الجامعة التقنية الوسطى كلية التقنيات الصحية والطبية/ بغداد قسم المختبرات

Diagnostic microbiology

الطبية المادة: Diagnostic Microbiology المرحلة: الرابعة

Title: lecture 1 العنوان:

Name of the instructor: اسم المحاضر:

أ.م.د.امال عزيز كريم

Target population: الفئة المستهدفة:

طلبة المرحلة الرابعة

Introduction:

المقدمة:

Diagnostic microbiology: purpose and philosophy

Manifestations of Infection

The clinical presentation of an infectious disease reflects the interaction between the host and the microorganism. This interaction is affected by the **host immune status and microbial virulence factors**. Signs and symptoms vary according to the site and severity of infection. Diagnosis requires a composite of information, including history, physical examination, radiographic findings, and laboratory data.

Microbial Causes of Infection

Infections may be caused by bacteria, viruses, fungi, and parasites. The pathogen may be **exogenous** (acquired from environmental or animal sources or from other persons) or **endogenous** (from the normal flora).

Diagnostic Microbiology is the tool that makes it possible to identify the exact pathogens of infectious diseases and the most optimal therapy at the level of individual patients.

Conventional methods require time to grow the microbes in vitro under specific conditions and not all microbes can easily be cultured. This is followed by biochemical methods for identification which further makes the process lengthy.

Transport of the specimens under less than ideal conditions, prior use of antibiotics and small number of organisms are among the factors that render culture-based methods less reliable.

Newer methods depend on **amplification of nucleic acids** followed by use of probes for identification. This mitigates the need for higher microbial load, presence of metabolically active viable organisms and shortens the time. These methods can be used to detect antibiotic resistance genes directly from the specimen and help direct targeted therapy with efficacy. Since these methods will not fulfill all the diagnostic needs, a second approach is being

used to shorten the time to identification after the organism has already grown.

Microbial colonization

Microbial colonization may result in:

1) Elimination of the microorganism without affecting the host.

2) Infection in which the organisms multiply and cause the host to react by making an immune or other type of response.

3) A transient or prolonged carrier state. Infectious disease occurs when the organism causes tissue damage and loss function of body systems.

So that that the purpose and the philosophy of diagnostic bacteriology is depending on identifying the causative microorganism by different laboratory methods which is usually essential for effective antimicrobial and supportive therapy. Through that initial treatment may be empiric, based on the microbiologic epidemiology of the infection and the patient's symptoms. However, definitive microbiologic diagnosis of an infectious disease usually involves one or more of the following **five basic laboratory techniques**, which guide the physician along a narrowing path of possible causative pathogens:

1. Morphologic identification of the agent in stains of specimens or sections of tissues (light and electron microscopy).

2. Cultivation and identification of the organisms.

3. Detection of microbial antigens by immunologic assay (latex agglutination, enzyme immunoassay [EIA].

4. Detection of microbial DNA or RNA.

5. Detection of an inflammatory or host immune response to the pathogenic agents. As show in figure (1).

After obtaining the proper specimens and informing the laboratory of the

careful clinical diagnosis, the clinician should begin treatment with drugs aimed at the organism thought to be responsible for the patient's illness.

As the laboratory staff begins to obtain results, they inform health care providers, who can then reevaluate the diagnosis and clinical course of the patient and perhaps make changes in the therapeutic program. This "**feedback**" information from the laboratory consists of earliest reports of the results of individual steps in the isolation and identification of the causative agent.

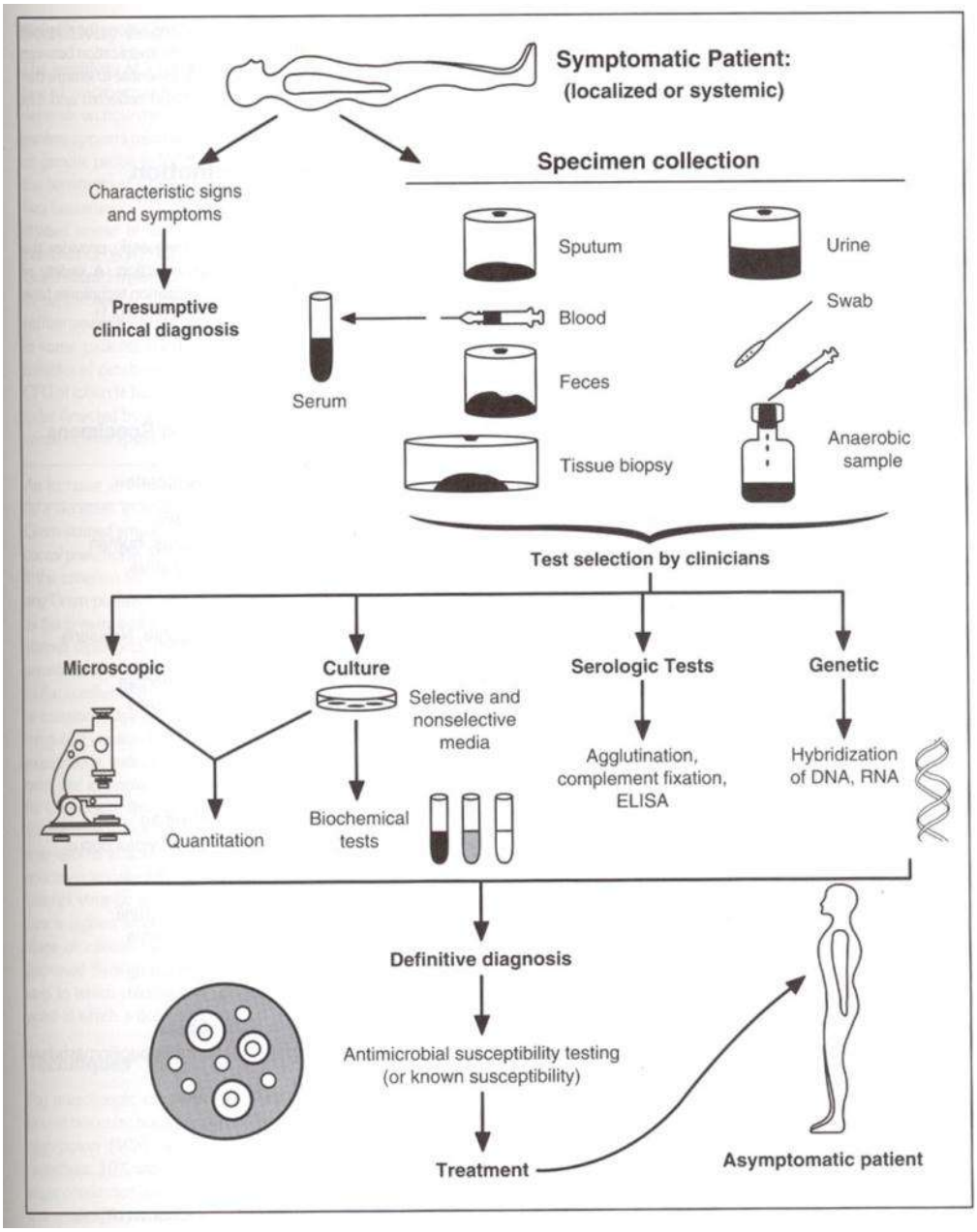

Figure (1): Represent human specimens collection and diagnoses

Pretest: **الاختبار القبلي:**

Give the purpose of study diagnostic bacteria ?

Scientific Content: **المحتوى العلمي:**

-infectious disease initiation.
-Basic laboratory techniques.
- Microbial Causes of Infection

Posttest **الاختبار البعدي:**

What are the five basic laboratory techniques ?

References: **المصادر :**

1. Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
2. Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.
3. Bailey & scott' s (2017): Diagnostic microbiology,fourteenth edition
4. Jeffrey C. Pommerville. (2018): Fundamentals of microbiology, Eleventh edition.
5. Prof.Dr.Mohammed Shammkhi Jeber.(2019):Notes of Diagnosis Microbiology

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المادة: Diagnostic Microbiology المرحلة: الرابعة

Title: **Lecture 2**

العنوان :

Laboratory safety

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

أ.م.د.امال عزيز كريم

Target population: **الفئة المستهدفة :**

طلبة المرحلة الرابعة

Introduction: **المقدمة:**

Laboratory safety

Microbiology laboratory include admonitions such as the necessity to (1) wear gloves, (2) wash hands after working with infectious materials, (3) disinfect all instruments immediately after use, (4) use water to moisten specimen labels rather than the tongue, (5) disinfect all contaminated waste before discarding, and (6) report to appropriate personnel all accidents or exposures to infectious agents.

Safety

Programs have been expanded to include the proper handling of biologic hazards encountered in processing patient specimens and handling infectious microorganisms; fire and electrical safety; the safe handling, storage, and disposal of chemicals and radioactive substances; and techniques for safely lifting or moving heavy objects.

Sterilization, Disinfection, and Decontamination

Sterilization is a process that kills all forms of microbial life, including bacterial endospores.

Disinfection is a process that destroys pathogenic organisms, but not necessarily all microorganisms, endospores, or prions. However, some disinfectants will kill endospores with prolonged exposure times.

Decontamination is the removal of pathogenic microorganisms so items are safe to handle or dispose of.

Many factors limit the success or degree of sterilization, disinfection, or decontamination in a health care setting, such as organic load (organisms and other contaminating materials such as blood or body fluids), the type of organisms present, the concentration and exposure time to the germicide, the physical and chemical nature of the surface (hinges, cracks, rough or smooth surfaces),temperature, pH, humidity, and presence of a biofilm. These

processes may be accomplished by a variety of physical or chemical methods.

Methods of Sterilization

The physical methods of sterilization include:

- Incineration
- Moist heat
- Dry heat
- Filtration
- Ionizing (gamma) radiation
- Chemicals (ethylene oxide gas, hydrogen peroxide gas plasma, vaporized hydrogen peroxide, and other liquid chemicals).

Chemical safety of diagnostic microbiologically laboratory should have a chemical hygiene plan that includes guidelines on proper labeling of chemical containers, manufacturers material safety data sheets (MSDSs), and the written chemical safety training and retraining programs.

Fire safety is an important component of the laboratory safety program. Each laboratory is required to post fire evacuation plans that are essentially strategies for finding the nearest exit in case of fire.

Electrical safety

Electrical cables should be checked regularly for fraying and replaced when necessary. All plugs should be the three-prong, grounded type. All sockets should be checked for electrical grounding and leakage at least annually. No extension cables should be used in the laboratory.

Handling of compressed gases

Compressed gas cylinders (CO_2, anaerobic gas mixture) contain pressurized gases and must be properly handled and secured.

Biosafety

Individuals are exposed in various ways to laboratory acquired infections in

microbiology laboratories, through that risks from a microbiology laboratory may extend to adjacent laboratories and to the families of those who work in the microbiology laboratory.

Individuals are exposed in various ways to health care– associated infections, transporting specimens and in public areas such as elevators or cafeterias, by:

• Rubbing the eyes or nose with contaminated hands

• Inhaling aerosols produced during centrifugation, mixing with a vortex, or spills of liquid cultures

• Accidentally ingesting microorganisms by putting pens or fingers in the mouth

• Receiving percutaneous inoculation (i.e., through puncture from an accidental needle stick)

• Manipulating or opening bacterial cultures in liquid media or on plates, creating potentially hazardous aerosols,outside of a biosafety hood

• Failure to wash hands upon leaving the restroom or other public areas before entering the laboratory

Figure (2):Autoclave bags.

Microbiologists should wear laboratory coats over their street clothes, and these coats should be removed before leaving the laboratory. Most exposures to blood-containing fluids occur on the hands or forearms, so gowns with

closed wrists or forearm covers and gloves that cover all potentially exposed skin on the arms are most beneficial. If the laboratory protective clothing becomes contaminated with body fluids or potential pathogens, it should be sterilized in an autoclave immediately and cleaned before reusing. The institution or a uniform agency should clean laboratory coats; it is no longer permissible for microbiologists to launder their own coats. Alternatively, disposable gowns may be use.

Pretest: **الاختبار القبلي :**

What are Laboratory safety?

Scientific Content: **المحتوى العلمي:**

-Sterilization and disinfection
- Chemical safety
-Fire safety
-Electrical safety
-Handling of compressed gases
-Biosafety
-Dispersal hazardous waste

Posttest **الاختبار البعدي:**

-How can you avoid bacterial infection in laboratory ?
-Write the physical methods for sterilization ?

References: المصادر :

1. Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
2. Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.
3. Bailey & scott' s (2017): Diagnostic microbiology,fourteenth edition
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الطبية المادة: الاحياء المجهرية التشخيصي المرحلة: الرابعة

Selection, collection, and transport of specimens for microbiological examination

Title: Lecture 3,4,5

العنوان :

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

أ.م.د أحمد سالم محمد

Target population: **الفئة المستهدفة :**

طلبة المرحلة الرابعة

Introduction: **المقدمة:**

The role of the laboratory in the diagnosis of infections can be considered a major steps of diagnostic microbiology. If pathogens are to be isolated successfully, the type of specimen, its collection, time and method of its dispatch to the laboratory must be correct.
(Figure- 1**)**
A detailed request form that accompanies a clinical sample is pivotal in ensuring the appropriate diagnostic procedures are undertaken; information should include:
1. Patient details.
2. Clinical diagnosis.
3. Onset of symptoms
4. Sample type.
5. Time of collection.
6. Treatment history and concurrent antimicrobial therapy.
7. Other health and safety issues.
Supporting clinical information, including travel history and contact with infected individuals, is also important.

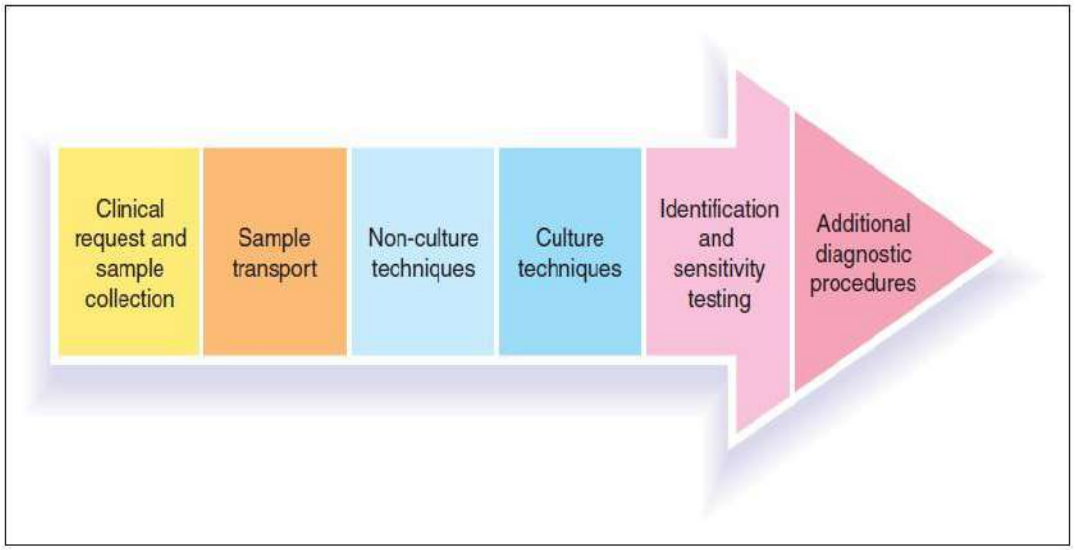

Figure (1**): Laboratory diagnostic pattern.**

Sample collection

Samples for microbiological investigation require careful collection, without contamination from external sources or the patient's own flora.

When taking clinical samples, it is important to: use sterile, leak-proof containers; label the specimen correctly; take care whilst obtaining samples through an area containing a normal flora like vein puncture through skin to obtain blood culture in this instance the skin should be decontaminated

with an appropriate antiseptic prior to collecting blood. If possible, specimens for microbiological culture should be taken prior to commencement of antibiotic therapy, to avoid false-negative results.

Transport of microbiological specimens

Rapid transport of samples to the microbiology laboratory is essential, as many fastidious microorganisms, such as *Neisseria gonorrhoeae* and *Haemophilus influenzae*, die during transit.

Furthermore, overgrowth by contaminating normal flora confusing the pathogen may also occur. To minimize these complications, the microbiology department may adopt several strategies such as:

1. Specimen should reach to the laboratory as soon as possible or a suitable preservative or transport medium must be used.
2. Refrigeration of sample at 4 ºC can help to preserve cells and reduce the multiplication of commensals in unpreserved specimens.
3. Freezing at -70 ºC or below in the presence of a stabilizing fluid, like glycerol or serum.
4. Ensure that the specimen container is free from cracks, and the cap is leak proof.
5. Seal round the container cap with adhesive tape to prevent loosening and leakage during transit.
6. If the container is glass tube or bottle, use sufficient packaging material to prefect a specimen.
7. If the specimen is fluid, use sufficient absorbent material to absorb it.
8. Mark all specimen that may contain highly infectious organism "HIGH RISK".

