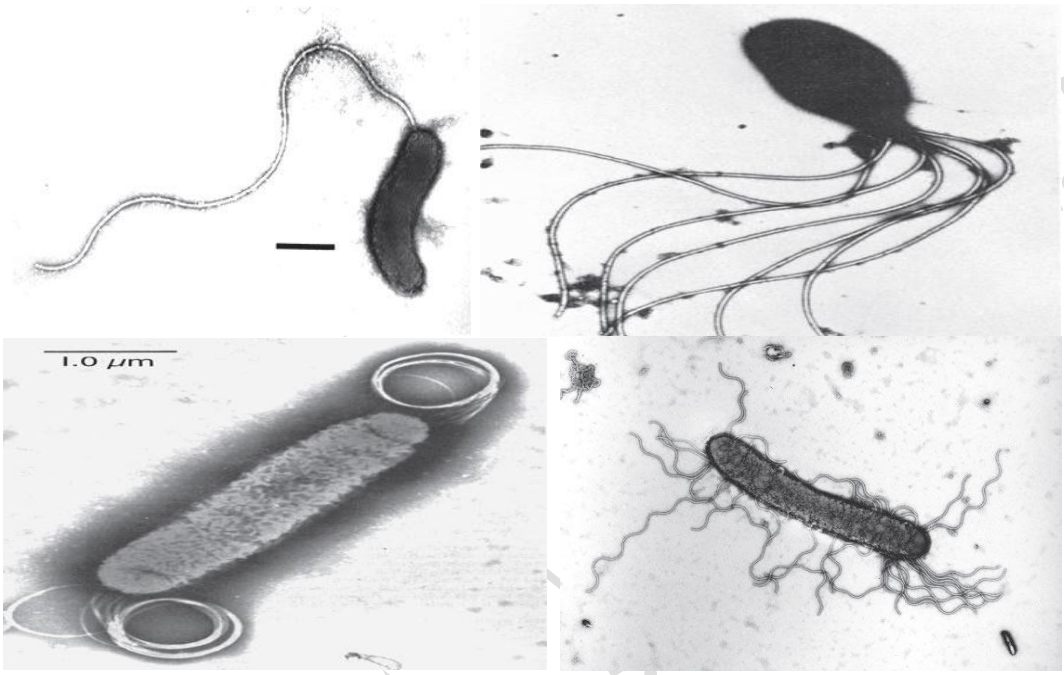
D-The appendages of bacteria

Pili(Fimbriae)

Many Gram-negative bacteria possess rigid surface appendages called **pili** (L "hairs") or **fimbriae** (L "fringes"). They are shorter and thinner than flagella; similar to flagella, they are composed of structural protein subunits termed **pilins**. Some pili contain a single type of pilin, others more than one. Minor proteins termed **adhesins** are located at the tips of pili and are responsible for the attachment properties. Two classes can be distinguished: ordinary pili, which play a role in the adherence of symbiotic and pathogenic bacteria to host cells; and sex pili, which are responsible for the attachment of donor and recipient cells in bacterial conjugation a means of DNA transfer (sex pili).

provide motility (flagella) Bacterial flagella are thread-like appendages composed entirely of protein called flagellin , approximately 20 nm in diameter. They are the organs of locomotion for the forms that possess them. motility is restored within 3–6 minutes. The flagellins of different bacterial species presumably differ from one another in primary structure. They are highly antigenic **(H antigens)**, and some of the immune responses to infection are directed against
Flagella vary in number and arrangement as well as in the type and rate of motion they produce.

a. b.



c. d.

Electron micrographs depicting types of flagellar arrangements. (a) Monotrichous flagellum **(b)** Lophotrichous flagella **(c)** amphitrichous (and lophotrichous) in arrangement and coil up into tight loops. **(d)** peritrichous flagella

2- CONTENTS OF THE CELL CYTOPLASM

Encased by the cell membrane is a dense, gelatinous solution referred to as **cytoplasm,** which is another prominent site for many of the cell's biochemical and synthetic activities. Its major component is water (70–80%), which serves as a solvent for the **cell pool,** a complex mixture of nutrients including sugars, amino acids, and salts. The components of this pool serve as building blocks for cell synthesis or as sources of energy. The cytoplasm also contains larger, discrete cell masses such as the chromatin body, ribosomes, mesosomes, and granules.

A-Bacterial Chromosomes and Plasmids: The Sources of Genetic Information

The hereditary material of bacteria exists in the form of a single circular

strand of DNA designated as the **bacterial chromosome.*** By definition, bacteria do not have a nucleus; that is, their DNA is not enclosed by a nuclear membrane but instead is aggregated in a dense area of the cell called the **nucleoid.*** The chromosome is actually an extremely long molecule of DNA that is tightly coiled around special basic protein molecules so as to fit inside the cell compartment.

many bacteria contain other, nonessential pieces of DNA called **plasmids.*** These tiny, circular extra-chromosomal strands can be free or integrated into the chromosome; they are duplicated and passed on to offspring. They are not essential to bacterial growth and metabolism, but they often confer protective traits such as resisting drugs and producing toxins and enzymes.

B-Ribosomes: Sites of Protein Synthesis

A bacterial cell contains thousands of tiny, discrete units called **ribosomes.*** ribosomes show up as fine, spherical specks dispersed throughout the cytoplasm that often occur in chains (polysomes).

Chemically, a ribosome is a combination of a special type of RNA called ribosomal RNA, or rRNA (about 60%), and protein (40%). The essential function of ribosomes is protein synthesis.

C-**Inclusions, or Granules: Storage Bodies**

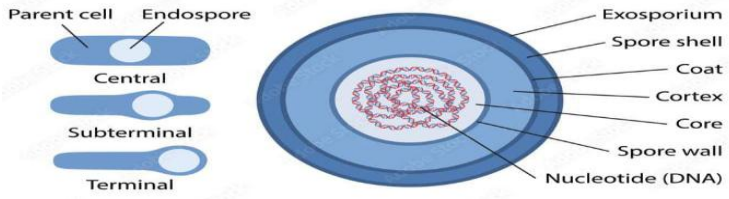
Most bacteria are exposed to severe shifts in the availability of food.

During periods of nutrient abundance, they compensate by laying down nutrients intracellularly in **inclusion bodies,** or **inclusions,*** of varying size, number, and content.

Other inclusions, also called granules, contain crystals of inorganic compounds and are not enclosed by membranes. Sulfur granules of photosynthetic bacteria polyphosphate granules of *Corynebacterium* and *Mycobacterium* are of this type. The latter represent an important source of building blocks for nucleic acid and ATP synthesis. They have been termed **metachromatic*** **granules** because they stain a contrasting color (red, purple) in the presence of methylene blue dye.

D- BACTERIAL ENDOSPORES:

Endospores are dormant bodies produced by the grampositive genera *Bacillus, Clostridium,* and *Sporosarcina.* These bacteria have a two-phase life cycle—a vegetative cell and an endospore.The depletion of nutrients, especially an adequate carbon or nitrogen source, is the stimulus for a vegetative cell to begin spore formation.



Post test
Compare between Gram positive and Gram negative cell wall?

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المادة :الأحياء المجهرية

المرحلة :الثانية

Title: **العنوان:**

Microbial Growth and Metabolism Lec 4

Name of the instructor: **اسم المحاضر:**

Dr. Mohammed Flaih **ا.د محمد فليح طريف**

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

The division of a bacterial cell occurs mainly through **binary, or transverse, fission;** *binary* means that one cell becomes two, and *transverse* refers to the division plane forming across the width of the cell. During binary fission, the parent cell enlarges, duplicates its chromosome, and forms a central transverse septum that divides the cell into two daughter cells. This process is repeated at intervals by each new daughter cell in turn, and with each successive round of division, the population increases in size, number, and mass.

Pretest: **الاختبار القبلي:**

Q\ Enumerate factors effecting in bacterial growth?

Scientific Content: **المحتوى العلمي:**

Microbial Growth and Metabolism

The division of a bacterial cell occurs mainly through **binary, or transverse, fission;** *binary* means that one cell becomes two, and *transverse* refers to the division plane forming across the width of the cell. During binary fission, the parent cell enlarges, duplicates its chromosome, and forms a central transverse septum that divides the cell into two daughter cells. This process is repeated at intervals by each new daughter cell in turn, and with each successive round of division, the population increases in size, number, and mass.

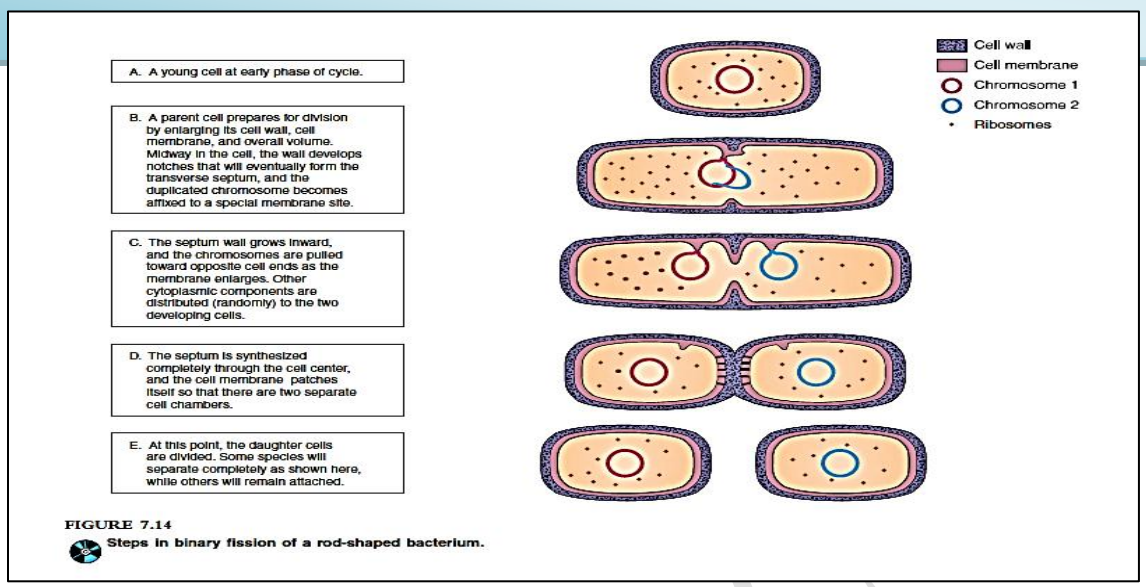

FIGURE 7.14
Steps in binary fission of a rod-shaped bacterium.

Pretest: **الاختبار القبلي:**

Q\ Enumerate steps of binary fission of a rod shaped bacterium?

STAGES IN THE NORMAL GROWTH CURVE

The system of batch culturing described as *closed*, meaning that nutrients and space are finite and there is no mechanism for the removal of waste products. Data from an entire growth period of 3 to 4 days typically produce a curve with a series of phases termed

1- The lag phase
2- The exponential growth (log) phase
3- The stationary phase
4- The death phase

1- The **lag phase** is a relatively "flat" period on the graph when the population appears not to be growing or is growing at less than the exponential rate. Growth lags primarily because: (a) The newly inoculated cells require a period of adjustment, enlargement, and synthesis; (b) the cells are not yet multiplying at their maximum rate; and (c) the population of cells is so sparse or dilute that the sampling misses them. The length of the lag period varies somewhat from one population to another. The cells reach the maximum rate of cell division during the

2-exponential growth (log) phase, a period during which the curve increases geometrically. This phase will continue as long as cells have adequate nutrients and the environment is favorable.

3-**stationary growth phase,** the population enters a survival mode in which cells stop growing or grow slowly. The curve levels off because the rate of cell inhibition or death balances out the rate of multiplication. The decline in the growth rate is caused by depleted nutrients and oxygen, excretion of organic acids and other biochemical pollutants into the growth medium, and an increased density of cells. As the limiting factors intensify, cells begin to die in exponential numbers and they are unable to multiply.

4-death phase The curve now dips downward as the death. The speed with which death occurs depends on the relative resistance of the species and how toxic the conditions are, but it is usually slower than the exponential growth phase. Viable cells often remain many weeks and months after this phase has begun.

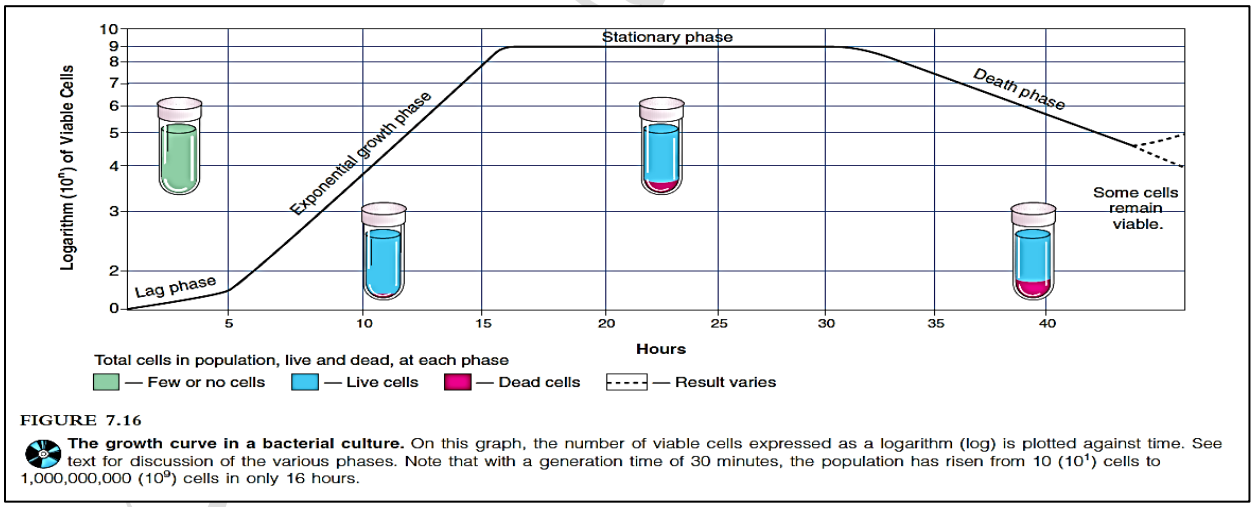

FIGURE 7.16

The growth curve in a bacterial culture. On this graph, the number of viable cells expressed as a logarithm (log) is plotted against time. See text for discussion of the various phases. Note that with a generation time of 30 minutes, the population has risen from 10 (10^1) cells to 1,000,000,000 (10^9) cells in only 16 hours.

Surface growth

If a single bacterial cell is placed on a solid nutrient agar surface progressive of this cell remain close to the site of deposition and eventually form a compact macroscopic mass of cells called colony. For rapidly growing species over night

incubation at 30 ℃ to 37℃ is sufficient to produce millions of cells. The colonies morphology include color, shape, adherence, smell, and surface texture can used guide for identification of the bacterial species.

Cell numbers can be counted directly by

1- a microscope counting chamber.
2- Coulter counter or flow cytometer.
3- Cell growth can also be determined by turbidometry.
4- total cell count.

The Meaning of Bacterial Death

For a microbial cell, death means the irreversible loss of the ability to reproduce (grow and divide). the empirical test of death is culture of cells on solid media: A cell is considered dead if it fails to give rise to a colony on appropriate medium. Obviously, then, the reliability of the test depends on the choice of medium and conditions: For example, a culture in which 99% of the cells appear "dead" in terms of the ability to form colonies on one medium may prove to be 100% viable if tested on another medium. Furthermore, the detection of a few viable cells in a large clinical specimen may not be possible by directly plating a sample because the sample fluid itself may be inhibitory to microbial growth. In such cases, the sample may have to be diluted first into liquid medium, permitting the outgrowth of viable cells before plating.

The conditions of incubation in the first hour after treatment are also critical in the determination of "killing." For example, if bacterial cells are irradiated with ultraviolet light and plated immediately on any medium, it may appear that 99.99% of the cells have been killed. If such irradiated cells are first incubated in a suitable medium for 20 minutes, plating may indicate only 10% killing. In other words, irradiation determines that a cell will "die" if plated immediately but will live if allowed to repair radiation damage before plating. A microbial cell that is not physically disrupted is thus "dead" only in terms of the conditions used to test viability.

Post test

Q\ Enumerate steps of growth curve?

References

1- Foundations in Microbiology
4th Edition
Kathleen Park Talaro Pasadena City College
Arthur Talaro Pasadena City College 2001
2- Todar's Online Textbook of Bacteriology
Dedication to Hans Zinsser 2005
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المادة :الأحياء المجهرية

المرحلة :الثانية

Title:

العنوان:

Bacterial Cultivation **Lec 5**

Name of the instructor: **اسم المحاضر:**

Dr. Mohammed Flaih **ا.د محمد فليح طريف**

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

Biologists studying large organisms such as animals and plants can, for the most part, immediately see and differentiate their experimental subjects from the surrounding environment and from another.

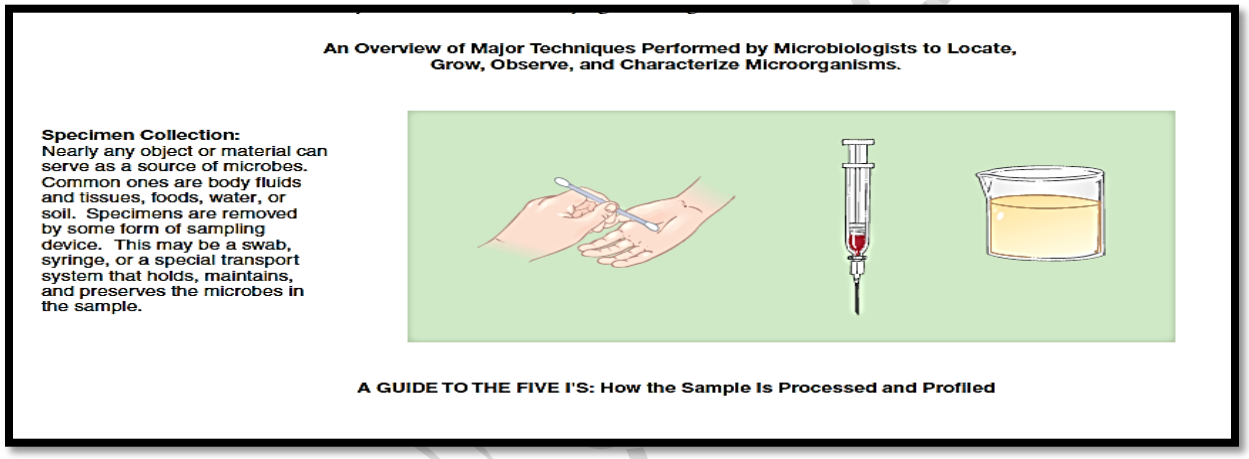
In fact, they can use their senses of sight, smell, hearing, and even touch to detect and evaluate identifying characteristics and to keep track of growth and developmental changes.

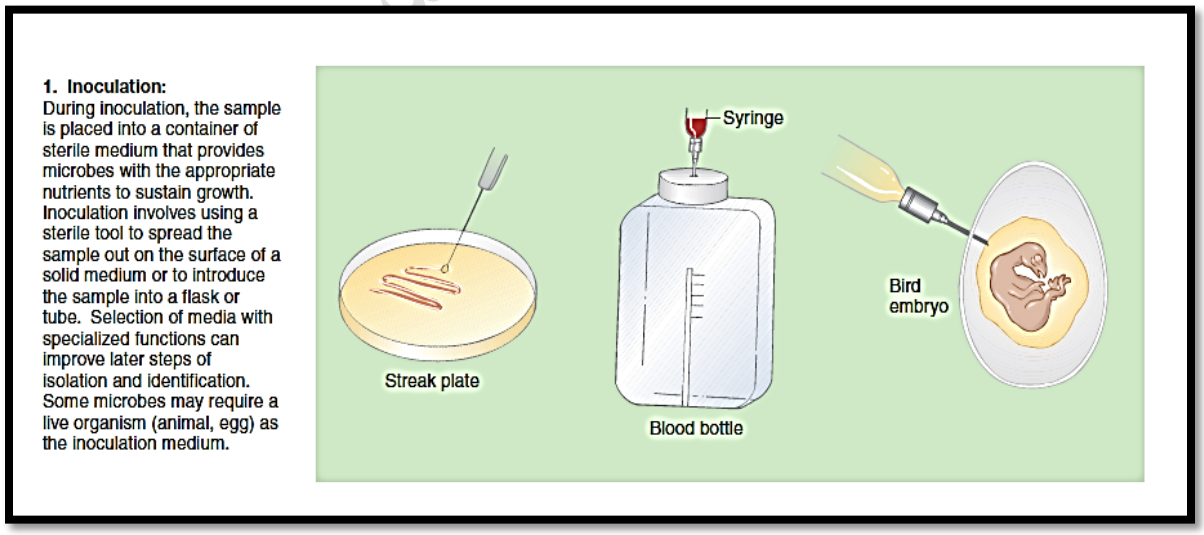
First, most habitats (such as the soil and the human mouth) harbor microbes in complex associations, so it is often necessary to separate the species from one another.
Second, to maintain and keep track of such small research subjects, microbiologists usually have to grow them under artificial conditions.
A **third** difficulty in working with microbes is that they are invisible and widely distributed, and undesirable ones can be introduced into an experiment and cause misleading results.

Scientific content: المحتوى العلمي:

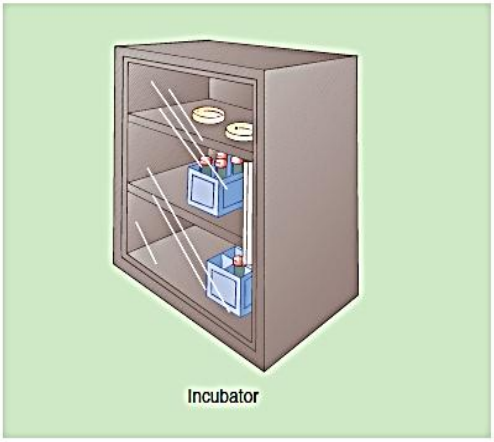
Microbiologists use five basic techniques to manipulate, grow, examine, and characterize microorganisms in the laboratory these are :

1-inoculation
2- incubation
3- isolation
4-inspection
5-identification

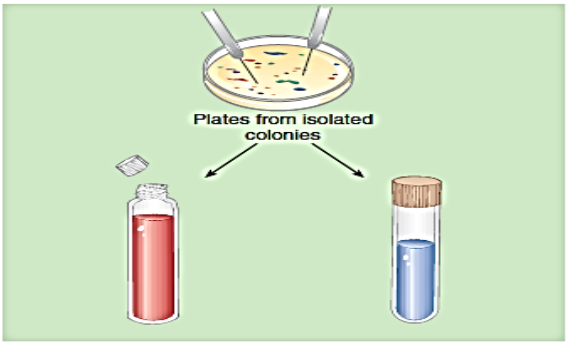




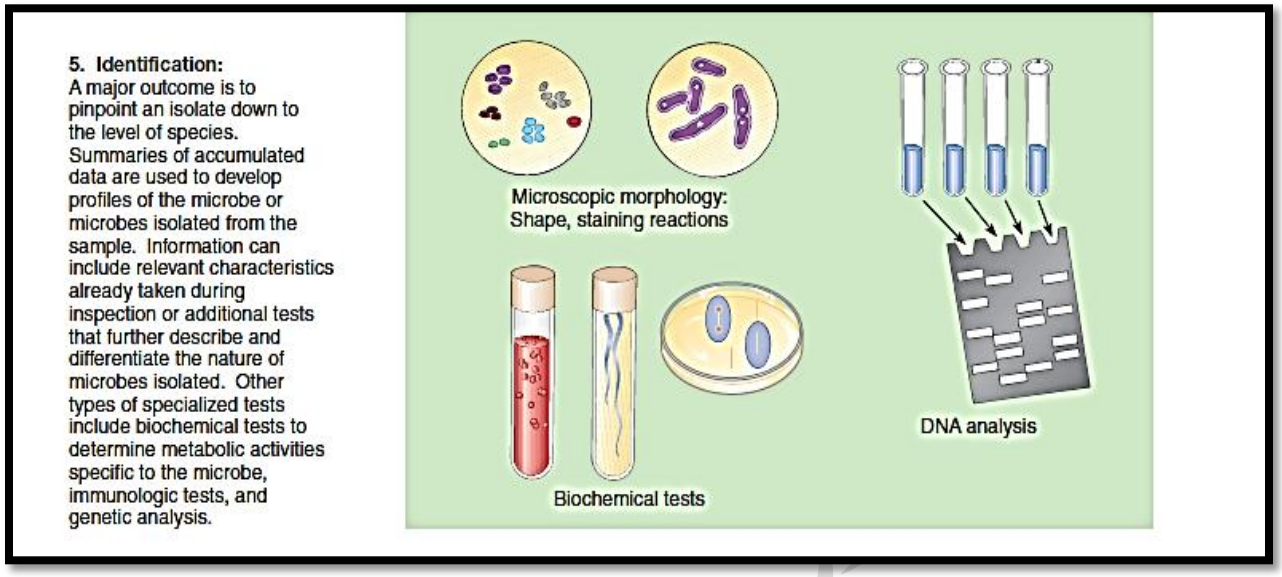
2. Incubation:
An incubator can be used to adjust the proper growth conditions of a sample. Setting the optimum temperature and gas content promotes multiplication of the microbes over a period of hours, days, and even weeks. Incubation produces a culture—the visible growth of the microbe in the medium.


Incubator

3. Isolation:
The end result of inoculation and incubation is isolation of the microbe in macroscopic form. The isolated microbes take the form of separate colonies (discrete mounds of cells) on solid media, or turbidity in broths. Further isolation, also known as subculturing, involves taking a tiny bit of growth and inoculating an additional culture of it. This is one way to make a pure culture that contains only a single species of microbe.



4. Inspection:
The cultures are observed macroscopically for obvious growth characteristics (color, texture, size) that could be useful in analyzing the specimen contents. Slides are made to assess microscopic details such as cell shape, size, and motility. Staining techniques may be used to gather specific information on microscopic morphology.



Types of culture media used in microbiology

Media contains:

- carbon source e.g., Glucose
- various salts and minerals
- amino acids and nitrogen source e.g., beef, yeast extract
- water

Media are of different types on consistency and chemical composition. **A. On Consistency:**

1**. Solid Media**. Advantages of solid media: (a) Bacteria may be identified by studying the colony character, (b) Mixed bacteria can be separated. Solid media is used for the isolation of bacteria as pure culture. 'Agar' is most commonly used to prepare solid media. Agar is polysaccharide extract obtained from seaweed. Agar is an ideal solidifying agent in **1.5-2.5%** percentage as it is :

(a) Bacteriologically inert, i.e. no influence on bacterial growth, (b) It remains solid at 37°C, and (c) It is transparent.

2**. Liquid Media**. It is used for profuse growth, e.g. blood culture in liquid media, **without agar** . Mixed organisms cannot be separated.

3. Semi-solid. it is used for detected motile bacteria, in **0.5% agar**

B. On Chemical Composition :

1. Routine Laboratory Media 2. Synthetic Media. These are chemically defined media prepared from pure chemical substances. It is used in research work.

ROUTINE LABORATORY MEDIA

These are classified into six types:

(1) Basal media, (2) Enriched media, (3) Selective media, (4) Indicator media, (5) Transport media, and (6) Storage media.

1. BASAL MEDIA. Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water. *Staphylococcus* and Enterobacteriaceae grow in these media.

2. ENRICHED MEDIA. The media are enriched usually by adding blood, serum or egg. Examples: Enriched media are blood agar and Lowenstein-Jensen media. Streptococci grow in blood agar media.

3. SELECTIVE MEDIA. These media favour the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: MacConkey agar, Lowenstein-Jensen media, tellurite media (Tellurite inhibits the growth of most of the throat organisms except diphtheria bacilli). Antibiotic may be added to a medium for inhibition.

4. INDICATOR (DIFFERENTIAL) MEDIA. An indicator is included in the medium. A particular organism causes change in the indicator, e.g. blood, neutral red, tellurite. Examples: Blood agar and MacConkey agar are indicator media.

5. TRANSPORT MEDIA. These media are used when specie-men cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium.

6. STORAGE MEDIA. Media used for storing the bacteria for a long period of time. Examples: Egg saline medium, chalk cooked meat broth, Brain heart infusion broth.

COMMON MEDIA IN ROUTINE USE

A- Nutrient Broth. Uses: (1) As a basal media for the preparation of other media, (2) To study soluble products of bacteria.

B- Nutrient Agar. It is solid at 37°C.

C- Peptone Water. Peptone 1% and sodium chloride 0.5%. It is used as base for sugar media and to test indole formation.

D- Blood Agar. Most commonly used medium. 5- 10% defibrinated sheep or horse blood is added to melted agar at 45-50°C. Blood acts as an enrichment material and also as an indicator. Certain bacteria when grown in blood agar produce haemolysis around their colonies. Certain bacteria produce no haemolysis. Types of changes : (a) beta β-haemolysis. The colony is surrounded by a clear zone of complete haemolysis, e.g. Streptococcus pyogenes is a beta haemolytic streptococci, (b) Alpha α-haemolysis. The colony is surrounded by a zone of greenish discolouration due to formation of biliverdin, e.g. Viridans streptococci, (c) Gamma γ-haemolysis, or, No haemolysis. There is no change in the medium surrounding the colony,

E- Chocolate Agar or Heated Blood agar: Prepared by heating blood agar. It is used for culture of pneumococcus, gonococcus, meningococcus and Haemophilus. Heating the blood inactivates inhibitor of growths.

F- MacConkey Agar. Most commonly used for enterobacteriaceae. It It is a selective and indicator medium : (1) Selective as bile salt does not inhibit the growth of enterobactericeae but inhibits growth of many other bacteria.
(2) Indicator medium as the colonies of bacteria that ferment lactose take a pink colour due to production of acid. Acid turns the indicator neutral red to pink. These bacteria are called 'lactose fermenter', e.g. *Escherichia coli*. Yellow or Colorless colony indicates that lactose is not fermented, e.g. Salmonella. Shigella, Vibrio.

G- Mueller Hinton Agar. Disc diffusion sensitivity tests for antimicrobial drugs should be carried out on this media as per WHO recommendation to promote reproducibility and comparability of results.

Post test: **الاختبار البعدي:**

Q/ Write types of media classified depended consistency?

References

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المادة :الأحياء المجهرية

المرحلة :الثانية

Title
Metabolism (energy production) Lec 6,7

Name of the instructor: **اسم المحاضر:**

Estabraq Ali AL-Sodani **د. استبرق علي السوداني**

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

1-Aerobic Respiration

Aerobic respiration is a series of enzyme-catalyzed reactions in which electrons are transferred from fuel molecules such as glucose to oxygen as a final electron acceptor. This pathway is the principal energy-yielding scheme for aerobic heterotrophs, and it provides both ATP and metabolic intermediates for many other pathways in the cell, including those of protein, lipid, and carbohydrate synthesis Aerobic respiration in microorganisms can be summarized by an equation:

Glucose ($C_6H_{12}O_6$) _ 6 O_2 _ 38 ADP _ 38 Pi →6 CO_2 _ 6 H_2O _ 38 ATP

2-Anaerobic respiration

Some bacteria have evolved an anaerobic respiratory system that functions like the aerobic cytochrome system except that it utilizes oxygen-containing salts, rather than free oxygen, as the final electron acceptor. Of these, the nitrate (NO_3_) and nitrite (NO_2_) reduction systems are best known. The reaction in species such as *Escherichia coli* is represented as:

Nitrate reductase

↓

$NO_3 + NADH \rightarrow NO_2 + H_2O + NAD\_2+$

3-Fermentation

Of all the results of pyruvate metabolism, probably the most varied is fermentation. Technically speaking, **fermentation*** is the incomplete oxidation of glucose or

other carbohydrates in the absence of oxygen, a process that uses organic compounds as the terminal electron acceptors and yields a small amount of ATP.
Fermentation is also what bacteriologists call the formation of acid, gas, and other products by the action of various bacteria on pyruvic acid. The process is a common metabolic strategy among bacteria.

Scientific content: المحتوى العلمي:

The Metabolism of Microbes

Metabolism, from the Greek term *metaballein*, meaning change, pertains to all chemical reactions and physical workings of the cell. Although metabolism entails thousands of different reactions, most of them fall into one of two general categories.

Anabolism,* sometimes also called *biosynthesis*, is any process that results in synthesis of cell molecules and structures. It is a building and bond making process that forms larger molecules from smaller ones, and it usually requires the input of energy.

Catabolism* is the opposite, or complement, of anabolism. Catabolic reactions are degradative; they break bonds, convert larger molecules into smaller components, and often produce energy.The linking of anabolism to catabolism ensures the efficient completion of many thousands of cellular processes.

Metabolism is a self-regulatory process that maintains the stability of the cell. Metabolism of nutrients can extract energy in the form of adenosine triphosphate (ATP), or other high energy compounds, that can be channeled into such processes as biosynthesis, transport, growth, and motility.

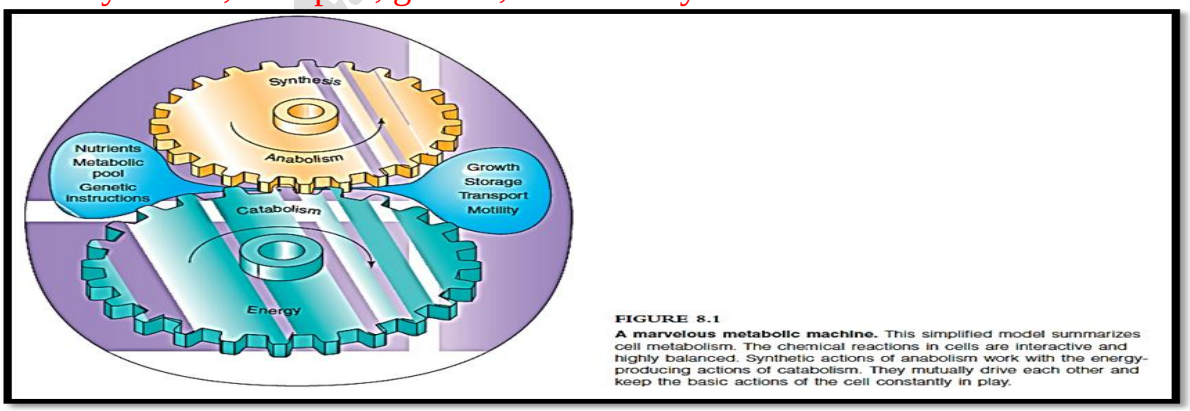


FIGURE 8.1
A marvelous metabolic machine. This simplified model summarizes cell metabolism. The chemical reactions in cells are interactive and highly balanced. Synthetic actions of anabolism work with the energy-producing actions of catabolism. They mutually drive each other and keep the basic actions of the cell constantly in play.

ENZYMES: CATALYZING THE CHEMICAL REACTIONS OF LIFE

chemical reactions of life, even when highly organized and complex, cannot proceed without a special class of proteins called **enzymes.***Enzymes are a remarkable example of **catalysts,*** chemicals that increase the rate of a chemical reaction without becoming part of the products or being consumed in the reaction. Do not make the mistake of thinking that an enzyme creates a reaction. Because of the great energy of some molecules, a reaction could occur spontaneously at some point even without an enzyme, but at a very slow rate. enzymes, which speed up the rate of reactions, are necessary to life.

How Do Enzymes Work?

We have said that an enzyme speeds up the rate of a metabolic reaction, but just how does it do this? During a chemical reaction, reactants are converted to products by bond formation or breakage. A certain amount of energy is required to initiate every such reaction, which limits its rate. This resistance to a reaction, which must be overcome for a reaction to proceed, is measurable and is called the **energy of activation**. At the molecular level, an enzyme promotes a reaction by serving as a physical site upon which the reactant molecules, called **substrate.**

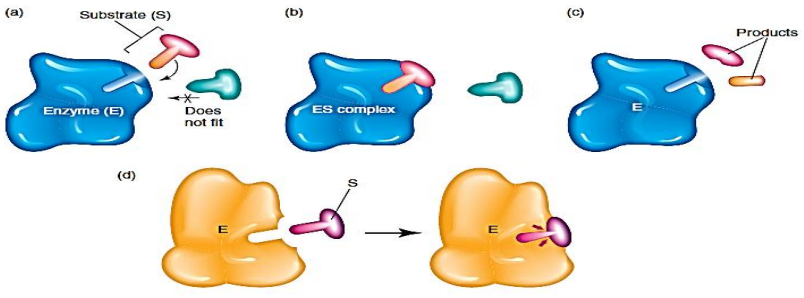


FIGURE 8.5
Enzyme-substrate reactions. (a) When the enzyme and substrate come together, the substrate (S) must show the correct fit and position with respect to the enzyme (E). **(b)** When the ES complex is formed, it enters a transition state. During this temporary but tight interlocking union, the enzyme participates directly in breaking or making bonds. **(c)** Once the reaction is complete, the enzyme releases the products. **(d)** The induced fit model proposes that the enzyme recognizes its substrate and adapts slightly to it so that the final binding is even more precise.

Enzyme Structure

The primary structure of all enzymes is protein (with some exceptions—Microbits), and they can be classified as simple or conjugated.1- **Simple** enzymes consist of protein alone, whereas 2-**conjugated** enzymes contain protein and non protein molecules. A conjugated enzyme, sometimes referred to as a **holoenzyme,*** is a combination of a protein, now called the **apoenzyme,** and one or more **cofactors.** Cofactors are either organic molecules, called **coenzymes,** or inorganic

elements(metal ions).

The Role of Microbial Enzymes in Disease

Many pathogens secrete unique exoenzymes that help them avoid host defenses or promote their multiplication in tissues. Because these enzymes contribute to pathogenicity, they are referred to as virulence factors, or toxins in some cases.

1-Streptococcus pyogenes (a cause of throat and skin infections) produces a **streptokinase** that digests blood clots and apparently assists in invasion of wounds. *2-Pseudomonas aeruginosa,* a respiratory and skin pathogen, produces **elastase** and **collagenase,** which digest elastin and collagen. These increase the severity of certain lung diseases and burn infections.

3-Clostridium perfringens, an agent of gas gangrene, synthesizes **lecithinase C**, a lipase that profoundly damages cell membranes and accounts for the tissue death associated with this disease.

The Sensitivity of Enzymes to Their Environment

The activity of an enzyme is highly influenced by the cell's environment.

In general, enzymes operate only under the natural temperature, pH, and osmotic pressure of an organism's habitat. When enzymes are subjected to changes in these normal conditions, they tend to be chemically unstable, or **labile.** Low temperatures inhibit catalysis, and high temperatures denature theapoenzyme. **Denaturation** is a process by which the weak bonds that collectively maintain the native shape of the apoenzyme are broken. This disruption causes extreme distortion of the enzyme's shape and prevents the substrate from attaching to the active site. Such nonfunctional enzymes block metabolic reactions and thereby can lead to cell death. Low or high pH or certain chemicals (heavy metals, alcohol) are also denaturing agents.

CELL ENERGETICS

Cells manage energy in the form of chemical reactions that change molecules. This often involves activities such as the making or breaking of bonds and the transfer of electrons. Not all cellular reactions are equal with respect to energy. Some release energy, and others require it to proceed. For example, a reaction that proceeds as follows:

$X + Y \xrightarrow{Enz}$ Z+Energy **exergonic.***

Energy $+A + B \xrightarrow{Enz} C$ **endergonic,***

Exergonic and endergonic reactions are coupled, so that released energy is immediately put to use.

Summaries of metabolism might make it seem that cells "create" energy from nutrients, but they do not. What they actually do is extract chemical energy already present in nutrient fuels and apply that energy toward useful work in the cell.

At the simplest level, cells possess specialized enzyme systems that trap the energy present in the bonds of nutrients as they are progressively broken. During exergonic reactions, energy released by electrons is stored in various high-energy phosphate molecules such as ATP. As we shall see, the ability of ATP to temporarily store and release the energy of chemical bonds fuels endergonic cell reactions. Before discussing ATP, let us examine the process behind electron transfer: redox reactions.

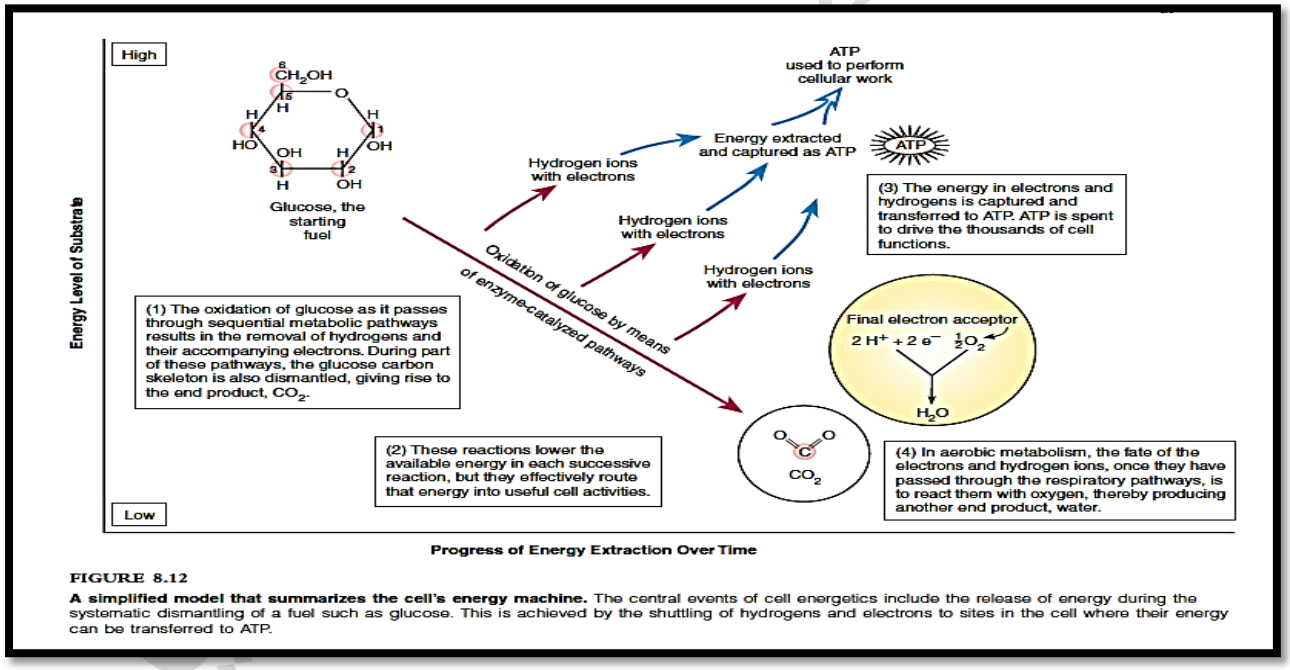


FIGURE 8.12

A simplified model that summarizes the cell's energy machine. The central events of cell energetics include the release of energy during the systematic dismantling of a fuel such as glucose. This is achieved by the shuttling of hydrogens and electrons to sites in the cell where their energy can be transferred to ATP.

Nutrient cycles and regulation Lec 7 د.محمد فليح د.استبرق السوداني

Pathways of Bioenergetics

One of the scientific community's greatest achievements was deciphering the biochemical pathways of cells. Initial work with bacteria and yeasts, followed by studies with animal and plant cells, clearly demonstrated metabolic similarities and strongly supported the concept of the universality of metabolism. The study of the production and use of energy by cells is called **bioenergetics,** including **catabolic routes that degrade nutrients and anabolic routes that are involved in cell synthesis.**

Acquisition of Nutrients

Bacteria use various strategies for obtaining essential nutrients from the external environment and transporting these substances into the cell's interior. For nutrients to be internalized, they must cross the bacterial cell wall and membrane. These complex structures help protect the cell from environmental insults, maintain intracellular equilibrium, and transport substances into and out of the cell. Although some key nutrients (e.g., water, oxygen, and carbon dioxide) enter the cell by simple diffusion across the cell membrane, the uptake of other substances is controlled by membrane selective permeability; still other substances use specific transport mechanisms.

Active transport is among the most common methods used for the uptake of nutrients such as certain sugars, most amino acids, organic acids, and many inorganic ions. The mechanism, driven by an energy-dependent pump, involves carrier molecules embedded in the membrane portion of the cell structure. These carriers combine with the nutrients, transport them across the membrane, and release them inside the cell.

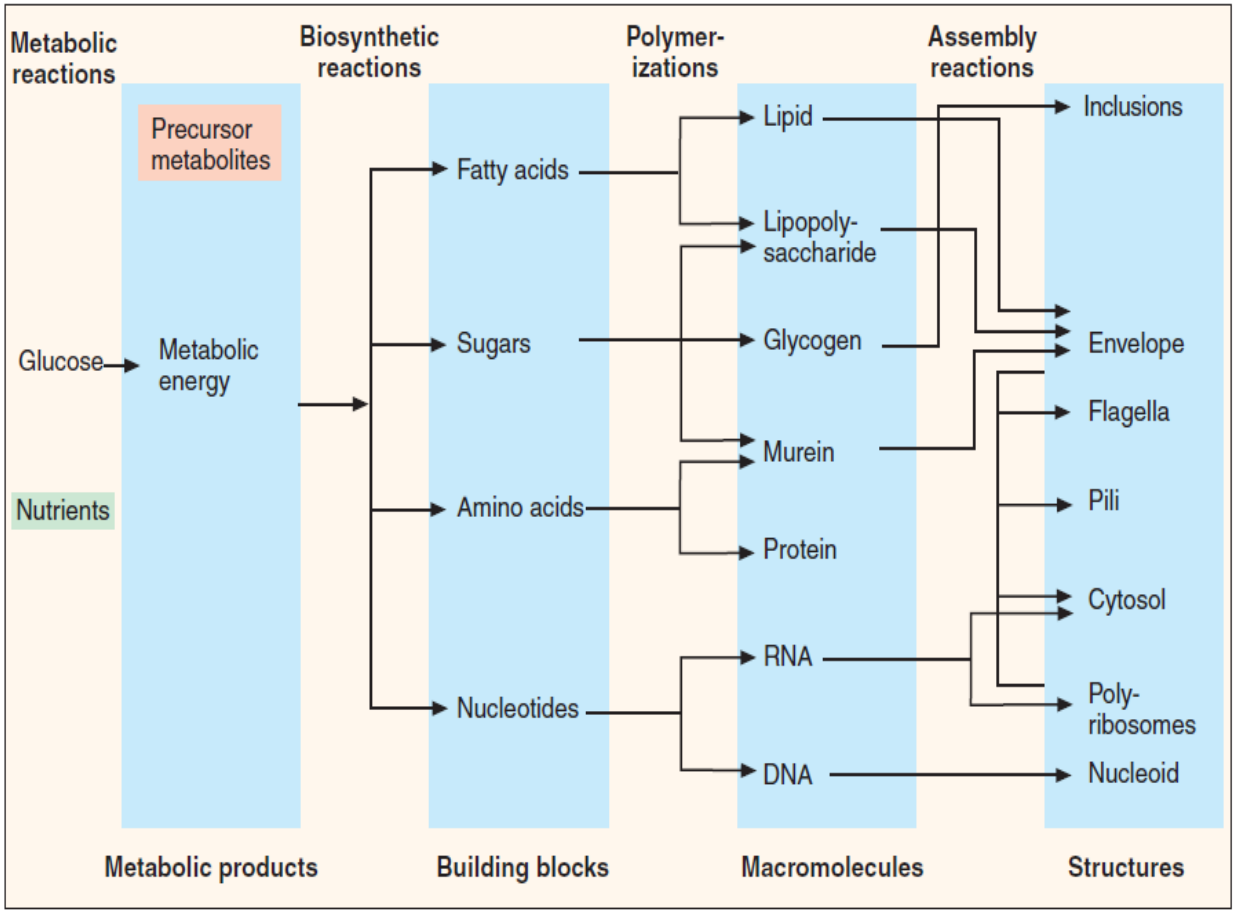
Group translocation is another transport mechanism that requires energy but differs from active transport in that the nutrients being transported undergoes chemical modification. Many sugars, purines, pyrimidines, and fatty acids are transported by this mechanism.

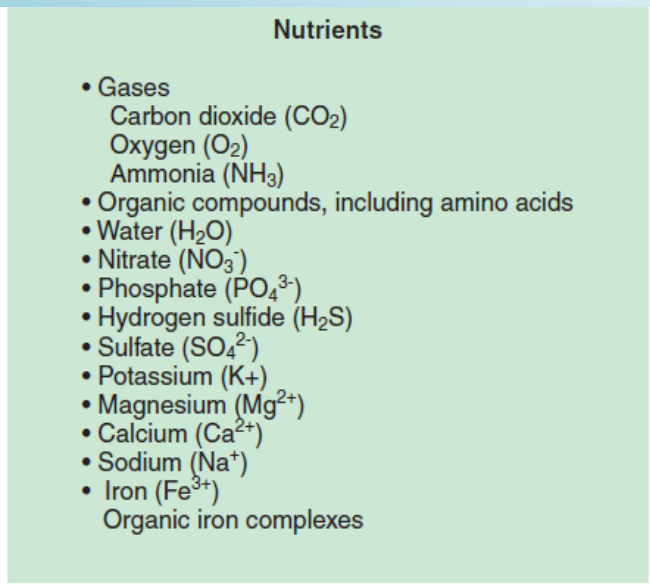
Production of Precursor Metabolites

Once inside the cell, many nutrients serve as the raw materials from which precursor metabolites for subsequent biosynthetic processes are produced. These metabolites, listed in the Figure below, are produced through two central pathways: the Embden-Meyerhof-Parnas (EMP) pathway
(glycolysis) and the tricarboxylic acid (TCA) cycle.

Precursor metabolites

- Glucose 6-phosphate
- Fructose 6-phosphate
- Pentose 5-phosphate
- Erythrose 4-phosphate
- 3-Phosphoglycerate
- Phosphoenolpyruvate
- Pyruvate
- Acetyl CoA
- α-Ketoglutarate
- Succinyl CoA
- Oxaloacetate





• **Figure 2-11** Overview of bacterial metabolism, which includes the processes of fueling, biosynthesis, polymerization, and assembly. (Modified from Niedhardt FC, Ingraham JL, Schaechter M, editors: *Physiology of the bacterial cell: a molecular approach,* Sunderland, MA, 1990, Sinauer Associates.)

The two major pathways and their relationship to one another are shown in this Figure, also the figure not shown are the alternative pathways (e.g., the Entner-Doudoroff and the pentose phosphate pathway) that play key roles in redirecting and replenishing the precursors as they are used in subsequent processes.

The Entner-Doudoroff pathway catalyzes the degradation of gluconate and glucose. The gluconate is phosphorylated, dehydrated, and converted into pyruvate and glyceraldehyde, leading to ethanol production.

Alternatively, the pentose phosphate pathway uses glucose to produce reduced nicotinamide adenine dinucleotide phosphate (NADPH), pentoses, and tetroses for biosynthetic reactions such as nucleoside and amino acid synthesis.

The production efficiency of a bacterial cell resulting from these precursor-producing pathways can vary substantially,

depending on the growth conditions and availability of nutrients. This is an important consideration, because the accurate identification of medically important bacteria often depends heavily on methods that measure the presence of products and byproducts of these metabolic pathways.

Energy Production

The third type of fueling pathway is one that produces the energy required for nearly all cellular processes, including nutrient uptake and precursor production. Energy production is accomplished by the breakdown of chemical substrates (i.e., chemical energy) through the degradative process of catabolism coupled with oxidation-reduction reactions.

In this process, the energy source molecule (i.e., substrate) is oxidized as it donates electrons to an electronacceptor molecule, which is then reduced. The transfer of electrons is mediated through carrier molecules, such as nicotinamide-adenine-dinucleotide (NAD_) and nicotinamide-adenine-dinucleotide-phosphate (NADP_).

The energy released by the oxidation-reduction reaction is transferred to phosphate-containing compounds, where highenergy phosphate bonds are formed. **ATP** is the most common of such molecules. The energy contained in this compound is eventually released by the hydrolysis of ATP under controlled conditions. The release of this chemical energy, coupled with enzymatic activities, specifically catalyzes each biochemical reaction in the cell and drives cellular reactions.

The two general mechanisms for ATP production in bacterial cells are **substrate-level phosphorylation** and electron transport, also referred to as **oxidative phosphorylation.**

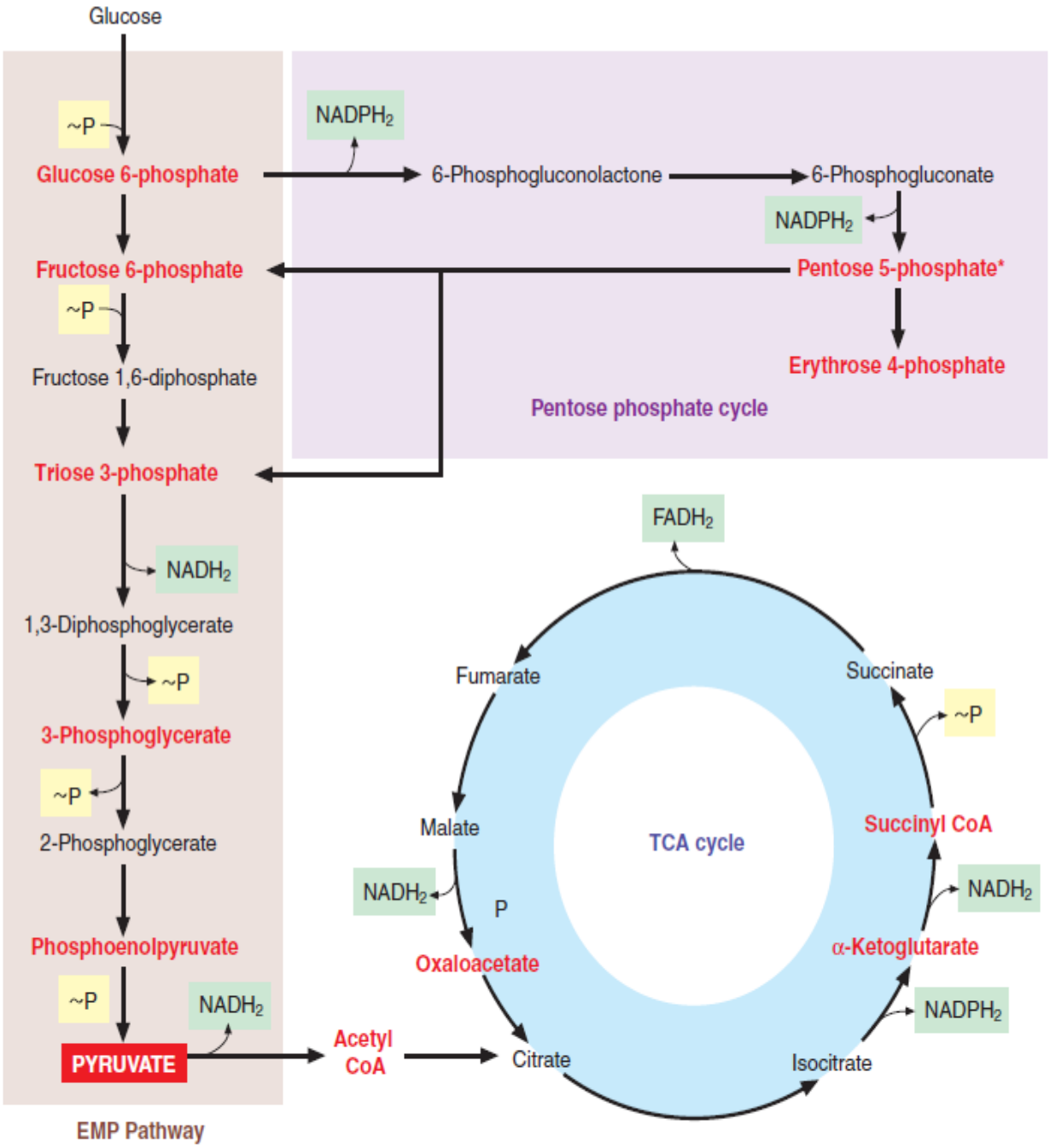
In substrate-level phosphorylation, high-energy phosphate bonds produced by the central pathways are donated to adenosine diphosphate (ADP) to form ATP directly from the substrate as opposed to generation via the electron transport chain. In addition, pyruvate, a primary intermediate in the central pathways, serves as the initial substrate for several other pathways to generate ATP by substrate-level phosphorylation.

These other pathways constitute **fermentative metabolism,** which does not require oxygen and produces various end products, including alcohols, acids, carbon dioxide, and hydrogen.

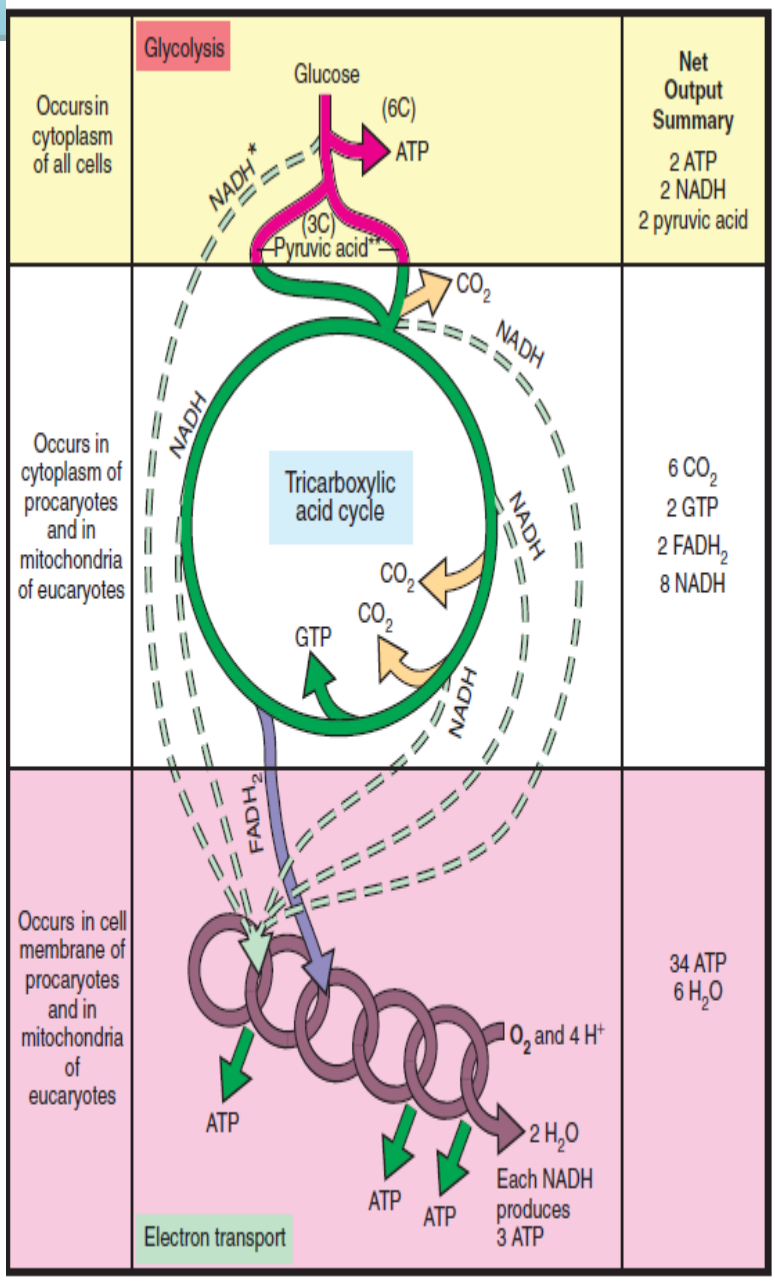
The specific fermentative pathways and the end products produced vary with different bacterial species. Detection of these products is an important basis for laboratory identification of bacteria.

Oxidative Phosphorylation

Oxidative phosphorylation involves an electron transport system that conducts a series of electron transfers from reduced carrier molecules such as NADH2, NADPH2 and FADH2 (flavin adenine dinucleotide), produced in the central pathways (as shown in the Figure below), to a terminal electron acceptor.



• **Figure 2-12** Overview of the central metabolic pathways (Embden-Meyerhof-Parnas [EMP], the tricarboxylic acid [TCA] cycle, and the pentose phosphate shunt). Precursor metabolites (see also Figure 2-11) that are produced are highlighted in *red;* production of energy in the form of ATP *(~P)* by substrate-level phosphorylation is highlighted in *yellow;* and reduced carrier molecules for transport of electrons used in oxidative phosphorylation are highlighted in *green.* (Modified from Niedhardt FC, Ingraham JL, Schaechter M, editors: *Physiology of the bacterial cell: a molecular approach,* Sunderland, MA, 1990, Sinauer Associates.)



*Note that the NADH ═══ transfers H^+ and e^- from the first 2 pathways to the 3rd.

Glycolysis divides the glucose into two 3-carbon fragments called pyruvic acid and produces a small amount of ATP. It does not require oxygen.

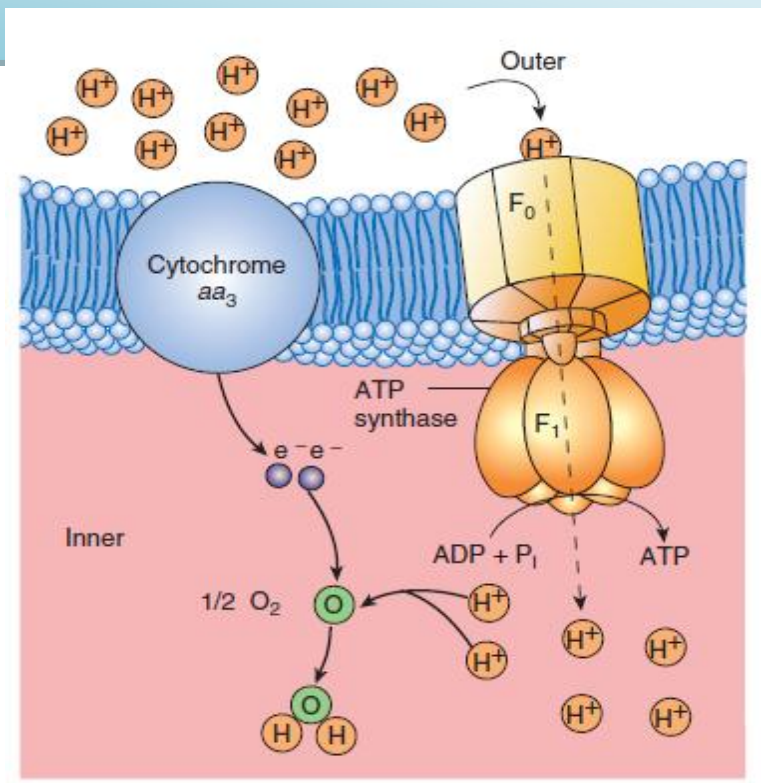
The tricarboxylic acid (TCA) cycle receives these 3-carbon pyruvic acid fragments and processes them through redox reactions that extract the electrons and hydrogens. These are shuttled into electron transport to be used in ATP synthesis. CO_2 is an important product of the TCA cycle.

**All reactions in TCA must be multiplied by 2 for summary because each glucose generates 2 pyruvic acids.

Transport of electrons in the third phase generates a large amount of ATP; in the final step, the electrons and hydrogens are received by oxygen, which forms water. Both TCA and electron transport require oxygen to proceed.

FIGURE 8.18

Overview of the flow, location, and products of pathways in aerobic respiration. Glucose is degraded through a gradual stepwise process to carbon dioxide and water, while simultaneously extracting energy.



(c) The distribution of electric potential across the membrane drives the synthesis of ATP by ATP synthase. The rotation of this enzyme couples diffusion of H^+ to the inner compartment with the bonding of ADP and P_i. The final event of electron transport is the reaction of the electrons with the accumulated H^+ and O_2 to form metabolic H_2O. This step is catalyzed by cytochrome oxidase (cytochrome aa_3)

Summary of Aerobic Respiration for One Glucose Molecule

	Glycolysis*	Net Output	TCA Cycle*	Net Output	Respiratory Chain	Net Output	Total Net Output per Glucose
ATP produced	2 × 2 =	4	1 × 2 =	2	17 × 2 =	34	40 − 2 (used) = 38**
ATP used	2		0		0		

There are several important facets of aerobic respiration:

1. The total yield of ATP is 40: 4 from glycolysis, 2 from the TCA cycle, and 34 from electron transport. However, since 2 ATPs were expended in early glycolysis, this leaves a maximum of **38 ATPs.**

2. Six carbon dioxide molecules are generated during the TCA cycle.
3. Six oxygen molecules are consumed during electron transport.
4. Six water molecules are produced in electron transport and 2 in glycolysis,but because 2 are used in the TCA cycle, this leaves a net number of 6.

The energy produced by the series of oxidation-reduction reactions is used to generate ATP from ADP. When oxidative phosphorylation uses oxygen as the terminal electron acceptor, the process is known as **aerobic respiration.**
Anaerobic respiration refers to processes that use final electron acceptors other than oxygen.
A knowledge of which mechanisms bacteria use to generate ATP is important for designing laboratory protocols for cultivating and identifying these organisms. For example,

1- some bacteria depend solely on aerobic respiration and are unable to grow in the absence of oxygen (**strictly aerobic bacteria**).
2- Others can use either aerobic respiration or fermentation, depending on the availability of oxygen (**facultative anaerobic bacteria**).
3- For still others, oxygen is absolutely toxic (**strictly anaerobic bacteria**).

Biosynthesis

The fueling reactions essentially bring together all the raw materials needed to initiate and maintain all other cellular processes. The production of precursors and energy is accomplished through catabolic processes and the degradation of substrate molecules.
The three remaining pathways for **biosynthesis, polymerization, and assembly** depend on anabolic metabolism. In **anabolic metabolism,**
precursor compounds are joined for the creation of larger molecules (polymers) required for assembly of cellular structures (Figure 2-11).
Biosynthetic processes use the precursor products in dozens of pathways to produce a variety of building blocks, such as amino acids, fatty acids, sugars, and nucleotides (Figure 2-11).
Many of these pathways are highly complex and interdependent, whereas other pathways are completely independent. In many cases, the enzymes that drive the individual pathways are encoded on a single mRNA molecule that has been transcribed from contiguous genes in the
bacterial chromosome (i.e., an operon).

As previously mentioned, bacterial genera and species vary extensively in their biosynthetic capabilities. Knowledge of these variations is necessary to use optimal conditions for growing organisms under laboratory conditions.
For example, some organisms may not be capable of synthesizing an essential amino acid necessary as a building block for proteins. Without the ability to synthesize the amino acid, the bacterium must obtain the building block from the environment. Thus if the organism is cultivated in the microbiology laboratory, the amino acid must be provided in the culture medium.

Polymerization and Assembly

Various anabolic reactions assemble (polymerize) the building blocks into macromolecules, including lipids, lipopolysaccharides, polysaccharides, proteins, and nucleic acids.
This synthesis of macromolecules is driven by energy and enzymatic activity in the cell. Similarly, energy and enzymatic activities also drive the assembly of various macromolecules into the component structures of the bacterial cell. Cellular structures are the product of all the genetic and metabolic processes discussed.

Post test: **الاختبار البعدي:**

Reference: **المصادر:**

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قسم تقنيات المختبرات الطبية

المادة :الأحياء المجهرية

المرحلة :الثانية

Title: العنوان:

Bacterial genetics **Lec 8**

Name of the instructor: اسم المحاضر:

Estabraq Ali AL-Sodani د. استبرق علي السوداني

Target population: الفئة المستهدفة:

Second stage students

Introduction: المقدمة:

Genetics* is the study of the inheritance, or **heredity,*** of living things. It is a wide-ranging science that explores the transmission of biological properties (traits) from parent to offspring, the expression and variation of those traits, the structure and function of the genetic material, and how this material changes. Organism genetics observes the heredity of the whole organism or cell; chromosome genetics examines the characteristics and actions of chromosomes; and molecular genetics deals with the biochemistry of the genes. All of these levels are useful areas of exploration, but in order to understand the expressions of microbial structure, physiology, mutations, and pathogenicity, we need to examine the operation of genes at the cellular and molecular levels. The study of microbial genetics provides a greater understanding of human genetics and an increased appreciation for the astounding advances in genetic engineering we are currently witnessing.

Pre test: الاختبار القبلي:

Q\ Define Genetics and gene?

Scientific content: **المحتوى العلمي:**

THE NATURE OF THE GENETIC MATERIAL

For a species to survive, it must have the capacity of self replication. In single-celled microorganisms, reproduction involves the division of the cell by means of binary fission, budding, or mitosis, but these forms of reproduction involve a more significant activity than just simple cleavage of the cell mass. Because the genetic material is responsible for inheritance, it must be accurately duplicated and separated into each daughter cell to ensure its normal function.

The Levels of Structure and Function of the Genome

The **genome** is the sum total of genetic material of a cell. Although most of the genome exists in the form of chromosomes, genetic material can appear in non chromosomal sites as well. For example, bacteria and some fungi contain tiny extra pieces of DNA (plasmids), and certain organelles of eucaryotes (the mitochondria and chloroplasts) are equipped with their own genetic programs. Genomes of cells are composed exclusively of DNA, but viruses contain either DNA or RNA as the principal genetic material. Although the specific genome of an individual organism is unique, the general pattern of nucleic acid structure and function is similar among all organisms.

In general, a **chromosome** is a discrete cellular structure composed of a neatly packaged elongate DNA molecule. The chromosomes of eucaryotes and bacterial cells differ in several respects.

1- The structure of eucaryotic chromosomes consists of a DNA molecule tightly wound around histone proteins, whereas a bacterial chromosome (chromatin body) is condensed and secured into a packet by means of histone like proteins.

2-Eucaryotic chromosomes are located in the nucleus; they vary in number from a few to hundreds; they can occur in pairs(diploid) or singles (haploid); and they appear elongate. In contrast, most bacteria have a single, circular chromosome, although exceptions exist in a few bacteria that have linear or multiple chromosomes.

The chromosomes of all cells are subdivided into basic informational packets called genes.

A **gene** can be defined from more than one perspective. In classical genetics, the term refers to the fundamental unit of heredity responsible for a given trait in an organism. In the molecular and biochemical sense, it is a site on the chromosome that provides information for a certain cell function. More specifically still, it is a certain segment of DNA that contains the necessary code to make a protein or RNA molecule. For an analogy that clarifies the relationship of the genome, chromosomes,
genes, and DNA.

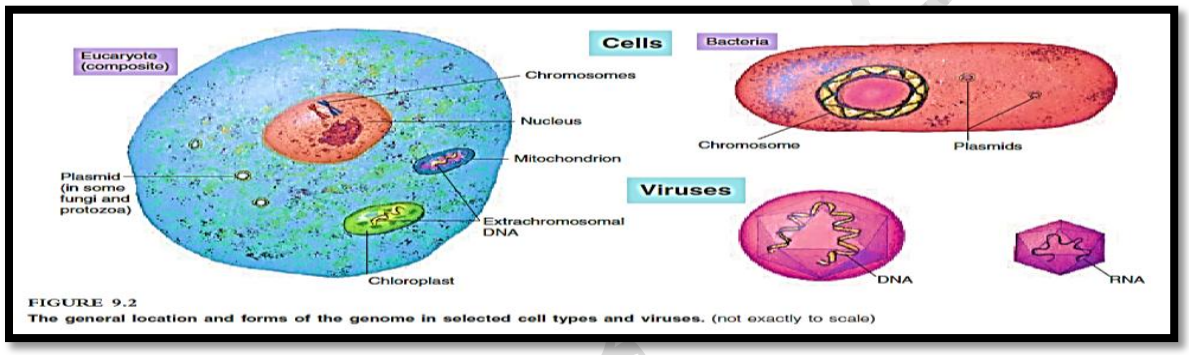

FIGURE 9.2
The general location and forms of the genome in selected cell types and viruses. (not exactly to scale)

Nonchromosomal Elements

Although the bacterial chromosome represents the majority of a cell's genome, not all genes are confined to the chromosome. Many genes may also be located on **plasmids** and **transposable elements.** Both of these extrachromosomal elements are able to replicate and encode information for the production of various cellular products. Many of these elements replicate by integration into the host chromosome, whereas others, referred to as **episomes,** are capable of replication independently of the host chromosome. Although considered part of the bacterial genome, they are not as stable as the chromosome and may be lost during cellular replication, often without any detrimental effects on the viability of the cell.

Plasmids exist as double-stranded, closed, circular, autonomously replicating extrachromosomal genetic elements ranging in size from 1 to 2 kilobases up to 1 megabase or more. The number of plasmids per bacterial cell varies extensively, and each plasmid is composed of several genes.

Some genes encode products that mediate plasmid replication and transfer between bacterial cells, whereas others encode products that provide a **specialized function, such as a determinant of antimicrobial resistance or a unique**

metabolic process. Unlike most chromosomal genes, plasmid genes do not usually encode for products essential for viability. Plasmids, in whole or in part, may also become incorporated into the chromosome.

Transposable elements are pieces of DNA that move from one genetic element to another, from plasmid to chromosome or vice versa. Unlike plasmids, many are unable to replicate independently and do not exist as separate entities in the bacterial cell. The two types of transposable elements are the **simple transposon** or **insertion sequence (IS)** and the **composite transposon.** Insertion sequences are limited to containing the genes that encode information required for movement from one site in the genome to another. Composite transposons are cassettes (grouping of genes) flanked by insertion sequences. The internal gene embedded in the insertion sequence encodes for an accessory function, such as antimicrobial resistance. Plasmids and transposable elements coexist with chromosomes in the cells of many bacterial species. **These extrachromosomal elements play a key role in the exchange of genetic material throughout the bacterial microbiosphere, including genetic exchange among clinically relevant bacteria.**

Replication and Expression of Genetic Information

Replication

Bacteria multiply by **binary fission** (a form of cell division), resulting in the production of two daughter cells from one parent cell. As part of this process, the genome must be replicated so that each daughter cell receives an identical copy of functional DNA. **Replication** is a complex process mediated by various enzymes, such as DNA polymerase and cofactors; replication must occur quickly and accurately. For descriptive purposes, replication may be considered in four stages as show in figure

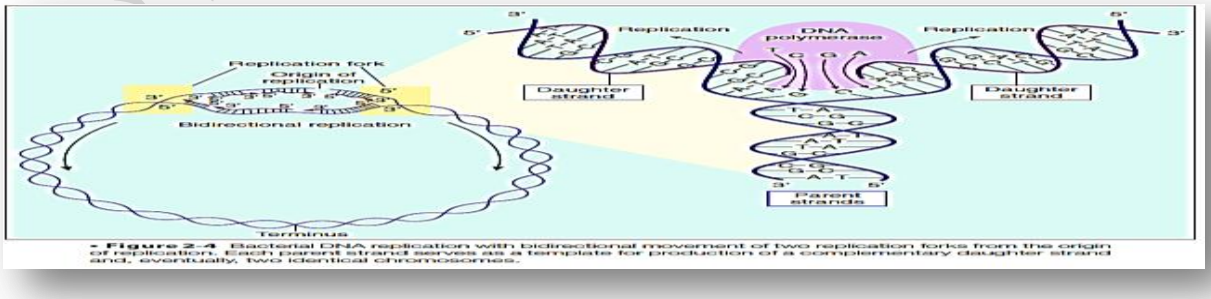

Figure 2-4 Bacterial DNA replication with bidirectional movement of two replication forks from the origin of replication. Each parent strand serves as a template for production of a complementary daughter strand and, eventually, two identical chromosomes.

1. Unwinding or relaxation of the chromosome's supercoiled DNA
2. Separation of the complementary strands of the parental DNA so that each may serve as a **template** (i.e., pattern) for synthesis of new DNA strands, referred to as **semiconservative replication**
3. Synthesis of the new (i.e., daughter) DNA strands
4. Termination of replication, releasing two identical chromosomes, one for each daughter cell.

Relaxation of supercoiled chromosomal DNA is required so that enzymes and cofactors involved in replication can access the DNA molecule at the site where the replication process will originate (i.e., origin of replication). The 1-**origin of replication** (a specific sequence of approximately 300 base pairs).

2-replication fork two bidirectional forks are involved in the replication process.

3-DNA polymerase playing a central role.Using each parental strand as a template, DNA polymerase adds nucleotide bases to each growing daughter strand in sequence that is complementary to the base sequence of the template (parent) strand.

The process generally takes approximately 20 to 40 minutes in rapidly growing bacteria such as *E. coli*. The replication time for a particular bacterial strain can vary depending on environmental conditions, such as the availability of nutrients or the presence of toxic substances (e.g., antimicrobial agents).

Genetic alterations and diversity in bacteria are accomplished by three basic mechanisms: **mutation**, **genetic recombination**, and **genetic exchange** between bacteria, with or without recombination.

Mutation

Mutation is defined as an alteration in the original nucleotide sequence of a gene or genes within an organism's genome; that is, a change in the organism's genotype. This alteration may involve a single DNA base in a gene, an entire gene, or several genes. Mutational changes in the sequence may arise spontaneously, perhaps by an error made during DNA replication. Alternatively, mutations may be induced by **mutagens** (i.e**., chemical or physical factors**) in the environment or by biologic factors, such as the introduction of foreign DNA into the cell. Alterations in the DNA base sequence can result in changes in the base sequence of mRNA during

transcription. This, in turn, can affect the types and sequences of amino acids that will be incorporated into the protein during translation.

Genetic Exchange

An organism's ability to undergo recombination depends on the acquisition of "foreign" DNA from a donor cell. The three mechanisms by which bacteria physically exchange DNA are

1-**transformation .**

2-transduction.

3-conjugation.

Transformation.

Transformation involves recipient cell uptake of naked (free) DNA released into the environment when another bacterial cell (i.e., the donor) dies and undergoes lysis.

This genomic DNA exists as fragments in the environment. Certain bacteria are able to take up naked DNA from their surroundings; that is, they are able to undergo transformation. Such bacteria are said to be **competent.** Among the bacteria that cause human infections, competence is a characteristic commonly associated with members of the genera*Haemophilus, Streptococcus,* and *Neisseria.*

Transduction

Transduction is a second mechanism by which DNA from two bacteria may come together in one cell, thus allowing for recombination. This process is mediated through viruses capable of infecting bacteria (i.e.,**bacteriophages**). In their "life cycle," these viruses integrate their DNA into the bacterial cell's chromosome, where viral DNA replication and expression occur. When the production of viral products is complete, viral DNA is excised (cut) from the bacterial chromosome and packaged within a protein

coat. The excision process is not always accurate, resulting in the removal of genetic material that contains both the bacterial and viral DNA. The newly formed recombinant virion, along with the additional multiple virions (virus particles), is released when the infected bacterial cell lyses.

Conjugation

The third mechanism of DNA exchange between bacteria is conjugation. This process occurs between two living cells, involves cell-to-cell contact, and requires mobilization of the donor bacterium's chromosome. The nature of intercellular

contact is not well characterized in all bacterial species capable of conjugation. However, in *E. coli*, contact is mediated by a sex pilus

الاختبار البعدي: **Post test:**

Q\ Enumerate steps of replication?

Reference

الجامعة التقنية الوسطى
كلية التقنيات الصحية والطبية \بغداد
قسم تقنيات المختبرات الطبية

المادة :الأحياء المجهرية

المرحلة :الثانية

Title:

العنوان:

Pathogenicity of bacteria Lec 9

Name of the instructor: **اسم المحاضر:**

Estabraq Ali AL-Sodani **د. استبرق علي السوداني**

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

A pathogenic M. is defined **as one that is capable of causing disease, some m. are obvious pathogenic, where as others (the majority) are generally harmless.**

Microbial infections result when a microorganism **penetrates** host defenses, **multiplies**, and **damages** host tissue. The **pathogenicity** of a microbe refers to its ability to cause infection or disease. The **virulence** of a pathogen refers to the degree of damage it inflicts on the host tissues.**True pathogens** cause infectious disease in healthy hosts, whereas **opportunistic pathogens** become infectious only when the host immune system is compromised in some way.

The site at which a microorganism first contacts host tissue is called the **portal of entry**. Most pathogens have one preferred portal of entry, although some have more than one An organism may invade an individual without causing infectious disease when the host's defense mechanisms are successful the occurrence of such infections can be recognized by the presence of antibody against the organism in the patient's serum.

Pretest **الاختبار القبلي:**

Q\ Define pathogenicity and virulence of pathogen?

Scientific content

Bacterial pathogenesis

Although the mechanism of infectious process may vary among bacteria, the methods by which **bacteria cause disease can** in general be divided in several stages, incubation period, invasion period, and convalescent period.