Non-culture techniques

Following receipt of the sample, the microbiology laboratory may utilize non-culture techniques to provide rapid clinical information, which may benefit patient management. There are several situations where non-

culture techniques are of importance:

. Microorganism cannot be readily cultured in vitro;

. Microorganism is slow-growing;

. Rapid laboratory diagnosis significantly influences clinical management of the patient.

Non-culture techniques include direct microscopy, immunological methods, serology, and nucleic acid amplification techniques (NAAT).

Pretest: **الاختبار القبلي :**

1-How you can collect the specimen from patient?
2- Specimen labeling include -----,------,-----,------,------.

Scientific Content: المحتوى العلمي:

-Sample collection
- Transport of microbiological specimens
- Non-culture techniques

Posttest الاختبار البعدي:

1- Draw the Laboratory diagnostic pattern?

3. Numerate the Transport of microbiological specimens steps?

References: المصادر :

1. Apurba S. S; Sandhya Bhat K. Review of Microbiology and Immunology. 4th Edition. The Health Sciences Publisher. 2015
2. **Orekan J. et al. (2021):Clinical Microbiology and Infection 27 .1400-1408.**
3. **Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.**
4. **Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.**
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المادة: الاحياء المجهرية التشخيصي المرحلة: الرابعة

Title: **Lecture 6**

العنوان:

Cultivation and Isolation of Viable Pathogen

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

أ.م.د أحمد سالم محمد

Target population: طلبة المرحلة الرابعة **الفئة المستهدفة:**

طلبة المرحلة الرابعة

Introduction: المقدمة:

Laboratory media

Culture media are required to isolate the bacteria from the clinical specimens; following which the appropriate biochemical tests can be performed to identify the causative agent.

Constituents of culture media:

The basic constituents of culture media are:

- Water: Distilled water or potable water with low mineral content is suitable for culture media preparation.
- Electrolyte: Sodium chloride or other electrolytes.

 Peptone: It is a complex mixture of partially digested proteins.

• Source: It is obtained from lean meat or other protein material, such as heart muscle, casein or fibrin, or soya flour usually by digestion with proteolytic enzymes, such as pepsin

• Constituents: 1t contains proteoses, aminoacids, inorganic salts (phosphates, potassium and magnesium), accessory growth factors like nicotinic acid and riboflavin.

 Agar: It is used for solidifying the culture media. It is commercially available in powder form; melts in water after boiling and jellifies after cooling also called 'agar-agar' is prepared from the cell wall of variety of seaweeds (red algae of species *Gelidium* and *Gracilaria)*

Preparation of agar media: The appropriate amount of agar powder is added to water and the mixture is dissolved and then sterilized by placing it in an autoclave. When the temperature of the molten agar comes down to 45°C, it is poured to the Petri dishes and then allowed to set for 20 minutes.

 Meat extract: It is a commercial preparation of highly concentrated meat stock, usually made from beef. It contains protein degradation products, inorganic salts, carbohydrates and growth factors.

 Yeast extract: Itis prepared commercially from washed cells of Baker's yeast. It contains amino acids, inorgani c salts (potassium and phosphates) and carbohydrates. (Figure-1)

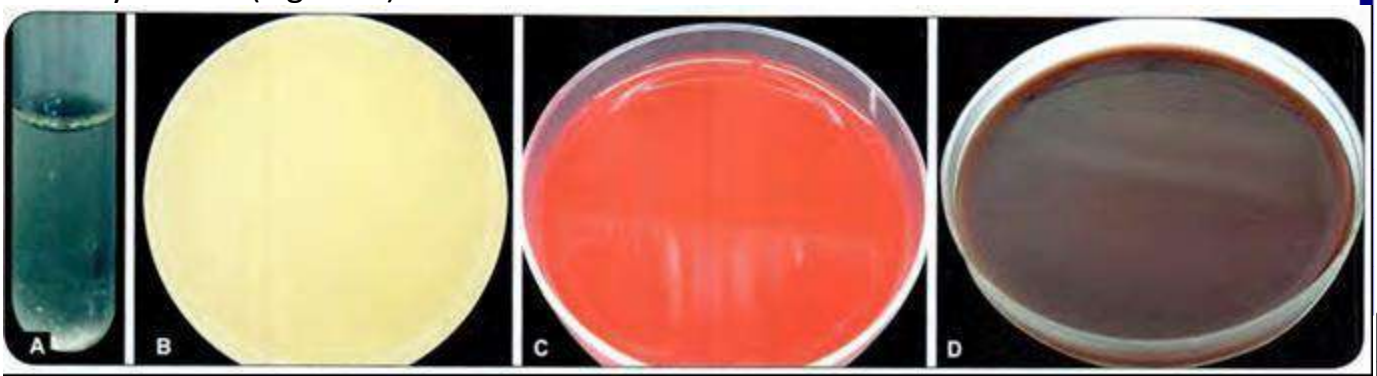

Figure (1): A. Peptone water; B. Nutrient agar; C. Blood agar; D. Chocolate agar.

- **Malt extract: It consists of maltose (about 50%), starch, dextrin, glucose and 5% protein products.**
- **Blood and serum: They are important components of enriched media and provide extra nutrition to fastidious bacteria. Usually 5- % of sheep blood is used. Horse, ox or human blood can also be used.**

Types of culture media:

Bacteriological culture media can be classified in two ways: A. Based on consistency, culture media are grouped into:

1. Liquid media (or broth)
2. Semisolid media
3. Solid media

B. Based on the growth requirements, culture media are classified as:

1. Routine laboratory media: They are prepared from nutrients, such as aqueous extract of meat, peptone, etc. They can further be classified into various types based on functional use or application, as Simple/basal media; Enriched media; Enrichment broth; Selective media; Differential media; Transport media; Anaerobic media.
2. Defined or synthetic media: They are prepared from pure chemical substances and the exact composition of the media is known, Simple synthetic media and Complex synthetic media.

Simple media

Many bacteria will grow in or on simple media such as nutrient broth/nutrient agar that contains 'peptone' (polypeptides and amino acids from the enzymatic digestion of meat) and 'meat extract'(water-soluble components of meat containing mineral salts and vitamins).

Enriched media

These contain additional nutrients for the isolation of more fastidious bacteria that require special conditions for growth like agar containing whole blood (blood agar) or agar containing lysed blood (chocolate agar). (Figure-2)

Figure (2): A. Brain-heart infusion broth; B. Biphasic medium (Brain-heart infusion broth/agar); C. Robertson's cooked meat medium; D. Thioglycollate broth

Selective media

These are designed to facilitate growth of some bacteria, while suppressing the growth of others, and include:

 Mannitol salt agar which contains increased NaCl (salt) concentration for the recovery of staphylococci;
 MacConkey agar, which contains bile salts and allows the growth of bile-tolerant bacteria only; and
 Antibiotics, which are frequently added to media to allow only certain bacteria to grow while suppressing or killing others.

Indicator media

These are designed to aid the detection and recognition of particular pathogens. They are often based on sugar fermentation reactions that result in production of acid and the subsequent colour change of a pH indicator, such as MacConkey agar contains lactose and a pH indicator (neutral red); lactose-fermenting bacteria (*Escherichia coli*) produce acid and form pink colonies, whereas non-lactose fermenting bacteria (*Salmonella spp.*) do not produce acid and form pale yellow colonies. This property facilitates the recognition of possible Salmonella colonies among normal bowel flora. Note that indicator media may also contain selective agents including antibiotics or substances such as bile salts and crystal violet to suppress growth of most Gram- positive microorganisms. MacConkey agar is therefore both a selective medium and an indicator medium.

Pretest: **الاختبار القبلي:**

-How u can cultivate the bacteria in Lab. ?

Scientific Content: **المحتوى العلمي:**

- Laboratory media
- Constituents of culture media:
- Types of culture media?

Posttest الاختبار البعدي:

1- Constituents of culture media are ---------,----------

2- Which are common pathogenic bacteria isolated form urinary tract?

References: المصادر:

1- Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
2-Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.
3-Bailey & scott' s (2017): Diagnostic microbiology,fourteenth edition
4-Jeffrey C. Pommerville. (2018): Fundamentals of microbiology, Eleventh edition.
5-Orekan J. et al. (2021):Clinical Microbiology and Infection 27 .1400-1408.
6-Cynthia Nau Cornelissen (2015): Lippincott Illustrated Reviews Flash Cards MICROBIOLOGY .Third Edition

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المادة: Diagnostic Microbiology المرحلة: الرابعة

Title: Lecture 7,8,9

العنوان:

Microbiological methods for identification of microorganisms

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

أ.م.د.امال عزيز كريم

Target population: **الفئة المستهدفة:**

طلبة المرحلة الرابعة

Introduction: **المقدمة:**

Microbiological methods for identification of microorganisms

Various methods used to identify organisms cultivated from patient specimens, associated with bacterial identification.The procedures to diagnosis of infectious diseases is as follows:

1. Direct examination of patient specimens
2. Growth and cultivation of the agents from the specimens
3. Analysis of the cultivated organisms to establish their identification and other pertinent characteristics such as susceptibility to antimicrobial agents.

Macroscopic Observation

It provide useful information to both the microbiologist and the physician the macroscopic observation should include the following:

- Swab or aspirate
- Stool consistency (formed or liquid)
- Blood or mucus present
- Volume of specimen
- Fluid—clear or cloudy

The gross examination also allows the processor to determine the adequacy of the specimen and the need for special processing. Areas of blood and mucus are selected for culture and direct microscopic examination. Anaerobic cultures may be indicated if gas, foul smell, or sulfur granules are present.

Microscopic Observation

Microscopy is defined as the use of a microscope to magnify (i.e., visually enlarge) Because most infectious agents cannot be detected with the unaided eye, microscopy plays a pivotal role in the laboratory.

Types of Microscope are:

Bright-field microscopy (also known as light microscopy) Many bacteria are difficult to see well because of their lack of contrast with the surrounding medium. Dyes (stains) can be used to stain cells or their organelles and

increase their contrast so that they can be more easily seen in the bright-field microscope.

The phase-contrast microscope was developed to improve contrast differences between cells and the surrounding medium, making it possible to see living cells without staining them; with bright-field microscopes, killed and stained preparations must be used.

Dark-field this technique has been particularly useful for observing organisms such asn*Treponema pallidum*, a spirochete that is smaller than 0.2 μm in diameter and therefore cannot be observed with a brightfield or phase-contrast microscope

Fluorescence microscopy is widely used in clinical diagnostic microbiology. For example, the fluorochrome auramine O, which glows yellow when exposed to ultraviolet light, is strongly absorbed by the cell envelope of *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis.When the dye is applied to a specimen suspected of containing *M. tuberculosis* and exposed to ultraviolet light, the bacterium can be detected by the appearance of bright yellow organisms against a dark background.

Differential Interference Contrast Microscope

Structures, such as spores, vacuoles, and granules, appear three dimensional. DIC microscopy is particularly useful for observing unstained cells because of its ability to generate images that reveal internal cell structures that are less apparent by bright-field techniques.

The Electron Microscope

There are two types of electron microscopes in general use: The **transmission electron microscope (TEM)**, which has many features in common with the light microscope; and the **scanning electron microscope (SEM)**. is particularly useful for providing threedimensional images of the surface of microscopic objects.

Scanning Probe Microscopes

A new class of microscopes, called **scanning probe microscopes**, measures surface features by moving a sharp probe over the object's surface. The

(A) Phase Contrast
Cells (dark) are contrasted from the lighter dots (spores).

(B) Dark Field
Unstained cells are seen against a dark background.

(C) Fluorescence
Cells "glow" due to the presence of a fluorescent antibody that binds to the cells.

Direct and Indirect Smears

A direct smear is a preparation of the primary clinical sample received in the laboratory for processing. A direct smear provides a mechanism to identify the number and type of cells present in a specimen, including white blood cells, epithelial cells, and predominant organism type.

Indirect smear organisms obtained after purification or growth on artificial media. Indirect smears may include preparation from solid or semisolid media or broth. Care should be taken to ensure the smear is not too thick when preparing the slide from solid media.

Staining:

A specimen must contain at least 10^5 organisms per milliliter before it is likely that organisms will be seen on a smear. Liquid medium containing 10^5 organisms per milliliter does not appear turbid to the eye. Specimens containing **10^2–10^3 organisms per milliliter** produce growth on solid media, there are many types of stains, each with specific applications.

- **Simple stains** are directed toward coloring the forms and shapes present,
- **differential stains** are directed toward coloring specific components of the elements present,
- **diagnostic antibody or DNA probe–mediated stains** are directed specifically at identification of an organism.

Staining techniques

Structural details of bacteria cannot be seen under light microscope due to lack of contrast. Hence, it is necessary to use staining methods to produce color contrast and thereby increase the visibility. Before staining, the fixation of the smear to the slide is done.

Fixation is the process by which the **internal and external structures** of cells are preserved and fixed in position. It also **inactivates the enzymes** that might disrupt cell morphology. It toughens (**hardens**) cell structure so that they do not change during staining. It kills and fixes the cells on to the slide.

There are two types of fixation as follows:

1. Heat fixation: It is usually done for bacterial smears by gently flame heating an air-dried film of bacteria. This adequately **preserves** overall **morphology but not structures** within the cells.
2. Chemical fixation: It can be done using ethanol, acetic acid, mercuric chloride, formaldehyde, methanol and glutaraldehyde. They are used to protect the fine internal structure of the cells. This is useful for examination of blood smears. The fixed smear is stained by appropriate staining technique.

Common staining techniques used on microbiology:

- **Simple stain**: Basic dyes, such as **methylene blue or basic fuchsin** are used as simple stains. They provide the color contrast, but impart the same color to all the bacteria in a smear.
- **Negative staining**: A drop of bacterial suspension is mixed with dyes, such as **India ink or nigrosin**.The background gets stained black whereas **unstained bacterial/yeast capsule** stand out in contrast.

  It is very useful in the demonstration of bacterial/yeast capsules which do not take up simple stains.
- **Impregnation methods**: Bacterial cells and structures that are too thin to be seen under the light microscope, are **thickened by impregnation**

of silver salts on their surface to make them visible, e.g. for demonstration of **bacterial flagella** and **spirochetes**.

- **Differential stain**: two stains are used which impart different colors to different bacteria or bacterial structures, which help in differentiating bacteria.The most commonly employed differential stains are:

1. Gram stain: It differentiates bacteria into gram positive and gram negative groups (G+ or G- bacteria)
2. Acid-fast stain: It differentiates bacteria into acid fast and nonacid fast groups
3. Chromatic granules from other bacteria that do not have them.

Single Enzyme Tests (biochemical tests)

Several tests are commonly used to determine the presence of a single enzyme. These tests usually provide rapid results because they can be performed on organisms already grown in culture.

Catalase Test

The enzyme **catalase** catalyzes the release of water and oxygen from hydrogen peroxide (H_2O_2 _ catalase H_2O _O_2); its presence is determined by direct analysis of a bacterial culture .The rapid production of bubbles when bacterial growth is mixed with a hydrogen peroxide solution is interpreted as a positive test.

Oxidase Test

Cytochrome oxidase participates in electron transport and in the nitrate metabolic pathways of certain bacteria. Testing for the presence of oxidase can be performed by flooding bacterial colonies on the agar surface with 1% **tetramethylp-phenylenediamine dihydrochloride.** Alternatively, a sample of the bacterial colony can be rubbed onto filter paper impregnated with the reagent .A positive reaction is indicated by the development of a purple color.

Indole Test

The enzyme **tryptophanase** are able to degrade the amino acid tryptophan into pyruvic acid,ammonia, and indole. **Indole** is detected by combining with an indicator Kovac’ s reagent), which results in a pink to red color formation.

Urease Test

Urease hydrolyzes the substrate urea into ammonia, water, and carbon dioxide. The presence of the enzyme is determined by inoculating an organism to broth (Stuart' s urea broth) or agar (Christensen' s urea agar) containing urea as the primary carbon source followed by detecting the production of ammonia. Ammonia increases the pH of the medium so its presence is readily detected using a pH indicator. Change in medium pH is a common indicator of metabolic process and, because pH indicators change color with increases (alkalinity) .The urease test helps identify *Proteus* spp., and other important bacteria such as *Corynebacterium urealyticum* and *Helicobacter pylori.*

Oxidation and Fermentation Tests

Bacteria utilize of carbohydrates (e.g., sugar or sugar derivatives) and protein substrates. **Oxidatio Oxidation-fermentation** determinations are usually accomplished using a special semisolid medium (oxidative fermentative [O-F] medium) that contains low concentrations of peptone and a single carbohydrate substrate such as glucose.