Pathogenicity of m. depends on its success in execution some or all these stages.

The infectious dose, or ID, refers to the minimum number of microbial cells required to initiate infection in the host. The ID varies widely among microbial species.

Virulence factors

Are those characteristics of a bacterium that enhance its pathogenicity, that is properties that enable a microorganism to establish itself and replicate on or with in a specific host.

***1-Entry into the host :**

The first step of the infectious process is the entry of the M.O. into the host by one of several parts via the respiratory, gastrointestinal tract, or urogenetal tract or through the skin that been cut, punctured or *burned.*

Once entry is achieved , the pathogen must over come diverse host defenses before it can establish it self these include:.

1- Phagocytosis .

2-acidic environment of the stomach and urogenetal tract.

3-various hydrolytic and proteolytic enzyme found in the saliva, stomach, and small intestine.

Bacteria that have an outer polysaccharide capsule for example (*Streptococcus pneumonia* and *Neisseria meningitids*) have a better chance of surviving these primary host defenses.

***2- Adhesion :**

is the ability of bacteria to adhere to host cell and resist physical removal, Fimbriae, flagella, hooks, and adhesive capsules are types of adherence factors by which pathogens physically attach to host tissues some bacteria for example

Escherichia coli use **pili** to adhere to the surface of host cells .

Group A Streptococci have similar structures (fimbriae).

Other bacteria have cell surface adhesion molecules or particularly hydrophobic cell wall that allow them to adhere to the host cell membrane.

In each case adherence enhances virulence by preventing the bacteria from being carried away by mucous or washed from organs with significant fluid flow such as the urinary and GI tract.

***3- Invasion:**

The ability of M.O to enter or penetrate mucosal surface ,multiply their and spread to other tissue.

Invasion is facilitated by several bacterial enzymes the most notable of which are **Collagenase(break down collagen)and Hyaluronidase (break down hyaluronic acid).**

These enzymes degraded component of the extra cellular matrix, providing the bacteria with easier access to host cell surface.

Invasion is followed by **inflammation,** which can be either **pyogenic (involving pus formation) or granulomatous(having nodular inflammatory lesion) depending on the organism.**

Pus of pyogenic inflammations contains mostly**, neutrophils**, where as granulomatous lesion contain **fibroblast, lymphocyte and macrophage.**

***4-Iron sequesterating:**

The ability of the M.O to compete for iron and other nutrients as they are essential for bacteria and cell growth (iron is an essential nutrient for most bacteria) to obtain the iron required for growth bacteria produce iron binding compounds called **Siderophores**.(these compounds capture iron from the host by chelation, and then ferrated-siderophore bind to specific receptors on the bacterial surface.

***5-Antiphagocytosis:**

The most important antiphagocytosis structure is the **Capsule** extracellular to the cell wall such as *Streptococcus pneumonia* , and *Neisseria meningitides.*

A second group of antiphagocytic factors are the **cell wall proteins of gram positive cocci such as protein A of *Staphylococcus* and M protein of group A Streptococci .**

***6- Bacterial toxins:**

Some bacteria cause disease by producing toxic substance, there are to two general types **(Exotoxins and Endotoxins)**

Exotoxins: a protein substances secreted by both G- and G+ bacteria these include some of the most poisonous substances known**.**
It is estimated that as little as 1µg of tetanus exotoxins can kill adult human.
Exotoxin proteins generally have two polypeptide components.
One is responsible for binding the protein to the host cells and one to the toxic effect.

Endotoxin :

Which are **Lipopolysaccharides(LPS)** also known as **Lipoglycans are large molecules consisting of a lipid and polysaccharide composed of O-antigen outer core and inner core joined by covalent bond they are found in the outer membrane of G- bacteria (but not G+ bacteria).**

LPS acts as the prototypical endotoxin because it binds receptor in many cell types especially in monocytes, dendritic cells, macrophages and B cells, which promotes the secretion of pro-inflammatory cytokines.

TABLE 13.7
Differential Characteristics of Bacterial Exotoxins and Endotoxins

Characteristic	Exotoxins	Endotoxins
Toxicity	Toxic in minute amounts	Toxic in high doses
Effects on the Body	Specific to a cell type (blood, liver, nerve)	Systemic: fever, inflammation
Chemical Composition	Polypeptides	Lipopolysaccharide of cell wall
Heat Denaturation at 60°C	Unstable	Stable
Toxoid Formation	Convert to toxoid*	Do not convert to toxoid
Immune Response	Stimulate antitoxins**	Do not stimulate antitoxins
Fever Stimulation	Usually not	Yes
Manner of Release	Secreted from live cell	Released by cell during lysis
Typical Sources	A few gram-positive and gram-negative	All gram-negative bacteria

*A toxoid is an inactivated toxin used in vaccines.
**An antitoxin is an antibody that reacts specifically with a toxin.

Resistance of host immunity

Its ability to resist host immune defence by

1-Immunoglobulin A protease

Lysis IgA which allow the organisms to adhere to mucous membrane

2-Leukocidin

destroy both neutrophils and macrophage.

3-Coagulase enzyme

accelerate the formation of fibrin clot from fibrinogen this clot can protect the bacterial from phagocytosis .

Koch's postulates are the following:

Heinrich Hermann Robert Koch:11 December 1843 – 27 May 1910) was a German physician and microbiologist. As one of the main founders of modern bacteriology, he identified the specific causative agents of tuberculosis, cholera, and anthrax and also gave experimental support for the concept of infectious disease,[1]

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be re isolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Post test

Q\ Enumerate Koch's postulates?

References

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قسم تقنيات المختبرات الطبية

المادة :الأحياء المجهرية

المرحلة :الثانية

Title:

العنوان:

Normal flora Lec 10

Name of the instructor: **اسم المحاضر:**

Estabraq Ali AL-Sodani **د. استبرق علي السوداني**

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

The human body exists in a state of dynamic equilibrium with microorganisms. In the healthy individual, this balance is maintained as a peaceful coexistence and lack of disease. But on occasion, the balance tips in favor of the microorganism, and an infection or disease results.

• Humans are constantly exposed to microbes in their environment, mostly without harm.

• When microbes colonize the human body, sometimes they become part of the normal flora and other times, they may cause infection and disease.

• Normal flora are bacteria, fungi, and protozoa that reside naturally in the skin, gastrointestinal tract, mucous membranes, and genitourinary tracts, where they provide a stabilizing balance.

Pre test: **الاختبار القبلي:**

Q\ Define the normal flora?

Scientific content **المحتوى العلمي:**

• Pathogens invade the body and cause harm to the tissues by means of virulence factors.

• An infection occurs when an adequate dose of pathogens gains access to the body through a certain route, and subsequently adheres, grows, and disrupts tissues.

• Types of infections include local, systemic, blood, latent, primary, secondary, acute, chronic, and asymptomatic.

• Infections and diseases often follow predictable patterns involving an incubation period, invasion period, and convalescent period.

*except for occasional transient invaders the internal organs and system are sterile as show in this table:

TABLE 13.2
Sterile (Microbe-Free) Anatomical Sites and Fluids

All Internal Tissues and Organs
Heart and circulatory system
Liver
Kidneys and bladder
Lungs
Brain and spinal cord
Muscles
Bones
Ovaries/testes
Glands (pancreas, salivary, thyroid)
Sinuses
Middle and inner ear
Internal eye
Fluids Within an Organ or Tissue
Blood
Urine in kidneys, ureters, bladder
Cerebrospinal fluid
Saliva prior to entering the oral cavity
Semen prior to entering the urethra
Amniotic fluid surrounding the embryo and fetus

A healthy new born enter the world in essentially sterile conditions, but after birth it rapidly acquires normal flora from food and environment including from other humans as shown in this figure.

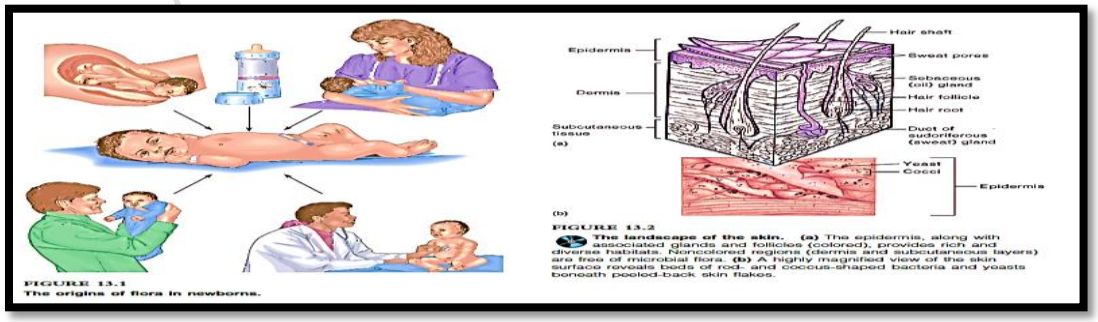

FIGURE 13.1
The origins of flora in newborns.

FIGURE 13.2
The landscape of the skin. (a) The epidermis, along with associated glands and follicles (colored), provides rich and diverse habitats. Noncolored regions (dermis and subcutaneous layers) are free of microbial flora. (b) A highly magnified view of the skin surface reveals beds of rod- and coccus-shaped bacteria and yeasts beneath peeled-back skin flakes.

Distribution of normal flora in the human body

1-Skin

Skin can acquire any bacteria that happen to be in the immediate environment, but this transient. Flora either **die or removable by washing(ex. *Staphylococcus epidermidis***)

2-Eye

The conjunctiva of the eye is colonized primarily by(***Staphylococcus epidermidis***, ***Staphylococcus aureus***)

3-Mouth and Nose

The mouth and nose harbor many m.o both aerobic and anaerobic (ex ***Corynebacterium*** spp., ***Staphylococcus epidermidis***, ***Staphylococcus aureus*** as aerobic bacteria). In addition the teeth and gingival tissue are colonized by ***Streptococcus mutans***. Some normal residents of Nasopharynx can also cause disease for ex. ***Streptococcus pneumonia***, can cause acute bacterial pneumonia especially in older adults.

4-Intestinal tract

In an adult M.O. concentration in the **stomach** is relatively low (10^3-10^5) per gram of the contents due to gastric enzymes and acidic ph .

Number of M.O. increase along the **alimentary canal** reaching (10^8-10^{10}) bacteria per gram of the contents in **the ileum** and **large intestine** 10^{11} per gram ex. ***Bacteroides spp. Bifidobacterium, Fusobacterium,*** **and** ***Clostridium*** anaerobic***).*** **Coliforms* such as *Escherichia coli, Enterobacter,* and *Citrobacter*** are present in smaller numbers. Many species ferment waste materials in the feces, generating vitamins (B12, vitamin K, pyridoxine, riboflavin, and thiamine) and acids (acetic, butyric, and propionic acids) of potential value to the host.

5-Urogenital tract

The low ph of the vagina is maintained by presence of ***Lactobacillus spp***. which are primary components of normal flora.

Yeast like fungi ***Candida albicans*** can grow in acidic conditions which it self a minor member of the normal flora of the vagina, mouth and small intestine **.**

The urine in the kidney and bladder is sterile but become contamination in the lower urethra by some organism that inhabit the outer layer of the skin perineum area.

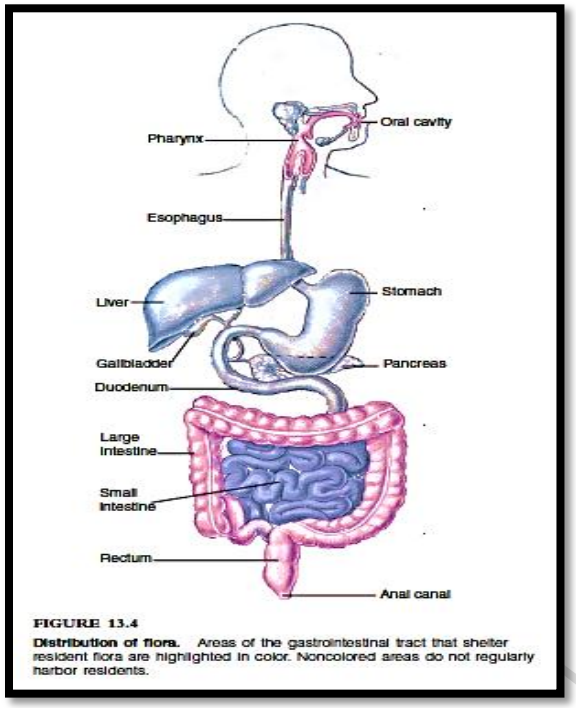

FIGURE 13.4
Distribution of flora. Areas of the gastrointestinal tract that shelter resident flora are highlighted in color. Noncolored areas do not regularly harbor residents.

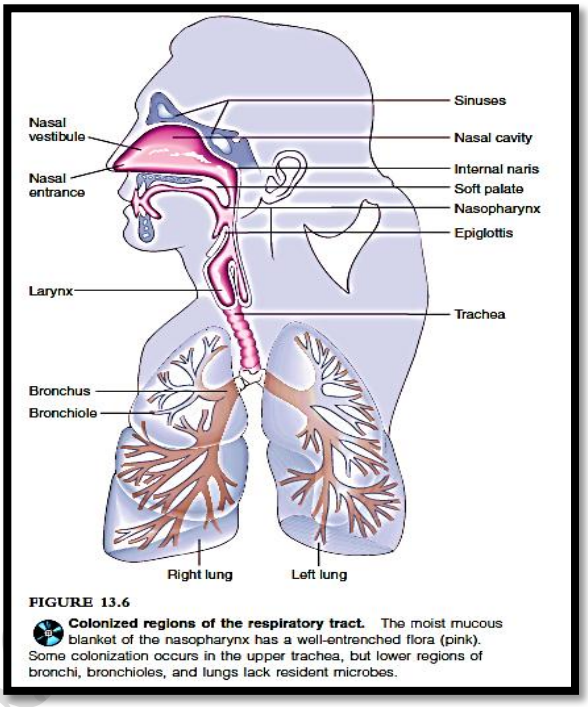

FIGURE 13.6
Colonized regions of the respiratory tract. The moist mucous blanket of the nasopharynx has a well-entrenched flora (pink). Some colonization occurs in the upper trachea, but lower regions of bronchi, bronchioles, and lungs lack resident microbes.

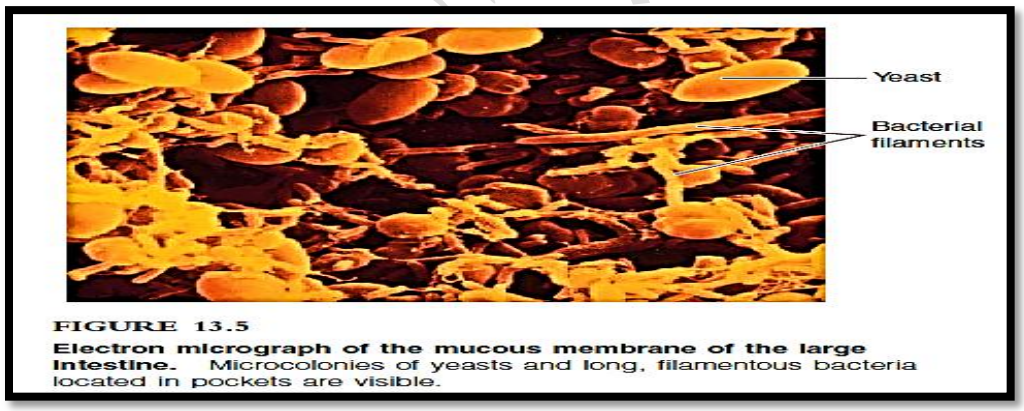

FIGURE 13.5
Electron micrograph of the mucous membrane of the large intestine. Microcolonies of yeasts and long, filamentous bacteria located in pockets are visible.

Post test **الاختبار البعدي:**

Q\ Enumerate distribution of human body normal flora?

References

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المادة :الأحياء المجهرية

المرحلة :الثانية

Title: **العنوان:**

Chemotherapy and Antibiotics Resistance **Lec 11,12**

Name of the instructor: **اسم المحاضر:**

Estabraq Ali AL-Sodani **د. استبرق علي السوداني**

Target population: **الفئة المستهدفة:**

Second stage student

Introduction: **المقدمة:**

A hundred years ago in the United States, one out of three children was expected to die of an infectious disease before the age of five. Early death or severe lifelong debilitation from **scarlet fever, diphtheria, tuberculosis, meningitis, and many other bacterial diseases** was a fearsome yet undeniable fact of life to most of the world's population. The introduction of modern drugs to control infections in the 1930s began as a medical revolution that has added significantly to the lifespan and health of humans. It is no wonder that for many years, antibiotics in particular were regarded as the miracle cure-all for infectious diseases.

Pre test: **الاختبار القبلي:**

Q\ Define Antibiotics and enumerate their types?

Scientific content: **المحتوى العلمي:**

Any chemical used in treatment, relief, or **prophylaxis*** of disease is defined as a **chemotherapeutic drug** or agent. When chemotherapeutic drugs are given as a means to control infection, the practice is termed **antimicrobial chemotherapy.*** Antimicrobial drug (also termed anti-infective drugs) are a special class of compounds capable even in high dilutions of destroying or inhibiting microorganisms.

Antibiotics are substances produced by the natural metabolic processes of some microorganisms bacteria and fungi that can inhibit or destroy other microorganisms.

This process can be visualized more tangibly as a series of stages (1) The drug is administered to the host via a designated route. Delivery is primarilyby oral, circulatory, muscular, and cutaneous routes. (2) The drug is dissolved in body fluids. (3) The drug is delivered to the infected area (extracellular or intracellular). (4) The drug destroys the infectious agent or inhibits its growth. (5) The drug is eventually excreted or broken down by the host's organs, ideally without harming them.

Antimicrobial chemotherapy is the treatment and prevention of infectious diseases by means of chemicals called drugs.

• Narrow-spectrum antimicrobial drugs affect a small range of microbes, and broad-spectrum drugs affect a wider range of microbes.

• Chemotherapy involves a complex interaction between the microbe, drug, and host.

• The best antimicrobial drugs have low toxicity to humans and lack other side effects such as drug resistance, allergy, and disruption of naturalflora.

• The primary action of antimicrobial drugs is to interfere with some specific component of the microbe's structure, its enzymes, or synthesis of proteins and other molecules.

• Drug resistance is a process by which microbes develop genetic changes that allow them to circumvent the effects of a drug.

• Hundreds of drugs have been developed for treating bacterial, fungal, protozoan, helminthic, and viral infections.

• The predominant antibacterial drug classes are the penicillins, cephalosporins, tetracyclines, sulfa drugs, and fluoroquinolones.

• Selecting a drug for therapy is based upon the microbe's sensitivity to the drug, the drug's toxicity, and the health of the patient.

• Adverse side effects of drugs include damage to skin, liver, kidney, circulatory system, nervous system, and gastrointestinal tract.

• Drugs may cause allergies and disrupt the host's normal flora, leading to other infections.

History of antibiotics - 4

Penicillin- the first antibiotic - 1928

- **Alexander Fleming** observed the killing of staphylococci by a fungus (*Penicillium notatum*)
- observed by others - never exploited
- Florey & Chain purified it by freeze-drying (1940) - Nobel prize 1945
- first used in a patient: 1942
- World War II: penicillin saved 12-15% of lives

Classification of Antibiotics according to effects on bacteria

Bacteriocidal which kill bacteria and cause no growth and division.

Bacteriostatic which stop the growth of bacteria without killed it.

Classification of Antibiotics according to nature

1-natural drugs

2-semi-synthetic drugs

3-synthetic drugs

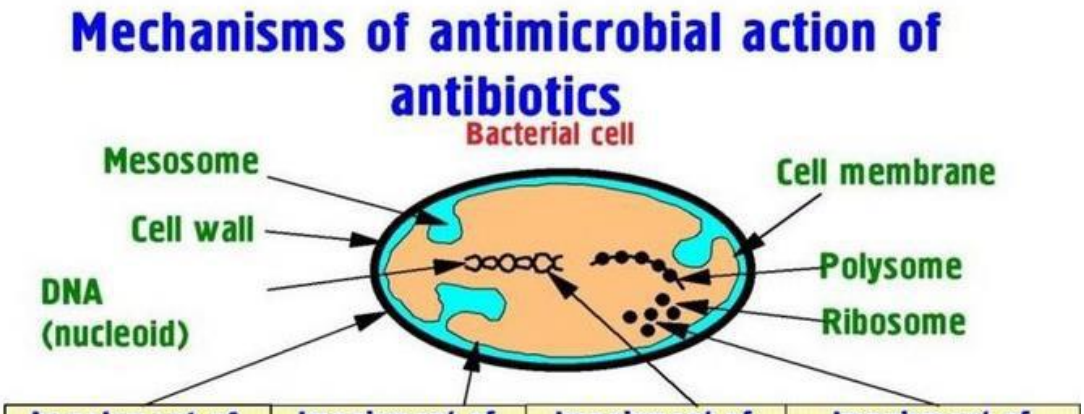
Characteristics of the Ideal Antimicrobial Drug

• Selectively toxic to the microbe but nontoxic to host cells
• Microbicidal rather than microbistatic
• Relatively soluble and functions even when highly diluted in body fluids
• Remains potent long enough to act and is not broken down or excreted prematurely
• Not subject to the development of antimicrobial resistance
• Complements or assists the activities of the host's defenses
• Remains active in tissues and body fluids
• Readily delivered to the site of infection
• Not excessive in cost
• Does not disrupt the host's health by causing allergies or predisposing the host to other infections.

MECHANISMS OF DRUG ACTION

Antimicrobial drugs function specifically in one of the following ways :-

(1) They inhibit cell wall synthesis
(2) They inhibit nucleic acid synthesis or function
(3) They inhibit protein synthesis
(4) They interfere with the function of the cell membrane.



Impairment of synthesis of the cell wall	Impairment of permeability of the cell membrane	Impairment of synthesis of RNA and DNA*	Impairment of protein synthesis in ribosomes
Penicillins, Cephalosporins, Carbapenems, Monobactams, Glycopeptides, Cycloserine	Polymyxines, Antifungal polyene macrolides,	Rifamycins, Antitumoral antibiotics, Fluorquinolones*	Tetracyclines, Chloramphenicol Macrolides, Lincosamides, Aminoglycosides, Streptogramines

Post test **الاختبار البعدي:**

Q\ Enumerate mechanism action of antibiotics ?

References

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المادة :الأحياء المجهرية

المرحلة :الثانية

Title:

العنوان:

Vaccines and Vaccination

Lec 13

Name of the instructor:

اسم المحاضر:

Estabraq Ali AL-Sodani **د. استبرق علي السوداني**

Target population:

الفئة المستهدفة:

Second stage students

Introduction:

المقدمة:

Vaccination is a simple, safe, and effective way of protecting you against harmful diseases, before you come into contact with them. It uses your body's natural defenses to build resistance to specific infections and makes your immune system stronger.Vaccines train your immune system to create antibodies, just as it does when it's exposed to a disease. However, because vaccines contain only killed or weakened forms of germs like viruses or bacteria, they do not cause the disease or put you at risk of its complications. Vaccines reduce risks of getting a disease by working with your body's natural defenses to build protection. When you get a vaccine, your immune system responds It:

- Recognizes the invading germ, such as the virus or bacteria.
- Produces antibodies. Antibodies are proteins produced naturally by the immune system to fight disease.
- Remembers the disease and how to fight it. If you are then exposed to the germ in the future, your immune system can quickly destroy it before you become unwell.

Pre test:

الاختبار القبلي:

Define vaccination and enumerate steps action of vaccines?

Scientific content: المحتوى العلمي:

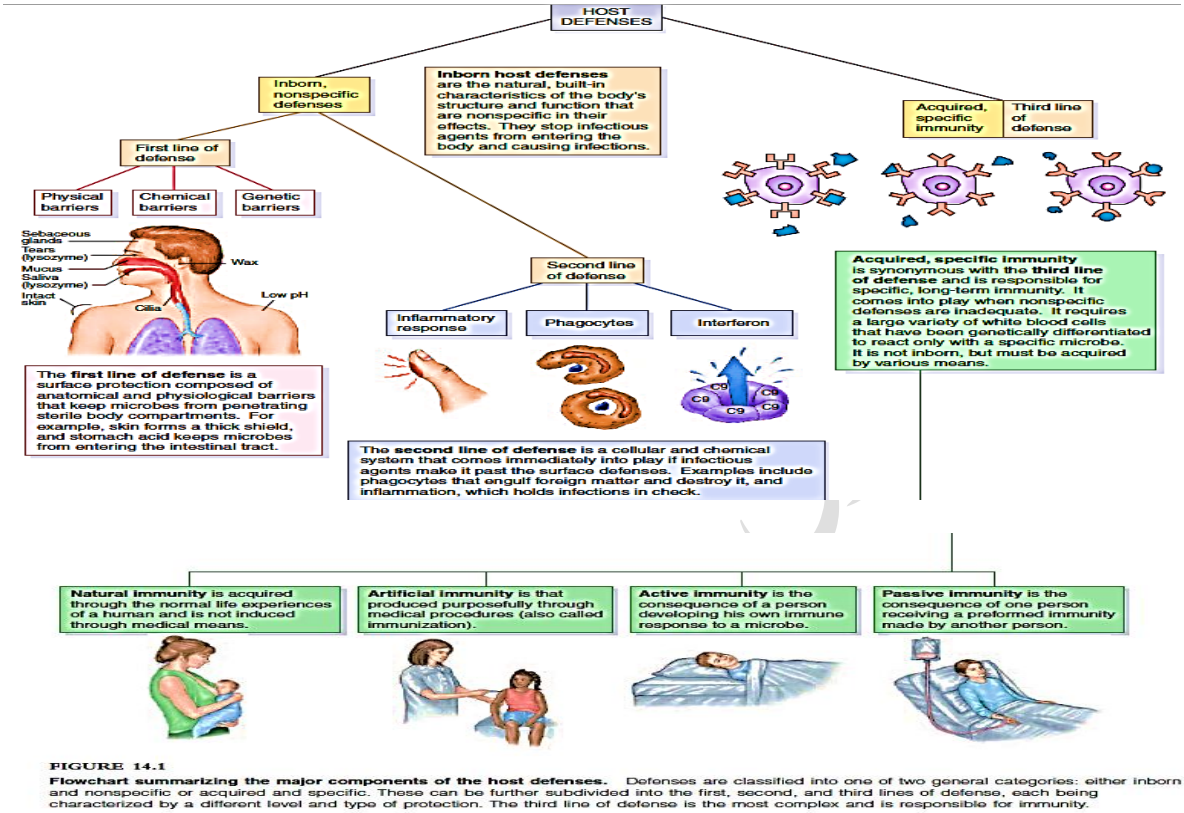

FIGURE 14.1
Flowchart summarizing the major components of the host defenses. Defenses are classified into one of two general categories: either inborn and nonspecific or acquired and specific. These can be further subdivided into the first, second, and third lines of defense, each being characterized by a different level and type of protection. The third line of defense is the most complex and is responsible for immunity.

An expanded knowledge of immune function has yielded significant break throughs in medical technology for manipulating and monitoring the immune system. One of the most practical benefits of immunology is administering1- vaccines and other immune treatments against common infectious diseases such as hepatitis and tetanus that once caused untold sickness and death. 2-Another valuable application of this technology is testing the blood for signs of infection or disease. It is routine medical practice to diagnose such infections as HIV, syphilis, hepatitis B, and rubella by means of immunologic analysis.

• Immunization may be administered by means of passive and active methods.

• Passive immunity is acquired by infusing antiserum taken from other patients' blood that contains high levels of protective antibodies.This form of immunotherapy is short-lived.
• Active methods involve administering a vaccine against an infectious agent that may be encountered in the future. This form of protection provides a longer-lived immunity.
• Vaccines contain some form of microbial antigen that has been altered so that it stimulates a protective immune response without causing the disease.
• Vaccines can be made from whole dead or live cells and viruses, parts of cells or viruses, or by recombinant DNA techniques.
• Reactions between antibodies and antigens provide specific and sensitive tests that can be used in diagnosis of disease and identification of pathogens.
• Serology involves the testing of a patient's blood serum for antibodies that can indicate a current or past infection and the degree of immunity.
• Tests that produce visible interactions of antibodies and antigens include agglutination, precipitation, and complement fixation.
• Assays can be used to separate antigens and antibodies and visualize them with radioactivity or fluorescence. These include immunoelectrophoresis, the Western blot, and direct and indirect immunoassays.

Passive Immunity

The first attempts at passive immunization involved the transfusion of horse serum containing antitoxins to prevent tetanus and to treat patients exposed to diphtheria. Since then, antisera from animals have been replaced with products of human origin that function with various degrees of specificity. **Immune serum globulin (ISG),** sometimes called *gamma globulin,* contains immunoglobulin extracted from the pooled blood of at least 1,000 human donors. The method of processing ISG concentrates the antibodies to increase potency and eliminates potential pathogens (such as the hepatitis Band HIV viruses). It is a treatment of choice in preventing measles and hepatitis A and in replacing antibodies in immunodeficient patients. Most forms of ISG are injected intramuscularly to minimize adverse reactions, and the protection it provides lasts 2 to 3 months. A preparation called **specific immune globulin (SIG)** is derived from a more defined group of donors. Companies that prepare SIG obtain serum from patients who are convalescing and

in a hyper immune state after such infections as **pertussis, rabies, tetanus, chickenpox, and hepatitis B.**

Active Immunity

Active immunity can be conferred artificially by **vaccination**—exposing a person to material that is antigenic but not pathogenic.

The discovery of vaccination was one of the farthest reaching and most important developments in medical science.

The basic principle behind vaccination is to stimulate a primary and secondary an amnestic response as show in figure.

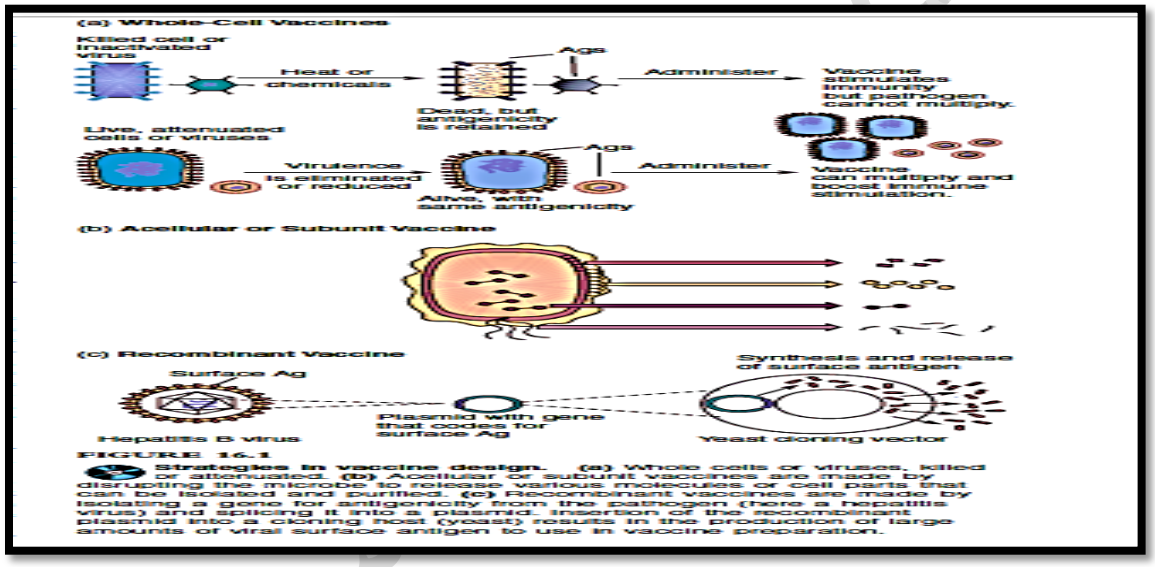

FIGURE 16.1

Strategies in vaccine design. (a) Whole cells or viruses, killed or attenuated. (b) Acellular or subunit vaccines are made by disrupting the microbe to release various molecules or cell parts that can be isolated and purified. (c) Recombinant vaccines are made by isolating a gene for antigenicity from the pathogen (here a hepatitis virus) and splicing it into a plasmid. Insertion of the recombinant plasmid into a cloning host (yeast) results in the production of large amounts of viral surface antigen to use in vaccine preparation.

Vaccines have profoundly reduced the prevalence and impact of many infectious diseases that were once common and often deadly.

Principles of Vaccine Preparation

A vaccine must be considered from the stand points of antigen selection, effectiveness, ease in administration, safety, and cost. In natural immunity, an infectious agent stimulates appropriate B and T lymphocytes and creates memory clones. In artificial active immunity, the objective is to obtain this same response with a modified version of the microbe or its components. A safe and effective vaccine should mimic the natural protective response, not cause a serious infection or other disease, have long-lasting effects in a few doses, and be easy to administer. Most vaccine preparations contain one of the following antigenic stimulants

(1) killed whole bacterial cells or inactivated viruses.
(2) live, attenuated bacterial cells or viruses.
(3) antigenic components of cells or viruses.
(4) genetically engineered microbes or microbial antigens.

1- Killed microorganisms

Killed vaccines have the advantage over attenuated m. in that they pose no risk of vaccine associated infection. Killed organisms often provide a weak or short lived immune response. Some vaccines such as polio and typhoid vaccines, are available both in live and killed versions.

2- Live pathogens

When live pathogens are used they are attenuated (weakened) to **preclude** clinical **consequences** of infection.

Attenuated microbes reproduce in the recipient typically leading to a more robust and long-lasting immune response than can be obtained through vaccination with killed organisms.

3- **Microbial extracts**

Instead of using whole O. vaccine can be composed of **antigen** molecules (often those located on the surface of the M. ex **flagella , fimbriae).** Extracted from the pathogen or prepared by recombination DNA technique.

The efficacy of these vaccines varies. In some instances the vaccine antigen is present on all strains of the organism, and the vaccine, thus protected against by all strains.

4- Toxoids

These are derivatives of bacterial exotoxins produced by chemically altering the natural toxin or by engineering bacteria to produce harmless variants of the toxin.

Vaccines containing toxoid are used when the pathogenicity of the O. is a result of the secreted toxins.

Depending on the specific vaccine, administration is generally via intramuscular or subcutaneous.

*** Bacterial Vaccines ***

Vaccines with more specialized indications are described bellow:

1- Anthrax (*Bacillus anthracis*)
2- Cholera (*Vibrio cholera*)
3- Typhoid fever (*Salmonella typhi*)
4- Plague *(Yersinia pestis*)

Viral Vaccines

1- Hepatitis A
2- Hepatitis B
3- Varicella zoster
4- Polio
5- Influenza
6- Measles, mumps and rubella
7- Human papilloma virus vaccine
8- Triple vaccine

Posttest **الاختبار البعدي**

Q\ explain Principles of Vaccine Preparation?

References

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المادة :الأحياء المجهرية

المرحلة :الثانية

Title:

العنوان:

Medical Bacteriology Gram positive cocci **Lec 14**

Name of the instructor: **اسم المحاضر:**

Estabraq Ali AL-Sodani **د. استبرق علي السوداني**

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

Clinical or medical **bacteriology is the science that studies all pathogenic bacteria which cause diseases and problems to humans and animals. Pathological Genera**

Gram-positive cocci

Staphylococcus , Streptococcus and Enterococcus

are significant causes of infections and disease in the community and clinical settings.

The organisms are aerobic or facultative anaerobic with the exception of *Staphylococcus aureus* subsp. *anaerobius* and *Staphylococcus saccharolyticus*, which are obligate anaerobes, and may be catalase negative. However, only those belonging to the genus *Staphylococcus* are of primary clinical significance. *Staphylococcus* are nonmotile and non– spore forming organisms. Several of the coagulase-negative staphylococci (CoNS or non–*S. aureus*) species listed may be encountered in clinical specimens. The CoNS have been subdivided into two groups based on their novobiocin susceptibility pattern. these genera will be associated with skin lesions and are more commonly isolated from immunocompromised patients.

Pretest: **الاختبار القبلي:**

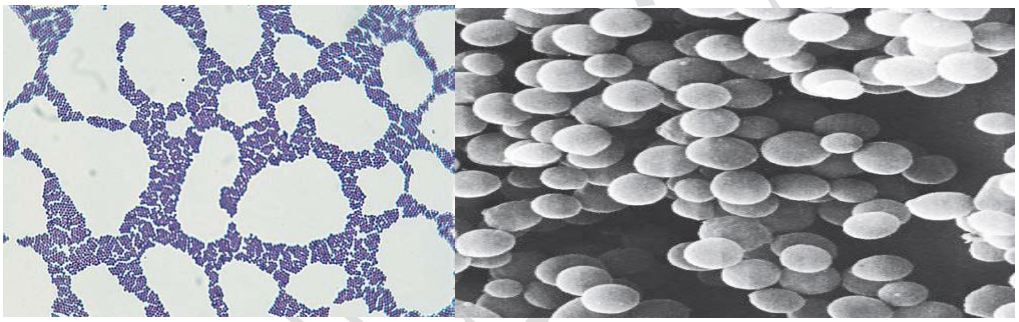
Q\ Define bacteriology

Scientific content: **المحتوى العلمي:**

Staphylococcus

• Members of the genus *Staphylococcus* **are gram-positive cocci arrangement as cluster grape like that commonly inhabit the human skin and mucous membranes yet are resistant enough to survive drying, heat, and other harsh environmental conditions. Staphylococcus have 31 species most of these are human commensals, but others may be pathogenic species**

1- ***Staphylococcus aureus***
2- ***Staphylococcus epidermidis***
3- ***Staphylococcus saprophyticus*** , and others may be pathogenic species



Staphylococcus aureus

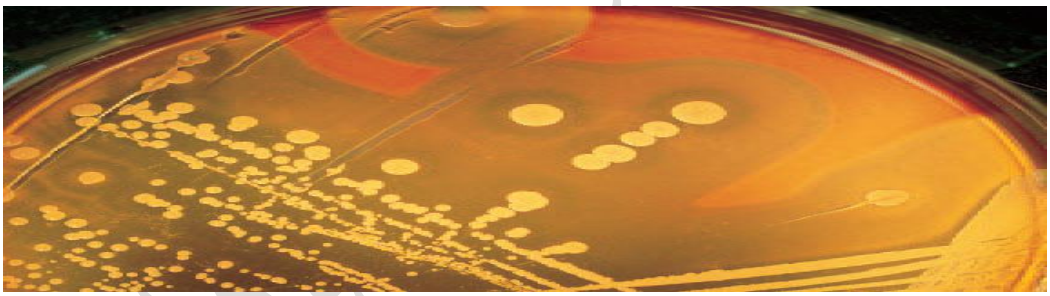
GROWTH AND PHYSIOLOGICAL CHARACTERISTICS

1- *Staphylococcus aureus* grows in **large, round, opaque colonies** at an optimum of 37_C.

2-The species is a **facultative anaerobe** whose growth is enhanced in the presence of O2 and CO2.

3-Its nutrient requirements can be satisfied by **routine laboratory media,** and most strains are metabolically versatile; that is, **they can digest proteins and lipids and ferment a variety of sugars such as Mannitole and produce golden colony**

4-This species is considered the most resistant of all **non-spore-forming** pathogens, with well-developed capacities to withstand **high salt (7.5–10%), called Halophilic bacteria**
5- extremes in pH, and high temperatures (up **to 60_C for 60 minutes**).
6-It also remains viable after months of air drying and **resists the effects of many disinfectants and antibiotics.**
7- *S. aureus* as a troublesome **hospital pathogen**.
The Enzymes of *S. aureus
1-**Catalase which converts H_2O_2 to water and oxygen .**
2-Coagulase an enzyme that cause plasma to clot.
3-Hyaluronidase analyzes hyaluronic acid found in cartilage and connective tissue.
4-Fibrinolysin analyzes fibrin.
5-Penicillinase analyzes penicillin antibiotic.
*** The toxins of *S. aureus***
1-Hemolysins lyse red blood cells by disrupting their membranes, and produce a zone of hemolysis in blood agar.



Blood agar plate growing *Staphylococcus aureus.* Some strains show two zones of hemolysis. The relatively clearer inner zone is caused by **α_-toxin,** whereas the outer zone is fuzzy and appears only if the plate has been refrigerated. This outer zone is the **β_, or "hot-cold," hemolysin** that shows up when the plate is refrigerated.