The glucose fermentative or oxidative capacity is generally used to separate organisms into major groups (e.g., *Enterobacteriaceae* are fermentative; *Pseudomonas* spp. are oxidative).

Amino Acid Degradation

The amino acid substrates most often tested include lysine, tyrosine, ornithine, arginine, and phenylalanine.(The indole test for tryptophan cleavage is presented.) Decarboxylases cleave the carboxyl group from amino acids so that amino acids are converted into amines; lysine is converted to cadaverine, and ornithine is converted to putrescine. Because amines increase medium pH, they are readily detected by color changes in a pH indictor indicative of alkalinity. **Decarboxylation** is an anaerobic process that requires an acid environment for activation, the amino acid substrate of interest (i.e., lysine, ornithine, or arginine), and a pH indicator.

***Complementary diagnostic methods are epi test and VITEC system .**

There some non-traditional methods for identification of pathogens or their products include:

- Molecular testing.
- Saliva based testing.
- Chromogeneic testing.
- Rapid immunochromatographic testing.

All these tests are expensive and not used as ordinary laboratory tests.

Pretest: الاختبار القبلي:

What are the basic flow of procedures involved in the laboratory diagnosis of infectious?

Scientific Content: المحتوى العلمي:

1-Staining techniques.
2-Macroscopic observation.
3- Microscopic observation.
4-Bacterial cultivation.
5-Biochemical tests.
6- Other diagnostic methods.
7-Antibiotic susceptibility tests.

Posttest **الاختبار البعدي:**

Draw the schism of pre diagnosis bacteria isolated from urine?

References: **المصادر:**

1. Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
2. Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.
3. Bailey & scott' s (2017): Diagnostic microbiology,fourteenth edition
4. Jeffrey C. Pommerville. (2018): Fundamentals of microbiology, Eleventh edition.
5. Prof.Dr.Mohammed Shammkhi Jeber.(2019):Notes of Diagnosis Microbiology

الجامعة التقنية الوسطى كلية التقنيات الصحية والطبية/ بغداد قسم المختبرات الطبية

المادة: الاحياء المجهرية التشخيصى المرحلة: الرابعة

Title: Lecture 11

العنوان:

Antibiotic susceptibility tests

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

أ.م.د أحمد سالم محمد

Target population: **الفئة المستهدفة:**

طلبة المرحلة الرابعة

Introduction: **المقدمة:**

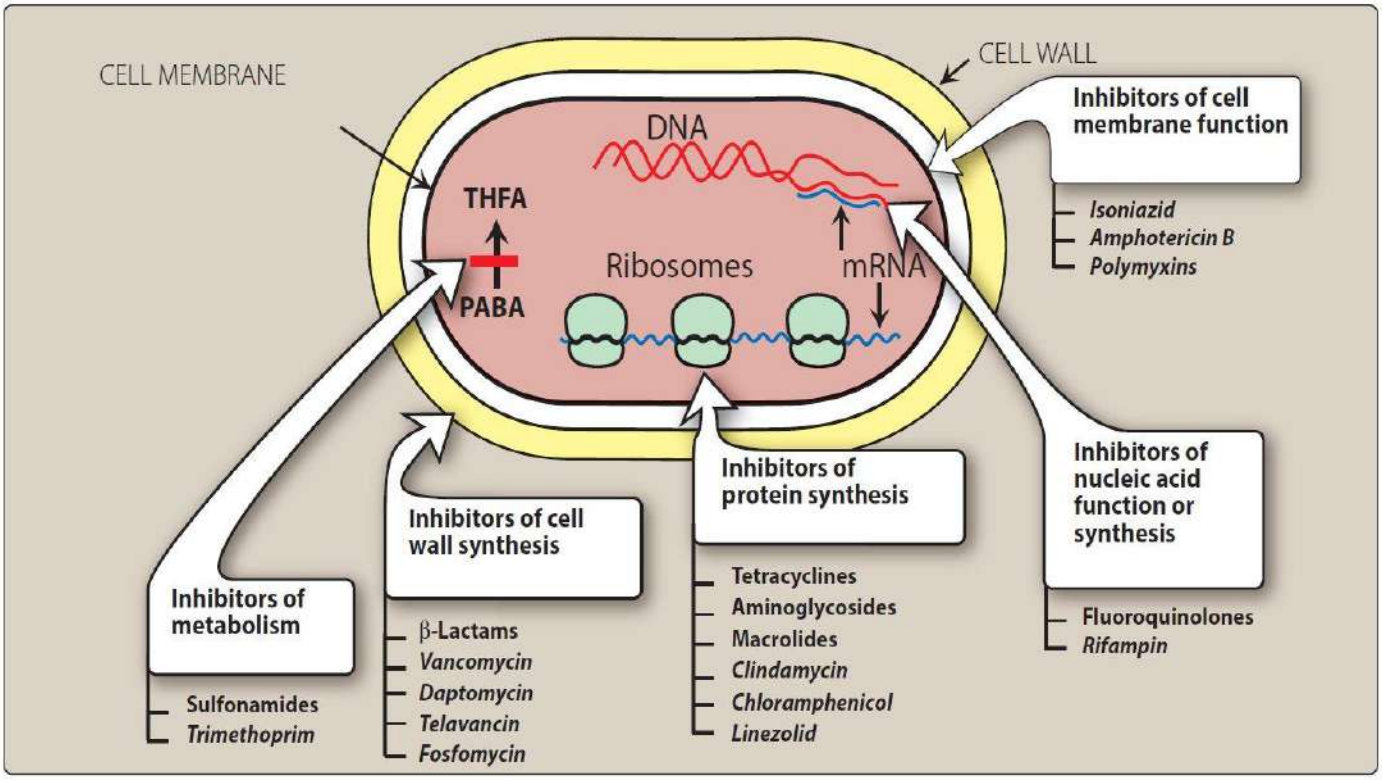
Antibiotic susceptibility tests

Antimicrobial agents are nontoxic antimicrobial therapeutic agents, which include antiseptics, antibiotics, preservatives, sterilants, and disinfectants; all have the capacity to kill or suppress th growth of microorganisms. Antimicrobial agents are an essential components of the practice of medicine. They are used to **treat**, **prevent**, and control the distribution of bacterial pathogens. The term **antibiotic** has been traditionally reserved for compounds that are naturally produced l **living microorganisms**, such as bacteria and fungi. The term has come to be more widely applied to any natural, semisynthetic, or synthetic molecule used to treat or prevent disease.

Antibiotics Mode of Action:

Antibiotics target anabolic **cellular processes** such as:

1. Cell wall synthesis.
2-Cell membrane synthesis
2. DNA replication.
3. RNA transcription.
4. Protein synthesis
5-cell metabolism



Classification of some antimicrobial agents by their sites of action. (THFA = tetrahydrofolic acid; PABA = *p*-aminobenzoic acid.)

Source : Lippincott Illustrated Reviews, Pharmacology - Whalen, Karen

Fig1: Antibiotics Mode of action

Antibiotic susceptibility testing is performed on bacteria **isolated** from **clinical specimens** to determine which antimicrobial agents might be effective in treating infections caused by the bacteria. Only bacteria that are likely to be contributing to an infection should be tested. Testing bacteria that are not involved in the infection would be misleading to the physician and could lead to a **more serious infection** with development of **antimicrobial resistance**. One of the major challenges in clinical microbiology is the identification of the bacterium that caused infections.

Often, these bacteria **need** to be **distinguished** from **normal flora** that may be present in at the site of the infection normally, although in some situations the microbial flora that reside at the

site of the infection may be **contributing to the infection**. Therefore, thought needs to go into determining which bacteria from a specimen will be tested for susceptibility to antimicrobials. Most microbiology laboratories have guidelines for determining when and on which bacteria susceptibility testing will be done. When in doubt about the significance of a bacteria from a specimen, it is best to discuss the **situation** with the attending **physician**.

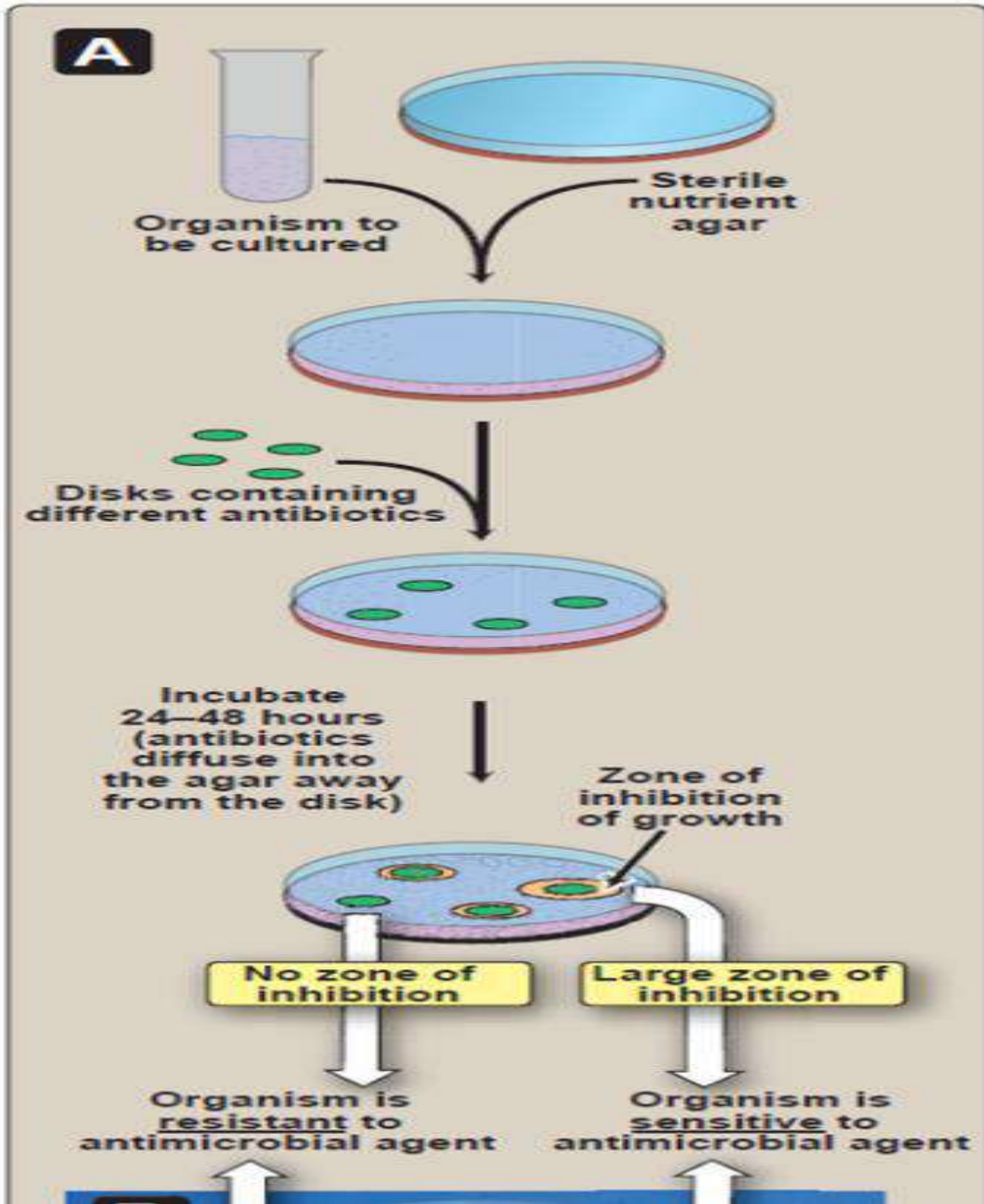
In clinical laboratories, susceptibility testing is usually performed by a disk diffusion or and minimal inhibitory concentration [MIC] methods. Standards that describe these methods are published and frequently updated by the **Clinical and Laboratory Standards Institute** (CLSI) formerly the **National Committee for Clinical Laboratory Standards** [NCCLS].

After a pathogen is cultured, its sensitivity to specific antibiotics serves as a guide in choosing antimicrobial therapy. Some pathogens, such as *Streptococcus pyogenes* and *N. meningitidis*, usually have predictable sensitivity patterns to certain antibiotics. In contrast, most gram-negative bacilli, enterococci, and staphylococcal species show unpredictable sensitivity patterns to various antibiotics and require susceptibility testing to determine appropriate antimicrobial therapy.

There are many methods for detecting this bacterial susceptibility pattern like:

1. Disk-diffusion method

The classic qualitative method to test susceptibility to antibiotics has been the Kirby-Bauer disk diffusion method, in which disks with exact amounts of different antimicrobial agents are place on culture dishes inoculated with the microorganism to be tested. The micro-organism's growth (resistance to the drug) or lack of growth (sensitivity to the drug) is then monitored (Figure-2).


A
Organism to be cultured
Sterile nutrient agar
Disks containing different antibiotics
Incubate 24–48 hours (antibiotics diffuse into the agar away from the disk)
Zone of inhibition of growth
No zone of inhibition
Large zone of inhibition
Organism is resistant to antimicrobial agent
Organism is sensitive to antimicrobial agent

Pretest: **الاختبار القبلي:**

- Define Antibiotic
-How you can test the bacterial antibiotic susceptibility in the Lab.

Scientific Content: **المحتوى العلمي:**

- Antibiotic susceptibility tests
- Antibiotics Mode of Action
- Disk-diffusion method
- Minimal inhibitory concentration
- Bacteriostatic versus bactericidal drugs
- Laboratory strategies for antimicrobial susceptibility testing

Posttest **الاختبار البعدي:**

Fill in the blank :

-The Antibiotic mode of actions are ------- ,--------and ---------.

- describe Bacteriostatic versus bactericidal drugs

-what is MIC.

References: المصادر :

1- Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
2-Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.
3-Bailey & scott' s (2017): Diagnostic microbiology,fourteenth edition
4-Jeffrey C. Pommerville. (2018): Fundamentals of microbiology, Eleventh edition.
5- Apurba S. S; Sandhya Bhat K**.** Review of Microbiology and Immunology. 4th Edition. The Health Sciences Publisher. 2015
6-Cynthia Nau Cornelissen (2015): Lippincott Illustrated Reviews Flash Cards MICROBIOLOGY .Third Edition

الجامعة التقنية الوسطى كلية التقنيات الصحية والطبية/ بغداد قسم المختبرات الطبية المادة: Diagnostic Microbiology المرحلة: الرابعة

Title: **Lecture 14,15**

العنوان:

Blood Stream infections

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

أ.م.د.امال عزيز كريم

Target population: **الفئة المستهدفة:**

طلبة المرحلة الرابعة

Introduction: **المقدمة:**

Blood Stream infections

Blood is a combination of plasma and cells that circulate through the entire body. It is a specialized bodily fluid that supplies essential substances around the body, such as sugars, oxygen, and hormones.

In healthy subjects, the blood is sterile

- There are various routes that organisms take to reach the blood.
- **Pneumococcus** colonizing the upper airways could be aspirated into the lungs during sleep and go on to cause a lobar pneumonia; from here it can enter the blood.**The presence of bacteria in the blood requires identification of the likely source.**There is the obvious association of *Escherichia coli* in blood and an ascending urinary tract infection (UTI).

The patient with endocarditis caused by a streptococcus of the mouth flora, such as Streptococcus sanguinis, can have poor dentition (Poor oral health), and this needs to be addressed as part of the patient's management, usually involving the maxilla-facial surgical team and also called periodontal organisms of dental infections.

- **More unusual situations** occur, and one is the identification of *Streptococcus gallolyticus* in blood culture. This organism is a minor member of the normal flora of **the colon.**
- **However, it is recognized that there is an association that can develop between it and a large bowel malignancy, likely due to a specific interaction between the organism and these malignant cells.**
- The *Streptococcus* gains a selective growth advantage, from where it accesses the blood. Once in the blood it has the potential to initiate infective endocarditis.
- The finding of *Streptococcus gallolyticus* in blood culture, often in the setting of endocarditis, is an alert to investigate this malignancy; if

found this is removed before any valve surgery.

Blood is cultured to detect and identify bacteria or other cultivable microorganisms (yeasts, filamentous fungi). The presence of such organisms in the blood is called bacteraemia or fungaemia, and is usually pathological.

bacteraemia defines the presence of bacteria as detected by the culture of blood.