2-**Leukocidins which damages cell membranes of neutrophils and macrophages, causing them to lyse.**
3- Enterotoxins that act upon the gastrointestinal tract of humans. caused food poisoning.
4- Exfoliative This toxin is responsible for staphylococcal scalded skin syndrome, SSSS in which the skin looks burned.

*Pathogenesis of *S. aureus* *

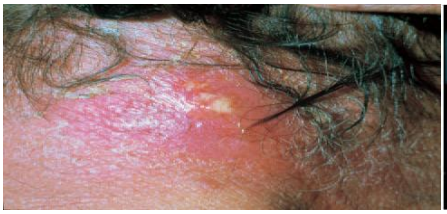
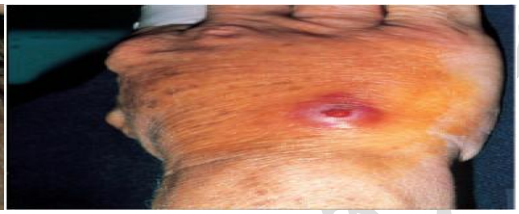
This bacteria found as a flora in human body (skin, ear, eye) and cause different diseases when the immunity depressed by other infections.

***Types of infections ***

A-Superficial infections

1-skin pustules

Carbuncle **furuncle**

2-boils
3-impetigo
4-blepharitis
5-conjunctivitis
6-wound infections
7-burn infections

B-Subcutaneous and Systemic infections

1-finger abscess
2-osteochondritis
3-mastitis
4-bronchopneumonia
5-food poisoning
6-septicemia

***Laboratory diagnosis**

1-clinical samples (pus from wound or burn)
2-direct exam stained by Gram stain
3-cultured on blood agar and mannitol salt agar 24hr/37C
4-diagnosis by biochemical tests API Staph
5-culture in Moller-Hinton agar for sensitivity
6-treatment by cephalosporins, nafcillin, sulfa drugs, vancomycin.

Post test: **الاختبار البعدي:**

Q\ Enumerate types of infections caused by *Staphylococcus aureus* ?

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المادة :الأحياء المجهرية

المرحلة :الثانية

Title:

العنوان:

Streptococcus **Lec 15**

Name of the instructor: **اسم المحاضر:**

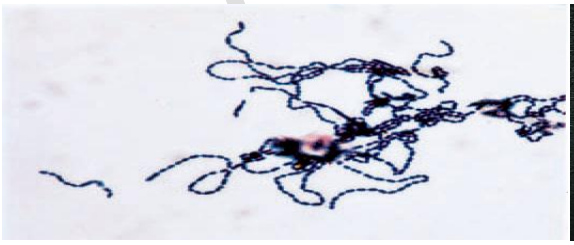
Estabraq Ali AL-Sodani **د. استبرق علي السوداني**

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

Genus Streptococcus The genus ***Streptococcus**** includes a large and varied group of bacteria. Some are normal residents or agents of disease in humans and animals ***General Features***Streptococci are **non-spore-forming** and **non motile** (except for an occasional flagellated strain), The general shape of the cells is **spherical**, but they can also appear **ovoid or rod like** especially in actively dividing young cultures, also produce **chain** in different long . They can form **capsules and slime** layers. They are **facultative anaerobes** that **ferment a variety of sugars**, usually with the production of **lactic acid**. Streptococci do not form **catalase(-),** but they do **have a peroxidase s**ystem for inactivating hydrogen peroxide, which allows their survival even in the presence of oxygen. Most parasitic forms are **fastidious in nutrition and require enriched media** for cultivation. Colonies are usually **small, non pigmented, and glistening**. Most members of the genus are quite **sensitive to drying, heat, and disinfectants.**



Pre test: **الاختبار القبلي:**

Q\ Enumerate general features of *Streptococcus*

Scientific content: **المحتوى العلمي:**

Despite the large number of streptococcal species, human disease is most often associated with ***S. pyogenes, S. agalactiae,*** **viridans streptococci,** ***S. pneumoniae,*** and ***Enterococcus faecalis*** **.**

***Classification of Streptococci**

Streptococci can be classified by several schemes, for example :

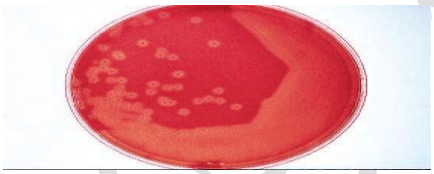
1-Hemolytic properties of the organisms.

2-According to the presence of specific surface antigens determined by immunological assays.

A-Hemolytic properties on blood agar

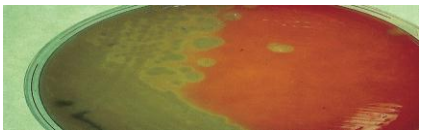
1-β-Hemolytic Streptococci :

Cause lysis of red blood cells resulting in a clear ring around the colony for example *Streptococcus pyogenes* .



2-α-Hemolytic Streptococci :

Cause chemical changes in the hemoglobin of RBCs in blood agar resulting in the appearance of green color that forms a ring around the colony for ex. *Streptococcus pneumonia* .



3-γ-Hemolytic Streptococci

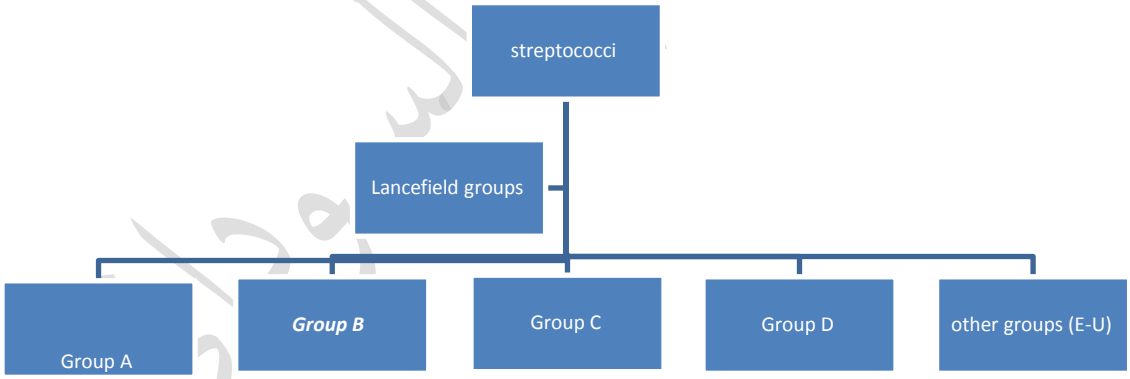
Is a term applied to Streptococci that no cause color changes or lysis of RBCs. *Streptococcus mutans* and *Streptococcus bovis.*

B-Serologic (Lancefield) groupings Streptococci display numerous surface antigens. **C-carbohydrates**, specialized **polysaccharides** or **teichoic acids** found on the surface of the **cell wall**, are the basis for **Lancefield groups.**

Their apparent contribution to pathogenesis is to protect the bacterium from being dissolved by the lysozyme defense of the host**. Lipoteichoic acid accounts for the adherence of *S. pyogenes* to epithelial cells in the skin or pharynx.**

The Lancefield scheme classified primarily β-hemolytic Streptococci in to groups **A** TO **U** on the basis of their C- substance.

The clinically most important groups of β-hemolytic Streptococci type A and B.



S. pyogenes *S. agalactiae* *S. equisimitis* *Enterococcus*

Non groupable streptococci **S. *pneumoniae* (pneumonia disease)

***Viridans streptococci *S. mutans* causing dental carries**

Another type-specific molecule is the **M-protein**, of which about **80** different subtypes exist. This substance is the main component of fimbriae, the spiky surface projections that contribute to virulence by resisting phagocytosis and improving adherence. A **Capsule** is formed by most *S. pyogenes* strains, but it remains attached to the cell surface only during the rapid growth phase of a culture and is probably not as important to virulence as are other factors.

*** General features of *S. pyogenes***

1-β-hemolytic 2-G+ cocci 3-non motile, non spore forming, capsule during the rapid growth phase 4-grow in different selective media such as blood agar 5-produce different **enzymes and toxins:**

a-haemolysin lysis RBCs
b-streptolysin O and streptolysin S lysis RBCs
c-streptokinase lysis fibrin
d-hyaluronidase lysis hyaluronic acid
e-DNAase lysis DNA
f-leucocidine lysis WBCs

Diseases caused by *S. pyogenes*

Respiratory diseases

1-tonsillitis 2-throat infection 3-pharyngitis

Another diseases

1-Scarlet fever 2- Impetigo 3-Rheumatic fever 4-Vaginitis

5-Meningitis 6-Burn and Wound infections 7- Puerperal fever 8-Otitis media

Genus Enterococcus

Enterococcus faecalis, E. faecium, and *E. durans* are called the enterococci because they are normal colonists of the human large intestine.

The group D species *Enterococcus faecalis* causes opportunistic infections of wounds, blood, endocardium, and the urinary and gastrointestinal tracts.More than 35 *Enterococcus* species exist, including commensals that lack potent toxins and other well-

defined virulence factors. Virulence factors associated with enterococc continue to be a topic of increasing research interest because of an increasing likelihood to cause health care–associated infections, especially *E. faecium*. Some of the virulence factors identified in *Enterococcus* species include,
aggregation substance, capsular polysaccharides, surface carbohydrates, ability to translocate across intact intestinal mucosa, hemolysin, lipoteichoic acid, gelatinase, superoxide production, peptide inhibitors, and ability to adhere to extracellular matrix proteins.
Compared with other clinically important gram-positive cocci, *Enterococcus* (especially *E. faecium* and *E. faecalis)* isintrinsically more resistant to the antimicrobial agents commonly used in acute and long-term health care settings.
This genus is the first clinically relevant group of gram-positive cocci to acquire and disseminate resistance to vancomycin, thus the name **vancomycin-resistant Enterococcus (VRE),** which was discovered in the late 1980s. Vancomycin is an antibiotic used to treat infections caused by gram-positive bacteria.

LABORATORY IDENTIFICATION TECHNIQUES for *Streptococcus*

1-Clinical Sample
Different samples reach to lab. Such as swabs from tonsil, wound, burn
With different other samples.
2-Direct Exam stained by Gram stain
3-Cultured on blood agar (for see type of hemolytic)
4-identification by biochemical or API Strep or other serological test
5-CAMP factor test : detection the production of diffusible thermostable, extracellular protein produced by group B Streptococci *S. agalactiae*
6-sensitivity test in Mueller-Hinton agar

Post test: الاختبار البعدي:

Q\ Enumerate laboratory identification techniq for *Streptococcus spp.?*

References

الجامعة التقنية الوسطى
كلية التقنيات الصحية والطبية \بغداد
قسم تقنيات المختبرات الطبية

المادة :الأحياء المجهرية

المرحلة :الثانية

Title: العنوان:

Gram positive spore forming bacilli **Lec 16**

Clostridium and Bacillus

Name of the instructor: اسم المحاضر:

ا.م.د جليل نجاح جليل

Target population: الفئة المستهدفة:

Second stage students

İntroduction	المقدمة

- Gram-positive, bacilli (rod) shape.
- obligate anaerobes meaning that oxygen is poisonous to the cells.
- Capable of producing endospores.
- Clostridium endospores have a distinct bowling pin or bottle (Figure) shape, distinguishing them from other bacterial endospores, which are usually **ovoid in shape**.

Pre-test

Q1: What is the difference between bacillus and clostridium?

A. Clostridium grows in anaerobic conditions; Bacillus grows in aerobic conditions.

B. Clostridium forms **bottle-shaped** endospores; Bacillus forms **oblong** endospores.

1. *C.botulinum*

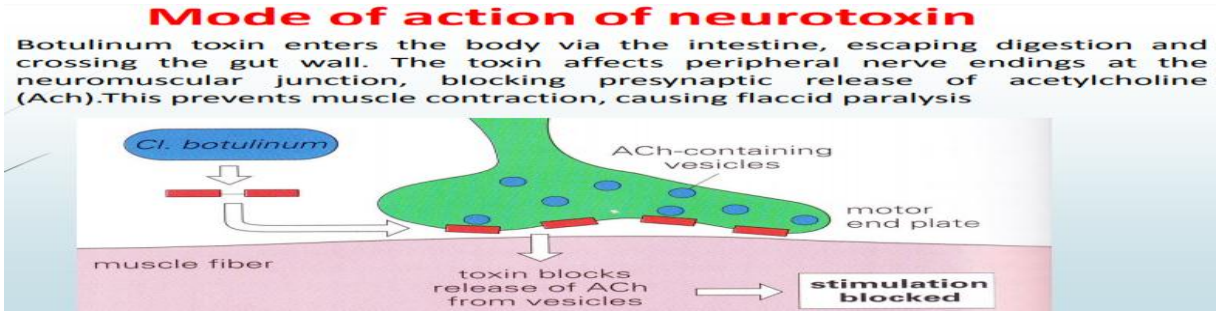
A. Virulence factor: include

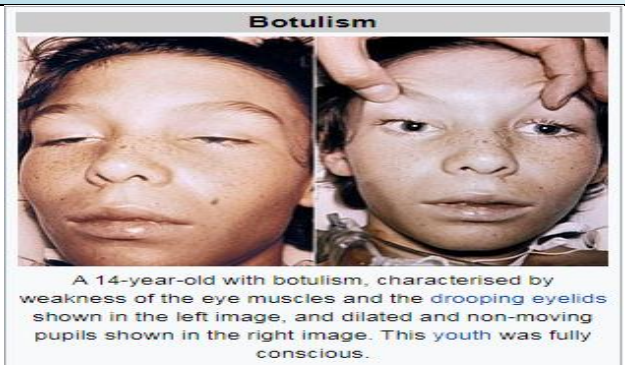
1. Produces extremely potent neurotoxins.

- *C. botulinum* is able to produce the neurotoxin only during sporulation, which can hap only in an anaerobic environment**.**
- They are resistant to degradation by enzymes found in the gastrointestinal tract. T allows for ingested toxin to be absorbed from the intestines into the bloodstream.

B. Spectrum of disease and infection

- ✓ Acute food poisoning that is caused by **botulinum toxin** produced in food by (*Clostridiun botulinum*) and is characterized by
  - Muscle weakness (flaccid paralytic) and paralysis ((Figure).
  - disturbances of vision,
  - swallowing,
  - and speech (شلل رخوي)
- ✓ Absorption of the toxin leads to nearly complete paralysis of respiratory and other essential muscle groups.
- ✓ The most potent toxin known to humankind, natural or synthetic, with a lethal dose of 1.3–2.1 (ng/kg) in humans.





A 14-year-old with botulism, characterised by weakness of the eye muscles and the drooping eyelids shown in the left image, and dilated and non-moving pupils shown in the right image. This youth was fully conscious.

hology

1. **Food borne botulism**
   - Signs and symptoms of foodborne botulism typically begin between **18** and **36** hours a the toxin gets into your body,
   - But can range from a few hours to several days, depending on the amount of tc ingested.
2. **Infant botulism**

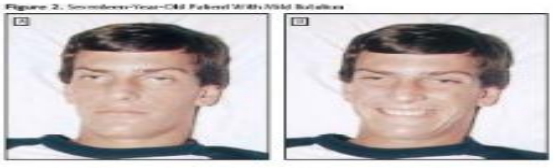
If infant botulism is related to food, such as **honey**, problems generally begin within **18 to 36** hours a the toxin enters the baby's body (can occur when the organism elaborates the toxin after it has coloni the gastrointestinal tract of infants).

3. **wound botulism** :

Most people who develop wound botulism inject drugs several times a day, so it's difficult to determ how long it takes for signs and symptoms to develop after the toxin enters the body. Most commoı people who inject black tar heroin.

Signs of Food-borne and Wound Botulism

- Ventilatory (respiratory) problems
- Eye muscle paresis/paralysis (extra ocular, eyelid)
- Dry mucous membranes in mouth/throat
- Dilated, fixed pupils
- Ataxia
- Hypotension
- Nystagmus
- Decreased to absent deep tendon reflexes


A. Patient at rest. Note bilateral mild ptosis, dilated pupils, disconjugate gaze, and symmetric facial muscles. B, Patient was requested to perform his maximum smile. Note absent smile creases, ptosis, minimally asymmetric smile.

✓ *botulinum* is commonly associated with bulging canned food; bulging, misshapen cans are due to an internal increase in pressure caused by gas produced by the bacteria.

C. perfringens (formerly known as C. welchii)

The specific name *perfringens* is derived from the Latin *per* (meaning "through") and *frango* ("burs referring to the disruption of tissue that occurs during gas gangrene**.**

A. Virulence factor:

1. Alpha toxins is the most important and mediates destruction of host cell membranes; Is a Lecithir or (phospholipase - C) that

lyses erythrocytes (RBCs),

platelets,

Leukocytes.

and endothelial cell (الخلايا البطانية).

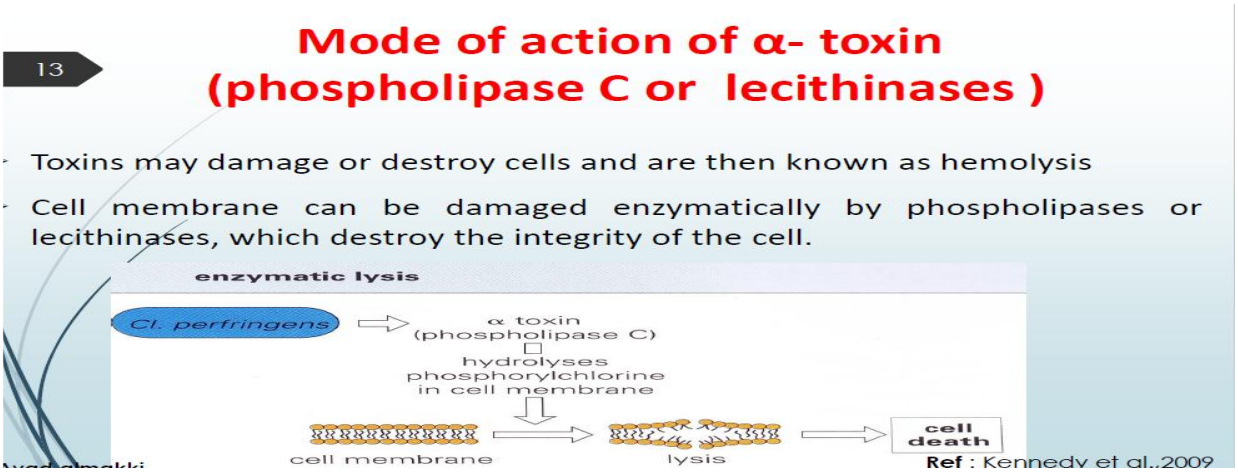
- ❖ Alpha toxin mediates:
  - massive hemolysis,
  - Increased vascular permeability and bleeding (augmented by destruction of platelets),
  - tissue destruction (as found in myonecrosis),

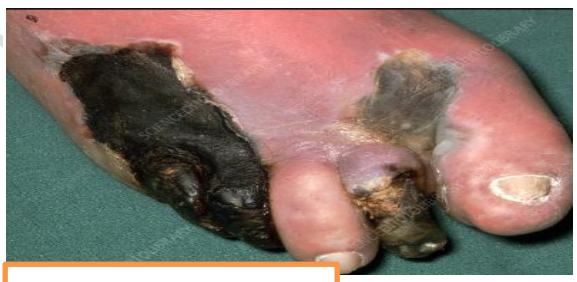
- hepatic toxicity,
- and myocardial dysfunction (bradycardia, hypotension)
- dead of tissue and release of gas (**Gas gangrene**).

2. Enterotoxin inserts and disrupts membranes of mucosal cells.

B. <u>Spectrum of disease and infection</u>

- ✓ **Gas gangrene**; a life-threating, toxin-mediated destruction of muscle and other tissues follow traumatic introduction of the organism.
- ✓ Food poisoning caused by release of the toxin after ingestion of large quantities of organisms.
- ✓ Disease is usually self-limiting and benign and in manifested by
  - Abdominal cramps,
  - Diarrhea, and
  - Vomiting.





Gas gangrene of toe

at are the symtoms of gas gangrene?

ıptoms of gas gangerene often include:

- Fever
- air under the skin
- pain in the area around a wound
- swelling un the area around a wound
- pale skin that quickly turns gray,dark red,purple , or black
- blister with foul-smelling discharge
- excessive sweating
- increase heart rate
- vomiting
- yellow skin and eyes (jaundice) is a late sign.

.tetani:

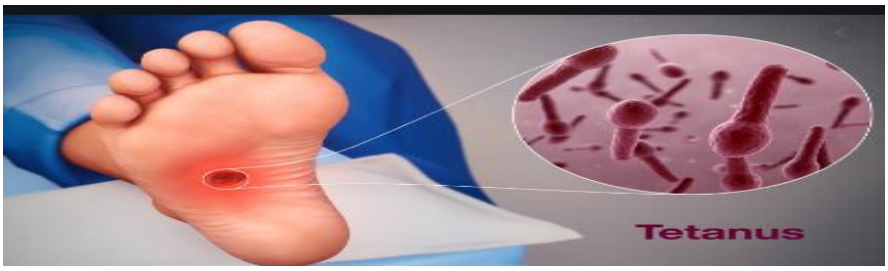
A. Virulence factor: include

1. Tetanospasmin; a neurotoxin exotoxin that disrupts nerve impulses to muscles.

○ **Tetanospasmin** inactivates proteins that regulate release of the inhibitory neuro transmitt glycine and gamma-aminobutryic (GABA) –unregulated excitatory synaptic activity the motor neur resulting in **Spastic paralysis** (الشلل التشنجي).

B. Spectrum of disease and infection

- ✓ ***C.tetani*** (commonly known as *lockjaw*) (Figure)
- ✓ Organism establishes a wound infection and elaborates the potent toxin that medi generalized muscle spasms.
- ✓ If untreated, spasms continue to be triggered by even minor stimuli, leading to exhaustion eventually respiratory failure.



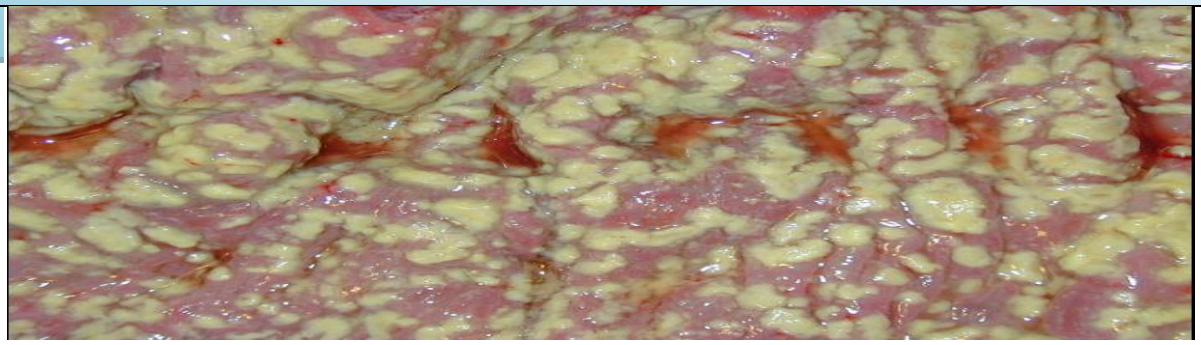
4. C.difficile:

A. Virulence factor: include

1. Exotoxin A, which is an enterotoxin that is thought to be primarily responsible for the gastrointestinal disease.
2. Toxin B, a cytotoxin, has a less clear role in C.difficiles.

B. <u>Spectrum of disease and infection</u>

- ✓ *C. difficile* infection is spread by bacterial spores found within <u>feces</u>.
- ✓ Organism required diminution of normal flora by the activity of various antimicrobial agent become established in the gut of hospitalized patients.
- ✓ Once established, elaboration of toxin (s) results in diarrhea (i.e., antibiotic-associated diarrh
- ✓ Or potentially life-threatening inflammation if the colon.
- ✓ When the surface of the inflamed bowel is overlaid with a"pseudommembrane" composed of:
  - Necrotic debris,
  - while blood cells,
  - and fibrin, the disease referred to as pseudomembranous colitis.



reatment

- In general, the treatment of clostridial infection is high-dose penicillin G, to which the organism has remained susceptible.
- *C. welchii* and *C. tetani* respond to sulfonamides.
- Clostridia are also susceptible to to
  - tetracyclines,
  - carbapenems (imipenem),
  - metronidazole,
  - vancomycin,
  - and chloramphenico.

---

ost test

Q1: What are the causes canned food; bulging

Reference

1. Bailey & Scott's diagnostic microbiology-E-Book. Elsevier Health Sciences; 2015 Dec 28.

İntroduction	المقدمة

The genera *Bacillus* and *Clostridium* constitute the family *Bacillaceae*.

Bacillus bacteria.

General Characteristics

- Bacillus spp. and related genera Brevibacillus and paenibacillus all are:
  - aerobic,
  - Gram positive rode,
  - Are able to survive in adverse environmental condition by formation of
  - Oval endospores and can remain in this dormant state for years.
  - Only one endospore is formed per cell.

Pre-test

Q1: What is the function of spore in bacteria

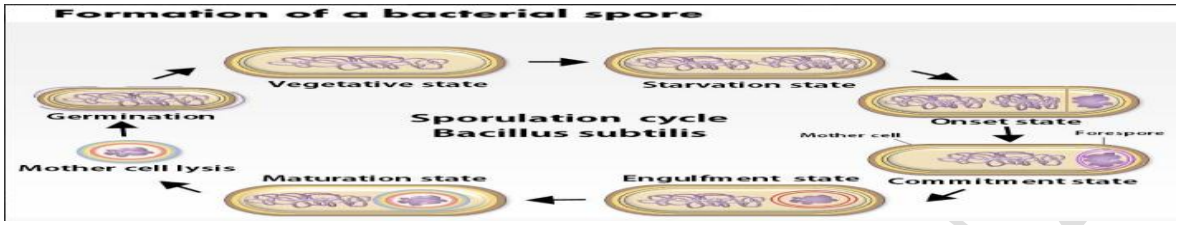
A. Reproduction
B. Protection

Q2: example of sporluated bacteria

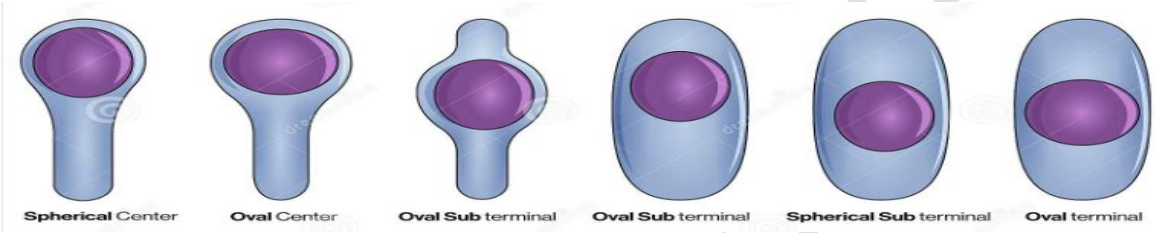
C. Bacillus and closteridium
D. *Salmonella typhi* and *Vibrio cholera*

Scientific Content:	المحتوى العلمي

ıdospore formation is usually triggered by a lack of nutrients: the bacterium divides with its cell wall, and one side then engulfs the other.



ınd positions of spore.



obligate aerobes: oxygen dependent;

or facultative anaerobes: having the ability to continue living in the absence of oxygen.

two *Bacillus* species are medically significant:

***B. anthracis* causes anthrax; and *B. cereus* causes food poisoning.**

B.anthracis the most notorious pathogens genus, inhibit the soil.

Human quire infections when they are (inoculate with spores, either:

Inhalation during exposure to contaminated animal product such as (hide)

Or by traumatic introduction.

All other Bacillus spp. are generally considered to be opportunistic pathogens of low virule ıd are usually only associated with compromised patients exposed to contaminated materials.

Pathogenesis and virulence factors

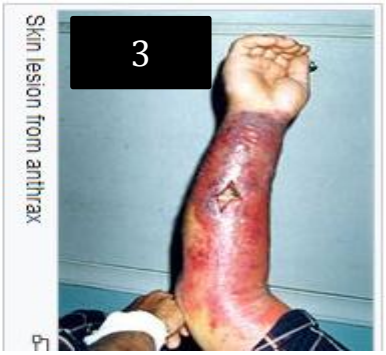
1. Pathogenesis of B.anthracis

- ✓ Is the most highly virulent species for humans and is the causative agent on (anthrax) which there are three (3) form

1. **Cutaneous anthrax** : known as hide-porter's disease occur at the site of sp

penetration **2** to **5 days** after exposure and is manifested by

- Presents as a boil-like skin lesion that eventually forms an ulcer with a black center (escl (Figure 1, 2,3). May progress to toxemia and death.



Pulmonary anthrax: also known as " wool sorter's" disease , follows inhalation of spore During the first few days of illness, most people have fever, chills, and fatigue
These symptoms may be accompanied by cough, shortness of breath, chest pain, a nausea or vomiting,
In progressive stage a condition called hemorrhagic mediastinitis, causing bloody fluid accumulate in the chest cavity, therefore causing shortness of breath.

3. **Gastrointestinal anthrax**:
   - caused by consuming anthrax-infected meat and is characterized by diarrh potentially with blood, abdominal pains,
   - Acute inflammation of the intestinal tract, and loss of appetite.
   - Occasional vomiting of blood can occur.

- Most patients die from toxemia and overwhelming sepsis.

❖ **Virulence factors of B. anthrax**

1. Production of antiphagocytic capsule.
2. and potent exototins (i.e, edema toxin and lethal toxin) that mediate cell and tis destruction.

2. Pathogensis of B. cereus

✓ Food poising of two types;
- diarrheal type, which is characterized by abdominal pain and watery diarrheal;
- emetic type, which is manifested by profuse vomiting.

✓ B. cereus is the most commonly encountered species of Bacillus opportunistic infecti that include:
- Posttraumatic eye infections,
- endocarditis, and
- Bacteremia.

✓ Infections of other sites are rare and usually involve
- Intravenous drug abuser or
- Immunocompromised patients.

❖ **Virulence factors of B. cereus**

1. Production of enterotoxin , and
2. Pyogenic toxin.
3. Pathogenesis of other species includes B. subtilis, B. circulans, B.licheniformis, ot

spp.,Brevibacillus sp.,and Parenibacillus spp.

- ✓ Food poisoning has been associated with some species but is not common.
- ✓ These organisms may also be involved in opportunistic infections similar to th described for B.cereus.

ɔoratory Diagnosis:

nples: no special consideration is required for specimen collection and transportation.

1. **Specimen processing**
   - ✓ Special processing procedure are required for food implicated B.cerus in food pois outbreaks and animal hides or products, and environmental samples, for the isolat of B.anthracis.
   - ✓ The organisms will be present as spores in these specimens, so initial processing v involve either
     - Heat or
     - Alcohol shock before platting on solid media. The shock will allow only the sp forming bacilli to survive; thus this is an enrichment and selection techni designed to increase the chance for laboratory isolation of these species.
     - Species are identified by using morphologic and biochemical criteria.

ture media of Bacillus

1. 5 % sheep blood agar.
2. Chocolate agar.
3. Routin blood culture media.
4. Nutrient agar.
5. Not grow on MaConkey.
6. Phenylethyl alcohol agar (PEA).
7. Bicarbonate agar used to induce B. anthracis capsule formation

Post test

Q1: In which stage sporulated bacteria produce forespore

A. Starvation state

B. Onset state

C. Commitment state.

Reference

P. Bailey & Scott's diagnostic microbiology-E-Book. Elsevier Health Sciences; 2015 Dec 28.

الجامعة التقنية الوسطى
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قسم تقنيات المختبرات الطبية

المادة :الأحياء المجهرية

المرحلة :الثانية

Title:

العنوان:

CORYNEBACTERIUM Lec 17

Name of the instructor: **اسم المحاضر:**

Name of the instructor: Assistant Professor Dr. Issam Jumaa

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

Introduction:

CORYNEBACTERIUM DIPHTHERIAE

Morphology and Identification

Corynebacteria are 0.5–1 µm in diameter and several micrometers long. Characteristically, they possess irregular swellings at one end that give them the "club-shaped" appearance Irregularly distributed within the rod (often near the poles) are granules staining deeply with aniline dyes (metachromatic granules) that give the rod a beaded appearance. Individual corynebacteria in stained smears tend to lie parallel or at acute angles to one another. True branching is rarely observed in cultures. On blood agar, the C diphtheriae colonies are small, granular, and gray with irregular edges and may have small zones of hemolysis. On agar containing potassium tellurite, the colonies are brown to black with a brown-black halo because the tellurite is reduced intracellularly (staphylococci and streptococci can also produce black colonies).

Pretest:

1-Describe the clinical infections associated with Corynebacterium diphtheriae.

2-Discuss several nondiphtheria Corynebacterium spp. that are capable of causing clinical infection in humans.

3-Discuss Corynebacterium diphtheriae Virulence Factors (Diphtheria toxin)

Scientific Content:

Pathogenesis and Virulence Factors.

Virulence Factors. Diphtheria toxin is the major virulence factor associated with C. diphtheriae. This toxin is produced by strains of C. diphtheriae infected with a lysogenic β-phage, which carries the tox gene for diphtheria toxin. Nontoxigenic strains can be converted to tox+ by infection with the appropriate β-phage. Only toxin-producing C. diphtheriae causes diphtheria; however, C. ulcerans and C. pseudotuberculosis, which belong

to the “C. diphtheriae group,” can also produce the toxin when they become infected with the tox-carrying β-phage. Diphtheria toxin is a protein of 62,000 Da. It is composed of two fragments, A and B, which are linked together by a disulfide bridge. The toxin is exceedingly potent and is lethal for humans

in amounts of 130 ng/kg of body weight. The toxicity is due to the ability of diphtheria toxin to block protein synthesis in eukaryotic cells. The toxin is secreted by the bacterial cell and is nontoxic until exposed to trypsin. Trypsinization cleaves the toxin into the two fragments. Both fragments are necessary for cytotoxicity. Fragment A is responsible for the cytotoxicity, and fragment B binds to receptors on the eukaryotic cells and mediates the entry of fragment A into the cytoplasm. On reaching the cytoplasm, fragment A disrupts protein synthesis. Fragment A splits nicotinamide adenosine dinucleotide to form nicotinamide and adenosine diphosphoribose (ADPR). ADPR binds to and inactivates elongation factor 2 (EF-2), an enzyme required for elongation of polypeptide chains on ribosomes.

Production of the toxin in vitro depends on numerous environmental conditions, including an alkaline pH (7.8 to 8.0), oxygen, and, most importantly, the iron concentration in the environment. The amount of iron needed for optimal toxin production is less than the amount needed for optimal growth. The toxin is released in significant amounts only when the available iron in the culture medium is exhausted.

Laboratory Diagnosis

Dacron swabs from the nose, throat, or other suspected Specimens should be inoculated to a blood agar plate (to rule out hemolytic streptococci) and a selective medium such as a tellurite plate (eg, cystine-tellurite blood agar [CTBA] or modified Tinsdale’s medium) and incubated at 37°C in 5% CO2. Plates should be examined in 18–24 hours. In 36–48 hours, the colonies on tellurite medium are sufficiently definite for recognition of C diphtheriae. On cystine tellurite agar, the colonies are black with a brown halo. A presumptive C diphtheriae isolate should be subjected to testing for toxigenicity. Such tests are performed only in reference public health laboratories. There are several methods, as follows:

1. Modified Elek immunoprecipitation method described by the World Health Organization Diphtheria Reference Unit. A filter paper disk containing antitoxin (10 IU/disk) is placed on an agar plate. The cultures to be tested (at least 10 colonies should be chosen) for toxigenicity are spot inoculated 7–9 mm away from the disk. After 48 hours of incubation, the antitoxin diffusing from the paper disk has precipitated the toxin diffusing from toxigenic cultures and has resulted in precipitin bands between the disk and the bacterial growth.

2. Polymerase chain reaction (PCR)–based methods have been described for detection of the diphtheria toxin gene (tox). PCR assays for tox can also be used directly on patient specimens before culture results are available. A positive culture result confirms a positive PCR assay. A negative culture result after antibiotic therapy along with a positive PCR assay result suggests that the patient probably has diphtheria.
3. Enzyme-linked immunosorbent assays can be used to detect diphtheria toxin from clinical C diphtheriae isolates.
4. An immunochromatographic strip assay allows detection of diphtheria toxin in a matter of hours. This assay is highly sensitive

Treatment and vaccination

The treatment of diphtheria rests largely on rapid suppression of toxin-producing bacteria by antimicrobial drugs and the early administration of specific antitoxin against the toxin formed by the organisms at their site of entry and multiplication. antimicrobial drugs (penicillin, macrolides) inhibit the growth of diphtheria bacilli. Although these drugs have virtually no effect on the disease process, they arrest toxin production and assist public health efforts. They also help to eliminate coexistent streptococci and C diphtheriae from the respiratory tracts of patients or carriers. DPT is a class of combination vaccines against three infectious diseases in humans: diphtheria pertussis (whooping cough), and, tetanus.

Posttest:

1- Skin diphtheria as occurs in children in tropical areas typically

(A) Does not occur in children who have been immunized with diphtheria toxoid

(B) Is clinically distinct from skin infections (pyoderma, impetigo) caused by Streptococcus pyogenes and Staphylococcus aureus

(C) Is also common in northern latitudes

(D) Results in protective antitoxin levels in most children by the time they are 6–8 years old

(E) Yields toxin-mediated cardiomyopathy
Correct answer: D.
2- All of the following statements regarding Corynebacterium diphtheriae are true, except?
A. It can be identified by using tests for toxigenicity

B. The toxin inhibits protein synthesis
C. Toxin has adverse effects on cardiovascular and nervous systems
D. Native chromosome is responsible for toxin production
Correct answer: D.
3- Elek's gel preciptiation test is for
A- Gonococcus
B- Diphtheria
C- Hemophillus
D- Anthrax
Correct answer: B.
4- Diphtheria toxin acts by
A- Inhibiting acetylcholine release
B- Inhibiting glucose transport
C- Increasing levels of cyclic AMP
D- Inhibiting protein synthesis
Correct answer: D.

5- One of the following statements regarding diphtheria toxin is true:
A- Toxin is produced by all corynebacteria
B- Positive Schick test suggest immunity
C- Toxin production is influenced by critical concentration of iron
D- It is an endotoxin
Correct answer: C.

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Lec 19 **د. جليل نجاح جليل**

Pre-test

All are oxidase positive bacteria except:
a. *Neisseria*
b. *Escherichia coli*
c. *Campylobacter*
d. *Pseudomonas aeruginosa*

Gram Negative Cocci Bacteria

1. Clinical Species

- *N. gonorrhoeae*
- and *N. meningitidis* are the primary human pathogens of the genus.

2. General Characteristics

- ❖ Members of the genus *Neisseria*
- are aerobic, gram-negative
- cocci typically arranged in pairs diplococci) with adjacent sides flattened together (resembling coffee beans).

Neisseria meningitidis

- ➢ **Also called Meningococcus; Diplococcus intracellularis meningitidis**

A. Cultural Characteristics

- ✓ Meningococci have exacting growth requirements
- ✓ and do not grow on ordinary media.
- ✓ Growth occurs on media enriched with blood, serum or ascitic fluid.
- ✓ Strains will grow on Mueller–Hinton medium without the addition of blood or serum.
- ✓ They are strict aerobes, no growth occurring anaerobically.
- ✓ The optimum temperature for growth is 35–36°C.
- ✓ No growth takes place below 30°C.
- ✓ Optimum pH is 7.0–7.4.

Growth is facilitated by 5–10% CO2 and high humidity.

- ✓ Blood agar, chocolate agar and Mueller–Hinton starch casein hydrolysate agar are the media commonly used for culturing meningococci.
- ✓ Modified Thayer –Martin (with vancomycin, colistin and nystatin) is a useful selective medium.

Colony morphology of **Meningococcus are**

1. On **blood agar**
   - ✓ colonies are 1–2 mm in diameter, round, convex, gray, translucent and nonhemolytic.
2. **on chocolateagar-**
   - ✓ colonies are slightly larger on heated blood (chocolate) agar than on ordinary blood agar.