- **Septicemia** also defines the presence of bacteria in blood, but it signals a sense of urgency in the management of the patient.
- **The terms sepsis and septic shock** are also used and, with clinical parameters such as fever, hypotension, tachycardia, multiorgan failure and leucocytosis, alert the clinician to the severity of the situation, and the need for immediate action in the management of the patient.

Bacteremia

1.A transient bacteremia (a single episode lasting less than 30 minutes or so) can arise from **a pneumococcal pneumonia, or pyelonephritis caused by *Escherichia coli.***

2.An intermittent bacteremia manipulation (guidance) of **an extravascular** site, such as a ***Staphylococcus aureus* abscess,** where bacteria enter the lymphatics at irregular intervals, and from there, to the blood.

3.A continuous bacteremia an **intravascular** source, and endocarditis is the most important example.

- Once bacteria enter the blood, they have the potential to settle (become down) in other sites of the body, and set up another focus of infection.
- A ***Staphylococcus aureus*** bacteremia arising from an infected **peripheral venous cannula** (PVC) site can **result in** bacteria attaching to a heart valve to initiate **endocarditis,** or settling in the **spine** and

causing an abscess there.

- **The bacteria can cross the synovial membrane of a joint to initiate septic arthritis**. These examples underline the critical importance of full clinical assessment of the septic or bacteremic patient.

Blood collection

blood should be taken before antibiotics are administered.It is recommended that two or preferably three blood cultures be obtained.

- **Tryptic soy broth (TSB)** should be able to support growth of all clinically significant bacteria.
- the blood should be mixed with 10 times its volume of broth (5 ml of blood in 50 ml of broth) to **dilute any antibiotic present and to reduce the bactericidal effect of human serum**. **Any medium showing turbidity should not be used**
- If strictly aerobic bacteria **(*Pseudomonas, Neisseria*)** the bottle should be **vented** as soon as it is received in the laboratory, by inserting a sterile cotton-wool-plugged needle through the previously disinfected diaphragm.**the use of a diphasic blood-culture bottle, with a broth phase and a solid-slant phase** on one of the flat surfaces of the bottle (Castaneda bottle), is recommended for the cultivation of Brucella spp.
- Blood-culture bottles should be incubated at 35–37 ◦C and routinely inspected twice a day (at least for the first 3 days) for signs of microbial growth.
- A sterile culture usually shows a layer of sediment red blood covered by a pale yellow transparent broth.
- Whenever **visible growth appears**, the bottle should be opened aseptically, a small amount of broth removed with a sterile loop or

Pasteur pipette, and a Gram-stained smear examined for the presence of microorganisms.

Table (3): Summary of bacterial blood infections.

Infection	Most Important Pathogens	Laboratory diagnosis
Endocarditis	*Streptococcus* spp. (60–80%) *Staphylococcus* spp. (20–35%) Gram-negative rods (2–13%) Numerous other bacterial spp. (5%) Fungi (2–4%) Culture negative (5–25%)	**Blood culture**, three sets from three different sites, within 1–2 h, before antimicrobials if possible. 10–20 ml venous blood into one aerobic and one anaerobic bottle, respectively.
Bacteria	***Staphylococcus aureus*** ***Streptococcus pneumoniae*** ***Enterobacteriaceae*** ***Mycobacterium tuberculosis***	Microscopy and culture from punctate DNA test from punctate if required
	Mycoplasma pneumoniae	Serology; culture from punctate
	Neisseria spp. Gram-negative anaerobes *Actinomyces* spp. *Nocardia* spp.	Microscopy and culture from punctate
	Rickettsia spp. *Chlamydia trachomatis*	Serology

Pretest: **الاختبار القبلي:**

Blood is cultured to detect and identify bacteria or other cultivable microorganisms. Explain?

Scientific Content: **المحتوى العلمي:**

-septicemia
-bacteremia
-blood collection and culturing.
- Subcultures are performed by streaking a loopful on appropriate media
- Blind subcultures and final processing
-blood contamination .

Posttest **الاختبار البعدي:**

Fill in the blank :

- For routine examinations, it is not necessary to incubate blood cultures beyond ------- days.
- A sterile culture usually shows a layer of sediment -------- covered by a ---------- transparent broth.
- (Castaneda bottle), is recommended for the cultivation of----------.

References: المصادر :

1- Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
2-Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.
3-Bailey & scott' s (2017): Diagnostic microbiology,fourteenth edition
4-Jeffrey C. Pommerville. (2018): Fundamentals of microbiology, Eleventh edition.
5-Orekan J. et al. (2021):Clinical Microbiology and Infection 27 .1400-1408.
6-Cynthia Nau Cornelissen (2015): Lippincott Illustrated Reviews Flash Cards MICROBIOLOGY .Third Edition
7-Prof.Dr.Mohammed Shammkhi Jeber.(2019):Notes of Diagnosis Microbiology

الجامعة التقنية الوسطى كلية التقنيات الصحية والطبية/ بغداد قسم المختبرات الطبية

المادة: الاحياء المجهرية التشخيصي المرحلة: الرابعة

Title: Lecture 16,17

العنوان :

Meningitis and other infections of the central nervous system

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

أ.م.د أحمد سالم محمد

Target population: **الفئة المستهدفة :**

طلبة المرحلة الرابعة

Introduction: المقدمة:

Diagnosis of bacterial brain abscess and Anaerobic infections:

Brain abscess is a serious and deadly clinical body. Pyogenic infection of brain parenchyma begins with a localized area of inflammatory change referred to as cerebritis. This early stage of infection has characterized by increased blood vessel permeability without angiogenesis. When unrecognized, this process will progress to an immature capsular stage and then to brain abscess, a condition defined by an area of parenchymal infection containing pus encapsulated by a vascularized membrane.

Anaerobic and microaerophilic cocci, gram-negative and gram-positive anaerobic bacilli

were the predominating bacterial isolates. **Many brain abscesses have mixed bacterial infections**. The predominant organisms include: *Staphylococcus aureus*, aerobic and anaerobic streptococci (especially *Streptococcus intermedius*), *Bacteroides*, and *Fusobacterium* species, Enterobacteriaceae, *Pseudomonas* species, and other anaerobes. Less common organisms include; *Haemophillus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitides*. Also bacterial abscess caused by *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella* spp., *Proteus* spp., *Enterobacter* spp., *Bacteroides* spp. and *Propionibacterium* spp.

Cerebrospinal (CSF) is a watery fluid, continuously produced and absorbed, which flows in the ventricles (cavities) within the brain and around the surface of the brain and spinal cord.

Functions of CSF:

- Hydrolic shock absorber
- Regulation of intracranial pressure
- Impacts the hunger sensation and eating behaviours

Bacterial infection of CSF cause meningitis, which ranks high among medical emergencies, and early, rapid, and exact diagnosis, is more essential. Diagnosis of meningitis depends on maintaining a high index of thought, obtaining **adequate specimens properly, and examining the specimens quickly.**

The most urgent diagnostic issue is the differentiation of acute purulent bacterial meningitis from aseptic (sterile) and granulomatous meningitis. The immediate decision usually based on the cell count, the glucose concentration in CSF and blood and protein content of cerebrospinal fluid, the results of microscopic examination for microorganisms. In addition, the results of culture, serologic tests, nucleic acid amplification tests, and other laboratory procedures.

Common Causes of Meningitis:

- Coagulase negative Staphylococci (especially *Staph. epidermidis*, *Staph. aureus*.
- Aerobic gram-negative bacilli, *Propionibacterium acnes*.
- Serogroup B streptococci *(Strep. agalactiae)* cause infection to neonates to age 3 months of age.
- *Escherichia coli* infect mainly neonates.
- *Listeria monocytogenes* also infect neonates; elderly; immunocompromised children and adults.
- *Haemophilus influenzae* infect children 6 months to 5 years
- *Neisseria meningitidis* infect all ages
- *Streptococcus pneumonia* infect all age groups; highest incidence in the young age.

Specimens

As soon as infection of the central nervous system has suspected, **blood samples** has taken for culture and **cerebrospinal fluid** (CSF) has obtained. To obtain cerebrospinal fluid, perform lumbar puncture with strict aseptic technique (Figure 1). Cerebrospinal fluid is usually collected in three to four portions of 2–5 ml each, in sterile tubes.

If bacterial meningitis has suspected, CSF is the best clinical specimen to use for isolation, identification, and characterization of the etiological agents. Suspected agents should include ***N. meningitidis, Strep. pneumoniae,* and *H. influenzae* and other pathogens in some cases.**

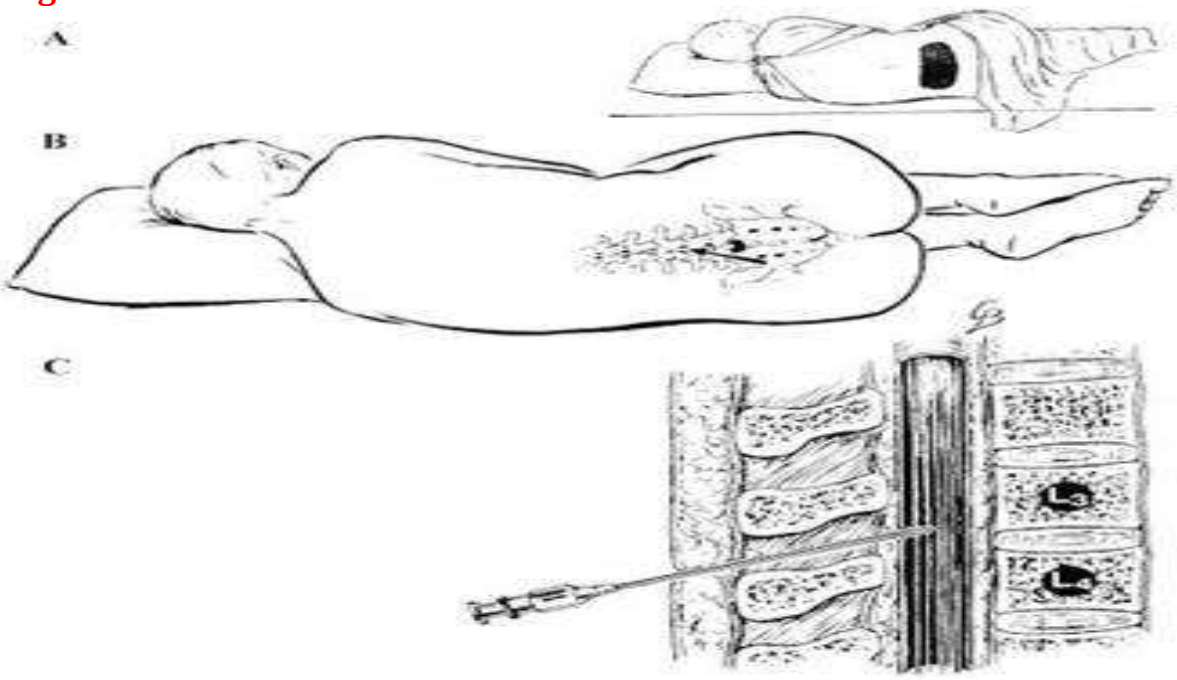


Figure (1): Collection of cerebrospinal fluid (CSF) by lumbar puncture.

Microscopic Examination

Smears have made from the sediment of centrifuged cerebrospinal fluid. Using a cytospin centrifuge to prepare the slides for staining has recommended because it concentrates cellular material and bacterial cells more effectively than standard centrifugation (Figure 2).

Smears have stained with **Gram stain**. Study of stained smears under the oil immersion objective may reveal intracellular gram-negative diplococci (meningococci), extracellular lancet-shaped gram-positive diplococci (pneumococci), or small gram-negative rods (*Hemophilus influenzae* or enteric gram-negative rods).

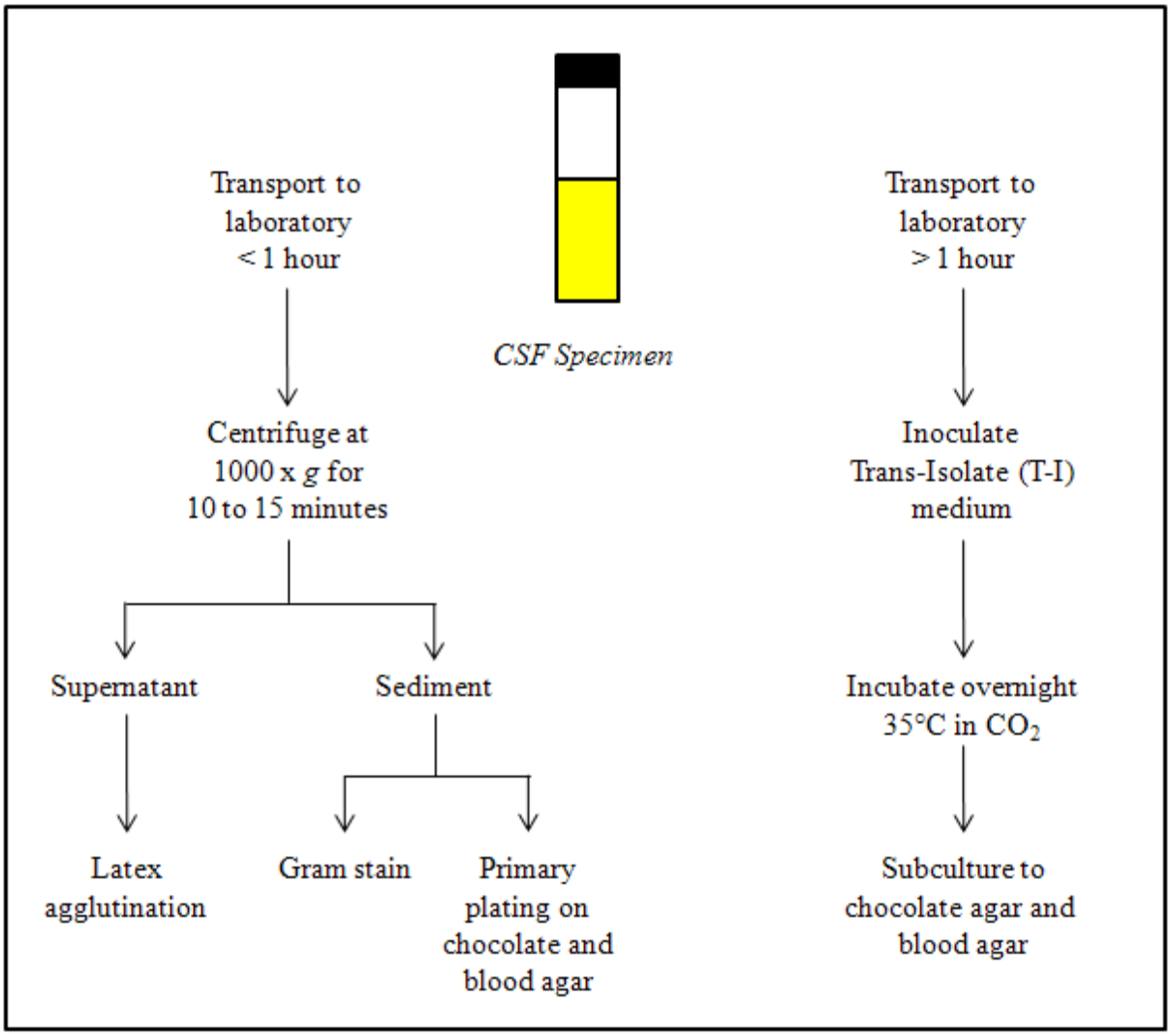


Figure (2): Cerebrospinal fluid (CSF) isolation and identification.

Culture

The culture methods used must help the growth of microorganisms most commonly encountered in meningitis. Sheep blood and chocolate agar together grow almost all bacteria that cause meningitis.

Follow-Up Examination of Cerebrospinal Fluid

The return of the cerebrospinal fluid glucose level and cell count toward normal is good evidence of adequate diagnosis and therapy.

Neisseria meningitids are gram-negative, coffee-bean shaped diplococci that may occur intracellularly or extracellularly in polymorphic nuclei (PMN) leukocytes. (**PMNs or neutrophils are often more than 1000 WBCs/cu mm**)

Neisseria meningitidis is a fastidious organism, aerobic diplococci, which grows best at 35-37°C with ~5% CO_2 (or in a candle-jar). It can grow on both a blood agar plate (BAP) and a

chocolate agar plate (CAP). Colonies of *N. meningitidis* are grey and unpigmented on a BAP and appear round, smooth, moist, shiny, and convex, with a clearly defined edge. ***N. meningitidis*** appear as large, colorless-to-grey, opaque colonies on a CAP (Figure 3, 4).

Biochemical tests have recommended confirming the identity of cultures that morphologically appear to be ***N. meningitidis*** such as oxidase test (+) and carbohydrate utilization (acid production from glucose, maltose). If the oxidase test is positive, carbohydrate utilization testing should have performed. If the carbohydrate utilization test indicates that the isolate may be *N. meningitidis*, serological tests to identify the serogroup should performed. Additional methods for identification and characterization of ***N. meningitidis*** using molecular tools like PCR technique.

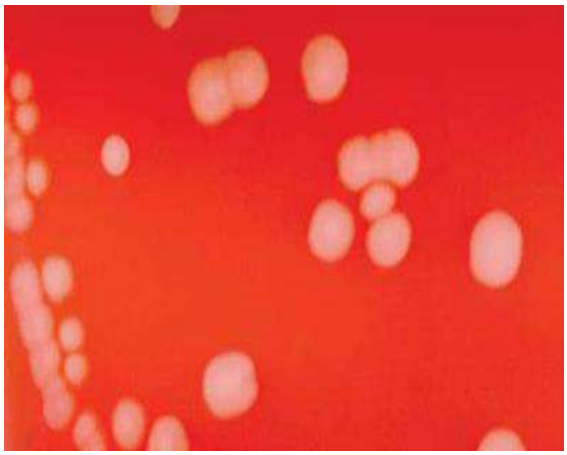


Figure (3): *N. meningitidis* colonies on a BAP

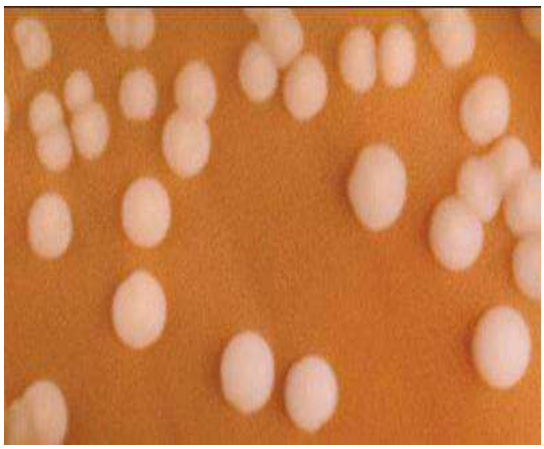


Figure (4): *N. meningitidis* colonies on a CAP

Streptococcus pneumoniae may occur intracellularly or extracellularly as gram-positive diplococci, but can also occur as single cocci or in short chains of cocci. ***Strep. pneumoniae*** is a fastidious bacterium, growing best at 35-37°C with ~5% CO_2 (or in a candle-jar). It is usually culturing on media that contain blood, but can also grow on a chocolate agar plate (CAP). On a blood agar plate (BAP), colonies of ***Strep. pneumoniae*** appear as small, grey, moist (sometimes mucoid), colonies and characteristically produce a zone of alpha-hemolysis (green) (**Figure 5**). The alpha-hemolytic property differentiates this organism from many species, but not from the commensal alpha-hemolytic (viridans) streptococci. Differentiating pneumococci from viridans streptococci is difficult as young pneumococcal colonies appear raised, similar to viridans streptococci. However, once the pneumococcal culture ages 24-48 hours, the colonies become flatten, and the central portion becomes depressed, which does not occur with viridans streptococci **(Figure 6).** When necessary, to obtain a pure culture. For the identification and characterization procedures, it is essential to test alpha-hemolytic colonies that are less than a day old, typically grown overnight at 35-37°C with ~5% CO_2 (or in a candle-jar).

The specialized tests have used to identify colonies on a BAP that resemble pneumococci

(Figure 7). ***Strep. pneumoniae*** can be identified using Gram stain, catalase (-), and optochin tests (see figure 8) (<14mm diameter) at the same time, with bile solubility (+) as a confirmatory test. If these tests indicate that, the isolate is *Strep. pneumoniae*, then serological tests used to identify the serotype caught performed. This sequence of testing is an efficient way to save costly serotyping reagents and time. Additional methods for identification and characterization of ***Strep. pneumoniae*** using molecular tools.

Figure (5): *Strep. pneumoniae* colonies with a surrounding green zone of alpha-hemolysis (black arrow) on a Blood Agar Plate.

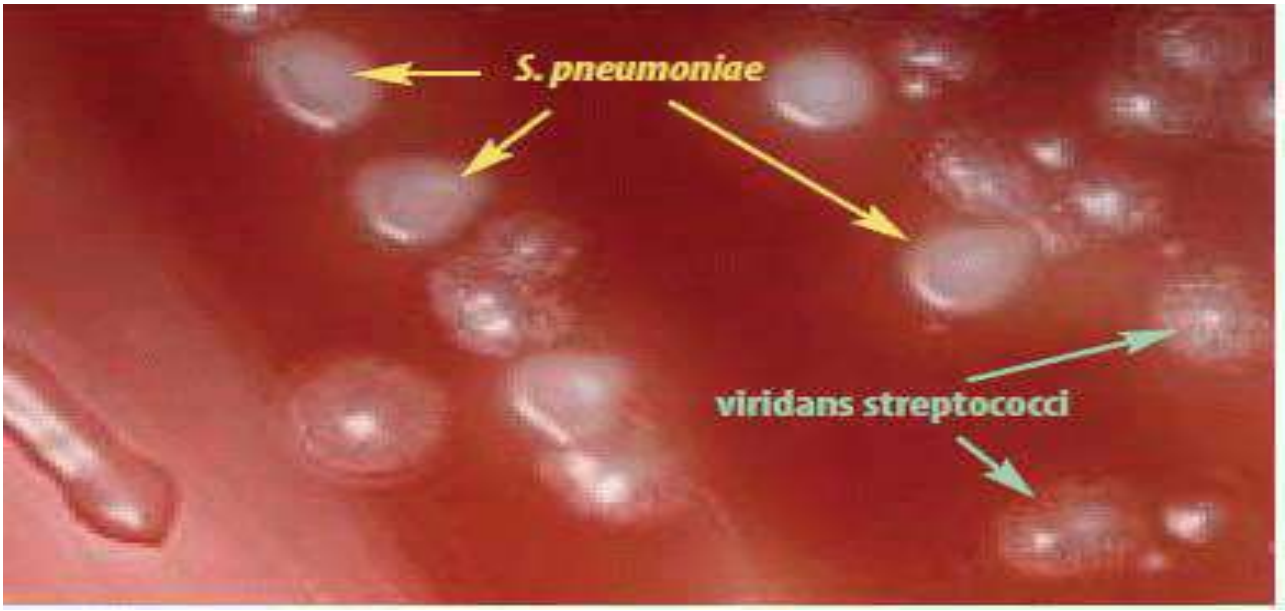


Figure (6): *Strep. pneumoniae* colonies have a flattened and depressed center after 24-

48 hours of growth on a BAP, whereas the viridans streptococci retain a raised center.

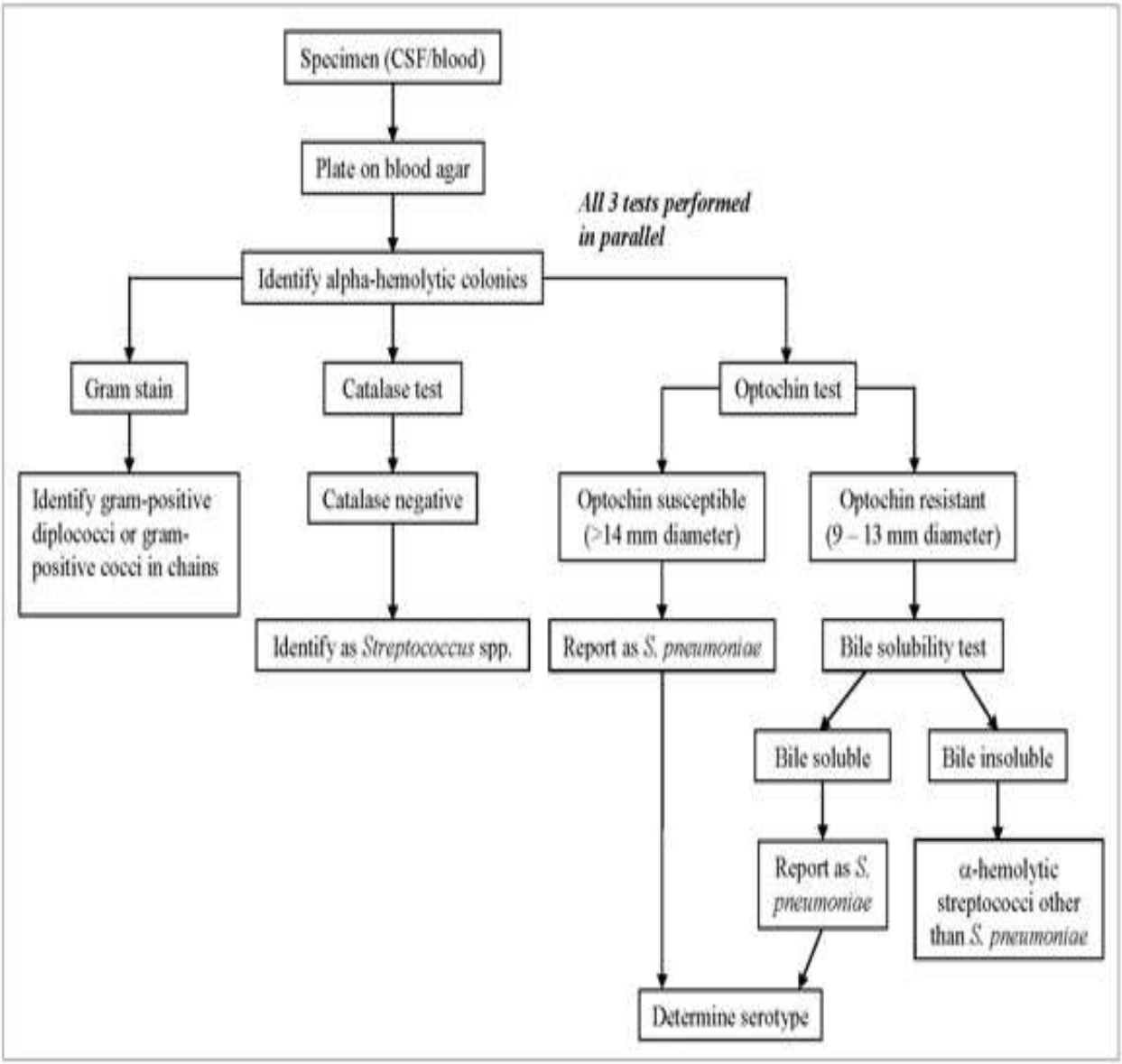


Figure (7): Flow chart for identification and characterization of a *Strep. pneumoniae* isolate.

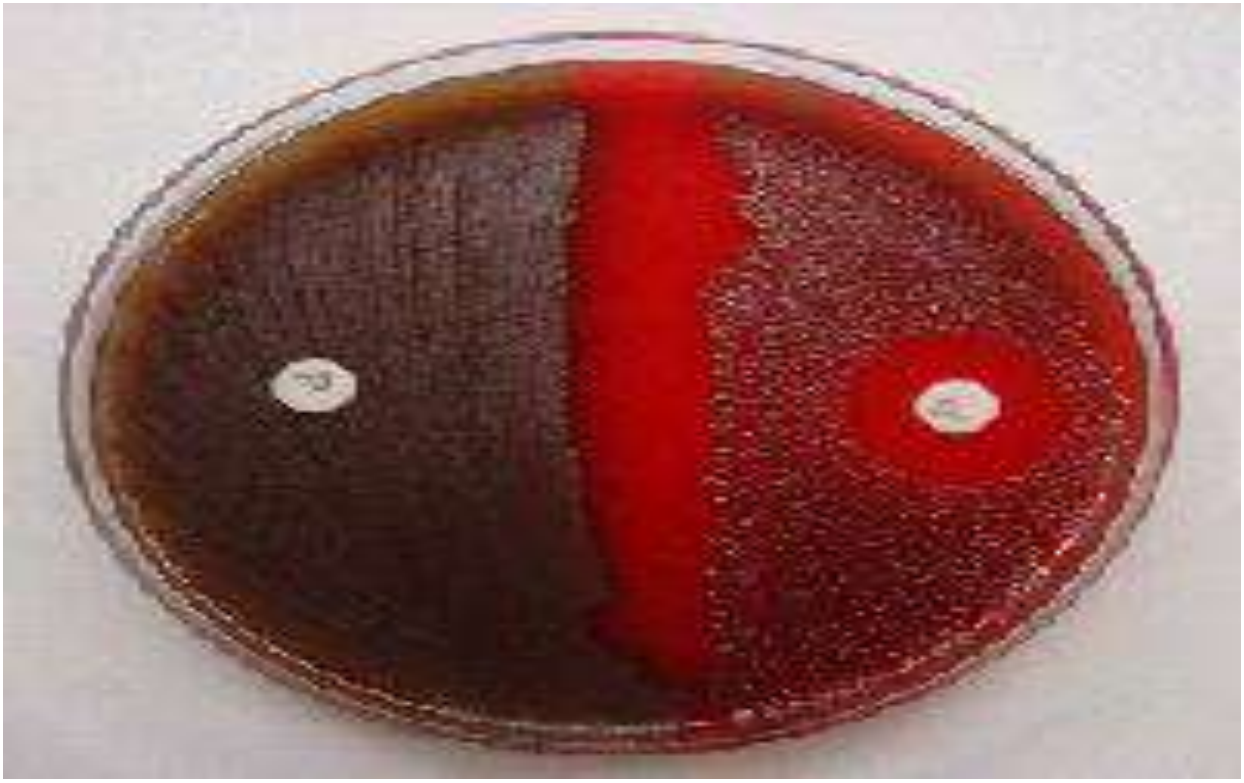


Figure (8): Optochin test for *Strep. pneumoniae* using optochin disks. The strain on the left is resistant to optochin with no zone of inhibition, and therefore is not a pneumococcus. The strain on the right is susceptible to optochin and is *Strep. pneumoniae*.

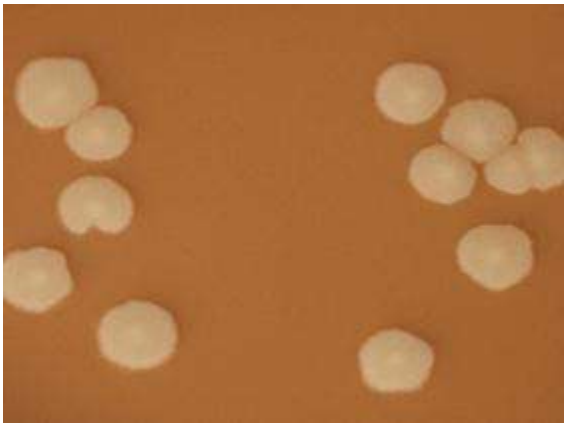
Haemophilus Influenzae are small, pleomorphic, gram-negative bacilli or coccobacilli with random arrangements. ***H. influenzae*** is a fastidious organism, which grows best at 35-37°C with ~5% CO_2 (or in a candle-jar) and requires hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD, also known as V factor) for growth. The standard medium used for growth of ***H. influenzae*** is a chocolate agar plate (CAP), which can be prepared with heat-lysed horse blood, a good source of both hemin and NAD, although sheep blood can also be used. Growth occurs on a CAP because NAD has released from the blood during the heating process of chocolate agar preparation and hemin is available from non-hemolyzed as well as hemolyzed blood cells.

H. influenzae appear as large, round, smooth, convex, colorless-to-grey, cloudy colonies on a CAP **(Figure 9).** ***H. influenzae*** produce a sharp indol smell, plates should not be opened in order to smell the cultures. ***H. influenzae*** cannot grow on an unsupplemented Blood Agar Plate. **(Figure 10).**

Biochemical tests have recommended confirming the identity of cultures that morphologically appear to be ***H. influenzae***. ***H. influenzae*** caught identified using Kovac's oxidase test and determining the necessity of hemin and **NAD as growth** requirements. If

the oxidase test is positive, hemin and NAD growth factor requirement testing should have performed. If the growth factor requirement test indicates that the isolate may be ***H. influenzae***, serological tests to identify the serotype should have performed. This sequence of testing is an efficient way to save costly antisera and time. Additional methods for identification and characterization of ***H. influenzae*** using molecular tools like PCR technique. Some of most common bacterial causes summarized at table (1).

Figure (9): *H. influenzae* colonies on a CAP



. **Figure (10): *H. influenzae* colonies on a CAP**

Table (1): Examples of bacterial nervous system infections.