B. **Morphology**

- ✓ Meningococci are **gram-negative** oval or spherical **cocci** (0.6–0.8 μm in size),
- ✓ Typically arranged in pairs, with the adjacent sides flattened or concave opposing edges and the long axes parallel (Figure-1).
- ✓ They are typically seen in large numbers inside polymorphonuclear leukocytes
- ✓ Most fresh isolates are capsulated (Based on their capsular polysaccharide antigens, meningococci are classified into at least 13).
- ✓ They are nonsporing and nonmotile.

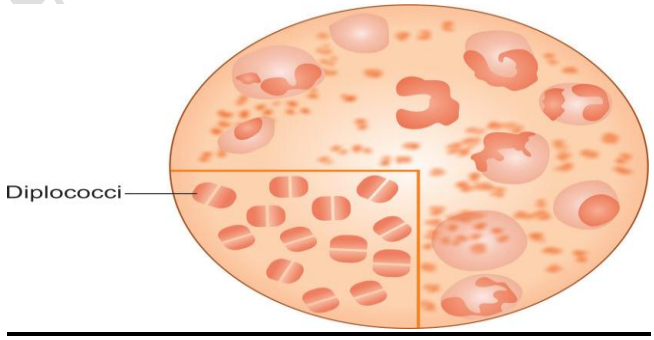

Figure-1 *Neisseria meningitidis* in cerebrospinal fluid (C.S.F). showing flat adjacent side of cocci.

C. Laboratory identification of *Neisseria meningitidis*

- They are **catalase and oxidase positive**.
- **Sugar fermentation**: Meningococci ferment
  - ✓ glucose and maltose with the production of acid but no gas,
  - ✓ but not lactose or sucrose (gonococci ferment glucose but not maltose).
- **Indole and hydrogen sulfide** are not produced and **nitrates** are not reduced.

D.Classification of N. meningitidis baesd on capsular antigen

- meningococci are classified into at least 13 serogroups including:

✓ A, B, C,D, X, Y, Z, Zl (29E) and W135, and H, I, K and L,and

✓ Groups A, B and C are the most important.

E. Pathogenicity

✓ Meningococci are strict human parasites inhabiting the nasopharynx. Infection is usually asymptomatic.

Stages of Meningococcal Infections

❖ There are three stages.

1. First Stage—Nasopharyngeal Infection

✓ The organisms appear in nasopharynx leading to **nasopharyngeal infection**, which is usuallyasymptomatic. (Remember to collect **nasopharyngeal swab)**

2. Second Stage—Meningococcal Septicemia

✓ In a small percentage of cases, the meningococci enter the bloodstream from the posterior nasopharynx. **(Remember to collect blood samples)**

✓ This stage is called **meningococcemia.** The patient develops

- fever,
- malaise
- and petechial skin lesions.

✓ The organisms may also cause lesions in the joints and lungs and rarely cause massive bilateral hemorrhages in the adrenals **(Waterhouse– Friderichsen syndrome)**.

3. Third Stage—Meningitis

- ✓ In the third stage of meningococcal infection, the organisms can cross the blood–brain barrier and infect the meninges. (Remember to collect C.S.F)
- ✓ The route of spread from the nasopharynx to the meninges is controversial.
- ✓ The spread may be directly along the perineural sheath of the olfactory nerve, through the cribriform plate to the subarachnoid space, or more probably, through the bloodstream.
- ✓ In certain cases, the site of entry of the *Meningococcus* may be the conjunctiva.
- ✓ On reaching the central nervous system, a suppurative lesion of the meninges is set up.
- ✓ Case fatality is variable but in untreated cases may be as high as 80%. Survivors may have sequelae such as
  - blindness
  - and deafness.
- ✓ The pathogenic agent in meningococcal disease appears to be the endotoxin (LPS) released by autolysis.
- ✓ The vascular endothelium is particularly sensitive to the endotoxin.

F. Laboratory Diagnosis

for diagnosis the meningococca we need to collect 4 types of smaple as following

1. Cerebrospinal fluid (CSF),
2. Blood for culture
3. Aspirate from skin lesions or pus from an infected joint.
4. Throat or nasopharyngeal swabs.

G. Detection of *N. meningitidis* from Cerebrospinal fluid (CSF)

1. **Cell counting:** polymorphonuclear in the the exudate smaple typically will seen.
2. **Cerebrospinal fluid (CSF) centrifugation**
   - ✓ After centrifuge the (CSF) a smear will make from (depostite), then stain with (Gram stain). CSF from a typical case of meningococcal meningitis will show gram negative diplococci inside a limited proportion of the pus cells; many are extracellular. Stain a
   - ✓

- ✓ second film with methylene blue to determine the cell type; occasionally, diplococci may be seen more easily with this stain.

3. Culture: Plate out the centrifuged deposit on both blood and heated blood agar (chocolate agar) and incubate at 37°C in 5–10% CO2. Colonies appear after 18–24 hours which may be identified by morphology and biochemical reactions

4. **Biochemical reactions including**

- ✓ **Sugar utilization tests** or commercial kits are used to identify any gram-negative diplococci**.**
- ✓ The oxidase test is performed on colonies on solid medium.

5. **Serogrouping** is performed by slide agglutination with hyperimmune sera that provide important epidemiological information

6. **Polymerase Chain Reaction (PCR*)*** Group specific diagnosis of infection can be made by detection of meningococcal DNA sequence in CSF or blood by PCR amplification.

Treatment

1. Penicillin 2. Chloramphenicol 3. **rifampicin or ciprofloxacin**

Neisseria gonorrhoeae (gonococcus)

N. gonorrhoeae causes the venereal disease gonorrhea. Gonococci resemble meningococci very closely in many properties.

Morphology

Morphology and staining of *N. gonorrhoeae* are identical to those of *N. meningitidis*. In smears from the urethral discharge in acute gonorrhea, the organism appears as a *Diplococcus* with the adjacent sides concave **(Fig. 27.2)**, being typically kidney-shaped. It is found predominantly within the polymorphs, some cells containing as many as a hundred cocci.

Cultural Characterstics

Gonococci are more difficult to grow than meningococci They are aerobic but may grow anaerobically also. Growth occurs best at pH 7.0– 7.4 and at a temperature of 35–36°C. It is

essential to provide 5–10% CO2. They grow well on **chocolate agar** and **Mueller– Hinton agar.** A popular selective medium is

the **Thayer–Martin medium (chocolate agar containing vancomycin, colistin and nystatin)** which inhibits most contaminants, including nonpathogenic *Neisseria*. Colonies are small, round, translucent, convex or slightly umbonate,

with finely granular surface and lobate margins after incubation for 24 hours..They are soft and easily emulsifiable. After 48 hours, the colonies are larger (1.5–2.5 mm), sometimes with a crenated margin and an opaque raised center.

Biochemical Reactions

- ✓ *Gonococcus* is oxidase positive and resembles meningococci except in the fermentation of maltose.
- ✓ Gonococci ferment only glucose and not maltose and neither species ferments lactose or sucrose.
- ✓ This can be remembered by G for *Gonococcus* and **M+G for *Meningococcus*.**

Antigenic Structure

The structure of *N. gonorrhoeae* is typical of gramnegative bacteria. The surface structures

include the following**:**

1. **Pili:** Pili are hair-like appendages that extend up to several micrometers from the gonococcal surface. They act as virulence factors by promoting attachment to host cells and inhibiting phagocytosis. The pili are composed of repeating protein subunits (pilins). Pili undergo antigenic and phase variation.

2. **Por proteins (protein I):** The Por proteins (formerly protein I) are porin proteins that form pores or channels in the outer membrane. Two classes of Por proteins (PorA and PorB), have been identified. Anyone strain carries only either IA or IB but not both.

3. **Opa protein (protein II):** These proteins facilitate bacterial adherence to each other and to eukaryotic cells and also for the clumping of cocci seen in urethral exudate smears.

4. **Rmp (protein III):** These proteins stimulate antibodies that block serum bactericidal activity against *N. gonorrhoeae.*

5. **Lipooligosaccharide (LOS):** This antigen possesses endotoxic activity.

6. **Other proteins:** Other important gonococcal proteins are **IgA1 protease**, β-**lactamase**, which degrades penicillin and **Fbp (ironbinding protein)**.

Pathogenesis

Gonorrhea

Gonorrhea is a venereal disease. The disease is acquired by sexual contact. The incubation period is 2–8 days.

A. Disease in men: The most common clinical presentation is **acute urethritis** in the male. **Dysuria** and a **purulent penile discharge** make most sufferers seek treatment rapidly. The infection extends along the urethra to the prostate, seminal vesicles and epididymis. **Chronic urethritis** may lead to stricture formation. The infection may spread to the periurethral tissues, causing abscesses and multiple discharging sinuses **('watercan perineum').**

B. Disease in women: Asymptomatic carriage in women is common, especially in the endocervical canal. The infection may extend to Bartholin's glands, endometrium and fallopian tubes to give rise to **acute salpingitis**, which may be followed by **pelvic inflammatory disease** and a high probability of sterility.

Peritoneal spread occasionally occurs and may produce a perihepatic inflammation **(Fitz–Hugh–Curtis syndrome).** Proctitis occurs in both sexes. Gonococcal pharyngitis may follow orogenital contact in either sex. Conjunctivitis may occur usually by autoinoculation with fingers.

C. Disseminated gonococcal disease: Blood invasion may occur from the primary site of infection and may lead to metastatic lesions such as arthritis, ulcerative endocarditis and very rarely meningitis.

D. Disease in childern

i. **Ophthalmic neonatorum:** A nonvenereal infection is **ophthalmia neonatorum** in the newborn, in which the eyes are coated with gonococci as the baby passes down the birth canal. A severe purulent eye discharge with periorbital edema occurs within a few days of birth. If untreated, ophthalmia leads rapidly to blindness. Once very common, this has been controlled by the practice of instilling 1% silver nitrate solution into the eyes of all newborn babies **(Crede's method).**

ii. **Vulvovaginitis:** In prepubertal girls, vulvovaginitis may be caused by gonococci. This occurs either in conditions of poor hygiene or by sexual abuse.

Laboratory Diagnosis

Diagnosis can be established readily in the acute stage but chronic cases sometimes present great difficulties.

1. Specimens

A. Specimens in Men

1. Urethra: In men, urethral samples usually suffice (with rectal cultures in homosexual males). In **acute gonorrhea**, the urethral discharge contains gonococci in large numbers. The meatus is cleaned with a gauze soaked in saline

and a sample of the discharge collected with a platinum loop for culture, or directly on slide for smears. Purulent discharge may be expressed at the anterior urethra and collected with a swab. In **chronic infections**, there may not be any

urethral discharge. The morning drop of secretion may be examined or some exudate may be obtained after prostatic massage. It may also be possible to demonstrate gonococci in the centrifuged deposits of urine in cases where

no urethral discharge is available.

2. Anal canal: In homosexual males.

B. Specimens in Women

1. **Endocervical swab:** In women, urethral, cervical and rectal specimens should always be examined. A single well taken endocervical swab will detect approximately 90% of gonococcal infections in women. A high vaginal swab is

not suitable.Throat infection also occurs and should be sought where appropriate.

2. Urethral

3. Anal canal and

4. Throat.

C. Blood, Swabs of skin lesions, or pus aspirated from a joint.

D. Conjunctival Swab: Particularly in neonatal ophthalmia.

E. Urine Specimen: Any urine specimen showing gram-negative diplococci in a Gram stain should

be cultured on an appropriate selective medium.

2. Transport

For culture, specimens should be inoculated on prewarmed plates, immediately on collection. If this is not possible, specimens should be collected with charcoal impregnated swabs and sent to the laboratory in Stuart's transport medium.

3. Direct Microscopy

Do the **Gram staining** shows characteristic kidneyshaped gram-negative diplococci lying within polymorphonuclear leukocytes with a few extracellular. Approximately 95% of infected men will yield a positive smear. It has to be emphasized that diagnosis of gonorrhea by smear examination is unreliable in women as some of the normal genital flora have an essentially similar morphology.

4. Culture

In acute gonorrhea, cultures can be obtained readily on chocolate agar or Mueller–Hinton agar incubated at 35–36°C under 5–10% CO2. In chronic cases, where mixed infection is usual and in the examination of lesions, such as proctitis; however, it is better to use a selective medium such as the Thayer–Martin medium. Examine plates after 24 hours incubation and the growth is identified by morphology and biochemical reactions. Incubation of primary isolation plates is continued for 48 hours.

Colonies

Are small, round, translucent, convex or slightly umbonate, with finely granular surface and lobate margins. They are soft and easily emulsifiable. After 48 hours, the colonies are larger (1.5–2.5 mm), sometimes with a crenated margin and an opaque raised center. Smear is made from the colony and Gram staining is done. Gonococci are gram-negative cocci arranged in pairs (diplococci) with adjacent sides concave (pear or bean shaped).

5. Identification

N. gonorrhoeae is identified preliminarily on the basis of the isolation of oxidase-positive, gram negative diplococci that grow on chocolate blood agar or on media that are selective for pathogenic *Neisseria* species.*N gonorrhoeae* is oxidase positive. It ferments glucose with acid only. It does not ferment maltose unlike meningococci.

6. Genetic Probes

Probes specific for the nucleic acids of *N. gonorrhoeae* have been developed for the direct detection

of bacteria in clinical specimens.

Post test

Q1:The specimen of choice for isolation of gonococci from women with gonorrhea is:

a. Vaginal swab.
b. Cervical swab.
c. Urethral swab.
d. Urine.

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Introduction

Miscellaneous Bacteria- Gram positive non-spor forming bacteria

These bacteria, not included in any other pathogenic groups, are not commonly isolated but may have importance in either animal or human disease, or as zoonotic infections.

Pre-test

Select the suitable answer from the following answer

Q:Tumbling motility is seen in:

a. *Listeria monocytogenes*
b. *Enterobacter cloacae*
c. *Proteus vulgaris*
d. *Salmonella Typhi*

LISTERIA MONOCYTOGENES

Organisms of the genus *Listeria* are nonsporing gram-positive bacilli. The genus contains eight species, but almost all cases of human listeriosis are caused by *L. monocytogenes.*

Morphology

Listeria monocytogenes

- ✓ İs a small, coccoid, Grampositive bacillus measuring approximately 0.5 × 2–3 μm.
- ✓ They occur singly or in pairs which are often angled at the point of contact and may resemble diphtheroids or diplococci.
- ✓ It exhibits a characteristic, slow; tumbling motility when grown at 25°C but at 37°C is nonmotile.
- ✓ This is because peritrichous flagella are produced by the bacillus optimally at 20–30°C but only scantily or not at all at 37°C.
- ✓ They are noncapsulate, nonsporing and nonacid-fast.

Cultural Characters

- ✓ Listeriae are aerobes and facultative anaerobes.
- ✓ They can grow over a temperature range of 2–43°C, the optimum temperature for the growth is 35–37°C.
- ✓ They can grow on ordinary media containing fermentable carbohydrate, but growth is better on blood agar or tryptose phosphate agar.
- ✓ After 24 hours incubation at 37°C, colonies are 0.5–1.5 mm in diameter, smooth, translucent and emulsifiable and non-pigmented.
- ✓ On blood agar, *L. monocytogenes* develops zones of slightly hazy β-hemolysis.

Biochemical Reactions

- ✓ *L. monocytogenes* ferments
  - glucose, maltose, *L. rhamnose* and alpha methyl D-mannoside, producing acid without gas.
  - It is catalase positive.
  - It grows in the presence of 0.1 % potassium tellurite, 10% salt and at pH 9.6.

Various Species

- ✓ On the basis of a few tests the genus *Listeria* can be divided into 8 species **(Table 1)**.
- ✓ Many serovars have been recognised.
- ✓ Two major tests of differentiation of various species include D-xylose fermentation and CAMP test with *Staph. aureus* and *Rhodococcus equi*. *L. monocytogenes* gives a positive CAMP test with *Staph. aureus.*

Table 1: Distinguishing characters of *Listeria* spp

Species	Hemolysis	CAMP test with		Acid production from				Nitrate reduction
		Staph. aureus	*Rhodococcus equi*	D-Mannitol	L-Rhamnose	D-Xylose	α-Methyl D-mannoside	
L. monocytogenes	+	+	–[a]	–	+	–	+	–
L. innocua	–	–	–	–	+/–	–	+	–
L. ivanovii	++	–	+	–	–	+	–	–
L. seeligeri	±	+	–	–	–	+	+/–	–
L. welshimeri	–	–	–	–	+/–	+	+	–
L. grayi	–	–	–	+	+/–	–	NK	+/–
L. murayi	–	–	–	+	V	–	NK	+
L. denitrificans	–	–	–	–	–	+	NK	+

[a]Regarded as ±; + = Positive reaction; – = Negative reaction; V = Variable; NK = Not known

Pathogenicity

- *Listeria monocytogenes* is commonly ingested in food.
- *L. ivanovii* and *L. seeligeri* have been associated with a very small number of human infections.
- Experimental inoculation in rabbits causes a marked monocytosis (hence the name *monocytogenes).*
- Monocytosis is a feature of human listeriosis also.

- Instillation into the eyes of rabbits produces keratoconjunctivitis *(Anton test).*
- Human infection is believed to result from contact with infected animals, inhalation of contaminated dust or ingestion of contaminated milk or food. Hospital acquired infections have also been reported.
- *L.monocytogenes* produces a hemolysin known as listeriolysin-O, which is a virulence factor antigenically related to streptolysin-O and pneumolysin.

Clinical Features

1. **Intrauterine and neonatal infection:**
   - ✓ Intrauterine infection of the fetus may result in abortion, stillbirth, premature delivery, or acute-onset disseminated infection in the newborn infant (including the form known as granulomatosis infantiseptica).
   - ✓ Asymptomatic infection of the female genital tract may cause infertility.
   - ✓ Meningitis or septicemia may ocur in neonates.
2. **Adult and juvenile infection:**
   - ✓ It may cause meningitis or meningoencephalitis, particularly in neonates and in the elderly.
3. **Disease in healthy adults:**
   - ✓ Most *Listeria* infections in healthy adults are asymptomatic or occur in the form of a mild influenza-like illness. Several foodborne outbreaks of acute gastroenteritis with fever have been described.
4. **Other infections:** Listeriosis may also present as
   - ✓ abscesses,
   - ✓ conjunctivitis,
   - ✓ pharyngitis,
   - ✓ urethritis,
   - ✓ pneumonia,
   - ✓ infectious mononucleosis like syndrome, endocarditis or septicemia.

Laboratory Diagnosis

1. Specimens

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

2. Microscopy

- ✓ If the Gram stain shows organisms, they are intracellular and extracellular gram-positive coccobacilli.

3. Culture

- ✓ Specimens should be inoculated on blood agar, chocolate agar and tryptose phosphate agar, and incubated at 35–37°C for 1–3 days.
- ✓ Greater success in isolation is achieved if the materials are stored in tryptose phosphate or thioglycollate broth at 4°C and subcultures are done at weekly intervals for 1–6 months *(cold enrichment*).
- ✓ Blood agar shows small colonies surrounded by a narrow zone of b-hemolysis.
- ✓ The bacteria are actively motile when grown at 25°C.
- ✓ The isolate is identified by its morphology and biochemical tests **(Table 1).**

Post test

Select the suitable answer from the following answer

Q: Which of the following bacteria does not produce pigment?

a. *Staphylococcus aureus*
b. *Chromobacterium violaceum*
c. *Flavobacterium meningosepticum*
d *Listeria monocytogenes*

Q:Which of the following properties is/are shown
by the organisms belonging to the family *Enterobacteriaceae?*
a. They are catalase positive
b. They are oxidase negative
c. They ferment glucose
d. All of the above

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Enteric Gram negtaive bacteria

The family Enterobacteriaceae is the largest, most heterogeneous collection of medically important Gram-negative bacilli. Because of their normal habitat in humans, these organisms are referred to as the "**enteric bacilli"** or "**enterics".**

1. Escherichia coli

This genus is named after the German pediatrician Theodar Escherich who first identified *Escherichia coli* (1885). The genus *Escherichia* consists of five species of which *E. coli* is the most common and clinically most important. Unlike other coliforms, *E. coli* is a obligate parasite living only in the human or animal intestine that cannot live free in nature.

Morphology

E. coli is a gram-negative, straight, rod measuring 1–3 × 0.4–0.7 μm arranged singly or in pairs. It is motile by peritrichate flagella, though some strains may be nonmotile. It is nonsporing and noncapsulated.

Cultural Characteristics

1. It is an aerobe and a facultative anaerobe. The temperature range is 10–40°C (optimum 37°C).
2. It can grow on ordinary media such as **nutrient agar.**
3. Colonies are large, thick, grayish white, moist, smooth opaque or partially translucent disks.
4. This description applies to the smooth (S) form seen on fresh isolation.
5. On **blood agar,** many strains, are hemolytic. On **MacConkey's medium**, colonies are red or pink due to lactose fermentation.
6. On selective media such as **DCA** or **SS agar** growth is largely inhibited used for the isolation of salmonellae and shigellae. In broth, growth occurs as general turbidity and a heavy deposit.

Biochemical Reactions

1. *E.coli* ferments glucose, lactose, mannitol, maltose and many other sugars with the production of acid and gas.

2. Typical strains do not ferment sucrose.
3. Indole and MR positive, and VP and citrate negative (IMViC + + – –)
4. It is negative for phenylalanine deaminasetest, urease test, H2S production, gelatin liquefication, growth in the presence of KCN, and malonate utilization.

Antigenic Structure

❖ Serotyping of *E. coli* is based on three antigens—

1. The flagellar antigen H, These are thermolbile. So far 75 antigens have been identified. Strains may need to be grown in semi-solid agar to induce flagella expression
2. somatic antigen O, These are heat-stable, lipopolysaccharide antigens of cell walls. Over 173 different O antigens have been described
3. **Capsular antigen (K antigen):** K antigen' refers to the acidic polysaccharide antigen located in the 'envelope' or microcapsule. (K for *Kapsel*, German for capsule). It encloses the O antigen and renders the strain in agglutinable by the O antiserum. It may also contribute to virulence by inhibiting phagocytosis.and the capsular antigen K as detected in agglutination assays with specific rabbit antibodies.
4. **Fimbrial antigen (F antigen):** These are thermolabile proteins. Heating the organisms at 100°C leads to detachment of fimbriae. The F antigen has no role in antigenic classification of *E. coli.*

Virulence Factors

❖ Two types of virulence factors have been recognized in *E. coli*—
A. surface antigens
B. and toxins.

A. Surface Antigens

1. **Somatic antigen (O antigen):** The somatic lipopolysaccharide surface O antigen, besides exerting endotoxic activity, also protects the bacillus from phagocytosis and the bactericidal effects of complement.

2. **K antigen:** Most strains of *E. coli* responsible for **neonatal meningitis** and **septicemia** carry the KI envelope antigen which is a virulence factor resembling the group B antigen of meningococcci.

3. **Fimbriae:** Like many other members of the Enterobacteriaceae, strains of *E. coli* exhibit common fimbriae which are chromosomally determined, present in large numbers and causing mannose sensitive hemagglutination and probably not relevant in pathogenesis. Filamentous protein structures resembling fimbriae cause mannose-resistant hemagglutination and play an important part in the pathogenesis of **diarrheal disease** and in **urinary tract infection**. They include the **K88 antigen** found in strains causing enteritis of pigs, the **K99 antigen** found in strains causing enteritis of calves and lambs, and the **colonization factor antigens** (CFAs) (CFAI, CFAII, CFA/III) expressed by enterotoxigenic *E. coli* (ETEC) causing diarrheal disease in humans.

B. Toxins

1. **Exotoxins:** *E. coli* produces two kinds of exotoxins *hemolysins* and *enterotoxins.*

a. **Hemolysins:** Hemolysins do not appear to be relevant in pathogenesis.

b. **Enterotoxins:** Three distinct types of *E. coli* enterotoxins have been identified:

i. **Heat-labile toxin (LT):** *Mechanism of action of LT: E. coli* LT is heat labile protein and is closely related to the toxin produced by strains of *Vibrio cholerae*. There are two main forms, termed LT-I and LT-II. Different forms of LT-1 have been described. Similarly, two forms of LT-II (LT-IIa and LT- lIb) have been detected. LT is a complex of polypeptide subunits each unit of the toxin consisting of one subunit A (A for *active)* and five subunits B (B for *binding).* The toxin binds to the Gm1 ganglioside receptor on intestinal epithelial cells by means of subunit B, following which the subunit A is activated to yield two fragments—A1 and A2. The A1 fragment activates adenyl cyclase in the enterocyte to form cyclic adenosine 5' monophosphate (cAMP), leading to increased outflow of water and electrolytes into the gut lumen, with consequent diarrhea. LT is a powerful antigen and can therefore, be detected by a number of
serological as well as biological tests.

ii. **Heat-stable toxin (ST):** The heat stable toxins of *E. coli* (ST) have a low molecular weight which is probably responsible for their heat stability and poor antigenicity. There are two major classes, designated ST-I (or STa) and ST-II (or STb). STa is associated with human disease.

a. **ST-I (or STa):** STa is a small, monomeric toxin that binds to guanylate cyclase, leading to an increase in the level of cyclic guanosine monophosphate and subsequent hypersecretion of fluids. ST-I is methanol soluble, is plasmid encoded.

b. **ST-II (STb)—ST-II (STb)** is distinguished from ST-I (STa) by its biological activity and by its insolubility in methanol. It stimulates fluid accumulation in ligated intestinal loops of young piglets (upto nine weeks) but not in the infant Mouse test. The mechanism of action is not known but it appears not to act via Camp or cGMP.

iii. **Verocytotoxin or Verotoxin (VT):** The biological properties, physical characteristics and antigenicity of VT are very similar to those of Shiga toxin (Stx), produced by strains of *Sh. dysenteriae* type 1 so it is also known as 'Shiga-like toxin' (SLT).

Clinical Infections

Four main types of clinical syndromes are caused by *E. coli* including

1. **Diarrhea**
2. **Urinary Tract Infection**
3. **Pyogenic Infections**
4. **Septicemia.**

1. Diarrhea

At least five different types of diarrheagenic *E. coli* are now recognized, each associated with specific serotypes and with different pathogenic mechanisms.

A. Enteropathogenic *E. coli* (EPEC): These have been associated mainly with diarrhea in infants and children usually occurring as institutional outbreaks. Certain strains belonging to characteristically EPEC serogroups, such as O26 and O111. **Pathogenesis of EPEC diarrhea:** EPEC neither ordinarily produce enterotoxins, nor are they invasive. In infantile enteritis, the bacilli are seen to be adherent to the mucosa of the upper small intestine, intimately attached to

cup-like projections (pedestals) of the enterocyte membrane, causing disruption of the brush border microvilli.

B. Enterotoxigenic *E. coli* (ETEC) Diarrhea caused by ETEC is endemic in the developing countries in the tropics, among all age groups in the local population. In developing countries. ETEC are a major cause of mortality in children under the age of 5 years.Persons from developed countries visiting endemic areas often suffer from ETEC diarrhea—a condition known as **'traveller's diarrhea'**. The **Pathogenesis**: organism must initially be able to adhere to the mucosal surface of the epithelial cells of the small intestine. This adhesion is usually mediated by fimbriae that bind to specific receptors in the intestinal cell membrane.

C. Enteroinvasive *E. coli* (EIEC) These are closely related by phenotypic and pathogenic properties to *Shigella*. Many of these, strains are nonmotile, do not ferment lactose or ferment it late with acid, but without producing any gas and do not form lysine decarboxylase.

Pathogenesis: EIEC, like those of Shigella species, can penetrate the epithelial cells of the large intestine and multiply intracellularly, giving rise to blood and mucus in the stool. Infection is by ingestion. Clinically, EIEC infection resembles shigellosis, ranging from mild diarrhea to frank dysentery, and occurs, in children as well as adults.

D. *E. coli* Verocytotoxin or Verotoxin (VT) *E. coli* verocytotoxin or verotoxin (VT) causes **hemorrhagic colitis** and **hemolytic uremic syndrome** Outbreaks of infection with VTEC have occurred in the community, in nursing homes for the elderly and in daycare centres for young children. The most severe clinical manifestations are usually seen in the young and the elderly.

E. Enteroaggregative *E. coli* (EAEC) These strains are so named because they are characterized by their ability to adhere to particular laboratory-cultured cells, such as HEp-2, in an aggregative or 'stacked brick' pattern. They have been associated with persistent diarrhea, especially in developing countries.

Diagnosis: The only methods currently available for detecting these bacteria are the HEp-2 cell test for determining the aggregative phenotype, and DNA probes. The HEp-2 cell test involves allowing strains of *E. coli* to adhere to cell monolayers *in vitro* and observing the pattern of adhesion by microscopy.

2. Urinary Tract Infection

E. coli and coliforms account for the large majority of naturally acquired urinary tract infections. Those acquired in the hospital, following instrumentation, are more often caused by other bacteria, such as *Pseudomonas* and *Proteus.* Most frequently encountered O serotypes of *E. coli* in UTI include 01, 02, 04, 06,07, 018 and 075. These are also known as nephritogenic strains.

3. Pyogenic Infections

E. coli form the most common cause of intraabdominal infections, such as peritonitis and abscesses resulting from spillage of bowel contents. They also cause pyogenic infections in the perianal area. They are an important cause of neonatal meningitis, but is much less so in older patients.

4. Septicemia

Bloodstream invasion by *E. coli* may lead to fatal conditions like septic shock and 'systemic inflammatory response syndrome' (SIDS). As *E. coli* commonly show multiple drug resistance, antibiotic sensitivity testing of strains is important in treatment.

Laboratory Diagnosis

a. Laboratory Diagnosis of EPEC: Fresh diarrheal feces is plated on blood agar and MacConkey
media. After overnight incubation, *E. coli* colonies are emulsified in saline on a slide and are examined by slide agglutination with polyvalent antisera belonging to EPEC-associated serogroups.

b. Laboratory Diagnosis of ETEC Diagnosis of ETEC diarrhea depends on the demonstration of enterotoxin in *E. coli* isolates by any of the methods listed in **(Table-1).**

Table-1: Methods for detection of ETEC enterotoxins

Assay	LT	ST
In vivo tests		
Ligated rabbit ileal loop		
Read at 6 hours	±	+
Read at 18 hours	+	–
Infant rabbit bowel	+	+
Infant mouse intragastric (4 hours)	–	+
Adult rabbit skin (vascular permeability factor)	+	–
In vitro tests		
i. Tissue culture tests		
ii. Rounding of Y1 mouse adrenal cells		
iii. Elongation of Chinese hamster ovary (CHO) cells	+	–
iv. Serological tests		
ELISA	+	(ST-ELISA with monocolonial antibody)
Passive agglutination tests, passive immune hemolysis, precipitin (Biken's) test	+	–
v. *Genetic tests*		
DNA probes	+	+

c. Laboratory Diagnosis of EIEC

i. Sereny test.
ii. Tissue culture and DNA hybridization methods.
iii. ELISA (VMA ELISA) test.

d. Laboratory Diagnosis of VTEC

i. **Demonstration of the bacilli or VT in feces:**
ii. **Sorbitol MacConkey medium:** Most VTEC strains belong to the serotype O157 H7 does not ferment sorbitol.
iii. **Serology.**

Laboratory Diagnosis of UTI

A. Collection of Specimen

i. Catheter specimen, Midstream urine specimen
iii. Suprapubic stab

B. Transport : Urine is a good medium for the growth of coliforms and other urinary pathogens. If delay of more than 1–2 hours is unavoidable, the specimen should be refrigerated at 4°C,

C. Microscopy of urine: The deposit of the centrifuged urine can be examined under microscope to find out the presence of pus cells, red blood cells and bacteria in it.

D. Semiquantitative culture: For quantitative culture, serial tenfold dilutions of urine are tested by the pour plate or surface culture methods.

Standard loop method: The most widely used technique employs a standard loop.Measured quantity of urine with the help of standarized loop is inoculated on blood agar and another loopful on MacConkey agar and

incubated overnight at 37°C. Blood agar medium gives a quantitative measurement of bacteriuria, while MacConkey agar enables a presumptive diagnosis of the bacterium. Other methods of semiquantitative estimation of bacterial counts are **filter paper method, dip spoon, dip slide methods**, etc.

Interpretation of results

Kass (1956) gave a criterion for active bacterial infection of urinary tract as follows:

Significant bacteriuria: When bacterial count is more than 105/mL of a single species.

Doubtful significance: Between 104 to 105 bacteria per mL. Specimen should be repeated for culture.

No significant growth: <103 bacteria per mL and are regarded as contaminant.

E. Identification: The organisms are identified by colony characters, Gram's staining, motility, biochemical reactions and slide agglutination test.

INFECTIONS

Laboratory Diagnosis of Septicemia

Diagnosis depends on the isolation of the organism by blood culture and its identification by colony morphology, staining, motility and biochemical
reactions.

Laboratory Diagnosis of Pyogenic Infection

Specimens: The specimens are usually pus and wound swab.

Culture: Cultures are made on MacConkey's agar.

Identification: The isolate is identified by colony morphology, staining, motility and biochemical reactions.

Post –test

Q1: Traveler's diarrhea is caused by

a. Enteropathogenic *Escherichia coli:*

b. Enterotoxigenic *Escherichia coli*

c. Enteroinvasive *Escherichia coli*

d. Verotoxigenic *Escherichia coli*

Pre-test

Q1: Which of the following is the example of encapsulated gram-negative bacteria?

a) *Klebsiella pneumoniae*
b) *Clostridium perfringens*
c) *Streptococcus pneumoniae*
d) *S.auerus*

Enteric Gram negtaive bacteria

The family Enterobacteriaceae is the largest, most heterogeneous collection of medically important Gram-negative bacilli. Because of their normal habitat in humans, these organisms are referred to as the "**enteric bacilli"** or "**enterics".**

2. *Klebsiella pneumoniae*

Introduction

- ✓ Members of the genus *Klebsiella* are gram-negative,
- ✓ nonsporing, non-motile bacilli that grow well on ordinary media,
- ✓ produce pink mucoid colonies on MacConkey's agar.
- ✓ They are usually found in the intestinal tract of humans and animals or freeliving in soil, water, and on plants.

Classification

- ✓ Their classification has undergone various modifications. The name *K. pneumoniae* is used for the species as a whole. It is further divided into 4 subspecies. The most frequently encountered, biochemically typical form of it is known as
  1. *K. pneumoniae* subsp. *aerogenes*,
  2. *K. pneumoniae* subsp. *ozaenae*,
  3. *K. pneumoniae* subsp. *pneumoniae*
  4. *K. pneumoniae* subsp. *rhinoscleromatis* **(Table -1)**.
  5. Indole-producing strains that resemble *K. pneumoniae subsp. aerogenes* biochemically are classified in a separate species, *K. oxytoca.*

Morphology

- ✓ They are short,plump, gram-negative, non-sporing, capsulated, non-motile bacilli, 1–2 μm long and 0.5–0.8 μm wide with parallel or bulging sides and slightly pointed or rounded ends.

Cultural Characteristics

- ✓ Klebsiellae grow well on ordinary media at a temperatures between 12°C and 43°C (optimum, 37°C) in 18–24 hours. On MacConkey agar, the colonies typically appear large, mucoid and red in colour. Mucoid nature of colonies is due to capsular material produced by the organism.

Biochemical Reactions

- ✓ They ferment sugars (glucose, lactose, sucrose,mannitol) with production of acid and gas.
- ✓ They are urease positive, indole negative, MR negative, VP positive and citrate positive (IMViC – – ++).
- ✓ These reactions are typical of *K. pneumoniae subsp. aerogenes.*

Glucose	Lactose	Sucrose	Mannitol	
+ (AG)	+	+	+	
Urease	**Indole**	**MR**	**VP**	**Citrate**
+	–	–	+	+

Antigenic Structure

Klebsiella possess capsular (K) and somatic(O) antigens.

1. **Capsular (K) antigen:** On the basis of capsular (K) antigens, the klebsiellae have been differentiated, into 80 serotypes. Members of capsular types 1–6 occur most frequently in the human respiratory tract. Capsular antigens are usually detected by means of the capsular 'swelling' reaction, countercurrent immunoelectrophoresis and enzyme-linked immunosorbent-assay (ELISA).
2. **Somatic (O) antigen:** Five different somatic or O antigens (01–05) occur in various combinations with the capsular antigens. Four of the five *Klebsiella* O antigens are identical to or related to *E. coli* O antigens.

Pathogenicity: *Klebsiella pneumoniae* can cause a primary community acquired pnuemonia, nosocomial infections, urinary tract infections, wound infections, bacteremia and meningitis and rarely diarrhea. In some hospital, *K. pneumoniae* has replaced *E. coli* as the leading blood culture isolate. As most strains are resistant to antibiotics, treatment poses serious problems.

1. **Pneumonia:** *Klebsiella* pneumonia is a serious disease with high case fatality. The typical patient is a middle- or older-aged man who have medical problems. Positive blood cultures can be obtained in about 25% of the cases.
2. **Diarrhea:** Some strains of *K. pneumoniae* isolated from cases of diarrhea have been shown to produce an enterotoxin very similar to the heat stable toxin of *E. coli. K. ozaenae* is a bacillus associated with ozena, an uncommon, chronic disease in which there is atrophy of the nasal mucosa characterized by foul smelling nasal discharge. *K. rhinoscleromatis* causes rhinoscleroma, a chronic granulomatous hypertrophy of the nose. *K. oxytoca* may be rarely isolated from clinical specimens **(Table -1)**.

Table -1 Distinguishing reactions of *Klebsiella* species

Tests	*K. pneumoniae subspecies*				K oxytoca
	aerogenes	*pneumoniae*	*ozaenae*	*rhinoscleromatis*	
Gas from glucose	+	+	V	–	+
Acid from lactose	+	+	V	–	+
Urease	+	+	V	–	+
Citrate	+	+	V	–	+
Malonate	+	+	–	+	+
MR	–	+	+	+	V
VP	+	–	–	–	V
Lysine decarboxylase	+	+	V	–	+
KCN	+	+	+	±	+

V, variable

Laboratory Diagnosis

Diagnosis is made by culturing appropriate specimens on

- ✓ blood agar
- ✓ and Mac Conkey agar and identifying the isolate by biochemical reactions.

Post test

Q1: Which of the following species/subspecies of *Klebsiella* produces indole?

a. *K. pneumoniae* subspecies *aerogenes*
b. *K. oxytoca*
c. *K. pneumoniae* subspecies *pneumoniae*
d. *K. pneumoniae* subspecies *rhinoscleromatis.*

Pre-test

Q1: A gram negative organism which produces swarming on culture medium is

A. Salmonella

B. b. Clostridium
C. c. Staphylococci
D. d. Proteus

3. Proteus

Introduction

- ✓ ***Proteus* Bacilli** *Proteus* bacilli are normal intestinal commensals and opportunistic pathogens like coliforms.
- ✓ The name '*Proteus*' refers to their pleomorphism, after the Greek god *Proteus* who could assume any shape.
- ✓ Genus *Proteus* has four species:
  1. *P. mirabilis*,
  2. *P. vulgaris*,
  3. *P. myxofaciens*
  4. and *P. penneri. P. mirabilis*,
- ✓ *P. vulgaris* are widely recognized as human pathogens. These are motile, gram-negative bacilli, characterized by *swarming* growth on agar.

Morphology

- ✓ They are gram-negative coccobacilli, 1–3 mm long and 0.6 mm wide. Pleomorphism is frequent—short
- ✓ coccobacilli to long filaments.
- ✓ In young swarming cultures, many of the bacteria are long, curved and filamentous.
- ✓ They may be arranged singly, in pairs or in short chains.
- ✓ They are actively motile with
- ✓ peritrichous flagella. They also have more type of fimbriae and are noncapsulated.

Cultural Characteristics

- They are aerobe and facultative anaerobes.
- All grow well on laboratory nutrient media.
- *Proteus* organisms are usually first recognized by their characteristic putrefactive odor described as **'fishy'** or **'seminal'** and swarming appearance on noninhibitory solid media, such as nutrient agar and blood agar.
- Swarming is a striking feature of *Pr. mirabilis* and *Pr. vulgaris*. Swarming of *Proteus* appears to be due to vigorous motility of the organisim although the exact cause is not yet established.
- Swarming growth is a problem in the laboratory when mixed growth is obtained in which *Proteus* bacilli are present with other bacteria.
- A number of methods have been devised to inhibit swarming.
- Swarming of *Proteus* can be inhibited by
- (i) increasing concentration of agar (6%) and
  by (ii) incorporation of chloral hydrale (1 :500), sodium azide (1:500), alcohol (5–6%), sulfonamide, surface. active agents or boric acid (1:1000).
- Swarming does not occur on MacConkey's medium, on which smooth colorless (NLF) formed. *Proteus* produces uniform turbidity with a slight

powdery deposit and an ammonical odor in liquid medium (peptone water).