Bacterial/mycobacterial pathogens that commonly cause meningitis

Pathogen	Risk Factor	Incidence
Streptococcus pneumoniae	Day care, HIV infection	Most common
Neisseria meningitidis	Crowded conditions	Outbreaks
Haemophilus influenzae		Significantly less common after vaccination
Listeria monocytogenes	Immune compromise, elderly	Less common
Group B streptococcus	Neonates	Decreased with antenatal detection of group B streptococcus
Escherichia coli	Neonates	Less common
Mycobacterium tuberculosis	Exposure, older age, immune compromise	Rare

Pretest: **الاختبار القبلي :**

1-what is CNS?
2- define CSF and numerate its functions in body

Scientific Content: المحتوى العلمي:

- Diagnosis of bacterial brain abscess and Anaerobic infections:
- Common Causes of Meningitis:
- Specimens
-Microscopic Examination
-Culture
- Follow-Up Examination of Cerebrospinal Fluid

Posttest الاختبار البعدي:

1- define meningitis, numerate some of bacrterial causes ?
3. Talk about the lab. Diagnosis of CNS infection with examples?

References: **المصادر :**

1- Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
2-Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.
3-Bailey & scott' s (2017): Diagnostic microbiology,fourteenth edition
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6-Cynthia Nau Cornelissen (2015): Lippincott Illustrated Reviews Flash Cards MICROBIOLOGY .Third Edition
7-Prof.Dr.Mohammed Shammkhi Jeber.(2019):Notes of Diagnosis Microbiology

الجامعة التقنية الوسطى كلية التقنيات الصحية والطبية/ بغداد قسم المختبرات الطبية

المادة: الاحياء المجهرية التشخيصى المرحلة: الرابعة

Title: Lecture 18,19

Diagnosis of bacterial respiratory tract infections

العنوان :

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

م.د.دنيا عبد الرزاق محمود

Target population: **الفئة المستهدفة :**

طلبة المرحلة الرابعة

Introduction: **المقدمة:**

Bacterial infections of respiratory tract

Respiratory system has divided into two major parts:

- Upper includes (nose and pharynx)
- Lower respiratory tract includes (larynx, trachea, bronchial tube and alveoli).

Each part or organ of this system has **own resident microflora**. Many factors play a vital role in challenging and limitation of **number and type of microflora colonizing**. Also each parts of respiratory tract **having physical factors** such as hair, mucus membrane lining the tract, cilia movement, sneezing, coughing besides oxygen tension in lung, which act all collectively as **unbreakable defense** line.

In addition, **innate immunity** and **circulating antibodies** stabilize natural balance, which represents equilibrium state between host immunity and **action of pathogens**.

Ear, eye and nose are all share common canal, so any infection of one of these parts may cause infection to others. **Nasal cavity** for example consider as a reservoir for genus Staphylococcus along with other gram-positive bacteria. Nasal cavity is the pathway for deeper parts of respiratory tract for example resident bacteria of **nasal cavity** may and **will find its way** to the system causing problems here location and to nervous system such as **meningitis**. Ear infection, on other hand may be the way for **enteric bacteria** to reach to un-limited area in respiratory or nervous systems. ***E. coli* meningitis** is one example among many of such cases.

Tonsils are the major front line of defense, yet, it is frequently had infected with so many species of bacteria, **Gram-negative** as well as **Gram-positive** bacteria.

Infection of respiratory tract sometimes classified as adult or childhood infections in this regard, ***Bordetella Pertussis*** the causative agents of whooping cough is the example of childhood infections. Respiratory infections may have classified as **accidental or seasonal infections**. The latter has associated with possible changes in the weather, from winter to summer and vice versa, bacterial infection may come **second to viral infection** in this aspect. Accidental infection is the infection that man acquired during daily life.

No limitation for the types of bacteria that may **cause** infection to **respiratory system** regardless the way that bacteria inter the system. Most of normal flora of upper respiratory tract play an important role in **causing opportunistic disease**. Staphylococus, Streptococcus, Haemophilus, Corynebacterium, Neisseria, Bacteroides, Fusobacterium, and Actinomyces, are typical examples for these bacteria.

Nearly any type of **gram-positive** or **negative** bacteria **Pneumonia**, **Mycoplasma** and **Chlamydia spp**., can cause respiratory infection. On other hand, may cause non-specific pneumonia, while **Tuberculosis** caused by Mycobacterium tuberculosis complex, both of these diseases involved **lower** respiratory tract.

Sore throat is a common infection of upper respiratory tract caused specially by **hemolytic Streptococci**, besides other **gram-positive cocci** or **gram-negative bacilli** (***Haemophilus spp*.**).

The middle and inner ear are normally sterile, while outer ear and auditory canal contain the **normal flora of mouth and nose**. When a person coughs, sneezes or blow the nose these microorganisms may reach middle or inner ear and causing infection. **Tears in eyes** decreases the number of microorganisms that may find its way to eye because it's content of **lysozyme that destroys bacterial cells**. (fig.1)

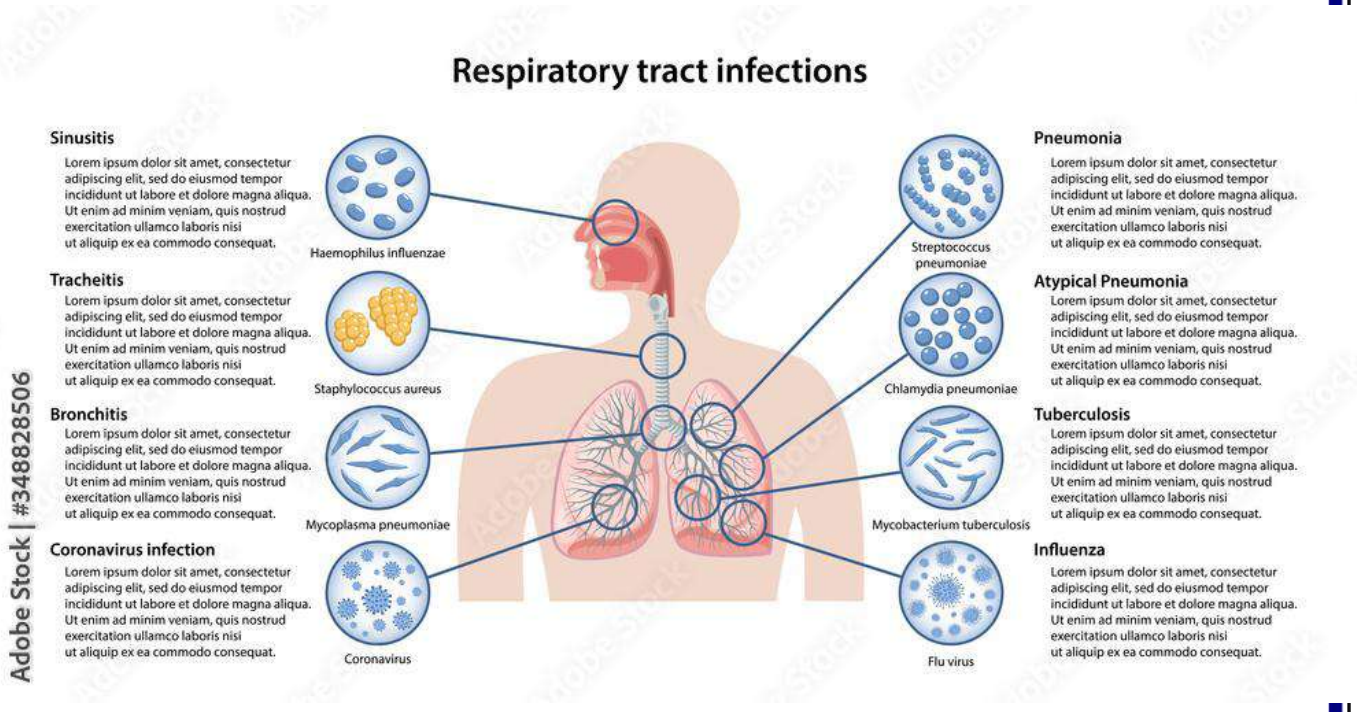

Fig.(1): Summary of bacterial respiratory tract infection

Bronchitis

1. Acute bronchitis

It is an acute inflammation of the tracheobronchial tree generally self-limited and with eventual (final) complete healing and return of function.

Causative agent: ***Mycobacterium pneumonia; Bordetella pertussis***

Laboratory diagnosis:

Specimen: **Sputum**

Procedure: **Gram staining**, **culture, biochemical** and **serological test** for microbe identification.

2. Chronic bronchitis

It has defined as chronic productive cough for at least three months in each of two successive years.

Causative factors: Cigarette smoking; Air pollution; Exposure to harmful stimuli

Bacteria that improve chronic bronchitis are:

Streptococcus pneumonia; Haempphilus influenza; Mycoplasma pneumoniae Branhamella catarrhalis.

Laboratory diagnosis:

Specimen: **Sputum**

Procedure: **Gram staining, culture, biochemical and serological test** for microbe identification.

Pneumonia

It is infection of the lung parenchyma.

Causative agents: ***Strep. pneumonia, Staph. aureus, Hemophillus influenzae*** and ***Mycoplasma pneumonia*.**

Route of entry of microbes to the lung:

- Aspiration of oral and gastric secretion
- Haematogenous spread from distant foci
- Direct inoculation and local spread from surrounding tissue
- Inhalation

Laboratory diagnosis:

Specimen: **Lower respiratory secretion** which indicated by **greater than 25 Neutrophils** and **less than 10 squamous epithelial cells** per high power field.
Procedure: Gram staining, culture, biochemical and serological test for microbe identification**.**

Bacterial Diagnosis of TB infection

Tuberculosis

It is a disease caused by group of ***Mycobacterium spp***., namely Mycobacterium tuberculosis complex. ***M. tuberculosis*** is of human origin, ***M. bovis*** is of cattle origin, ***M. avium*** is of bird origin.

The main problem of these bacteria is:

1. Their high resistance to environmental stress such as dryness.
2. Survive in dry sputum for months.
3. Members of genus mycobacterium are very resistant to chemical and antibiotic treatment.

All these features are because of their highly **contents of cell wall of lipids**. Cell wall lipid content makes these bacteria **difficult to stain** with ordinary stains. Therefore, special stain is required (Acid Fast Stain: AFS). **AFS** depends on **penetration of Carbol-fuchsin dye to cell wall with aid of heat**, once it is in there, a complex of stain and lipid of cell wall is formed, this complex is **not removed** by normal **decolorizing agent (alcohol**), it **resists even the decolorizing** with acid-alcohol from which it takes its name (Acid Fast Bacteria).

Air born **droplets**, **milk**, or even **prolonged contact** with sick peoples consist collectively the major pathways for **transmission of disease**, yet, **air born rout** is the **important rout of entry,** fine particles containing one or two TB. **Cells travels** from patient for a distance of one meter **to another person** (air born) will enough to cause a disease in susceptible individual; normally these bacteria are overcoming by **host defense**. If bacteria succeeded to penetrate host defense, then **alveoli** will the **battlefield (area)** of the disease.

Bacilli are **multiply in macrophages** protect themselves against killing process, in a self-protection process host try to limit the drastic (severe) effect of the pathogen by forming a **tubercle,** which is a **matrix tissues**, **exudates**, **WBC**s, and other materials. ***M. tuberculosis*** tend to arrange in cord formation, which increase the immune response of host resulting in what is called hypersensitivity reaction which lead ultimately to tissue damage.

Lab. diagnosis:

Mycobacterium may come from a wide range of samples, these include; sputum, lung wash,

urine, wound, CSF, lymph secretion, bone, gastro-intestinal material.
The prime diagnostic parameter is **culturing of materials** (regardless the origin of it) on suitable culture medium, the medium commonly used is (**L J M**), enriched media with **high contents** of **nutrition** to aid the **long period of incubation**. TB bacilli appear as **hydrophobic colonies with wrinkled (crumpled) surface**. Because of long time of incubation, **alternative diagnostic methods** have employed such as **PCR** or other methods.
Blood film might of little help in diagnosis of TB. Since **WBCs,** count may **still normal** with marked **elevation in number of monocytes**. ESR on the other hand might more evident in this regard, **ESR** is **shooting up reaching levels of 100 mm/h** or higher. Commercial kits for diagnosis of **IgM and IgG for TB**. Are available now in local markets.

AFB serves as a **screening test** in diagnosis of TB., the existence of **even a single** bacilli/ many microscopic fields is **enough to consider it " AFB positive**", yet the absence of AFB from the investigated sample does not mean that " patient has no TB. And vice-versa the existence of AFB does not mean that patient is a TB. Patient. Since may other bacteria such as **Nocardia** may show a similar appearance of TB.

Pretest: الاختبار القبلي :

1- classified of (RTI) ?
2- Symptoms and signs of RT disease may include -----,------,-----,------,------.

Scientific Content: المحتوى العلمي:

-Bacterial infections of respiratory tract
-Bronchitis
1. Acute bronchitis1-*Strep.pnemonia*
2. Chronic bronchitis
-Pneumonia
-Bacterial Diagnosis of TB infection
-Tuberculosis

Posttest الاختبار البعدي:

1- Enumerate the normal flora of URT which play an important role in causing opportunistic disease.
2- Explain bacterial diagnosis of TB infection and what the main problem of these bacteria ?
3. what are the micro organisms which are responsible of pulmonary abscess Necrotizing pneumonia
4. each parts of respiratory tract having physical factors ,what are ?

المصادر :
References:

1. Orekan J. et al. (2021):Clinical Microbiology and Infection 27 .1400-1408.
2. Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
3. Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.
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7.Prof.Dr.Mohammed Shammkhi Jeber.(2019):Notes of Diagnosis Microbiology

Bacterial infections of urinary tract systems

Title: Lecture 20,21

العنوان:

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

م.د.دنيا عبد الرزاق محمود

Target population: طلبة المرحلة الرابعة **الفئة المستهدفة:**

طلبة المرحلة الرابعة

Introduction: **المقدمة:**

Bacterial infections of urinary tract systems

Urinary tract consists of the kidney, ureters, bladder and urethra as shown in figure (2). The function of it is produce and process urine, which is **normal sterile**. Urinary tract infection has classified as; **upper** or **lower tract** infections based on the location of infections. The upper urinary tract consists of the kidneys and the ureters, and the lower urinary tract consists of the bladder and the urethra.

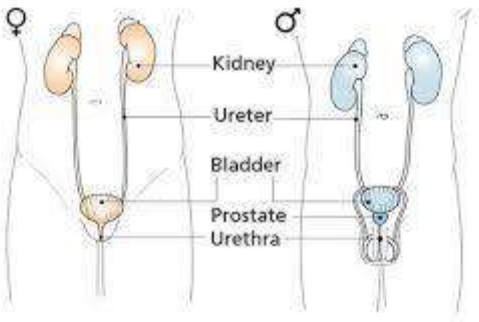

Figure (2): Male and female urinary tract system.

They are one of the most common types of infection and account for around 8.1 million **They are one of the most common types of infection and account for around** 8.1 million **visits to a p hysician every year. A proper classification has employed c urrently:** H hospital **or community acquired infections**, whatever the classification is an infection could affect ureters (ureteritis), or renal parenchyma cells (pyelonephritis), or urethra **urethritis), or the bladder (cystitis). Sometimes prostate gland might involve (prostitis). There are** t hree routes for b acteria to gain excess to UT**.: a scending, hematogenous and lymphatics.**

Routes of infection

1. **Ascending route (passage of bacteria from urethra to bladder and kidney).**
2. **Haematogenous route (source of infection is blood).**

Ascending route is the commonest route infection of the urinary tract.

Females are susceptible to get infection than men because of shorter urethra that allow pathogens to reach different site of urinary tract. The **only part of UT** has a limited number of resident bacteria is **urethra,** these microflorae colonize the epithelium in the **distal portion.**

Most UTIs go away after treatment. Chronic UTIs **either don't go away after treatment or keep recurring. Recurrent UTIs are common among women**

Bacterial species involved in community acquired UTI is by far *E. coli***, yet not all** E . coli **are capable causing UTI,** only those uropathogenic E. coli equipped **by** pili **are responsible for UTIs. Other microorganisms incriminated with UTIs are** *Proteus spp., Klebseilla sp., Enterobacter sp***. and** *Acinetobacter sp***.**

On the other hand, *Staph. saprophyticus* **is more efficient in attaching to UT epithelial** cells than **coagulase positive Staphylococcus or Staph. Epidermidis**. The former is associated with UTIs among females in reproductive ages. ***Proteus spp***. that produce urease **turns the environment alkaline which causing damage to tissues leading to** renal stone (normal v aginal pH level is between 3.8-4.5).

Lab. Diagnosis

The diagnosis of UTI include general examination **of urine then** culture **has done depending on findings of general examination. Other parameters of diagnosis might aid** the diagnosis of UTI, **biochemical parameters** are of great importance in diagnosis of complicated UTI, **hematological parameters** aid the diagnosis by showing of elevation **(raise) in number of** leucocytes in general and neutrophils in specific**.**

Culture, is on the **t op of all diagnostic tools**, final decision is going to be taken according to the out-come of culture. Different culture media are used to full-fill this purpose.**V itek** system, PCR**, or other techniques come to** confirm the diagnosis **in most cases.** system, PCR**, or other techniques come to** confirm the diagnosis **in most cases.**

The existence of pus **cells is the** guide **to culture urine sample, yet, this is not valid for every case, pus cells sometimes may reflect an inflammation act, i.e. culture shows no bacterial growth, or absence of pus** d oes not mean that the patient has no UTIs**.** *Proteus spp., Staphylococcus spp***. or any bacterial species** produces urease enzyme may destroy pus cells and give false negative results. *Mycoplasma***,** *Chlamydia spp.* are produced pus **with** no growth on culturing routinely**, so it very important to take care towards** these observations**. (Pus consists of a thin**, protein**-rich fluid and dead** leukocytes **from the body's** immune response **(mostly** neutrophils**)).**

The commonest causative agents of UTIs are gram-negative rods (as listed in

figure 20). These are: ***Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus spp., Enterobacter aerogens .***
Other important causative agents: ***Enterococci*** and ***Staphylococcus saprophyticus***.

Laboratory diagnosis:

Specimens: Clean midstream urine, Catheterized urine, Suprapubic aspiration.
Direct microscopic examinations: WBCs, RBCs, Epithelial cells at general urine analysis. The presence of more than five WBCs **and abundant** epithelial cells **per HPF (**high-power field**) supports infections of urinary trac**
Gram stain: The presence of one bacterium in u n-centrifuged gram stained urine confirms urinary tract infections.
Culture: Blood agar medium, MacConkey agar medium (see figure 2 1).

1. **Urethral and vaginal discharge**

Urethritis**: It manifests with urethral discharge, pain during urination and frequency of urination. These types are:**

a. Gonococcal urethritis

Causative agent: Neisseria gonorrhea
Incubation period is 2-7 days.
It accounts for 1/3 of urethritis cases.
Clinical findings: Yellowish purulent discharge and dysuria.

b. Non-gonococcal urethritis

Causative agents: Chlamydia trachomatis **(50%);** Ureaplasma urealyticum **(30%); and** Mycoplasma hominis**.**
Incubation p eriod about 2-3 weeks.
Clinical findings: White mucoid discharge

Laboratory diagnosis:

- Specimen: Urethral discharge or swab (Before urination or antibiotics)
- Gram stain: Gram-negative intracellular diplococcic
- Culture: Modified thayer-martin medium
- Biochemical and serology: Species identification

2. Cervicitis / Vaginitis
It manifests with vaginal discharge.
Causative agents: **Neisseria gonorrhea** (**Mucopurulent vaginal discharge).**
Non-specific vaginitis (Yellowish homogenous vaginal discharge). It is caused by

anaerobes and **Gardnerella vaginalis**

Laboratory diagnosis:

❖**Specimen: Vaginal discharge.**

❖Wet mount: Clue (indication) cells that distorted vaginal epithelial cells coated heavily with gram-negative coccobacilli which are diagnostic of infection with Gardnerella vaginalis

❖**Gram stain, culture, biochemical and serology for species identification.**

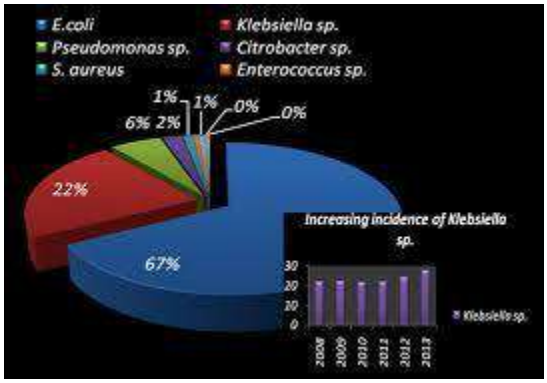

Figure (20): Percentages of UTIs bacterial causes.

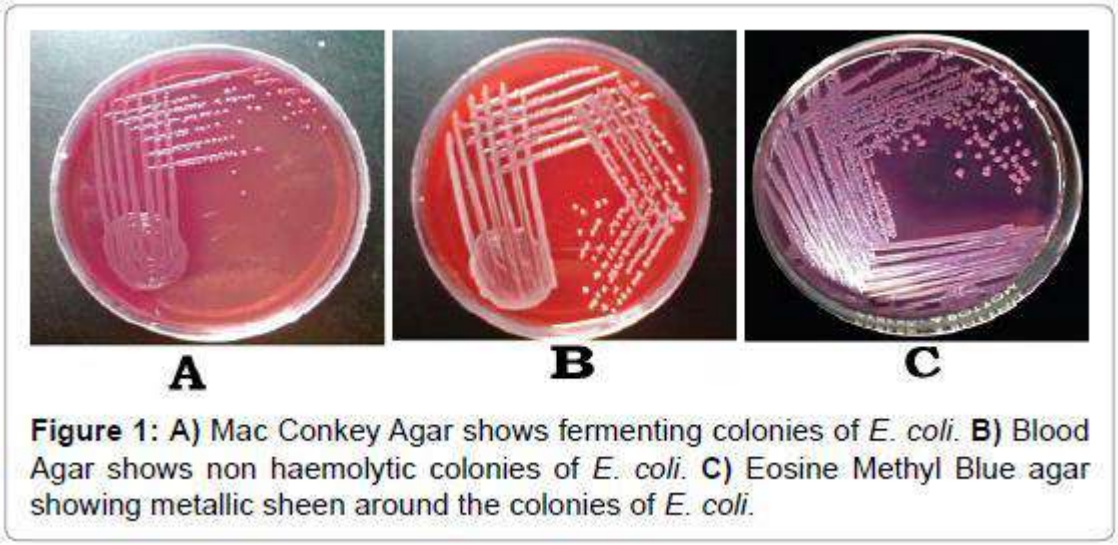

Figure 1: A) Mac Conkey Agar shows fermenting colonies of *E. coli*. **B)** Blood Agar shows non haemolytic colonies of *E. coli*. **C)** Eosine Methyl Blue agar showing metallic sheen around the colonies of *E. coli*.

Figure (21): E . coli on blood and MacConkey agar.

Pretest: الاختبار القبلي:

What are the excess to UT ?

Scientific Content: المحتوى العلمي:

-Chronic UTIs

- the diagnosis of (UTI)include general examination of urine and culture

- Escherichia coli, Klebsiella pneumonia, proteus spp, Enterobacter aerogens Pseudomonas aeruginosa* and *Neisseria gonorrhea

- gonococcal urethritis

-non gonococcal urethritis

Posttest الاختبار البعدي:

3- **What is the causative agents of gonococcal urethritis ?**

4- **Which are common pathogenic bacteria isolated form urinary tract ?**

5- **Pus cells sometimes may reflect an inflammation , why?**

6- **What is the most serious type of UTI?**

References: المصادر :

1- Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.

2-Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.

3-Bailey & scott' s (2017): Diagnostic microbiology,fourteenth edition

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الجامعة التقنية الوسطى كلية التقنيات الصحية والطبية/ بغداد قسم المختبرات الطبية

المادة Diagnostic Microbiology المرحلة: الرابعة

Title: Lecture 22

العنوان :

Diagnosis of bacterial genital tract infections

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

أ.م.د.امال عزيز كريم

Target population: **الفئة المستهدفة :**

طلبة المرحلة الرابعة

Introduction: **المقدمة:**

Diagnosis of bacterial genital tract infections

Sexually transmitted infections (STI), or sexually transmitted diseases (STD) and venereal diseases (VD), are infections that are commonly spread by sex, especially vaginal contact, anal sex. Most STIs initially do not cause symptoms. Symptoms and signs of disease may include vaginal discharge, penile discharge, ulcers on or around the genitals, and pelvic pain. Some STIs may cause problems with the ability to get pregnant.More than 30 different bacteria, viruses, and parasites can cause STIs.

Bacterial STIs include:

1. Chlamydia (*Chlamydia trachomatis*)
2. Gonorrhea (*Neisseria gonorrhoeae*
3. Granuloma inguinale or (*Klebsiella granulomatis*)
4. *Mycoplasma genitalium; Mycoplasma hominis*
5. Syphilis due to Spirochetes (*Treponema pallidum*)
6. Ureaplasma infection usually spread by sex, some STIs could also spread by non-sexual contact with contaminated blood and tissues, breastfeeding, or during childbirth.

The Male and Female reproductive systems have a set of natural defenses against infection

In the male only the urethra is colonized by resident microbes because mount local immune responses, providing antibodies along the entire length of the urethra and in the seminal fluid.

In the female reproductive tract, the vagina has an acidic pH because *Lactobacillus* and other acid-producing species produce lactic acid. The acidic environment discourages the growth of many potential pathogens. Because the cervix represents several antimicrobial defense mechanisms, including a mucociliary escalator; mucus that contains a variety of antimicrobial chemicals, including lysozyme and lactoferrin; and antibacterial peptides that can kill or inhibit the growth of many bacterial species.

***Chlamydia trachomatis*:**

The disease chlamydia or chlamydial urethritis is caused by *Chlamydia trachomatis*, an exceptionally small (0.35 μm), round to ovoid-shaped organism. Being an obligate, intracellular parasite, it has one of the smallest bacterial genomes, having about 600 genes (*Escherichia coli* has around 4,200 genes). Serotypes D–K of *C. trachomatis* are associated with NGU.

***C. trachomatis* has a biphasic and unique reproductive cycle** (Figure 11-1). There is a nonreplicating, extracellular, infectious elementary body (EB) and a replicating, intracellular, noninfectious reticulate body. Humans appear to be the only host for the organism. Chlamydial urethritis is the most common STI globally.

***C. trachomatis* is transmitted** by any sexually active individual can be infected through sexual contact with an infected individual. The disease has an incubation period of about 1 to 3 weeks. Chlamydia often is referred to as the "silent disease" because the organism does not cause extensive tissue injury directly. Thus, some 85% to 90% of infected individuals are asymptomatic, whereas others might not seek treatment and thus can unknowingly pass the disease on to others.

If symptoms do occur, females often note a slight cervical discharge (drainage of fluid from the complication is uncommon. Chlamydial pharyngitis or inflammation of the anus (proctitis) is possible through anal intercourse.

The reproductive cycle of the chlamydiae involves two types of cells. After infection, nonreplicating elementary bodies (EBs) reorganize into reticulate bodies (RBs), which divide to form additional RBs. Within 30 hours, the RBs begin to reorganize into EBs within an inclusion. The EBs are released directly from the inclusion body or by the spontaneous lysis of the inclusion and host cell.

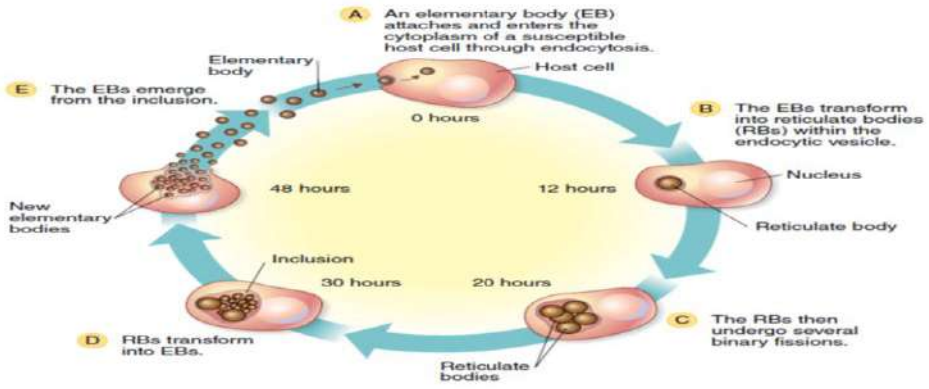

Figure (11-1): The Chlamydial Life Cycle.

Laboratory identification

Chlamydia trachomatis could have demonstrated in clinical material by several direct procedures and by culturing in human cell lines (tissue culture). Samples, particularly from the urethra and cervix in urogenital tract infection and conjunctivae in ocular disease, should obtained by cleaning away overlying exudate and gently scraping to collect infected epithelial cells.

1. **Direct tests:** Microscopic examination using direct fluorescent antibody staining reveals characteristic cellular cytoplasmic inclusions.*C. trachomatis* infections could have detected with high sensitivity and specificity using DNA amplification performed on urine specimens.
2. **Culturing methods:** *Chlamydia trachomatis* could have cultivated by tissue culture in several human cell lines. The presence of chlamydial inclusions could have demonstrated after 2 to 7 days of incubation.
3. **Detection of serotypes:** Serotypes of *Chlamydia trachomatis* could determine by immunofluorescence staining with monoclonal antibodies. However, the procedure is not widely used because it enhances little to clinical effects. **Serologic testing** for specific antibodies is similarly not helpful except in suspected lympho-granuloma venereum (LGV), in which a single high-titer response is diagnostic.

Neisseria gonorrhoeae

One of the most common STIs in men and women is gonorrhea caused by *Neisseria gonorrhoeae*, a small, unencapsulated, nonmotile, gram-negative diplococcus named for Albert Neisser, who isolated it in 1879. The organism, commonly known as the gonococcus, has a characteristic double-bean shape.The great majority of cases of gonorrhea are transmitted during sexual intercourse.

The organism is found only in humans, where it can infect the cervix, uterus, fallopian tubes, and urethra, as well as the mouth, throat, and anus.

Gonorrhea is the second most frequently reported nationally.

Virulence Factors

• Receptors for human transferrin
• Capsule (*N. meningitidis*)
• Pili (fimbriae)
• Cell membrane proteins
• **Lipooligosaccharide** (LOS) or endotoxin;

Clinical Presentation

Following attachment of *N. gonorrhoeae* by pili to the genital tract, the incubation period for gonorrhea ranges from 2 to 6 days. In females, the cervix becomes reddened, and a discharge might be expressed by pressure against the pubic area. Patients often report abdominal pain and a burning sensation on urination, and the normal menstrual cycle might be interrupted.

In some females, gonorrhea also spreads to the fallopian tubes. As these thin passageways become riddled with pouches and adhesions, salpingitis and

Gonorrhea is particularly dangerous to an infant born to an infected woman. The infant can contract gonococci during passage through the birth canal and develop neonatal conjunctivitis.

Symptoms of gonorrhea tend to be more acute in males than in females, and males thus tend to seek diagnosis and treatment more readily. When gonococci infect the mucus membranes of the urethra, symptoms include a tingling sensation in the penis, followed in a few days by pain when urinating.There is also a thin, watery discharge at first, followed later by more

obvious yellow, thick fluidnresembling semen. Frequent urination and an urge to urinate develop as the disease spreads further into the urethra.

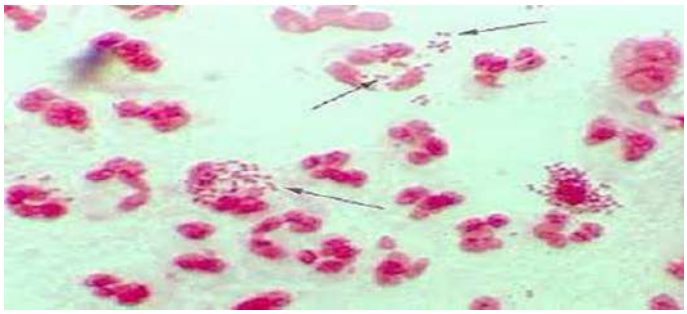

Figure (11-2):Gonococcal Urethral Smear. A gram-stained smear of discharge from the male urethra showing the gonococci (arrows) in the cytoplasm of white blood cells.

Laboratory identification:

In the male, the finding of numerous neutrophils containing gram negative diplococci in a smear of **urethral exudate** permits a temporary diagnosis of gonococcal infection and indicates that the individual should treated. In contrast, a positive culture has needed to diagnose gonococcal infection in the female as well as at sites other than the urethra in the male.

1. Growth conditions for culture: *N. gonorrhoeae* grows best under aerobic conditions, and most strains require enhanced CO_2. *N. gonorrhoeae* utilizes glucose as a carbon and energy source but not maltose, lactose, or sucrose. [Note: *N. meningitidis* utilizes both glucose and maltose. All members of the genus are **oxidase-positive**, that used to identify Neisseriae.