Biochemical Reactions

The distinctive characters of this genus are:
i. **PPA test**—Deamination of phenyl alanine to phenyl pyruvic acid (PPA test) is always positive.
ii. **Urea hydrolysis**—Urea hydrolysis by enzyme urease is another characteristic of *Proteus*, but is negative in some *Providencia* strains.
iii. All species of *Proteus* produce acid from glucose.
iv. Lactose is not fermented.
v. They are malonate utilization negative.
vi. Indole is formed by *Pr. vulgaris* but is negative in *Pr. mirabilis*.
vii. They are MR positive and VP negative.
viii. H2S is produced by *Pr. vulgaris* and *Pr. mirabilis*.
ix. Nitrate reduction positive.
Other biochemical characters of species of *Proteus* are given in (**Table-1).**

Pathogenesis

- ✓ *Proteus* bacilli are widely distributed in nature as saprophytes, being found in decomposing animal matter, in sewage, in manured soil and in human and animal fecesThey are frequently present on the moist areas of the skin. They are opportunistic pathogens, commonly responsible for urinary and septic infections, often nosocomial. *P. mirabilis* accounts for the majority of human infections seen with this group of organisms. All members of the tribe can cause
- ✓ **urinary tractinfections (UTI),**
- ✓ **wound infections,**
- ✓ **pneumonia,**
- ✓ **infection of the ear,**
- ✓ **respiratory tract infection,**
- ✓ **septicemia**
- ✓ **a**nd **nosocomial infections**.
- ✓ Strains of *Pr. mirabilis* are a prominent cause of urinary tract infection in children and in domiciliary practice.
- ✓ UTI caused by *Proteus* tends to be more serious than that caused by *E. coli* and other coliforms.
- ✓ It produces urease which splits urea into carbon dioxide and ammonia. Ammonia inactivates complement, damages renal epithelium and makes the urine alkaline.
- ✓ This increase in pH causes precipitation of calcium and magnesium salts from the urine and results in the formation of urinary calculi.

Table -1:Biochemcal features of species of *Proteus*

Test	*Pr. vulgaris*	*Pr. mirabilis*
Swarming	+	+
Gas from glucose	+	+
Indole	+	–
Phenyl pyruvic acid (PPA) test	+	+
Urease	+	+
H_2S production	+	+
Ornithine decarboxylase	–	+
Fermentation of adonitol	–	–
Fermentation of trehalose	±	+

Labortory Diagnosis

Culture: Laboratory diagnosis of the infections caused by species *Proteus* can be carried out by culture of the specimen on

1. MacConkey agar
2. or DCA.

Identification: The isolate is identified by its morphological, biochemical and agglutination reactions.

Treatment

Proteus bacilli are resistant to many of the common antibiotics. An exception is *P. mirabilis* which is
sensitive to ampicillin and cephalosporins.

Post test

Q1: Swarming of *Proteus* strains on solid media can be inhibited by:

a. Increasing the concentration of agar in the medium
b. Incorporation of chloral hydrate in the medium
c. Incorporation of sodium azide in the medium
d. All of the above methods.

Pre-test

Q1: What are the two main types of dysentery?

The first type, amoebic dysentery or intestinal amoebiasis, is caused by a single-celled, microscopic parasite living in the large bowel. The second type, bacillary dysentery, is caused by invasive bacteria. Both kinds of dysentery occur mostly in hot countries.

4. *Shigella*

INTRODUCTION

The genus *Shigella* is named after the Japanese microbiologist Kiyoshi Shiga, who first isolated the
organism in 1896.

Morphology

- ✓ Shigellae are nonsporing, noncapsulate, Gram negative rods, 2–4 × *0.6 μm,* nonmotile and nonflagellate.

Cultural Characteristics

- ✓ They are aerobes and facultative anaerobes, with a growth temperature range of 10–40 °C and optima of 37°C and pH 7.4.They grow well on conventional media.

Biochemical Reactions

- ✓ The shigellae are divided into four **groups**, or **species,** by their biochemical reactions and
- ✓ antigenic structure **(Table-1).** The groups A, B, C and D correspond to the species

1. *S. dysenteriae,*
2. *S. flexneri,*
3. *S. boydii*
4. and *S. sonnei.*

- ✓ Glucose is fermented with the production of acid, without gas, except for two serotypes, *S. flexneri* serotype 6 and *S. boydii* serotype 14, which form gas.
- ✓ Fermentation of mannitol is of importance in classification and shigellae have traditionally been divided into mannitol fermenting and nonfermenting species.

Classification

- ✓ Shigellae are classified into four species or subgroups (A,B,C,D) based on a combination of biochemical and serological characteristics.
- ✓ Serotypes are distinguished within the species. *S. sonnei* is serologically homogeneous and is classified by colicin typing **(Table-1)**.

Table -1 Distinguishing features of *Shigella* species

Subgroup	A	B	C	D
Species	*Sh. dysenteriae*	*Sh. flexneri*	*Sh. boydii*	*Sh. sonnei*
Mannitol	–	A	A	A
Lactose	–	–	–	A (Late)
Sucrose	–	–	–	A (Late)
Dulcitol	–	–	d	
Indole	d	d	d	
Ornithine decarboxylase	–	–	–	+
Serotypes	10	6 + variants	15	Only one

A, acid; d, delayed

Pathogenic Mechanisms

1. Surface properties: Lipopolysaccharide (LPS) has been implicated to entering intestinal cells.

2. Invasiveness: Invasive property is related to the presence in the bacillus of large plasmids coding for the outer membrane protein responsible for cell penetration. These proteins are called **'virulence marker antigens' (VMA)**.

3. Toxins: *S. dysenteriae* type 1 forms a produces a powerful exotoxin **(Shiga toxin**) (details see under heading group A *(S. dysenteriae)*.

Pathogenicity

- Shigellae cause bacillary dysentery.
- Humans are the only known reservoir of *Shigella* organisms infection occurs by ingestion.
- The minimum infective dose is low, as few as 10–100 bacilli being capable of initiating the disease.
- *Shigella* cause disease by invading and replicating in cells lining the colonic mucosa.
- After reaching the large intestine, the shigellae multiply in the gut lumen. The shigellae multiply within the epithelial cells and spread laterally into adjacent cells, where cell-to-cell passage occurs, and deep into the lamina propria.
- The infected epithelial cells are killed and the lamina propria and submucosa develop an inflammatory reaction with capillary thrombosis. Patches of nerotic epithelium are sloughed and ulcer form.
- The cellular response is mainly by polymorphonuclear leukocytes.
- The ulcers of the bacillary dysentery are much shallower than amoebic ulcers. Bacteremia may occur in severe infections.

✓ **Bacillary dysentery** has a short incubation period (1–7 days, usually 48 hours). The main clinical features are

- frequent passage of loose,
- scanty feces containing blood and mucus, along with abdominal cramps and tenesmus.
- Fever and vomiting may be present.
- Infection is usually self-limited.

- In dysentery caused by *S. dysenteria* type 1, patients experience more severe symptoms.
- Severe illness may also be caused by members of *S. flexneri* and *S. boydii* groups.
- In contrast dysentery associated with *S. sonnei* (*Sonne dysentery)* in an otherwise healthy person may be confined to the passage of a few loose stools with vague abdonimal discomfort and the patient often continues at school or work.

Laboratory Diagnosis

Diagnosis depends on isolating the bacillus from feces.

A. Specimens

- **i. Feces:** A specimen of feces is always preferable to a rectal swab
- **ii. Rectal swabs:** A direct swab may be taken from the ulcer by sigmoidoscopic examination.

B. Transport : Fresh feces should be inoculated without delay or transported in a suitable medium such as Sachs' buffered glycerol saline.

C. Microscopy

Make a wet film of a suspension of the feces in saline. This will show numerous erythrocytes and polymorphs and some macrophages.

D. Culture

- ✓ For inoculation it is best to use mucus flakes if they are present in the sample. The feces are inoculated on

1. **MacConkey agar**
2. and **DCA.**
3. **SS agar** and **XLD medium** can also be used.

- ✓ After overnight incubation at 37°C, the plates are inspected for pale (non-lactose-fermenting)

colonies on MacConkey agar and DCA, and red and colorless colonies with no blackening on XLD and SS agar, respectively.

Identification

i. **Biochemical reactions:** These are tested for motility and biochemical reactions. Any nonmotile bacillus that is urease, citrate, H2S and KCN negative should be further investigated by biochemical tests **(Table-1).**

ii. **Slide agglutination:** Identification is confirmed by slide agglutination with polyvalent and monovalent sera and then with type-specific sera (belonging to subgroups A, B, C) unless the strain is S. *sonnei.*

Post test

Q1: All the following media are employed for isolation of *Shigella* except:

a. Hektoen enteric agar
b. Salmonella-Shigella agar
c. Thiosulfate citrate bile salt agar
d. Xylose lysine deoxycholate agar.

Pre-test

Define typhoid fever: Is a bacterial infection caused by *Salmonella* Typhi which is a membrane of Enterobacteriaceae which caused a fever.

5. *Salmonella*

Introduction

Genus *Salmonella* consists of bacilli that parasitise the intestines of a large number of vertebrate species and infect human beings, leading to enteric fever, gastroenteritis, septicemia with or without focal suppuration, and the carrier state. The most important member of the genus is *Salmonella* Typhi, the causative agent of typhoid fever. Eberth (1880) first observed the typhoid bacillus. Salmon and Smith (1885) described a bacillus which was believed to cause hog cholera.

SALMONELLA

For practical purposes, they may be divided into two groups:

1. **The enteric fever group:** It consists of the typhoid and paratyphoid bacilli that are exclusively or primarily human parasites.
2. **The food poisoning group:** These are essentially animal parasites but which can also infect human beings, producing gastroenteritis, septicemia or localized infections.

Morphology

- ✓ Salmonellae are gram-negative bacilli, 2–4 × 0.6 μm in size.
- ✓ They are motile with peritrichous flagella except S. Gallinarum- Pullorum which is nonmotile.
- ✓ They are non-acid-fast, noncapsulate and nonsporing.

Cultural Characteristics

Salmonellae are aerobes and facultatively anaerobes, growing readily over a range of pH 6–8 and temperature 15–45° C (optimum 37°C). They can grow on simple laboratory media. Various media are as follows:

1. Nutrient agar and blood agar	2. Peptone water and nutrient broth	3.MacConkey agar
4. Deoxycholate-citrate agar (DCA)	5. Wilson and Blair's brilliant-green bismuth sulfite agar (BBSA	6. Xylose lysine deoxycholate (XLD) agar

ochemical Reactions

Salmonellae ferment glucose, mannitol, arabinose, maltose, dulcitol and sorbitol, forming
d and gas except S. Typhi, Gallinarum and rare anaerogenic variants in other serotypes form
ly acid and no gas.

Lactose, sucrose, salicin or adonitol are not fermented.

They are, indole positive, MR positive, VP negative and citrate positive (IMViC – + – +) except by S. phi and S. Paratyphi *A* which are citrate negative as they need tryptophan as the growth factor.
Hydrogen sulfide is produced except by S. Paratyphi A, S. Choleraesuis, S. Typhisuis and S. Sendai.
Urease is not hydrolyzed.
Salmonellae decarboxylate the amino acids lysine, ornithine and arginine. The enteric fever group may separated
ochemically **(Table-1)**.

ble- 1 Biochemical characters of typhoid and paratyphoid bacilli

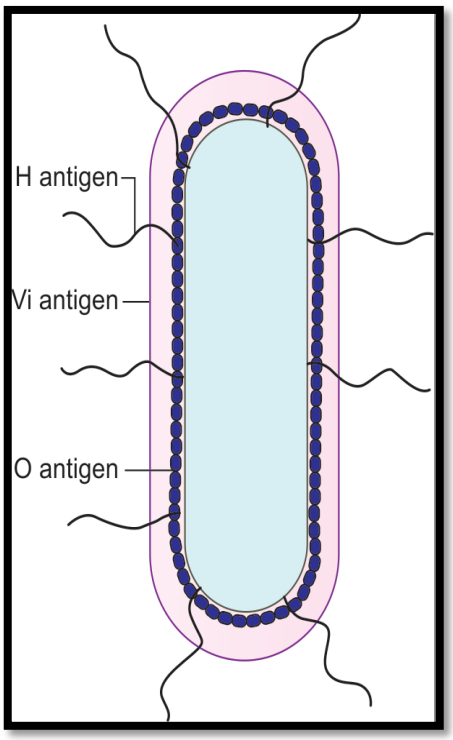
	Glucose	Xylose	d-Tartrate	Mucate
. Typhi	A	d	A	d
. Paratyphi A	AG	–	–	–
. Paratyphi B	AG	AG	–	AG
. Paratyphi C	AG	AG	AG	–

= Acid; AG = Acid and Gas; d = Delayed

Antigenic Structure

Antigenic structure of salmonellae is shown in **(Figure-1)** Salmonellae possess the following antigens based on which they are classified and identified:

1. Flagellar antigen H.
2. Somatic antigen O.
3. Surface antigen Vi, found in some species.



Pathogenesis

- Salmonellae are strict parasites of animals or humans.
- All vertbrates appear capable of harboring these bacteria in their gut and salmonellae can colonize virtually all animals.

Host-adapted Serotypes

- Some serotypes exhibit host specificity. S. Typhi, S. Paratyphi *A* and usually,but not invariably *S.* Paratyphi B are confined to human beings.
- Other salmonellae are parasitic in various animals—domestic animals, rodents, reptiles and birds.

Clinical Syndromes

Salmonellae cause the following four major syndromes:

1. Enteric fever.
2. Bacteremia with focal lesion.
3. Gastroenteritis or food poisoning.

4. Asymptomatic carrier state.

1. Enteric Fever

- ❖ The term enteric fever includes typhoid fever caused by
- S. Typhi and paratyphoid fever caused by Paratyphi A, B or C.
- The clinical features tend to be more severe' with S. Typhi (*typhoid fever*).

2. Bacteremia with Focal Lesion

- ✓ This is associated with S. Cholerae-suis but may be caused by any *Salmonella* serotype.
- ✓ Infection occurs by oral route and there is early invasion of the bloodstream with possible focal lesions and in particular may cause septicemic disease (with focal lesions, in lungs, bones, meninges, etc.)
- ✓ but intestinal manifectations are often absent. Blood culture are positive.

3. Gasteroenteritis or Food Poisoning

- ✓ This is the most common manifestation of Salmonella infection.
- ✓ In the USA, Salmonella Typhimurium and Salmonella enteritis are prominent (For detail see under the heading, "***Salmonella* gastroenteritis"**.)

4. Asymptomatic Carrier State

- ✓ Most people infected with *salmonella* continue to excrete the organism in their stools for days or weeks after complete clinical recovery, but eventual clearance of the bacteria from the body is usual.
- ✓ A few patients continue to excrete the salmonellae for prolonged periods.

Laboratory Diagnosis

- ✓ Bactriological dignosis of enteric fever consists of:

A. Isolation and identification of the bacilli.
B. Demonstration of circulating antigen.
C. Demonstration of antibodies in patient's serum.
D. Other laboratory tests.

A. Isolation and Identification of the Bacilli

- ✓ It may be done by culture of specimens such as patient's
  - blood, feces, urine, bone marrow, duodenal drainage rose spots etc.
- ✓ For the leborotatory diagnosis of enteric fever, selection of relevant specimens depends upon duration of illness, e.g. blood for culture must be taken repeatedly. Urine culture may be positive after second week and stool second or third week.

Blood Culture

- ✓ Blood cultures are positive in approximately 90% cases in the first week of fever,
- ✓ in approximately 75% of cases in the second week,

- ✓ 60% in the third week and 25% thereafter till the subsidence of pyrexia **(Table-1)**.

Table 1: Positivity of various specimens at different phases of enteric fever

Duration	Specimen	% positivity
1st week	Blood culture	90
	Feces culture	–
	Widal test	–
2nd week	Blood culture	75
	Feces culture	50
	Widal test	Low titer
3rd week	Blood culture	60
	Feces culture	80
	Widal test	80–100

Feces Culture

Salmonellae are shed in the feces throughout the course of the disease and even in convalescence. Hence, fecal cultures are almost as valuable as blood cultures in diagnosis.

Urine Culture

- ✓ Urine culture is less useful than the culture of blood and feces because salmonellae are shed in the urine irregularly and infrequently.
- ✓ Generally cultures are positive only in the second and third weeks and then only in about 25% of cases.
- ✓ The rate of isolation is improved by repeated sampling.
- ✓ Clean voided urine samples are centrifuged and the deposit inoculated into enrichment and selective media as for fecal culture.

Biochemical reactions:

- ✓ Salmonellae will be
  - indole and urease negative, catalase positive,
  - oxidase negative, nitrate reduction positive and
  - ferment glucose, mannitol and maltose but not lactose or sucrose.

S. Typhi will be anerogenic and ferments glucose and mannitol with production of acid only, while paratyphoid bacilli (S. Paratyphi A, B and C) will form acid and gas from sugars.

Identification: Identification of the isolate is by slide agglutination.

Demonstration of Antibodies in Patient's Serum

i. Widal test. This is a test for measurement of H and O agglutinins for typhoid and paratyphoid bacilli in the patient's serum

ii. Other serological tests. Enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination test and CIEP are other serological methods of diagnosis.

Post test

Q1: All the following enrichment media are used for isolation of *Salmonella* except:
a. Alkaline peptone water
b. Selenite F broth
c. Brilliant green tetrathionate broth
d. Tetrathionate broth

الجامعة التقنية الوسطى
كلية التقنيات الصحية والطبية \بغداد
قسم تقنيات المختبرات الطبية

المادة :الأحياء المجهرية

المرحلة :الثانية

Pseudomonas , Acinetobacter , Shigella , Salmonella **Lec 21**

Name of the instructor: **اسم المحاضر:**

Estabraq Ali AL-Sodani **د.جليل د. استبرق علي السوداني نجاح**

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

The *Pseudomonas* and *Acinetobacter* species are widely distributed in soil and in water. *Pseudomonas aeruginosa* sometimes colonizes humans and is the major human pathogen of the pseudomonads. *P. aeruginosa* is invasive and toxigenic, produces infections in patients with abnormal host defenses, and is an important nosocomial pathogen. Of the *Acinetobacter* species, *Acinetobacter baumannii* is responsible for most human infections. It is a significant nosocomial pathogen, especially in critical or intensive care units, and is frequently resistant to multiple antibiotics. *Burkholderia* consists of many species, but only *B. cepacia* complex, *B. pseudomallei*, *B. mallei*, and *B. gladioli* are notable human or animal pathogens. Like pseudomonads, *Bukholderia* are typically environmental organisms and opportunistic pathogens. *Stenotrophomonas maltophilia* is typically not pathogenic for healthy people; however, the organism is a well-known opportunistic and nosocomial pathogen.

Pre test **الاختبار القبلي**

Q\ Enumerate enteric gram negative rods?

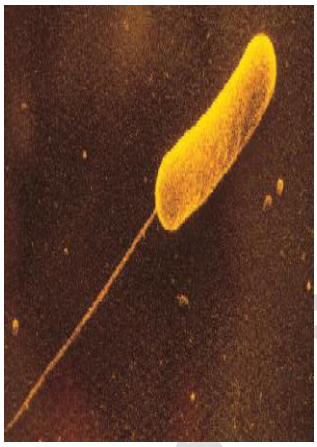
Scientific content المحتوى العلمي

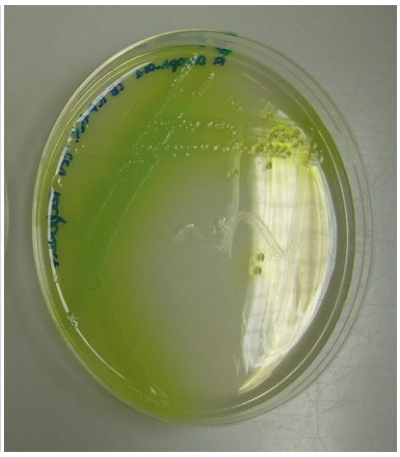
PSEUDOMONAS

The Pseudomonads are a large group of free-living bacteria that live primarily in soil, seawater, and fresh water. They also colonize plants and animals and are frequent contaminants in homes and clinical settings.

*Culture characteristics

These small, gram-negative rods have a single polar flagellum, produce oxidase and catalase, and do not ferment carbohydrates. Although they ordinarily obtain energy aerobically through oxidative metabolism, some species can grow anaerobically if provided with a salt such as nitrate. Many species produce green, brown, red, or yellow pigments that diffuse into the medium and change its color.





*Pseudomonas aeruginosa**

is a common inhabitant of **soil and water and an intestinal resident in about 10% of normal people. On occasion, it can be isolated from saliva or even the skin.** Because the species is **resistant to soaps, dyes, quaternary ammonium disinfectants, drugs, drying, and temperature extremes**, it is a frequent **contaminant of ventilators, intravenous solutions, and anesthesia equipment.**

Even disinfected instruments, utensils, bathroom fixtures, and mops have been incriminated in hospital outbreaks.

*Habitat Reservoir

Pseudomonas aeruginosa Environment (soil, water, plants); survives well in domestic environments (e.g., hot tubs, whirlpools, contact lens solutions) and hospital environments (e.g., sinks, showers, respiratory equipment); rarely part of normal microbiota of healthy humans

***Mode of Transmission**

Ingestion of contaminated food or water; exposure to contaminated medical devices and solutions; introduction by penetrating wounds; person-to-person transmission is assumed to occur.

Ps. aeruginosa is a **typical opportunist**. It is unlikely to cross healthy, intact anatomical barriers, thus its infectiousness **results from invasive medical procedures or weakened host defenses.**

***Ps. aeruginosa* expresses virulence factors including exotoxins, a phagocytosis-resistant slime layer, and various enzymes that degrade host tissues. It also causes endotoxic shock.**

Over 100,000 patients a year die from a condition called **septic shock.** Shock is a pathologic state of low blood pressure accompanied by a reduced amount of blood circulating to vital organs, particularly the brain, heart, lungs, and kidneys. Its major symptoms and signs are nausea, tachycardia, cold, clammy skin, and weak pulse. Damage to the organs can elicit respiratory failure, coma, heart failure, and death in a few hours. Although the endotoxins of all gram negative bacteria can cause shock, the most common clinical cases are due to gram-negative enteric rods.

The adverse factor in gram-negative sepsis is the presence of an endotoxin called lipopolysaccharide (LPS) in the outer membrane of the gram-negative cell wall. Lipopolysaccharide is a potent immune stimulator. The component that accounts for most of the adverse effects is *lipid A* embedded in the external layer of the membrane. This lipid is active only after it is liberated by bacteria that are growing or lysed by host defenses and other factors. In general, lipid A triggers the secretion of interleukins, tumor necrosis factor, and other cytokines by macrophages.

*Diseases caused by *Pseudomonas aeruginosa*

The most common nosocomial *Pseudomonas* infections occur in compromised hosts with **severe burns, neoplastic disease, and cystic fibrosis. Complications include pneumonia, urinary tract infections, abscesses, otitis, and corneal disease. *Pseudomonas* septicemia can give rise to diverse and grave conditions such as endocarditis, meningitis, and bronchopneumonia. Infection is especially virulent in premature infants and neonates. Healthy people are subject to outbreaks of skin rashes, urinary tract infections, and external ear infections from community hot tubs and swimming pools.**

***Ps. aeruginosa* infections are a grapelike odor and the noticeable color that appears in tissue exudates ("blue pus").**

The notorious **multidrug resistance** of *Ps. aeruginosa* makes testing.

Drugs found effective in controlling infections and treatment are the **third-generation Cephalosporins, aminoglycosides, carbenicillin, polymyxin, quinolones, amonobactams**.

Acinetobacter

The organisms are a diverse group of nonfermenting gram-negative bacilli. These emerging pathogens are clinically important because they can cause a wide range of infections and are often multidrug resistant (MDR). Similar to the *Enterobacteriaceae*, these organisms are oxidase negative and generally grow well on MacConkey agar, advanced molecular taxonomic studies, 34 unique species of *Acinetobacter* have been identified.

Of note, only *Acinetobacter* species and *Stenotrophomonas maltophilia* are routinely recovered from clinical specimens and will be discussed in depth; other less frequently encountered genera and species will be briefly mentioned. Through DNA homology studies, the genus *Acinetobacter* has been moved out of the *Neisseriaceae* family to the family *Moraxellaceae.*

Acinetobacter species are aerobic, catalase-positive, oxidasenegative, Gram-negative bacteria that are ubiquitous in nature and widely distributed in soil and water *A. baumannii* is the species most commonly isolated in clinical laboratories.

Other clinically relevant *Acinetobacter* species that are associated with health care infections include *A. nosocomialis*, *A. pittii*, and *A. ursingii*; *A. lwoffii* and *A. radioresistens* have been described to colonize human skin and cause occasionally infections in immunocompromised patients. Acinetobacters are usually coccobacillary or coccal in appearance, but rod-shaped form may also be seen. *Acinetobacter* may also appear as diplococci on smears and then resemble *Neisseria*; however, the *Neisseria* produces oxidase, and *Acinetobacter* does not. *Acinetobacter* grows well on most types of agar media used to culture specimens from patients. While most acinetobacters grown between 20°C and 35°C, the majority of medically important acinetobacters grow best at 35°C to 37°C; some strains of *A. baumannii* are capable of growing at 44°C. Accurate species-level identification of *Acinetobacter* using conventional biochemical tests and/or commercially available phenotypic identification systems can be challenging at time; newer test methods such as MALDI-TOF mass spectrometry have been shown to reliable identify various *Acinetobacter* species, specifically those that are medically relevant.

Acinetobacters are opportunistic pathogens, known to cause nosocomial infections. *A. baumannii* has been isolated from blood, sputum, skin, pleural fluid, and urine.

With increasing frequency, *Acinetobacter* has been isolated from hospitalized patients in the ICU or those requiring mechanical ventilation, leading to increased morbidity, length of stay and higher mortality.

Laboratory Diagnosis

Specimen

Urine, csf, blood, fluid, wound and burn swab, sputum.

Direct Detection Methods

Other than Gram stain of patient specimens, there are no specific procedures for the direct detection of these organisms in clinical material. *Acinetobacter* spp. are plump coccobacilli that tend to resist alcohol decolorization, thus appearing gram positive. They also may be mistaken for *Neisseria* spp. as a result of their similar microscopic appearance (i.e., shape and arrangement).

Cultivation

Media of Choice

All of the organisms discussed grow well on routine media such as Trypticase soy agar with 5% sheep blood and chocolate agars. common nutrient broths, such as thioglycollate and brain-heart infusion.

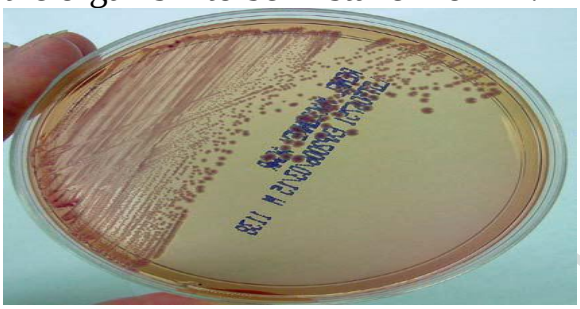
Incubation Conditions and Duration

growth on 5% sheep blood and chocolate agars when incubated at 35°C to 37°C in aerobic conditions or 5% carbon dioxide after 24 hours. MacConkey agar should be incubated only in ambient air.

Colonial Appearance

Acinetobacter spp. Blood Agar ---------- Smooth, opaque, raised, creamy, and smaller than *Enterobacteriaceae;* some genospecies are beta-hemolytic

MacConkey agar ------- NLF, but colonies exhibit a purplish hue that may cause the organism to be mistaken for LF.



Diseases caused by *Acinetobacter spp.*

1-pneumonia
2-bloodstream infections, and are commonly associated with intravascular devices.
3-meningitis
4-wound infections (eg, severe trauma and burn wounds)
5-urinary tract infections (associated with biofilm production on indwelling urinary catheters).

The pathogenicity of *Acinetobacter*

strains is related to the organism's ability to create biofilms on surfaces and human cells and its increasing antimicrobial resistance. Many of the acinetobacters isolated in the hospital setting are multidrug resistant, and therapy of these infections can be difficult. In many cases, the only active antimicrobial agent may be **colistin**. Such multidrug- resistant *A. baumannii* strains were a common cause

of serious wound infections among servicemen in Iraq who sustained traumatic injuries.

Post test **الاختبار البعدي**

Q\ Discuss pathogenicity of *Acinetobacter baumannii* ?

References **المصادر**

الجامعة التقنية الوسطى
كلية التقنيات الصحية والطبية \بغداد
قسم تقنيات المختبرات الطبية

المادة :الأحياء المجهرية

المرحلة :الثانية

Title: العنوان

Yersinia **Lec 22**

Name of the instructor: اسم المحاضر:

Estabraq Ali AL-Sodani د. استبرق علي السوداني
د.جليل نجاح

Target population: الفئة المستهدفة:

Second stage students

Introduction: المقدمة:

are short, pleomorphic Gram-negative rods that often exhibit bipolar staining. They are catalase positive and microaerophilic or facultatively anaerobic. While most of the organisms discussed here have animals as their natural hosts, they are known to cause zoonotic infections and produce at times serious disease in humans.

The genus *Yersinia* includes 17 different species; however, only three species are pathogenic to humans, whereas the other 14 are considered to be environmental species and nonpathogenic. The three human pathogens include:

Yersinia pestis
Yersinia enterocolitica
Yersinia pseudotuberculosis

These three *Yersinia* species generally cause disease in domestic and wild animals (eg, pigs, rodents, and birds) and humans are usually considered to be incidental hosts. *Y. pestis* is the cause of plague; *Y. enterocolitica* and *Y. pseudotuberculosis* are zoonotic food-borne pathogens, both typically causing a mild diarrheal disease, following ingestion of contaminated food and/or water.

Q\ *Yersinia* three human pathogens include:-

Scientific content المحتوى العلمي

YERSINIA PESTIS AND PLAGUE

While the epizootic cycle of plague has not been completely understood, infection with *Y. pestis* is fundamentally a disease of rodents, and plague is found in various endemic foci around the world. Humans are incidental, "dead-end" hosts that become infected when the plague bacillus is transmitted via flea bite or by exposure to fluids and tissues from an infected animal.

As a result of such exposure, a serious infection will develop, often with a high mortality (40–100%).
Plague "Black Death," has caused at least three major pandemics in previous centuries. The ability of this organism to be easily transmitted by aerosol and the severity and high mortality associated with pneumonic plague make *Y. pestis* quite suitable for being a potential agent of biowarfare.

Morphology and Identification

Y. pestis is a Gram-negative rod that exhibits striking bipolar staining with special stains such as Wright, Giemsa, Wayson,or methylene blue.

It is nonmotile. It grows as a facultative anaerobe on many bacteriologic media and can be readily isolated when sterile specimens such as blood or lymph node aspirates are plated onto sheep blood agar. Growth is more rapid when agar plates are incubated at **28°C.**
In cultures on sheep blood agar incubated at 37°C, colonies may be smaller

when compared to colonies from agar plates incubated at 28°C. To enhance the recovery of *Y. pestis* from a nonsterile site specimen (eg, sputum), it is

recommended to inoculate the specimen onto cefsulodin-irgasan-novobiocin (CIN) agar and incubate agar plates at 25–28°C. Colonies of *Y. pestis* are typically gray to white, sometimes opaque, and are 1–1.5 mm in diameter with irregular edges; the organism does not produce hemolysis.

Antigenic Structure

All Yersiniae possess 1- lipopolysaccharides that have endotoxic activity when released.

2-*Y. pestis* and *Y. enterocolitica* also produce antigens and toxins that act as virulence factors.

3-They have type III secretion systems that consist of a membranespanning complex that allows the bacteria to inject proteins
directly into cytoplasm of the host cells.

4-The virulent yersiniae produce V and W antigens, which are essential for virulence; the V and W antigens yield the requirement for calcium for growth at 37°C.

Virulence Factors

1- capsular and envelope proteins, which protect against phagocytosis and foster intracellular growth.

2- The bacillus also produces coagulase, which clots blood and is involved in clogging the esophagus in fleas and obstructing blood vessels in humans.

3-Other factors that contribute to pathogenicity are endotoxin and a highly potent murine toxin.

The Enteric *Yersinia* Pathogens

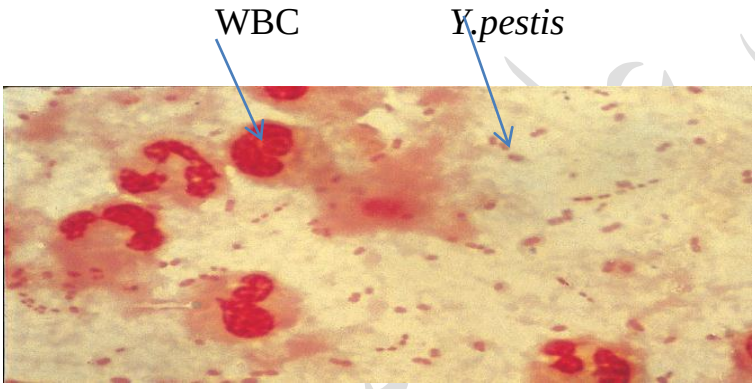
Although formerly classified in a separate category, the genus *Yersinia** has been placed in the Family Enterobacteriaceae on the basis of cultural, biochemical, and serological characteristics. All three species cause zoonotic infections called *yersinioses. Yersinia enterocolitica* and *Y. pseudotuberculosis* are intestinal inhabitants of wild and domestic animals that cause enteric infections in humans, and *Y. pestis* is the nonenteric agent of bubonic plague. *Yersinia enterocolitica* has been isolated from healthy and sick farm animals, pets, wild animals, and fish, as well as fruits, vegetables, and drinking water. During the incubation period of

about 4 to 10 days, the bacteria invade the small intestinal mucosa, and some cells enter the lymphatics and are harbored intracellularly in phagocytes. Inflammation

of the ileum and mesenteric lymph nodes gives rise to severe abdominal pain that mimics appendicitis. *Yersinia pseudotuberculosis* shares many characteristics with *Y. enterocolitica*, though infections by the former are more benign and center upon lymph node inflammation rather than mucosal involvement.

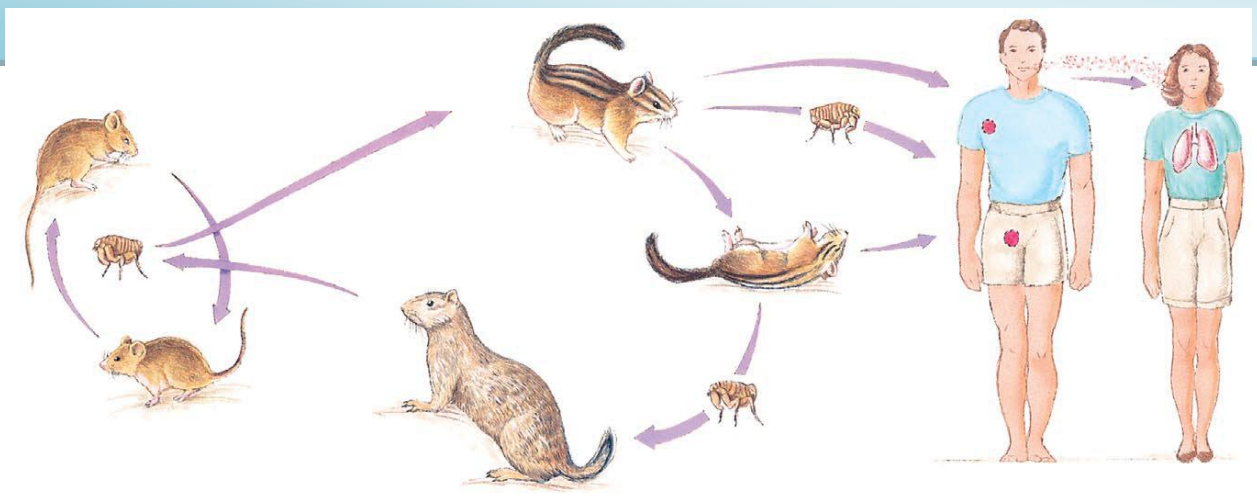
NONENTERIC *YERSINIA PESTIS* AND PLAGUE

The cause of this dread disease is a tiny, harmless-looking gram-negative rod called ***Yersinia pestis,*** formerly *Pasteurella pestis*, with unusual bipolar staining and capsules.



The Life Cycle of Plague

The plague bacillus exists naturally in many animal hosts, and its distribution is extensive, though the incidence of disease has been reduced in most areas.



The infection cycle of *Yersinia pestis* simplified for clarity.

Humans can develop plague through contact with wild animals **(sylvatic plague),** domestic or semidomestic animals **(urban plague),** or infected humans.

The Animal Reservoirs The plague bacillus occurs in 200 different species of mammals. **rodents** such as **mice** and **voles** that harbor the organism but do not develop the disease. These hosts spread the disease to other mammals called *amplifying hosts* that become infected with the bacillus and experience massive die-offs during epidemics.
These hosts, including rats, ground squirrels, chipmunks, and rabbits, are the usual sources of human plague.
The particular mammal that is most important in this process depends on the area of the world. Other mammals (camels, sheep, coyotes, deer, dogs, and cats) can also be involved in the transmission cycle.
Flea Vectors The principal agents in the transmission of the plague bacillus from reservoir hosts to amplifying hosts to human are fleas.

Clinical Findings

three forms of the disease have been described:
1-bubonic plague
2-pneumonic plague
3- septicemic plague

Bubonic plague is by far the most common clinical presentation of infection with *Y. pestis*. After an incubation period of 2–7 days, there is a sudden onset of high fever and development of painful lymphadenopathy, commonly with greatly enlarged, tender lymph nodes (buboes) in the neck, groin, or axillae.

Septicemic plague can occur spontaneously or as a complication of untreated bubonic plague. In this form of the disease, *Y. pestis* multiplies intravascularly and can be seen in blood smears. Patients typically present with a sudden onset of high fever, chills, and weakness, progressing rapidly to septic shock with associated disseminated intravascular coagulation, hypotension (septic shock), altered mental status, and renal and cardiac failure. Bleeding into skin and organs can also occur. Vomiting and diarrhea may develop during the early stages of septicemic plague. pneumonia and meningitis can appear. Primary pneumonic plague results from direct inhalation of organisms into the lung. This form of the disease typically occurs through close and direct contact with another patient who has pneumonic plague, and symptoms begin within 1–4 days after the exposure.

Diagnostic Laboratory Tests

A. Specimens

Blood is taken for culture and aspirates of enlarged lymph nodes for smear and culture. Acute and convalescent sera may be examined for antibody levels. In pneumonia, sputum is cultured; in possible meningitis, cerebrospinal fluid is taken for smear and culture.

B. Smears

Y. pestis are small Gram-negative bacilli that appear as single cells or as pairs or short chains in clinical material. Wright, Giemsa, or Wayson stains may be more useful when staining material from a suspected buboes or a positive blood culture result because of the striking bipolar appearance (safety pin shape) of the organism using these stains that is not evident on a possibly available through reference laboratories) include the use of **fluorescent antibody stains targeting the capsular F1 antigen.**

C. Culture

All materials are cultured on blood agar, chocolate, and Mac- Conkey agar plates and in brain–heart infusion broth. Incubation about 24-48hr Cultures can be tentatively identified by biochemical reactions. *Y. pestis* produces

nonlactose-fermenting colonies on MacConkey agar, and it grows better at 25°C than at 37°C. The organism is catalase positive; indole, oxidase, and urease negative; and nonmotile.

NOTE All cultures are highly infectious and must be handled with extreme caution inside a biological safety cabinet.

D. Serology

In patients who have not been previously vaccinated, a convalescent serum antibody titer of 1:16 or greater is presumptive evidence of *Y. pestis* infection.

A titer rise in two sequential specimens confirms the serologic diagnosis.

Treatment

The drug of choice is Streptomycin, but the more readily available Aminoglycoside Gentamicin has been shown to be as effective. Streptomycin is nephrotoxic and ototoxic and should therefore be cautiously used in elderly patients, pregnant women, and in children.

Q\Enumerate Diagnostic Laboratory Tests for *Yersinia pestis*

References **المصادر**

د.عصام جمعة ناصر

Title: **العنوان**

Vibrio **Lec 23**

Title: **Vibrio.**

Name of the instructor: Assistant Professor Dr. Issam Jumaa Nasser

Target population: Students of the second stage of the Department of Medical Laboratory Technologies

Introduction:

Vibrio is Gram-negative rods that are all widely distributed in nature. The vibrios are found in marine and surface waters. Aeromonads are inhabitants of aquatic ecosystems, worldwide, and are found in fresh and brackish waters. Vibrio cholerae produces an enterotoxin that causes cholera, a profuse watery diarrhea that can rapidly lead to dehydration and death.

Pretest:

1. Describe the general characteristics of the organisms discussed in this chapter, including natural habitat,

route of transmission, Gram stain reactions, and cellular morphology.

2. Describe the media used to isolate Vibrio spp. and the organisms' colonial appearance.

3. Correlate the patient's signs and symptoms and laboratory data to identify an infectious agent

Scientific Content:

THE VIBRIOS

Vibrios are among the most common bacteria in marine and estuarine waters, worldwide. They are comma-shaped, curved, and sometimes straight facultatively anaerobic, fermentative rods; they are catalase and oxidase positive, and most species are motile by means of monotrichous or multitrichous polar flagella. Vibrios can grow within a broad temperature range (14–40°C), and all species require sodium chloride (NaCl) for growth; hence the term halophilic ("salt loving"). *V. cholerae* serogroups O1 and O139 cause cholera in humans, and other vibrios, most commonly *V. parahaemolyticus* and *V. vulnificus*, are important human pathogens, causing skin and soft tissue infections, sepsis, or gastroenteritis.

General Characteristics

The organisms discussed in this chapter are considered together because they are all oxidase-positive, glucose fermenting, gram-negative bacilli capable of growth on MacConkey agar. Their individual morphologic and physiologic features are presented later in this chapter in the discussion of laboratory diagnosis. Other halophilic (salt-loving) organisms, such as *Shewanella algae,* require salt but do not ferment glucose, as do the halophilic *Vibrio* spp. *Aeromonas* spp. are gram-negative straight rods with rounded ends or coccobacillary facultative anaerobes that occur

singly, in pairs, or in short chains. They are typically oxidase and catalase positive and produce acid from oxidative and fermentative metabolism.

The family *Vibrionaceae* includes six genera, the *Photobacterium* and *Grimontia* each include a single species. The genus *Vibrio* consists of 10 species of gram-negative, facultative anaerobic, curved or comma-shaped rods. Most *Vibrio* spp. Require sodium for growth and glucose fermentation; with the exception of *Vibrio metschnikovii,* all species are motile and catalase and oxidase positive.

Organism	Human Disease
V. cholerae serogroups O1 and O139	Epidemic and pandemic cholera
V. cholerae serogroups non-O1/non-O139	Cholera-like diarrhea; mild diarrhea; rarely, extraintestinal infection
V. parahaemolyticus	Gastroenteritis, wound infections, septicemia
V. vulnificus	Gastroenteritis, wound infections, septicemia

Vibrio cholerae Enterotoxin

V. cholerae produce a heat-labile enterotoxin with a molecular weight (MW) of about 84,000, consisting of subunits A(MW, 28,000) and B. Ganglioside GM1 serves as the mucosal receptor for subunit B, which promotes entry of subunit A into the cell. Activation of subunit A1 yields increased levels of intracellular cyclic adenosine monophosphate (cAMP) and results in prolonged hypersecretion of water and electrolytes. There is increased sodium-dependent

chloride secretion, and absorption of sodium and chloride by the microvilli is inhibited. Electrolyte-rich diarrhea occurs with as much as 20–30 L/day, resulting in dehydration, shock,

acidosis, and death. The genes for V. cholerae enterotoxin are located on the bacterial chromosome. Cholera enterotoxin is antigenically related to LT of Escherichia coli and can stimulate the production of neutralizing antibodies. However, the precise role of antitoxic and antibacterial antibodies in protection against cholera is not clear.

Diagnostic Laboratory Tests

A. Specimens

As stated above, stool specimens should be collected early in the course of the diarrheal illness and inoculated within2–4 hours of collection onto appropriate agar media, to ensure optimal recovery of vibrios. If processing of specimens may be delayed, the stool specimen should be mixed in **a Cary-Blair transport medium and refrigerated.**

B. Smears

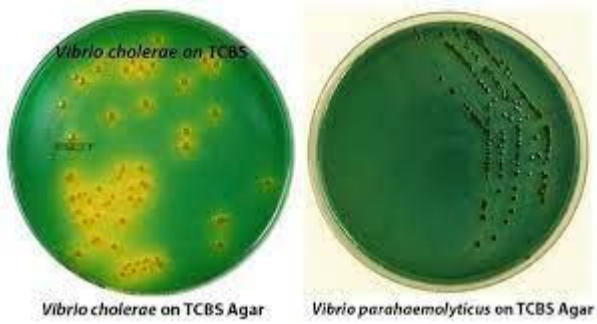
Direct detection of V. cholerae on smears made from stool samples is not distinctive of the organism, and therefore not routinely recommended. Dark-field or phase-contrast microscopy can be used to detect V. cholerae O1 directly from stool samples or the enrichment broth. Observation of “shooting star” motility is suggestive of V. cholerae O1; if the motility is extinguished after mixing the sample with a polyvalent O1 antiserum, the organism is confirmed as V. cholerae O1. However, if there is no motility or the type of

motility does not change after applying the antiserum, the organism is not V. cholerae O1.

C. Culture

Vibrios, including V. cholerae, grow well on most agar media (including MacConkey and blood agar) used in clinical laboratories. Some strains of V. cholerae may however be inhibited on MacConkey agar. Growth is rapid in alkaline peptone broth or water, containing 1% NaCl with a

pH of 8.5, or on TCBS agar; typical colonies can be picked in 18 hours of growth. For enrichment, a few drops of stool can be incubated for 6–8 hours in taurocholate peptone broth (pH, 8.0–9.0); organisms from this culture can then be stained or subculture onto other appropriate agar media. Accurate identification of vibrios, including V. cholerae, using commercial systems and kit assays is quite variable. MALDI-TOF MS is a promising newer methodology for identification of vibrios, and studies have shown rapid and reproducibly accurate identification for V. parahaemolyticus.



Posttest

Describe the media used to isolate Vibrio spp. and the organisms' colonial appearance

Explain the physiologic activity of the cholera toxin and its relationship to the pathogenesis of the organism.

Describe the media used to isolate Vibrio spp. and the organisms' colonial appearance

Explain the physiologic activity of the cholera toxin and its relationship to the pathogenesis of the organism.

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Lec 24 د. عصام جمعة ناصر

Title: العنوان

Title: Campylobacter and Helicobacter
Name of the instructor: Assistant Professor Dr. Issam Jumaa Nasser
Target population: Students of the second stage of the Department of Medical Laboratory Technologies

Introduction:

CAMPYLOBACTER

Campylobacters cause both diarrheal and systemic diseases and are among the most widespread causes of infection, worldwide. *Campylobacter* infections of wild and domesticated animals, which are also the natural reservoirs for these organisms, are also widespread. *C. jejuni* is the prototype organism in the group and is a very common cause of diarrhea in humans. Other campylobacters, less commonly isolated from humans, include *C. fetus*, *C. coli*, and *C. upsaliensis*.

Pretest:

1. List the *Campylobacter* species most often associated with infections in humans, and explain how they are transmitted.

2. Identify the culture methods for optimum recovery of *Campylobacter jejuni* and *Campylobacter coli,* including agar, temperatures, oxygenation, and length of incubation.

Scientific Content:

CAMPYLOBACTER JEJUNI

C. jejuni has emerged as a common human pathogen, causing mainly gastroenteritis and occasionally systemic infections.
This organism is the most common cause of bacterial gastroenteritis in the United States;

Morphology and Identification

A. Typical Organisms

C. jejuni and other campylobacters are curved, comma-, or S-shaped, Gram-negative, non-spore-forming

rods; they
have also been described as having “sea gull wing” shapes Campylobacters are motile, with a single polar flagellum at one or both ends, but some organisms may lack flagella all together.

B. Culture

Campylobacter species, including *C. jejuni*, multiply at a slower rate when compared to other Gram-negative, enteric
bacteria; therefore, selective media, containing various antibiotics (eg, Campy-Blood agar and Skirrow’s media) are
needed for isolation of campylobacters from stool specimens. *Campylobacter* species require a microaerobic atmosphere,
containing reduced O2 (5–7%) and increased 10% CO2 for incubation and optimal growth. A relatively simple way to

produce the incubation atmosphere is to place the plates in an anaerobe incubation jar without the catalyst and to produce the gas with a commercially available gas-generating pack or by gas exchange. Furthermore, most campylobacters growbest at 42°C, although growth can be seen on agar media with incubation between 36°C and 42°C. Incubation of primaryplates for isolation of *C. jejuni* should always be at 42°C. Several selective agar media are in widespread use for isolation of campylobacters; Skirrow’s medium contains vancomycin, polymyxin B, and trimethoprim to inhibit growth of other bacteria, but this medium may be less sensitive than other commercial products that contain charcoal, other inhibitory compounds, as well as cephalosporin antibiotics.

C. Growth Characteristics

Because of the selective media and incubation conditions for growth, an abbreviated set of tests is usually all that is necessary for further identification of campylobacters. *C. jejuni* as well as *C. coli* are positive for both oxidase and catalase. Campylobacters do not oxidize or ferment carbohydrates. Gramstained smears show typical morphology. Nitrate reduction, hydrogen sulfide production, hippurate hydrolysis tests, and antimicrobial susceptibilities can be used for further identification of species. A positive hippurate hydrolysis test distinguishes *C. jejuni* from the other *Campylobacter* species.

Antigenic Structure and Toxins

The campylobacters have lipopolysaccharides with endotoxic activity. Cytopathic extracellular toxins and enterotoxins
have been found, but the significance of the toxins in human disease is not well defined.

Pathogenesis and Pathology

The infection is acquired by the oral route from food, drink, or contact with infected animals or animal products, especially poultry. *C. jejuni* is susceptible to gastric acid, and ingestion of about 104 organisms is usually necessary to
produce infection.
This inoculum is similar to that required for *Salmonella* and *Shigella* infection but less than that for *Vibrio* infection. The
organisms multiply in the small intestine, invade the epithelium, and produce inflammation that results in the
appearance of red and white blood cells in the stools. Occasionally, the bloodstream is invaded, and a clinical picture of enteric fever develops. Localized tissue invasion coupled with the toxic activity appears to be responsible for the enteritis

Diagnostic Laboratory Tests

A. Specimens
Diarrheal stool is the preferred specimen when attempting to isolate campylobacters in patients with gastrointestinal illness. Rectal swabs may also be acceptable specimens. *C. jejuni* and *C. fetus* may occasionally be recovered from blood cultures usually from immunocompromised or elderly patients.

B. Smears
Gram-stained smears of stool may show the typical "gull wing"–shaped rods. Dark-field or phase-contrast microscopy
may show the typical darting motility of the organisms.
C. Culture
Culture on the selective media as described earlier is the definitive test to diagnose *C. jejuni* enteritis. If *C. fetus* or
another species of *Campylobacter* is suspected, an agar medium without cephalosporins should be used and incubation at 36–37°C is necessary

HELICOBACTER PYLORI

Members of the genus *Helicobacter* are usually spiral, curved, or fusiform rod-shaped Gram-negative bacteria. *Helicobacter* species have been isolated from the gastrointestinal and hepatobiliary tract of many different mammalian hosts, including humans, dogs, cats, pigs, cattle, and other domestic and wild animals. The various helicobacters can be divides into two groups: *Helicobacter* species that

primarily colonize the stomach (gastric helicobacters), and those that colonize the intestines (enterohepatic helicobacters). Humans are the primary host-reservoir for *H. pylori*, which is a spiral-shaped, Gram-negative, catalase- and oxidase-positive, and ureasepositive rod. *H. pylori* is associated with antral gastritis, duodenal (peptic) ulcer disease, gastric ulcers, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphomas.

Morphology and Identification

A. Typical Organisms

Helicobacter species, including *H. pylori*, have many characteristics in common with campylobacters. *Helicobacter*
species are motile and have single and/or multiple monopolar flagella that are typically sheathed and can vary greatly in
their flagellum morphology.

B. Culture

While *H. pylori* can be readily isolated from gastric biopsy specimens, culture sensitivity may be limited by several factors, including delayed specimen transport and processing, prior antimicrobial therapy, or contamination with other
mucosal bacteria. Special transport media (eg, Stuart's transport medium) should be used to main the organisms' viability when transport to the laboratory is anticipated to exceed2 hours. *H. pylori* usually grows within 3–6 days when incubated at 37°C in a microaerophilic and humid atmosphere; however, incubation of up to 14 days may be necessary before
resulting the culture as negative. To achieve a higher yield for recovery of the organism, the biopsy specimen may be
homogenized prior to streaking onto the agar plate. The agar media for primary isolation include enriched agar media
supplemented with blood and/or blood products (eg, chocolate agar) or antibiotic-containing media such as Skirrow's
medium, in order to suppress overgrowth by other competing bacterial flora. The colonies have varying

appearance on
blood agar ranging from gray to translucent and are 1–2 mm in diameter.

C. Growth Characteristics

H. pylori is oxidase positive and catalase positive, and has a characteristic Gram-stain morphology; the organism is motile, and is a strong producer of urease.

Pathogenesis and Pathology

H. pylori is able to survive in the acidic environment of the stomach and ultimately establish lifelong colonization of
the gastric mucosa in the absence of antimicrobial treatment. While *H. pylori* grows optimally at a pH of 6.0–7.0,
it would be killed or not grow at the pH within the gastric lumen (pH 1–3). Several factors contribute to the organism's
ability to overcome the acidic environment of the stomach, contributing to colonization, inflammation, changes in gastric acid production, and tissue destruction. Gastric mucus is relatively impermeable to acid and has a strong buffering capacity. On the lumen side of the mucus, the pH is low (1.0–3.0); on the epithelial side, the pH is about 5.0–7.0. After entering the stomach, *H. pylori* utilizes its urease activity to neutralize the gastric acid; intracellular urease activity as well
as urease located on the bacterial cell surface allow for the breakdown of urea into ammonia and CO2; NH3 is converted
to ammonium (NH4+) and extruded from the bacterial cell leading to neutralization of the gastric acid. Flagella-mediated motility then allows the organisms, protected from the gastric acid, to move through the gastric mucus toward the
epithelium. H. pylori is found deep in the mucous layer near the epithelial surface where a near physiologic pH is present.

Diagnostic Laboratory Tests

A. Specimens

Gastric biopsy specimens can be used for histologic examination or minced in saline and used for

culture. Blood is collected for determination of serum antibodies. Stool samples may be collected for *H. pylori* antigen detection.

B. Smears

The diagnosis of gastritis and *H. pylori* infection can be made histologically; this approach is generally more sensitive than

culture. A gastroscopy procedure with biopsy is required. Routine stains (eg, hematoxylin & eosin stain) demonstrate

acute/chronic gastritis, and Giemsa or special stains (eg, show the silver stains or immunohistochemical stains) can curved or spiral-shaped organisms.

C. Culture

Since *H. pylori* organisms adhere to the gastric mucosa, the bacteria cannot be recovered from stool specimens like other

gastrointestinal pathogens. As described above, culture is usually performed when patients are not responding to treatment, and there is a need to perform antimicrobial susceptibility testing. Tissue for culture is obtained by endoscopy and biopsy of the gastric mucosa.

D. Antibodies

Several assays have been developed to detect serum antibodies specific for *H. pylori*. While testing for IgG serum antibodies against *H. pylori* is useful to confirm the exposure to the organism, either for epidemiologic purposes or for the evaluation of a symptomatic patient, the antibody titers do not typically correlate with the severity of the disease

.

Posttest

3. Describe how to isolate *Campylobacter* from blood, including special stains, atmospheric conditions, and length of incubation.
4. List the colonial morphology, microscopic characteristics, and biochemical reactions of *Campylobacter* and *Helicobacter.*
5. List the key biochemical test to identify *Helicobacter pylori* in specimens.
6. Describe how *H. pylori* colonize in the stomach and how motility plays an important role in the pathogenesis of the organism.
7. Describe why therapy is often problematic for *H. pylori*

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المادة :الأحياء المجهرية

المرحلة :الثانية

د. عصام جمعة ناصر

Title: العنوان

Haemophyilus Bordetella and Brucella Lec 25

Title: Haemophilus, Bordetella and Brucella.
Name of the instructor: Assistant Professor Dr. Issam Jumaa Nasser
Target population: Students of the second stage of the Department of Medical Laboratory Technologies

Introduction:

THE HAEMOPHILUS SPECIES

This is a group of small, Gram-negative, pleomorphic bacteria that require enriched media, usually containing blood or its
derivatives, for isolation. *Haemophilus influenzae* is a major human pathogen; *Haemophilus ducreyi*, a sexually transmitted pathogen, causes chancroid; six other *Haemophilus* species are among the normal microbiota of mucous membranes and only occasionally cause human disease.
Haemophilus aphrophilus and *Haemophilus paraphrophilus* have been combined into a single new species *Aggregatibacter*
aphrophilus; likewise, *Haemophilus segnis*

Pretest:

1. List the general characteristics within the genus *Haemophilus,* including general habitat, atmosphere, and
temperature requirements.

2. Describe the infections caused by *Haemophilus influenzae* and *Haemophilus ducreyi.*

3. Describe the difference in the typeable and nontypeable categories of *Haemophilus,* their virulence factors, and the disease they cause

Scientific Content:

HAEMOPHILUS INFLUENZAE

H. influenzae is found on the mucous membranes of the upper respiratory tract in humans. It is an important cause of meningitis in unvaccinated children and causes upper and lower respiratory tract infections in children and in adults.

Morphology and Identification

A. Typical Organisms

In specimens from acute infections, the organisms are short(1.5 μm) coccoid bacilli, sometimes occurring in pairs or
short chains. In cultures, the morphology depends both on the length of incubation and on the medium. At 6-8 hours in rich medium, the small coccobacillary forms predominate. Later, there are longer rods and very pleomorphic forms.
Some organisms in young cultures (6-18 hours) on enriched medium express a definite capsule. The capsule is the antigen used for "typing" *H. influenzae*

B. Culture

On chocolate agar, flat, grayish, translucent colonies with diameters of 1-2 mm are present after 24 hours of incubation.
IsoVitaleX in media enhances growth. *H. influenzae* does not grow on sheep blood agar except around colonies of staphylococci ("satellite phenomenon"). *Haemophilus haemolyticus* and *Haemophilus parahaemolyticus* are hemolytic variants of *H. influenzae* and *Haemophilus parainfluenzae*, respectively.

C. Growth Characteristics

Identification of organisms of the *Haemophilus* group depends partly on demonstrating the need

for certain growth factors called X and V. Factor X acts physiologically as hemin; factor V can be replaced by nicotinamide adenine dinucleotide (NAD) or other coenzymes. Colonies of staphylococci on sheep blood agar cause the release of NAD, yielding the satellite growth phenomenon. The requirements for X and V factors of various *Haemophilus* Carbohydrate fermentation are useful in species identification as is the presence or absence of hemolysis.

Antigenic Structure

Encapsulated *H. influenzae* contains **capsular polysaccharides** (molecular weight > 150,000) of one of six types (a-f). The capsular antigen of type b is a polyribitol ribose phosphate (PRP). Encapsulated *H. influenzae* can be typed by slide agglutination, coagglutination with staphylococci, or agglutination of latex particles coated with type-specific antibodies A capsule swelling test with specific antiserum is analogous to the quellung test for pneumococci.

Pathogenesis

H. influenzae produces no exotoxin. The nonencapsulated organism is a regular member of the normal respiratory microbiota of humans. The capsule is antiphagocytic in the absence of specific anticapsular antibodies. The polyribose phosphate capsule of type b *H. influenzae* is the major virulence factor. The carrier rate in the upper respiratory tract for *H. influenzae* type b was 2-5% in the prevaccine era and is now less than 1%. The carrier rate for NTHi is 50-80% or higher. Type b *H. influenzae* causes meningitis, pneumonia and empyema, epiglottitis, cellulitis, septic arthritis, and

occasionally other forms of invasive infection. NTHi tends to cause chronic bronchitis, otitis media, sinusitis, and conjunctivitis after breakdown of normal host defense mechanisms. The carrier rate for the encapsulated types a and c to f is low (1-2%), and these capsular types rarely cause disease. Although type b can cause chronic bronchitis, otitis media, sinusitis, and conjunctivitis, it does so much less commonly than NTHi. Similarly, NTHi only occasionally causes invasive disease (~5% of cases).

Clinical Findings

H. influenzae type b enters by way of the respiratory tract. There may be local extension with involvement of the sinuses

or the middle ear. H. influenzae, mostly nontypeable, and pneumococci are two of the most common etiologic agents

of bacterial otitis media and acute sinusitis. Lower respiratory tract infections such as bronchitis and pneumonia may be seen in patients with conditions that diminish mucociliary clearance.

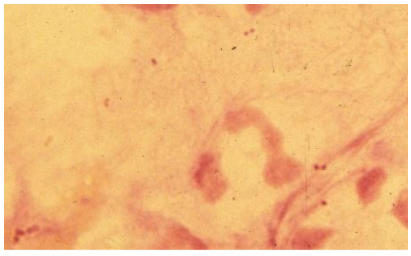
Diagnostic Laboratory Tests

A. Specimens

Specimens consist of expectorated sputum and other types of respiratory specimens, pus, blood, and spinal fluid for smears and cultures depending on the source of the infection.

B. Direct Identification

Commercial kits are available for immunologic detection of *H. influenzae* antigens in spinal fluid. These antigen detection tests generally are not more sensitive than a



Gram-stain and therefore are not widely used, especially because the incidence of *H. influenzae* meningitis is so low. Their use discouraged in all but limited resource settings where disease prevalence remains high. A Gram-stain of *H. influenzae* in sputum is depicted in Figure 18-1. Nucleic acid amplification methods have been developed by some laboratories and may soon be commercially available for direct detection for cerebrospinal fluid and lower respiratory tract infections

C. Culture

Specimens are grown on IsoVitaleX-enriched chocolate agar until typical colonies appear (see above). *H. influenzae* is differentiated from related Gram-negative bacilli by its requirements for X and V factors and by its lack of hemolysis on blood agar Tests for X (heme) and V (nicotinamide adenine dinucleotide) factor requirements can be done in several ways. The *Haemophilus* species that require V factor grow around paper strips or disks containing V factor placed on the surface of agar that has been autoclaved before the blood was added (V factor is heat labile). Alternatively, a strip containing X factor can be placed in parallel with one containing V factor on agar deficient in these nutrients. Growth of *Haemophilus* in the area between the strips indicates requirement for both factors. A better test for X factor requirement is based on the inability of *H. influenzae* (and a few other *Haemophilus* species) to synthesize heme from δ-aminolevulinic acid.

Gram-stain of *H. influenzae* in sputum. The organisms are very small (0.3 × 1 μm), Gram-negative coccobacilli(*small arrows*). The large, irregularly shaped objects (*large arrow*) are the nuclei of polymorphonuclear cells. Mucus is faintly stained pink in the background.

Posttest

1. A-Describe the Gram stain and colonial morphology of the various *Haemophilus* spp.

   B-. Describe the isolation requirements necessary for optimal recovery of *Haemophilus,* including any special specimen processing or transport requirements.

   C-. Explain the satellite phenomenon and the chemical basis for the phenomenon.

   D-. List the X and V factor requirements for *H. influenzae, Haemophilus parainfluenzae,*

2. A-. Explain the principle of the porphyrin test.

   B- Explain why routine susceptibility testing of clinical isolates for *H. influenzae* is only necessary on strains of clinical significance (i.e., sterile sites).

   C- Correlate patient signs, symptoms, and laboratory data to identify the most probable etiologic agent associated
   with an infection

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2011;60(RR-3):13-15.

THE BORDETELLAE

There are several species of *Bordetella*. *Bordetella pertussis*, a highly communicable and important pathogen of humans,
causes whooping cough (pertussis). *Bordetella parapertussis* can cause a similar disease. *Bordetella bronchiseptica* (*Bordetella bronchicanis*) causes diseases in animals, such as kennel cough in dogs and snuffles in rabbits, and only occasionally causes respiratory disease and bacteremia in humans. Newer species and their disease associations include *Bordetella hinzii* (bacteremia, respiratory illness, arthritis), *Bordetella holmesii* (bacteremia among immunosuppressed patients), and *Bordetella trematum* (wound infections and otitis media). *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* are closely related, with 72-94%
DNA homology and very limited differences in multilocus enzyme analysis; the three species might be considered three subspecies.

BORDETELLA PERTUSSIS

Morphology and Identification

A. Typical Organisms

The organisms are minute, Gram-negative coccobacilli resembling *H. influenzae*. A capsule is

present.

B. Culture

Primary isolation of *B. pertussis* requires enriched media. Bordet-Gengou medium (potato-blood-glycerol agar) that
contains penicillin G, 0.5 μg/mL, can be used; however, a charcoal-containing medium supplemented with horse blood, cephalexin, and amphotericin B (Regan-Lowe) is preferable because of the longer shelf life. The plates are incubated at 35-37℃ for 3-7 days aerobically in a moist environment (eg, a sealed plastic bag). The small, faintly staining Gramnegative rods are identified by immunofluorescence staining. B. pertussis is nonmotile.

C. Growth Characteristics

The organism is a strict aerobe and it is oxidase and catalase positive but nitrate, citrate, and urea negative, the results of
which are useful for differentiating among the other species of bordetellae. It does not require X and V factors on subculture.

Antigenic Structure and, Pathogenesis

B. pertussis produces a number of factors that are involved in the pathogenesis of disease. One locus on the *B. pertussis*
chromosome acts as a central regulator of virulence genes. This locus has two *Bordetella operons*, **bvgA** and **bvgS**. The
products of the A and S loci are similar to those of known two-component regulatory systems. *bvgS* responds to environmental signals, and *bvgA* is a transcriptional activator of the virulence genes. **Filamentous hemagglutinin**,
a large surface protein, and fimbriae (surface appendages) mediate adhesion to ciliated epithelial cells and are essential for tracheal colonization. **Pertussis toxin** (a classic A/B structure toxin) promotes lymphocytosis, sensitization to histamine, and enhanced insulin

secretion by means of adenosine diphosphate-ribosylating activity that disrupts function of signal transduction in many cell types.

Clinical Findings

After an incubation period of about 2 weeks, the "catarrhal stage" develops, with mild coughing and sneezing. During this stage, large numbers of organisms are sprayed in droplets, and the patient is highly infectious but not very ill. During the "paroxysmal" stage, the cough develops its explosive character and the characteristic "whoop" upon inhalation. This leads to rapid exhaustion and may be associated with vomiting, cyanosis, and convulsions. The "whoop" and major complications occur predominantly in infants; paroxysmal coughing predominates in older children and adults.

Diagnostic Laboratory Tests

A. Specimens

Nasopharyngeal (NP) swabs or NP aspirates using saline are the preferred specimens. Swabs should be either Dacron or rayon tipped and not calcium alginate, as it inhibits the polymerase chain reaction (PCR), nor cotton, as cotton kills the organisms. For adults, cough droplets expelled directly onto a "cough plate" held in front of the patient's mouth during a paroxysm is a less desirable method of specimen collection.

B. Direct Fluorescent Antibody Test

The fluorescent antibody (FA) reagent can be used to examine nasopharyngeal swab specimens. However, false-positive and false-negative results may occur; the sensitivity is about 50%. The FA test is most useful in identifying *B. pertussis* after culture on solid media.

C. Culture

NP aspirates or swabs are cultured on solid media. The antibiotics in the media tend to inhibit other respiratory microbiota but permit growth of *B. pertussis*. Organisms are identified by immunofluorescence staining or by slide agglutination with specific antiserum

D. Polymerase Chain Reaction

PCR and other nucleic acid amplification methods are the most sensitive methods to diagnose pertussis. Primers for both *B. pertussis* and *B. parapertussis* should be included. Where available, a nucleic acid amplification test should replace the direct FA tests. Existing primer targets may crossreact with other *Bordetella* species.

E. Serology

Production of IgA, IgG, and IgM antibodies occurs after exposure to *B. pertussis*, and these antibodies can be detected by enzyme immunoassays.

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THE BRUCELLAE

The brucellae are obligate parasites of animals and humans and are characteristically located intracellularly. They are relatively inactive metabolically. *Brucella melitensis* typically infects goats; *Brucella suis*, swine; *Brucella abortus*, cattle; and *Brucella canis*, dogs. Other species are found only in animals. Although named as species, DNA relatedness studies have shown there is only one species in the genus, *B. melitensis*, with multiple biovars. The disease in humans, brucellosis (undulant fever, Malta fever), is characterized by an acute bacteremic phase followed by a chronic stage that may extend over many years and may involve many tissues.

Morphology and Identification

A. Typical Organisms

The appearance in young cultures varies from cocci to rods1.2 μm in length, with short coccobacillary forms predominating. They are Gram-negative but often stain irregularly, and they are aerobic, nonmotile, and nonspore forming.

B. Culture

Small, convex, smooth colonies appear on enriched media in2-5 days.

C. Growth Characteristics

Brucellae are adapted to an intracellular habitat, and their nutritional requirements are complex. Some strains have been cultivated on defined media containing amino acids, vitamins, salts, and glucose. Fresh specimens from animal or human sources are usually inoculated on trypticase-soy agar or blood culture media. Whereas *B. abortus* requires 5-10% CO_2 for growth, the other three species grow in air.

Pathogenesis

The common routes of infection in humans are the intestinal tract (ingestion of infected milk), mucous membranes (droplets), and skin (contact with infected tissues of animals). Cheese made from unpasteurized goats' milk is a particularly common vehicle. The organisms progress from the portal of entry via lymphatic channels and regional lymph nodes to the thoracic duct and the bloodstream, which distributes them to the parenchymatous organs. Granulomatous nodules that may develop into abscesses form in lymphatic tissue, liver, spleen, bone marrow, and other parts of the reticuloendothelial system.

B. Culture

Brucella agar was specifically designed to culture *Brucella* species bacteria. The medium is highly enriched and-in reduced form-is used primarily in cultures for anaerobic bacteria. In oxygenated form, the medium grows *Brucella* species bacteria very well. However, infection with *Brucella* species is often not suspected when cultures of a patient's specimens are set up, and *Brucella* agar incubated aerobically is seldom used. *Brucella* species bacteria grow on commonly used media, including trypticase-soy medium with or without 5% sheep blood, brain-heart infusion medium, and chocolate agar. Blood culture media readily grow *Brucella* species bacteria. Liquid medium used to culture *Mycobacterium tuberculosis* also supports the growth of at least some strains. All cultures should be incubated in8-10% CO_2 at 35-37℃ and should be observed for 3 weeks before being discarded as being negative; liquid media cultures should be blindly subcultured during this time.

C. Serology

Laboratory diagnosis of brucellosis is most frequently accomplished by serologic testing. IgM antibody levels rise during the first week of acute illness, peak at 3 months, and may persist during chronic disease. Even with appropriate antibiotic therapy, high IgM levels may persist for up to 2 years in a small percentage of patients.

1- Agglutination test—To be reliable, serum agglutination tests must be performed with standardized heat-killed, phenolized, smooth *Brucella* antigens. IgG agglutinin titers above 1:80 indicate active infection.

2. Blocking antibodies-These are IgA antibodies that interfere with agglutination by IgG and IgM and cause a serologic test result to be negative in low serum dilutions(prozone), although positive in higher dilutions.

3. Brucellacapt (Vircell, Granada, Spain)—This is a rapid immunocapture agglutination method based on the Coombs test that detects nonagglutinating IgG and IgA antibodies. It is easy to perform and has a high sensitivity and specificity.

4. ELISA assays—IgG, IgA, and IgM antibodies may be detected using enzyme-linked immunosorbent assays (ELISA), which use cytoplasmic proteins as antigens. These assays tend to be more sensitive and specific than the agglutination test especially in the setting of chronic disease.

Posttest

1. Identify the primary routes of transmission for *Brucella* spp.

2. List the occupations at risk for developing brucellosis.

3. Identify the signs and symptoms associated with brucellosis.

4. State two reasons why the microbiology laboratory should be notified when *Brucella* spp. infection is suspected.

5. Describe the media and incubation requirements to isolate *Brucella* spp.

6. Describe the differential characteristics of *Brucella abortus, Brucella melitensis, Brucella suis,* and *Brucella canis*

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د.عصام جمعة ناصر

Title: العنوان

Chlamydia and Spirochetes Lec 26

Title: Chlamydia and Spirochaetes.
Name of the instructor: Assistant Professor Dr. Issam Jumaa Nasser
Target population: Students of the second stage of the Department of Medical Laboratory Technologies

Pretest:

1. Define the following: bubo, proctitis, bartholinitis, salpingitis, elementary body, reticulate body, Whipple disease, morulae, and Donovan body.

2. Describe the general characteristics for the organisms included in this chapter, including Gram stain characteristics, cultivation methods (media and growth conditions), transmission, and clinical significance.
3. Explain the mechanism and location for the replication of *Chlamydia* spp.

4. Compare the clinical manifestations and diagnosis of trachoma and other oculogenital infections associated with *Chlamydia*

Introduction:

Chlamydiae that infect humans are divided into three species, Chlamydia trachomatis, Chlamydia pneumoniae, and Chlamydia psittaci, on the basis of antigenic composition, intracellular inclusions, sulfadiazine susceptibility, and disease production. The separation of the genus Chlamydia into the genera Chlamydia and Chlamydophila remains controversial; in this chapter, the three chlamydiae that are pathogens of humans are considered to be in the genus Chlamydia in keeping with publications that do not support the new taxonomy. Other chlamydiae infect animals but rarely if ever infect humans. All chlamydiae exhibit similar morphologic features, share a common group antigen, and multiply in the cytoplasm of their host cells by a distinctive developmental cycle. The chlamydiae can be viewed as Gram-negative bacteria that lack mechanisms for the

production of metabolic energy and cannot synthesize adenosine triphosphate (ATP). This restricts them to an intracellular existence, where the host cell furnishes energy-rich intermediates. Thus, chlamydiae are **obligate intracellular pathogens**.

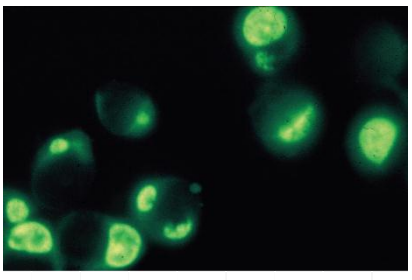
Developmental Cycle

All chlamydiae share a common and unique biphasicdevelopmental cycle. The environmentally stable infectious particle (transmissible form) is a small cell called the **elementary body** (**EB**). These are about 0.3 μm in diameter with an electron-dense nucleoid. The EB membrane proteins have highly cross-linked membrane proteins. The EBs have a high affinity for host epithelial
cells and rapidly enter them. The first step in entry involves interaction between outer membrane proteins of the EB and
heparin sulfate proteoglycan of the host cells. The second step involves additional and irreversible binding to a variety of other host cell receptors. There appear to be multiple adhesins, such as OmcB, the major outer membrane protein **MOMP**), glycosylated MOMP, and other surface proteins. Following adherence, the mechanisms thought to mediate (entry into the host cell also vary and involve cytoskeletal rearrangements and activation of type III secretion systems and other effectors.

Structure and Chemical Composition

In chlamydiae, the outer **cell wall** resembles the cell wall of Gram-negative bacteria. It has a relatively high lipid content
including lipopolysaccharide of low endotoxic activity. It is rigid but does not contain a typical bacterial peptidoglycan.

As mentioned above, another important structural component is the MOMP encoded by *ompA*. MOMP antigenic



variants of *C*

. trachomatis are associated with different clinical syndromes. Penicillin-binding proteins occur in chlamydiae, and chlamydial cell wall formation is inhibited by penicillins and other drugs that inhibit transpeptidation of bacterial peptidoglycan. Lysozyme has no effect on chlamydial cell walls. *N*-acetylmuramic acid appears to be absent from as DNA, whereas the EBs contain about equal amounts of RNA and DNA. In EBs, most DNA is concentrated in the electron-dense central nucleoid. Most RNA exists in ribosomes. The circular genome of chlamydiae is 1.04 megabases in length, encodes 900 genes, and is one of the smallest bacterial genomes.

Staining Properties

Chlamydiae have distinctive staining properties (similar to those of rickettsiae). Elementary bodies stain purple with
Giemsa stain-in contrast to the blue of host cell cytoplasm. The larger, noninfective RBs stain blue with Giemsa stain. The
Gram reaction of chlamydiae is negative or variable and is not useful in identification of the agents. Chlamydial particles and
inclusions stain brightly by immunofluorescence, with groupspecific, species-specific, or serovar-specific antibodies.

Similar growth of *C. trachomatis* in McCoy cells stained with a fluoresceinlabeled antibody against a *C. trachomatis* species antigen

Classification

Chlamydiae are classified according to their pathogenic potential, host range, antigenic differences, and other methods. Three species that infect humans have been characterized.

	Chlamydia trachomatis	*Chlamydia pneumoniae*	*Chlamydia psittaci*
Inclusion morphology	Round, vacuolar	Round, dense	Large, variable shape, d
Glycogen in inclusions	Yes	No	No
Elementary body morphology	Round	Pear shaped, round	Round
Susceptible to sulfonamides	Yes	No	No
Plasmid	Yes	No	Yes
Serovars	15	1	≥4
Natural host	Humans	Humans, animals	Birds
Mode of transmission	Person to person, mother to infant	Airborne person to person	Airborne bird excreta to
Major diseases	Trachoma, STDs, infant pneumonia, LGV	Pneumonia, bronchitis, pharyngitis, sinusitis	Psittacosis, pneumonia, unexplained origin

A. *Chlamydia trachomatis*

This species produces compact intracytoplasmic inclusions that contain glycogen; it is usually inhibited by sulfonamides.

It includes agents of human disorders such as trachoma, inclusion conjunctivitis, nongonococcal urethritis, salpingitis, cervicitis, pneumonitis of infants, and LGV.

B. *Chlamydia pneumoniae*

This species produces intracytoplasmic inclusions that lack glycogen; it is usually resistant to sulfonamides. It causes

respiratory tract infections in humans.

C. *Chlamydia psittaci*

This species produces diffuse intracytoplasmic inclusions that lack glycogen; it is usually resistant to sulfonamides. It

includes agents of psittacosis in humans, ornithosis in bird's feline pneumonitis, and other animal diseases.,

Laboratory Diagnosis

A. Specimen Collection

Proper specimen collection is the key to the laboratory diagnosis of chlamydia infection. Because the chlamydiae are

obligate intracellular bacteria, it is important that the specimens contain infected human cells as well as the extracellular material where they might also be present.

B. Nucleic Acid Detection

Nucleic acid amplification tests (NAATs) are the tests of choice for the diagnosis of

genital *C. trachomatis* infections.

C. Direct Cytologic Examination (Direct Fluorescent Antibody) and Enzyme-Linked Immunoassay

Commercially available direct fluorescent antibody (DFA) and enzyme-linked immunoassay (EIA) assays to detect
C. trachomatis continue to be marketed.

D. Culture

Culture of *C. trachomatis* has historically been used to diagnose chlamydia infections. Culture, however, is costly and arduous.
Results are delayed compared with the timeliness of NAATs and other tests. Culture is generally much less sensitive than
NAATs; the degree of lower sensitivity is largely dependent on the culture method used. Culture is now done in a limited
number of reference laboratories. A number of susceptible cell lines can be used, most often McCoy, HeLa 229, or HEp-2.
The cells are grown in monolayers on coverslips in dram or shell vials.

E. Serology

Because of the relatively great antigenic mass of chlamydiae in genital tract infections, serum antibodies occur much more
commonly than in trachoma and are of higher titer. A titer rise occurs during and after acute chlamydial infection.

Posttest

1. List the appropriate specimens used for the isolation of the Chlamydia trachomatis

2. Describe the correct collection method for a specimen to be submitted for *Chlamydia trachomatis* screening from the female genital tract.

3. Explain the three stages associated with lymphogranuloma venereum, and compare the disease with

other
genital infections

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Spirochetes:

Pretest:

1. Describe the bacterial agents discussed in this chapter in terms of morphology, taxonomy, and growth conditions.

2. Identify the four stages of syphilis (i.e., primary, secondary, latent, and tertiary) according to clinical symptoms, antibody production, transmission, infectivity, and treatment.
3. Explain congenital syphilis, including transmission and clinical manifestations.

Introduction:

The spirochetes are a large, heterogeneous group of spiral, motile bacteria. One family (Spirochaetaceae) of the order Spirochaetales consists of two genera whose members are human pathogens: *Borrelia* and *Treponema*. The other family (Leptospiraceae) includes one genus of medical importance: *Leptospira*. The spirochetes have many structural characteristics in common, as typified by *Treponema pallidum*

They are long, slender, helically coiled, spiral, or corkscrewshaped bacilli. *T. pallidum* has an **outer sheath** or glycosaminoglycan coating. Inside the sheath is the outer membrane, which contains peptidoglycan and maintains the structural integrity of the organisms. **Endoflagella** (axial filaments) are the flagella-like organelles in the periplasmic space encased by the outer membrane. The endoflagella begin at each end of the organism and wind around it, extending to and overlapping at the midpoint. Inside the endoflagella is the inner membrane (cytoplasmic membrane) that provides osmotic stability and covers the protoplasmic cylinder. A series of cytoplasmic tubules (body fibrils) are inside the cell near the inner membrane. Treponemes reproduce by transverse fission.

TREPONEMA PALLIDUM AND SYPHILIS

Morphology and Identification

A. Typical Organisms

T. pallidum are slender spirals measuring about 0.2 μm in width and 5-15 μm in length. The spiral coils are regularly
spaced at a distance of 1 μm from one another. The organisms are actively motile, rotating steadily around their endoflagella
even after attaching to cells by their tapered ends. The long axis of the spiral is ordinarily straight but may sometimes bend so
that the organism forms a complete circle for moments at a time, returning then to its

normal straight position.

The spirals are so thin that they are not readily seen unless immunofluorescent stain or dark-field illumination is

used. They do not stain well with aniline dyes, but they can be seen in tissues when stained by a silver impregnation method.

B. Culture

Pathogenic *T. pallidum* has never been cultured continuously on artificial media, in fertile eggs, or in tissue culture

In proper suspending fluids and in the presence of reducing substances, *T. pallidum* may remain motile for 3-6 days

at 25° C. In whole blood or plasma stored at 4° C, organisms remain viable for at least 24 hours, which is of potential importance in blood transfusions.

D. Reactions to Physical and Chemical Agents

Drying kills the spirochete rapidly, as does elevation of the temperature to 42° C. Treponemes are rapidly immobilized

and killed by trivalent arsenical, mercury, and bismuth(contained in drugs of historical interest in the treatment

of syphilis). Penicillin is treponemicidal in minute concentrations, but the rate of killing is slow, presumably because of the metabolic inactivity and slow multiplication rate of *T. pallidum* (estimated division time is 30 hours). Resistance

to penicillin has not been demonstrated in syphilis.

E. Genome

The *T. pallidum* genome is a circular chromosome of approximately 1,138,000 base pairs, which is small for bacteria.

Most pathogenic bacteria have transposable elements, but *T. pallidum* does not, which suggests

that the genome is highly conserved and may explain its continued susceptibility to penicillin. There are few genes involved in energy production and synthesis of nutrients, indicating that *T. pallidum* obtains these from the host.

Antigenic Structure

The fact that *T. pallidum* cannot be cultured in vitro has markedly limited the characterization of its antigens.

The outer membrane surrounds the periplasmic spaced the peptidoglycan-cytoplasmic membrane complex. Membrane proteins are present that contain covalently a bound lipids at their amino terminals. The lipids appear to anchor the proteins to the cytoplasmic or outer membranes and keep the proteins inaccessible to antibodies. The endoflagella are in the periplasmic space. *T. pallidum* has hyaluronidase that breaks down the hyaluronic acid in the ground substance of tissue and presumably enhances the invasiveness of the organism.

Pathogenesis, Pathology, and Clinical Findings

A. Acquired Syphilis

Natural infection with *T. pallidum* is limited to the human host. Human infection is usually transmitted by sexual contact, and the infectious lesion is on the skin or mucous membranes of genitalia. In 10-20% of cases, however, the primary lesion is intrarectal, perianal, or oral. It may be anywhere on the body. *T. pallidum* can probably penetrate intact mucous membranes, or the organisms may enter through a break in the epidermis. Based on experiments in rabbits, as few as four to eight spirochetes may cause infection

B. Congenital Syphilis

A pregnant woman with syphilis can transmit *T. pallidum* to the fetus through the placenta

beginning in the
10th-15th weeks of gestation. Some of the infected fetuses die, and miscarriages result; others are stillborn at term.
Others are born live but develop the signs of congenital syphilis in childhood, including interstitial keratitis, Hutchinson's teeth, saddlenose, periostitis, and a variety of central nervous system anomalies.

Diagnostic Laboratory Tests

A. Specimens

Specimens include tissue fluid expressed from early surface lesions for demonstration of spirochetes by either dark-field
microscopy or immunofluorescence; such specimens can also be tested by nucleic acid amplification. Blood can be
obtained for serologic tests; cerebrospinal fluid (CSF) is useful for Venereal Disease Research Laboratory (VDRL) testing

B. Dark-Field Examination

A drop of tissue fluid or exudate is placed on a slide, and a coverslip is pressed over it to make a thin layer. The preparation is then examined under oil immersion within 20 minutes of collection with dark-field illumination for typical motile

spirochetes. Dark-field microscopy should not be performed on lesions within the oral cavity because it is not possible to
differentiate pathogenic from commensal spirochetes. Treponemes disappear from lesions within a few hours after the beginning of antibiotic treatment.

C. Immunofluorescence

Tissue fluid or exudate is spread on a glass slide, air-dried, and sent to the laboratory. It is fixed, stained with a fluoresceinlabeled antitreponeme antibody and examined by means of immunofluorescence microscopy for typical fluorescent spirochetes

D. Serologic Tests for Syphilis

These tests use either nontreponemal or treponemal antigens.

1. Nontreponemal tests—The nontreponemal tests are universally used as screening tests for syphilis. The tests are widely available, lend themselves to automation with ease of performance in large numbers, and have a low cost. In addition to their function as screening tests, they can be used to follow the efficacy of therapy.

2. Treponemal antibody tests—The treponemal tests measure antibodies against *T. pallidum* antigens. The tests are used to determine if a positive result from a nontreponemal test is truly positive or falsely positive.

Posttest

1. Define reagin, cardiolipin, and biologic false positive.
2. Differentiate reagin and treponemal antibodies, including specificity and association with disease.
3. Identify the various serologic methods that use specific treponemal or nonspecific nontreponemal antigens

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الجامعة التقنية الوسطى

Title: **العنوان**

Mycobacterium **Lec 27**

Name of the instructor: **Assistant Professor Dr. Issam Jumaa**

Target population: **الفئة المستهدفة:**

Second stage students

Introduction: **المقدمة:**

Introduction:

The mycobacteria are rod-shaped, aerobic bacteria that do not form spores. Although they do not stain readily, after being stained, they resist decolorization by acid or alcohol and are therefore called "acid-fast" bacilli. Mycobacterium tuberculosis causes tuberculosis and is a very important pathogen of humans. Mycobacterium leprae causes leprosy. Mycobacterium avium-intracellulare (M avium complex, or MAC) and other nontuberculous mycobacteria (NTM) frequently infect patients with AIDS, are opportunistic pathogens in other immunocompromised persons, and occasionally cause disease in patients with normal immune systems. There are more than 200 Mycobacterium species, including many that are saprophytes.

Pretest:

1.Describe the general characteristics of the Mycobacterium spp., including oxygen requirements, staining patterns and cell morphology, artificial media required for cultivation and growth, and pigmentation

2. Compare the general characteristics of mycobacteria to those of other groups of bacteria.

3. Compare the different culture media used for the isolation of mycobacteria.
4. Discuss the different tests used to identify mycobacteria, isolation and identification of Mycobacterium tuberculosis from a sputum specimen.
5. Compare continuous monitoring systems to those of conventional media for detecting mycobacterial species in clinical samples.
6. Discuss the clinical disease caused by Mycobacterium tuberculosis and describe the use of the tuberculin skin test.

Scientific Content:

General Characteristics

Mycobacteria are slender, slightly curved or straight, rod-shaped organisms 0.2 to 0.6 µm × 1 to 10 µm in size. They are nonmotile and do not form spores. The cell wall has extremely high lipid content; thus, mycobacterial cells resist staining with commonly used basic aniline dyes, such as those used in the Gram stain, at room temperature. Mycobacteria take up dye with increased staining time or application of heat but resist decolorization with acid-ethanol. This characteristic is referred to as acid fastness—hence, the term AFB—and is a basic characteristic in distinguishing mycobacteria from most other genera. Mycobacteria are strictly aerobic, but increased carbon dioxide (CO_2) will enhance the growth of some species. The pathogenic mycobacteria grow more slowly than most other bacteria pathogenic for humans. The rapidly growing species generally grow on simple media in 2 to 3 days at temperatures of 20° C to 40°C. Most mycobacteria associated with disease require 2 to 6 weeks of incubation on complex media at specific optimal temperatures. One of the mycobacteria pathogenic for humans, M. leprae, fails to grow in vitro.

Mycobacterium spp. have been classified according to phenotypic characteristics. The MTB complex consists of M. tuberculosis, M. bovis (including the vaccination strain bacillus Calmette-Guérin), M.africanum, M. canettii, and M. microti. M. africanum has been associated with human cases of TB in tropical Africa, and M. microti has been linked to TB in immunocompetent and immunocompromised individuals. In 1959 Runyon classified Nontuberculous Mycobacteria (NTM)into four groups (Runyon groups I to IV) based on the phenotypic characteristics of the various species, most notably the growth rate and colonial pigmentation (Table1)

Runyon Group	Number Group	Name Description
I	Photochromogens	NTM colonies that develop pigment on exposure to light after being grown in the dark and take longer than 7 days to appear on solid media
II	Scotochromogens	NTM colonies that develop pigment in the dark or light and take longer than 7 days to appear on solid media.
III	Nonphotochromogens	NTM colonies that are nonpigmented regardless of whether they are grown in the dark or light and take longer than 7 days to appear on solid media
IV	Rapid growers	NTM colonies that grow on solid media and take fewer than 7 days to appear

(TABLE 1) Runyon Classification of Nontuberculous Mycobacteria (NTM

Tuberculosis Signs and Symptoms

There aren't any for latent TB. You'll need to get a skin or blood test to find out whether you have it. There are usually signs if you have active TB disease. They include:

• A cough that lasts more than 3 weeks

• Chest pain• Coughing up blood• Feeling tired all the time• Night sweats

• Chills• Fever• Loss of appetite• Weight loss

Modes of transmission

• Inhalation• Ingestion• Inoculation• Transplacental route

Spread

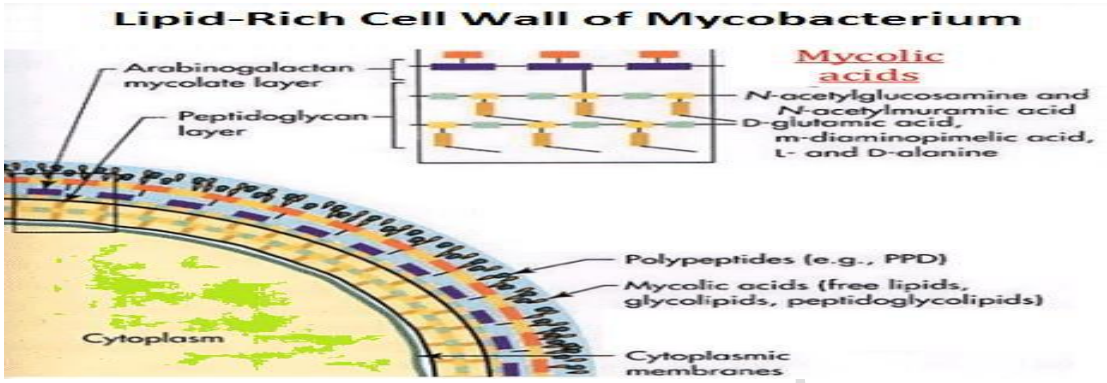
• Local• Lymphatic• Haematogenous

Constituents of Tubercle Bacilli

Lipids

Mycobacteria are rich in lipids. These include mycolic acids (long-chain fatty acids C78–C90), waxes, and phosphatides. In the cell, the lipids are largely bound to proteins and polysaccharides. Muramyl dipeptide (from peptidoglycan) complexed with mycolic acids can cause granuloma formation; phospholipids induce caseous necrosis. Lipids are to some extent responsible for acid fastness. Their removal with hot acid destroys acid fastness, which depends on both the integrity of the cell

wall and the presence of certain lipids. Acid fastness is also lost after sonication of mycobacterial cells.



Pathogenesis

Mycobacteria are emitted in droplets smaller than 25 µm in diameter when infected persons cough, sneeze, or speak. The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli. Inside the alveoli, the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages. Mycobacteria begin to multiply within macrophages. Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli. Pathogenic lesions associated with infection appear in the lung 1–2 months after exposure. Two types of lesions as described later under Pathology may develop. Resistance and hypersensitivity of the host greatly influence development of disease and the type of lesions that are seen.

Immunodiagnostic of Mycobacterium tuberculosis Infection

Skin Testing

The tuberculin skin test has been used for many years to determine an individual's exposure to *M. tuberculosis*. Protein extracted and purified from the cell wall of culture-grown *M. tuberculosis* is used as the antigen (i.e., purified protein derivative; PPD). A standardized amount of antigen is injected intradermally into the patient's forearm. Reactivity is read at 48 hours; in immunocompetent individuals, the presence of a raised firm area (induration) 10 mm or larger is considered reactive. A reactive tuberculin skin test indicates past exposure to *M. tuberculosis*;

other *Mycobacterium* spp. generally result in an induration smaller than 10 mm.

Immunocompromised patients with previous *M. tuberculosis* infection may also produce induration smaller than 10 mm. The skin test detects a patient's cellmediated immune response to the bacterial antigens in a type IV hypersensitivity reaction.

laboratory diagnosis of tuberculosis

AFB testing: used to detect several different types of acid-fast bacilli, most commonly active tuberculosis (TB) infection.

• **Ziehl Neelsen:** The reagents used are Ziehl–Neelsen carbol fuchsin, acid alcohol, and methylene blue. Acid-fast bacilli will be bright red.

• **Culture:** The first step is the decontamination of a non-sterile specimen such as sputum. This will then be grown in media.

• **Lowenstein-Jensen Media:** growth medium specially used for culture of Mycobacterium species, notably *Mycobacterium tuberculosis*. When grown on L.J medium, *M. tuberculosis* appears as brown, granular colonies.

• **Automated liquid culture system**

•**The interferon gamma release assay (IGRA) test** is a blood test used to see whether a person has been exposed to the tuberculosis (TB) bacteria. The IGRA test is used to diagnose TB infection. This is when the TB bacteria is in the body, but the person is not experiencing any symptoms suggestive of TB disease.

•**Nucleic Acid Amplification Tests (NAATs)**

NAATs are available for the rapid and direct detection of *M. tuberculosis* in clinical specimens. An advance PCR tests GeneXpert MTB/RIF test a real-time multiplex PCR method

Treatment

The two major drugs used to treat tuberculosis are INH and RMP. The other first-line drugs are pyrazinamide (PZA) and ethambutol (EMB). Second-line drugs are more toxic or less effective (or both), and they should be used in therapy only under extenuating circumstances (eg, treatment

failure, multiple drug resistance). Second-line drugs include kanamycin, capreomycin, ethionamide, cycloserine, ofloxacin, and ciprofloxacin

Pretest

1. Which of the following statements regarding interferon-γ release assays (IGRAs) is correct?

(A) They are useful for evaluating immunocompromised patients for active tuberculosis.

(B) They detect antigens present in all Mycobacterium species.

(C) They are not available yet for testing in the United States.

(D) They are performed using molecular probes that detect organism DNA.

(E) They are used as alternatives to the tuberculin skin test to evaluate for latent tuberculosis

Answer: (E)

2. The causative of tuberculosis is

(a) Virus

(b) Bacterium

(c) Malnutrition

(d) Protozoan

Answer: (b)

3. The first person who discovered Mycobacterium tuberculosis was

(a) Louis Pasteur

(b) Robert Koch

(c) Edward Jenner

(d) None of the above

Answer: (b)

4. Which of these is the culture medium for Mycobacterium tuberculosis?

(a) Wilson blair medium

(b) Löwenstein–Jensen medium

(c) Mac Conkey's medium

(d) None of the above

Answer: (b)

5. For Tuberculosis, the drugs used to combat it are

(a) Streptomycin, Pyrazinamide

(b) Isoniazid, Rifampicin

(c) Both (a) and (b)

(d) None of these

Answer: (c)

The definition of extensively drug-resistant (XDR) tuberculosis

includes

(A) Resistance to isoniazid

(B) Resistance to a fluoroquinolone

(C) Resistance to capreomycin, amikacin or kanamycin

(D) Resistance to rifampin

(E) All of the above

Answer: (E)

6. The BCG vaccine is administered for immunity against

(a) Malaria

(b) Tuberculosis

(c) Jaundice

(d) Hepatitis

Answer: (b)

7. A combination of medications which are applied to treat tuberculosis is

(a) to generate a better response

(b) to decrease the resistance of the entity to the treatment

(c) both (a) and (b)

(d) none of these

Answer: (c)

8. This is the reason why diagnosing tuberculosis is turning challenging

(a) disease takes years to become active

(b) symptoms are irregular, they appear and then vanish

(c) symptoms are not very obvious and prominent always

(d) both (b) and (c)

Answer: (a)

9. The causative of Tuberculosis produces Tuberculin, it is a/an

(a) enzyme

(b) hormone

(c) endotoxin

(d) exotoxin

Answer: (c)

10. This is the main symptom of Tuberculosis

(a) Liquid formation

(b) Tubercle formation

(c) both (a) and (b)

(d) None of these

Answer: (b)

11. Diagnosis of tuberculosis is done by

(a) Emulator and antiformin method

(b) Concentration method

(c) Petroff's method

(d) All of the above

Answer: (d)

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د.عصام جمعة ناصر

Title: **العنوان**

Introduction to medical Virology Lec 28

الجامعة التقنية الوسطى
كلية التقنيات الصحية والطبية/ بغداد
قسم: تقنيات المختبرات الطبية المادة: الاحياء المجهرية المرحلة: الثانية

Title: Introduction to Medical Virology

Name of the instructor: Assistant Professor Dr. Issam Jumaa
Target population: Students of the second stage of the Department of Medical Laboratory Technologies

Introduction:

Viruses are the smallest infectious agents (ranging from about 20 to 300 nm in diameter) and contain only one kind of nucleic acid (RNA or DNA) as their genome. The nucleic acid is encased in a protein shell, which may be surrounded by a lipid-containing membrane. The entire infectious unit is termed a virion. Viruses are parasites at the genetic level, replicating only in living cells and are inert in the extracellular environment. The viral nucleic acid

contains information necessary to cause the infected host cell to synthesize virus-specific macromolecules required for the production of viral progeny.

Pretest: List some of the viruses associated with the following clinical specimens: throat or nasopharyngeal swab or aspirate, urine, stool, lesion, blood, bone marrow, and stool or rectal swab

TERMS AND DEFINITIONS IN VIROLOGY

Schematic diagrams of viruses with icosahedral and helical symmetry are shown in Figure 29-1. Indicated viral components are described below.

Capsid: The protein shell, or coat, that encloses the nucleic acid genome.

Capsomeres: Morphologic units seen in the electron microscope on the surface of icosahedral virus particles. Capsomeres represent clusters of polypeptides, but the morphologic units do not necessarily correspond to the chemically defined structural units.

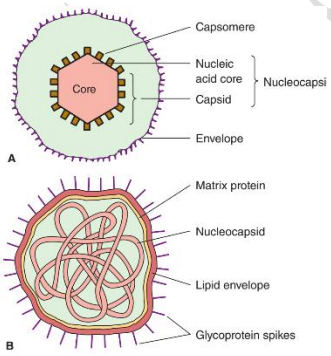
Defective virus: A virus particle that is functionally deficient in some aspect of replication.

Envelope: A lipid-containing membrane that surrounds some virus particles. It is acquired during viral maturation

by a budding process through a cellular membrane. Virus-encoded glycoproteins are exposed on the surface of the envelope. These projections are called **peplomers**.

Nucleocapsid: The protein–nucleic acid complex representing the packaged form of the viral genome. The term is commonly used in cases in which the nucleocapsid is a substructure of a more complex virus particle.

Structural units: The basic protein building blocks of the coat. They are usually a collection of more than one nonidentical protein subunit. The structural unit is often referred to as a **protomer**.



Subunit: A single folded viral polypeptide chain.

Virion: The complete virus particle. In some instances, eg, papillomaviruses and picornaviruses), the virion is identical with the nucleocapsid. In more complex virions (herpesviruses, orthomyxoviruses), this includes the nucleocapsid plus a surrounding envelope. This structure, the virion, serves to transfer the viral nucleic acid from one cell to another.

Schematic diagram illustrating the components of the complete virus particle (the virion). A: Enveloped virus with icosahedral symmetry. Not all icosahedral viruses have envelopes. B: Virus with helical symmetry.

CLASSIFICATION OF VIRUSES

Basis of Classification

The following properties have been used as a basis for the classification of viruses. The amount of information available in each category is not the same for all viruses. Genome sequencing is now often performed early in virus identification, and comparisons with databases provide detailed information on the viral classification, predicted protein composition, and taxonomic relatedness to other viruses.

1. Virion morphology, including size, shape, type of symmetry, presence or absence of peplomers, and presence or
absence of membranes.

2. Virus genome properties, including type of nucleic acid(DNA or RNA), size of the genome, strandedness (single or double), whether linear or circular, sense (positive, negative, ambisense), segments (number, size), nucleotide sequence, percent GC content, and presence of special features (repetitive elements, isomerization, 52-terminal cap, 52-terminal covalently linked protein, 32-terminal poly(A) tract.

3. Genome organization and replication, including gene order, number and position of open reading frames, strategy of replication (patterns of transcription, translation), and cellular sites (accumulation of proteins, virion assembly, virion release).

4. Virus protein properties, including number, size, amino acid sequence, modifications

(glycosylation, phosphorylation, myristoylation), and functional activities of structural and nonstructural proteins (transcriptase, reverse transcriptase, neuraminidase, fusion activities).

5. Antigenic properties, particularly reactions to various antisera.

6. Physicochemical properties of the virion, including molecular mass, buoyant density, pH stability, thermal stability,
and susceptibility to physical and chemical agents, especially solubilizing agents and detergents.

7. Biologic properties, including natural host range, mode of transmission, vector relationships, pathogenicity, tissue
tropisms, and pathology.

Universal System of Virus Taxonomy

A system has been established in which viruses are separated into major groupings—called **families**—on the basis of virion
morphology, genome structure, and strategies of replication. Virus family names have the suffix **-viridae**. Criteria used to define genera vary from family to family. Genus names carry the suffix *-virus*. In several families (Herpesviridae, Paramyxoviridae, Parvoviridae, Poxviridae

Viral Replication

Viruses are strict intracellular parasites that rely upon components of the host cell to replicate, and therefore are
capable of replication only within a host cell. The six steps of virus replication, called the **infectious cycle,** proceed as follows

1. **Attachment,** also referred to as adsorption, is the first step of the infectious cycle. It involves recognition of a
suitable host cell and specific binding between viral capsid proteins (often the glycoprotein spikes) and the carbohydrate receptor of the host cell. Each type of

virus specifically recognizes and attaches to a specific type of host cell, allowing infection of some tissues but not others (viral tropism, as described previously).

2. **Penetration** (also referred to as virus entry) is the process by which viruses enter the host cell. One mechanism of penetration involves fusion of the viral envelope with the host cell membrane. This method not only provides a mechanism for internalizing the virus, but also leads to fusion between the infected host and additional
nearby host cells, forming multinucleated cells called **syncytia**

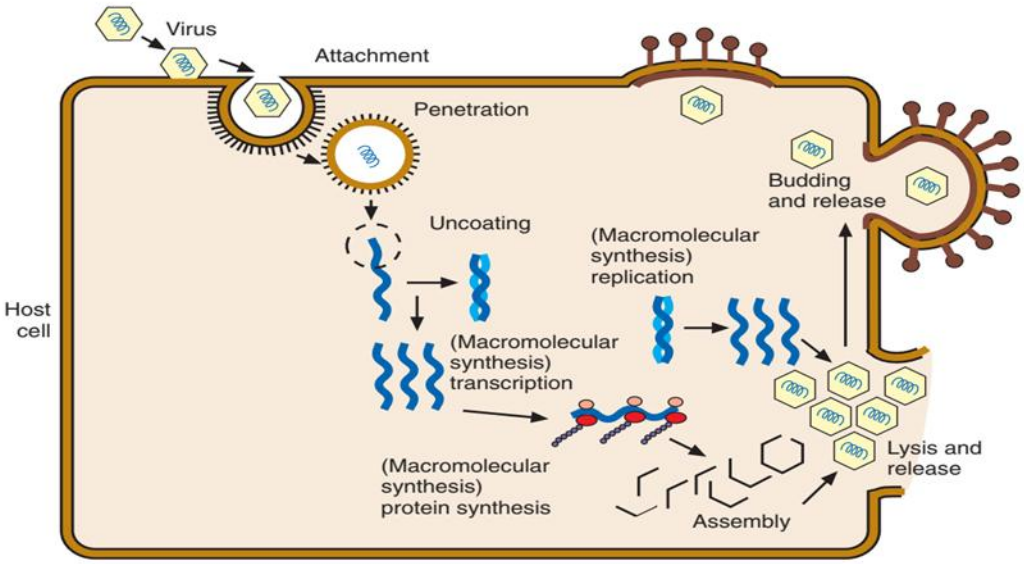
3. **Uncoating** occurs once the virus has been internalized. It is the process by which the capsid is removed; this may be by degradation of viral enzymes or host enzymes or by simple dissociation. Uncoating is necessary to release the viral genome for delivery of the viral DNA or RNA to its intracellular site of replication in the nucleus or cytoplasm.

4. **Macromolecular synthesis** involves the production of nucleic acids and protein polymers. Viral transcription leads to the synthesis of messenger RNA (mRNA), which encodes early and late viral proteins. Early proteins are nonstructural elements, such as enzymes, and late proteins are structural components. Rapid identification of virus in a cell culture can be accomplished by detecting early viral proteins in infected cells using immunofluorescent staining techniques. In addition, replication of viral nucleic acid is necessary to synthesize genomes that are incorporated into progeny virus particles. The mechanics of macromolecular synthesis varies depending on the organization of the viral genome (i.e., positive or negative sense RNA, single- or double-stranded nucleic acid genome).

5. **Viral assembly** is the process by which structural proteins, genomes, and in some cases viral enzymes are assembled into virus particles. Envelopes are

acquired during viral "budding" from a host cell membrane. Nuclear endoplasmic reticulum and cytoplasmic membranes are common areas for budding. Acquisition of an envelope is the final step in viral assembly.

6. **Release** of intact virus particles occurs after cell lysis (**lytic virus**) or by virus particle **budding** from cytoplasmic membranes. Release by budding may not result in rapid host cell death, as does release by cell lysis. Detection of virus in cell cultures is facilitated by recognition of areas of cell lysis. Detection of virus released by budding is more difficult, because the cell monolayer remains intact. Influenza viruses, which are released by budding with minimal cell destruction, can be detected in cell culture by an alternative technique called **hemadsorption.**



viral infectious cycle. (Modified from Murray PR, Drew WL, Kobayashi GS, et al, editors: Medical microbiology, St Louis, 1990, Mosby.)

Pathogenesis and Spectrum of Disease

Once introduced into a host, the virus infects susceptible cells and the infectious cycle begins. Viral infections may produce one of three characteristic clinical presentations: (1) **acute viral infection,** displaying evident signs and symptoms; (2) **latent infection,** which has no visible signs and symptoms, but the virus is still present in the host cell in a **lysogenic state** (inserted into the host genome in a resting state) or maintained as a nuclear or cytoplasmic **episome;** and (3) **chronic or persistent infection,** in which low levels of virus are detectable and the degree of visible signs or symptoms varies.

Laboratory Diagnosis of Viral Infections

Laboratories can provide different levels of services, based on the mission, financial resources, and need. All these must be balanced to provide the most cost-effective and complete diagnostics that will meet the needs of the clinical staff. Full-service virology laboratories provide viral culture and identification using different mammalian **cell cultures** to support the growth of viruses from clinical specimens. Although not all medical treatment facilities provide full virology services, these laboratories can still provide information about viral infections using rapid tests that detect specific viruses in clinical specimens. These tests can involve the detection of viral antigens by a number of methods, such as immunofluorescence (IF) or enzyme immunoassay (EIA). Some tests are Clinical Laboratory Improvement Act (CLIA)–waived, which brings viral identification services into physicians' offices and clinics. Other laboratories limit their virology services to viral serology—determining the patient's immune response to viruses—rather than detecting the viruses directly. While this is sometimes useful, it is usually 3 to 4 weeks after infection before these antibodies are produced, which may mean treatment is too late or not needed. Many new molecular methods based on nucleic acid detection and amplification are being used in more high-complexity, full-service virology laboratories. This technology can detect viral infections very early in infection, and many tests are completed in less than 2 hours.

Posttest:

1.Describe the physical components that make up a virion and list a function for each component.

2. Define the viral infectious cycle, including naming the six steps in this process.

3. Explain viral tropism and provide a specific example related to a human pathogen

4. Define the properties used to classify viruses, and how these properties can be used to predict infection before a confirmed laboratory result.

5. Explain the general steps in viral pathogenesis (e.g., of a. respiratory virus).

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الجامعة التقنية الوسطى

كلية التقنيات الصحية والطبية/ بغداد

قسم: تقنيات المختبرات الطبية المادة: الاحياء المجهرية المرحلة: الثانية

Title: Mycology

Name of the instructor: Assistant Professor Dr. Issam Jumaa

Target population: Students of the second stage of the Department of Medical Laboratory Technologies

Introduction:

Mycology is the study of fungi, which are eukaryotic organisms that evolved in tandem with the animal kingdom. However, unlike animals, most fungi are nonmotile and possess a rigid cell wall. Unlike plants, fungi are no photosynthetic. Approximately 80,000 species of fungi have been described, but only about 400 are medically important, and less than 50 are responsible for more than 90% of the fungal infections of humans and other animals. Rather, most species of fungi are beneficial to humankind. They reside in nature and are essential in breaking down and recycling organic matter. Some fungi greatly enhance our quality of life by contributing to the production of food and spirits, including cheese, bread, and beer.

Pretest:

Define the terms mycology; saprophytic; dermatophyte; and polymorphic, dimorphic, and thermally dimorphic fungi.

Scientific Content:

The term mycoses refer to infections that are caused by fungi. Most pathogenic fungi are exogenous, their natural

habitats being water, soil, and organic debris. The mycoses with the highest incidence—candidiasis and dermatophytosis—are caused by fungi that are frequent components of the normal human microbiota and highly adapted to survival on the human host.

Clinical Classification of the Fungi

The botanic taxonomic schema for grouping the fungi has little value in a clinical microbiology laboratory.

For clinicians, dividing the fungi into four categories of mycoses according to the type of infection is much more

useful. The fungi are categorized as follows:

- **Superficial (cutaneous) mycoses**
- **Subcutaneous mycoses**
- **Systemic mycoses**
- **Opportunistic mycoses**

The superficial, or cutaneous, mycoses are fungal infections that involve hair, skin, or nails without direct invasion of deeper tissue. The fungi in this category include the dermatophytes (agents of ringworm, athlete's foot) and agents of infections such as tinea, tinea nigra, and Piedra. All of these infect keratinized tissues.

General Clinical Classification of Pathogenic Fungi

Cutaneous	Subcutaneous	Opportunistic	Systemic
Superficial mycoses Tinea Piedra Candidosis	Chromoblastomycosis Sporotrichosis Mycetoma (eumycotic) Phaeohyphomycosis	Aspergillosis Candidosis Cryptococcosis Geotrichosis	Aspergillosis Blastomycosis Candidosis Coccidioidomycosis Adiaspiromycosis Emmonsiosis
Dermatophytosis		Mucormycosis Fusariosis Trichosporonosis Others*	Histoplasmosis Cryptococcosis Geotrichosis Paracoccidioidomycosis Mucormycosis Fusariosis Trichosporonosis

LABORATORY DIAGNOSIS OF MYCOSES

The vast majority of fungi have evolved to reside in various environmental niches where they grow readily on vicinal organic substrates and are protected from deleterious conditions. Although these exogenous fungi are unable to penetrate

the intact surfaces of healthy hosts, they may be acquired accidentally by traumatic exposure to resident fungi in soil, water,
air, or vegetation. Once fungal cells have breached the cutaneous or mucosal surfaces, such as the skin, or the respiratory,
urinary, or gastrointestinal tract, they are repelled by innate host defenses. Potentially pathogenic fungi must be able to
grow at 37° C, acquire essential nutrients from the host, and evade the immune responses.

A. Specimens

Clinical specimens collected for microscopy and culture are determined by the site(s) of infection and the condition of
the patient. All specimens should be obtained using aseptic technique, especially with specimens from normally sterile
sites, such as blood, tissue biopsies, and cerebrospinal fluid. Specimens from nonsterile body sites include skin and subcutaneous lesions, nasopharyngeal or genital swabs, sputa, urine,

B. Microscopic Examination

One or two drops of an aqueous or serous specimen, such as sputum, urine, spinal fluid, or aspirate, can be placed on a
glass slide in a drop of 10-20% potassium hydroxide (KOH), and after adding a coverslip, the slide is examined under the
microscope with the low- and high-power (450×) objectives. KOH dissolves any tissue cells, and the resistant, highly refractory fungal cell walls become more visible.

C. Culture

In most cases, the culture is more sensitive than the direct examination, and a portion of the material collected for
microscopy should be cultured. The traditional mycological medium, Sabouraud’s dextrose

agar (SDA), contains glucose
and modified peptone (pH 7.0), supports the growth of fungi, and restricts the growth of bacteria. The morphologic characteristics of fungi used for identification have been described
from growth on SDA.

D. Serology

The following sections of this chapter will explain how the detection of specific antibodies or antigens in serum or CSF
can provide useful diagnostic and/or prognostic information.

E. Molecular Methods

An increasing number of clinical laboratories have implemented methods based on the detection of fungal nucleic
acids, proteins, or antigens to identify pathogenic fungi in clinical specimens or after their recovery in culture. Multiple approaches have been published, and in-house as well as commercial systems are available, but none have been widely
adopted.

F. Antifungal Susceptibility Testing

After the diagnosis of a systemic mycosis, appropriate antifungal chemotherapy is initiated. As discussed at the end
of this chapter, there are three major classes of antifungal drugs. However, many pathogenic fungi are capable of developing resistance to antifungal drugs, and the clinical microbiology laboratory is often required to assess in vitro the susceptibility (or resistance) of the patient’s fungal isolate versus a specific antifungal drug.

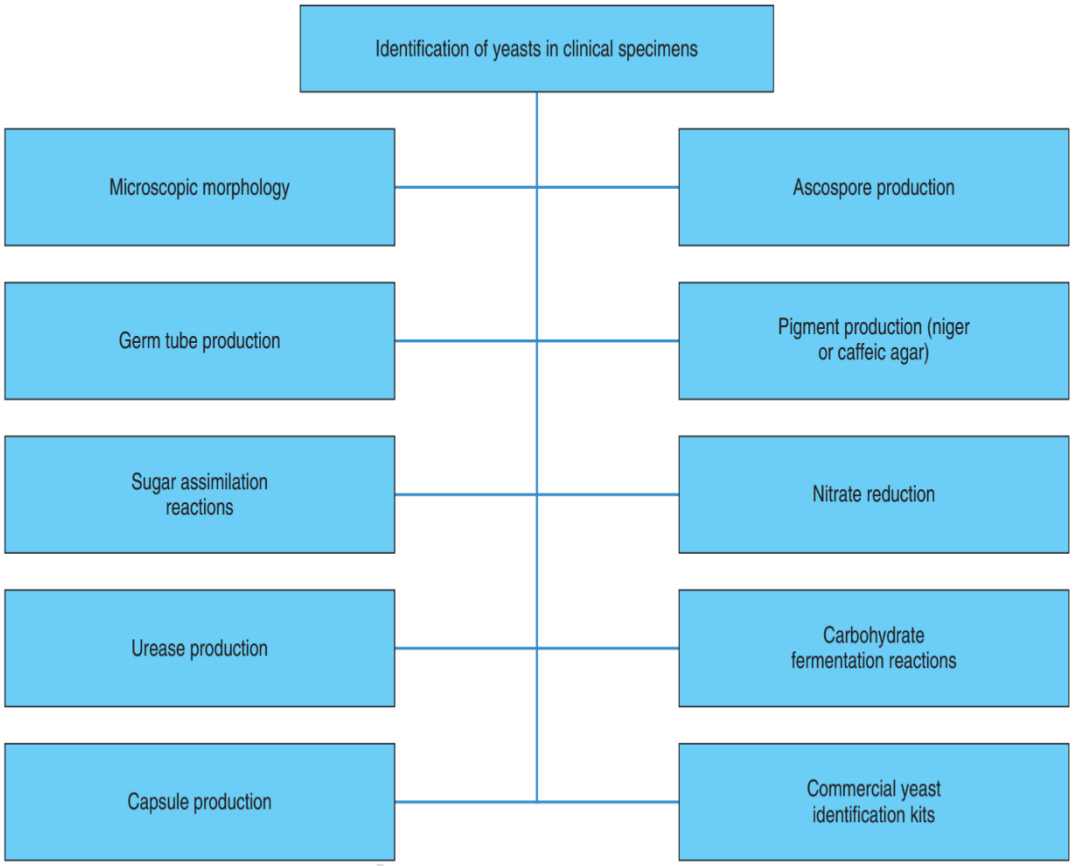

FIGURE 1 . Identification of yeasts in clinical specimens.

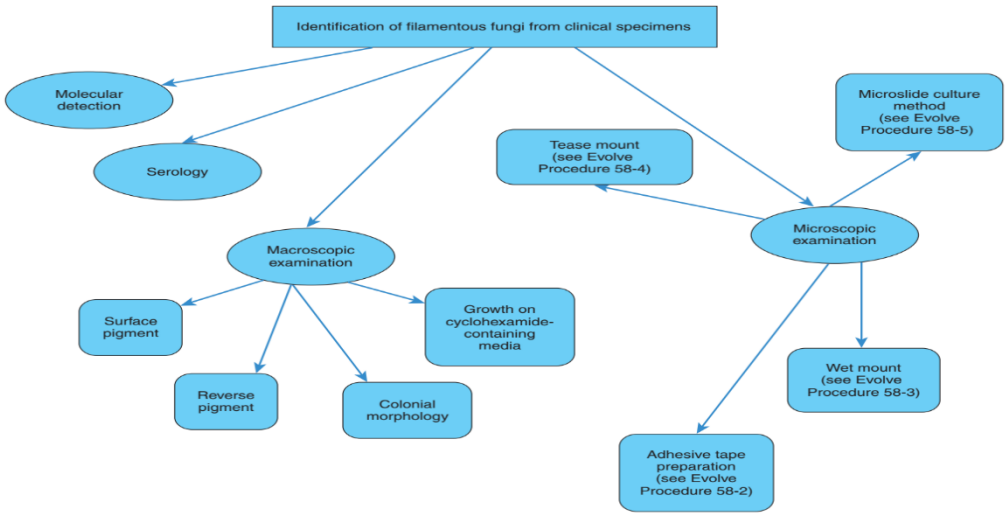

Figure 2 Identification of filamentous fungi from clinical specimens

Posttest:

1. Differentiate the colonial morphology of yeasts and filamentous fungi (moulds).

2. Define and differentiate anamorph, teleomorph, and synanamorph

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