2. Selective media: Gonococci, like pneumococci, are very sensitive to heating or drying. Cultures might have plated quickly or, if this is not possible, transport media might use to extend the viability of the organism to be cultured. Thayer-Martin medium (chocolate agar supplemented with several antibiotics that suppress the growth of nonpathogenic Neisseriae and other normal and abnormal flora) has typically used to isolate gonococci. On nonselective media, the normal flora overgrows the gonococci. Culture of *N. gonorrhoeae* on Thayer-Martin agar remains the "gold standard" for

diagnosis.

Treponema pallidum

Syphilis is caused by *Treponema pallidum* .This spirochete moves by means of endoflagella. Humans are the only host for *T. pallidum*, so the organism must spread by direct human-to-human contact, usually during sexual intercourse.

Syphilis is currently ranked among the top five most-reported microbial diseases in the United States. The cells of *T. pallidum* can evade immune defenses because the pathogen has very few surface proteins that the immune system can recognize. This invisibility allows the pathogen to persist in the body.

The pathogen penetrates the skin surface through the mucous membranes of the genitalia or via a wound, abrasion, or hair follicle. The variety of clinical symptoms accompanying the stages, and their similarity to other diseases, have led some physicians to call syphilis the "great imitator."
Untreated, the disease can progress through a number of stages, which can overlap.

Clinical Presentation

The incubation period for syphilis varies greatly (10 to 90 days), but it averages about 3 weeks.

_ **Primary Syphilis.** A lesion, called a chancre, which is a painless circular, purplish ulcer with a small, raised margin with hard edges is typical of primary syphilis .The chancre develops at the site of entry of the spirochetes, often the genital organs. However, any area of the skin can be affected, including the pharynx, rectum, or lips.

The chancre teems with spirochetes and represents the most infectious stage. It persists for 3 to 6 weeks and then heals spontaneously. However, the infection has not been eliminated, as the spirochetes have spread through the blood and lymph to other body organs.

_ **Secondary Syphilis.** Several weeks after the chancre of primary syphilis has healed, the patient develops a fever and a flu-like illness as well as swollen lymph nodes. With secondary syphilis, a skin rash develops,
which can be mistaken for measles, rubella, or chickenpox. The rash appears as reddish-brown spots on the palms, face, and trunk .Transmission can occur if there are moist lesions.

Tertiary Syphilis. About 40% of untreated patients develop tertiary syphilis. This stage occurs in many forms, but most commonly,it involves the skin, skeletal, or cardiovascular and nervous systems. The hallmark of
tertiary syphilis is the gumma, a soft, painless,gummy noninfectious granular lesion.

Congenital syphilis: is a serious problem in pregnant women because the *Treponema* spirochetes penetrate the placental barrier after the third or fourth month of pregnancy. Infection in the fetus can lead to death (stillbirth); surviving infants can develop skin lesions and open sores. Affected children often suffer poor bone formation, meningitis, or Hutchinson's triad, a combination of deafness, impaired vision, and notched, peg-shaped teeth.

Laboratory identification:
Syphilis is difficult to diagnose clinically early in its presentation. Confirmation is via either blood tests or direct **visual inspection** using microscopy. **Blood tests** have more commonly used, as they are easier to perform. Diagnostic tests are unable to distinguish between the stages of the disease.
Definitive diagnosis of syphilis has complicated by the inability to cultivate *Treponema pallidum* subsp *pallidum* in vitro. Clinical manifestations,
demonstration of treponemes in lesion material, and **serologic reactions** have used for diagnosis. If manifestations include one or more cutaneous exudative lesions, motile treponemes could visualized within lesion exudate by dark-field microscopy.

Treponema pallidum subsp *pallidum* is a fastidious organism that exhibits narrow optimal ranges of pH (7.2 to 7.4) and temperature (30 to 37°C). It is rapidly inactivated by mild heat, cold, desiccation, and most disinfectants.
Traditionally this organism had considered a strict anaerobe, but it is now known to be microaerophilic. The in vivo generation time is relatively long (30 hours). *T.pallidum* subsp *pallidum* had not successfully cultured in vitro. Viable organismscan be maintained for 18 to 21 days in complex media, while limited replication has been obtained by co-cultivation with tissue culture cells.

Blood tests

Blood tests have divided into non-treponemal and treponemal tests.
Because of the possibility of false positives with non-treponemal tests, confirmation is required with a treponemal test, such as treponemal pallidum particle agglutination (TPHA) or fluorescent treponemal antibody absorption test (FTA-Abs).
Treponemal antibody tests usually **become positive two to five weeks** after the initial infection. **Neurosyphilis is diagnosed** by finding high numbers of **leukocytes** (predominately lymphocytes) and high protein levels in the cerebrospinal fluid (CSF) in the setting of a known syphilis infection.

Direct testing

Dark ground microscopy of serous fluid from a chancre (painless ulcer) may be used to make an immediate diagnosis. Sensitivity has reported to be nearly 80%; therefore, the test can only use to confirm a diagnosis.
Two other tests can carried out on a sample from the chancre: direct fluorescent antibody testing and nucleic acid amplification tests.
Direct fluorescent testing uses antibodies tagged with fluorescein, which attach to specific syphilis proteins, while nucleic acid amplification uses techniques, such as the **polymerase chain reaction**, to detect the presence of specific syphilis genes.
These tests are not as time-sensitive, as they do not require living bacteria to make the diagnosis.

Pretest: **الاختبار القبلي :**

1-Define STD?

2- Symptoms and signs of STdisease may include -----,------,-----,------.

Scientific Content: **المحتوى العلمي:**

-Bacterial Vaginosis

-Bacterial STIs include:

1- Chlamydia (*Chlamydia trachomatis*)

2- Gonorrhea (*Neisseria gonorrhoeae*)

3- Syphilis due to Spirochetes (*Treponema pallidum*)

Posttest **الاختبار البعدي:**

1- Explain how the resident microbiome protects the vagina from infection.
2- What factors appear to be responsible for the overgrowth of the vagina by other members of the resident microbiome?
3. Why is chlamydial urethritis referred to as the “silent disease”?
4. Contrast the symptoms of gonorrhea in females and males.

References: **المصادر :**

1. Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
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3. Bailey & scott' s (2017): Diagnostic microbiology,fourteenth edition
4. Jeffrey C. Pommerville. (2018): Fundamentals of microbiology, Eleventh edition.
5. Prof.Dr.Mohammed Shammkhi Jeber.(2019):Notes of Diagnosis Microbiology

الجامعة التقنية الوسطى كلية التقنيات الصحية والطبية/ بغداد قسم المختبرات الطبية

المادة: الاحياء المجهرية التشخيصي المرحلة: الرابعة

Title: **Lecture 23,24**

العنوان :

Bacterial infections of gastrointestinal tract

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

أ.م.د أحمد سالم محمد

Target population: **الفئة المستهدفة :**

طلبة المرحلة الرابعة

Introduction: المقدمة:

Bacterial gastrointestinal tract infection has many causes, can range from mild to severe, and typically manifests with symptoms of nausea, vomiting, diarrhea, and abdominal discomfort. In reality, most such attacks have caused by enterotoxins, drugs, or systemic illnesses.

The lower bowel has an especially large normal bacterial microbiota. The most prevalent organisms are anaerobes (*Bacteroides*, gram-positive rods, and gram-positive cocci), gram-negative enteric organisms, and *Enterococcus faecalis.* Any effort to improve pathogenic bacteria from feces involves separation of pathogens from the normal

microbiota, usually through using of differential selective media and enrichment cultures. Important causes of **acute gastroenteritis** include viruses, toxins (of staphylococci, clostridia, vibrios, toxigenic *E. coli*), invasive enteric gram-negative rods, slow lactose fermenters, shigellae, salmonellae, and campylobacters (figure 1).

Diagnosis Gastrointestinal Tract Specimens

Specimens (on general)

Feces and rectal swabs are the most readily available specimens. The presence of blood, mucus, or helminths must note on gross inspection of the specimen.

Leukocytes seen in suspensions of stool examined microscopically which are useful means of differentiating invasive from noninvasive infectious diarrheas. However, it is important to note that leukocytes may be present in non-infectious, inflammatory conditions of the gastrointestinal tract.

Culture Media:

Specimens have suspended in broth and cultured on ordinary as well as differential media (MacConkey agar, EMB agar) to permit separation of non-lactose fermenting gram-negative rods from other enteric bacteria. If salmonella infection has suspected, the specimen has also placed in an enrichment medium (selenite broth) for 18 hours before has plated on differential media (Hektoen enteric or Shigella- Salmonella agar). Vibrios grow best on thiosulfate citrate bile salts sucrose agar. Thermophilic campylobacters are isolated on Skirrow's selective medium incubated at 40–42°C in 10% CO_2.

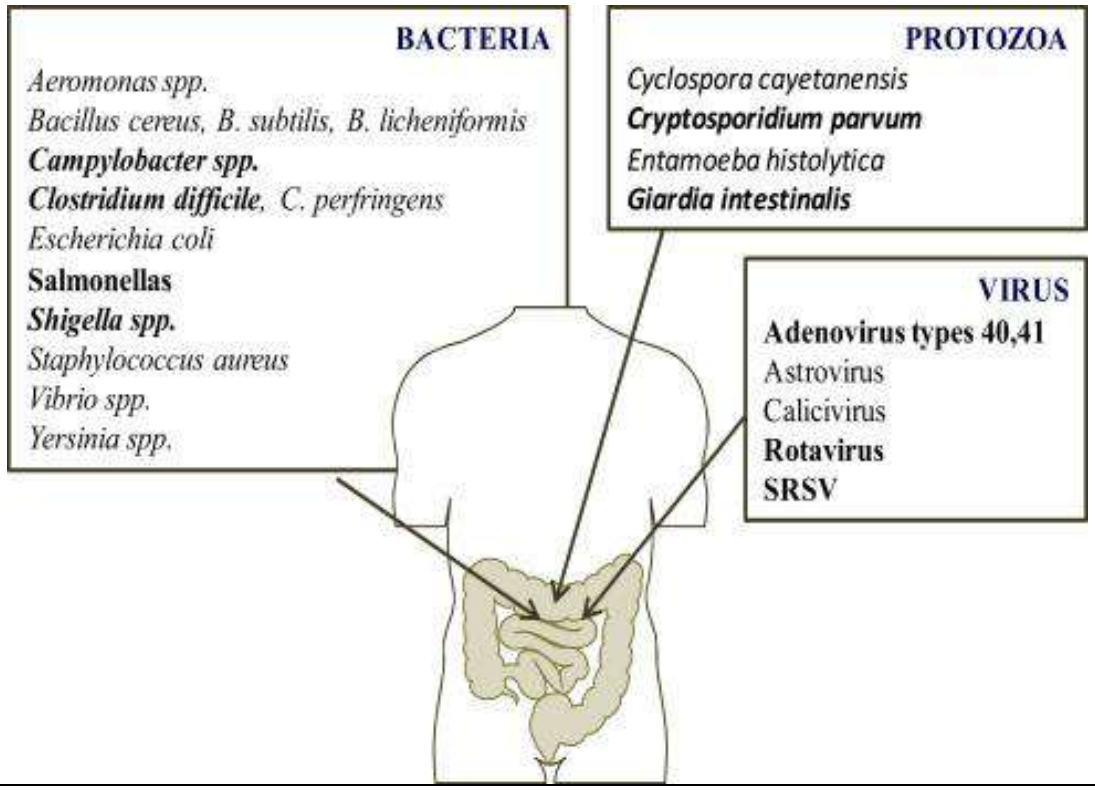

Fig (1):Microbial causes of GIT infections

Gastrointestinal Gram-negative rods

All organisms that have routinely found in the gastrointestinal (GI) tract of humans or other animals. Many also have alternative habitats in soil or water. All are relatively hardy but are sensitive to drying, and all grow in the presence or absence of oxygen, being facultative anaerobes. They contain lipopolysaccharide (LPS), which is both antigenic and an important virulence factor (endotoxin).
Different enteric gram-negative rods cause diseases within the GI tract, outside the GI tract, or in both locations.

Fecal contamination is commonly important in the transmission of those organisms that cause GI diseases.

Escherichia coli

Escherichia coli is part of the normal flora of the colon in humans and other animals but can be pathogenic both within and outside of the GI tract.
E. coli has fimbriae or pili that are important for adherence to host mucosal surfaces, and different strains of the organism may be motile or non-motile. Most strains can ferment lactose (Lac+) in contrast to the major intestinal pathogens, Salmonella and some strains of Shigella, which cannot ferment lactose (Lac –). ***E. coli*** produces both acid and gas during fermentation of carbohydrates.

Transmission of intestinal disease is commonly by the fecal–oral route, with contaminated food and water serving as vehicles for transmission. At least five types of *E. coli* that differ in pathogenic mechanisms have identified as:

1. Enterotoxigenic (ETEC), a common cause of traveler's diarrhea.
2. Enteropathogenic (EPEC), an important cause of diarrhea in infants.
3. Enterohemorrhagic (EHEC), associated with acute bloody diarrhea.
4. Enteroinvasive (EIEC), cause a dysentery-like syndrome.
5. Enteroaggregative (EAEC), cause traveler's diarrhea.

Laboratory identification

Intestinal disease: Because ***E. coli*** is normally part of the intestinal flora, detection in stool cultures of disease-causing strains is generally difficult. Many strains have detected on media such as MacConkey agar **(figure 2, 3).** Strains of ***E. coli*** could further characterize based on serologic tests. Current molecular techniques, such as PCR technique, might employed to identify ***E. coli*** strains.
General biochemical tests of ***E. coli*** are:

Short rods, Facultative anaerobe, Ferments glucose, Most strains ferment lactose, Catalase positive, Oxidase negative and Culture on MacConkey agar

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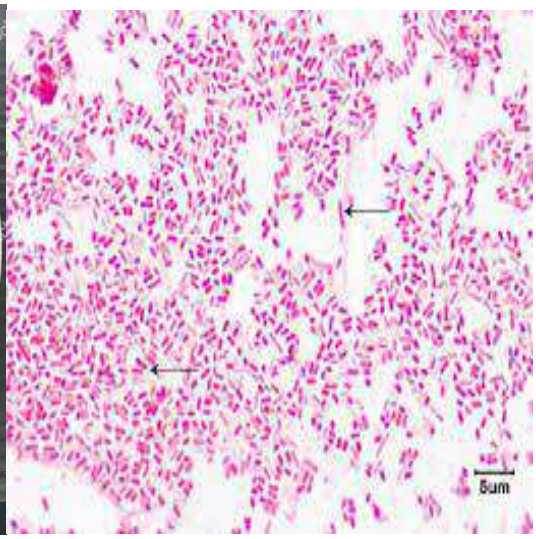


Figure (2): *E. coli* colony **Figure (3): *E. coli* Gram stain**

SALMONELLA (Salmonellosis)

Members of the genus Salmonella can cause a variety of diseases, including gastroenteritis and enteric (typhoid) fever. Although Salmonella classification has undergone numerous modifications, currently, all strains affecting humans have grouped in a single species, *Salmonella enteritidis*, which has approximately 2,500 different serotypes, or serovars, including the clinically significant serotypes Typhimurium and Typhi.

A **serotype** or **serovar** is a isolated variation within a species of bacteria or virus or among immune cells of different individuals. These microorganisms, viruses, or cells have classified together based on their cell surface **antigens**, allowing the **epidemiologic classification** of organisms to the sub-species level.

Serotyping often play an essential role in determining **species** and **subspecies**. ***Salmonella* genus** of **bacteria**, for example has determined to have over 2600 serotypes, including *Salmonella enterica* serovar **Typhimurium**, *S. enterica* serovar **Typhi**, and *S. enterica* serovar **Dublin**.

Most strains of Salmonella are Lactose negative and produce acid and gas during fermentation of glucose. They also produce H_2S from sulfur-containing amino acids.

Transmission

Salmonella are widely distributed in nature. Serovar Typhi is an absolutely human pathogen, whereas other strains are associated with animals and foods (for example, eggs and poultry). **Fecal–oral** transmission occurs and Salmonella serovar Typhi may involve **chronic carriers**. Young children and older adults are particularly susceptible to Salmonella infections. Individuals in crowded areas may also be involved in Salmonella epidemics.

Salmonella infection can cause both intestinal and extraintestinal diseases.

1. Gastroenteritis: This localized disease (salmonellosis) has caused primarily by serovars Enteriditis and Typhimurium. Salmonellosis is characterized by nausea, vomiting, and diarrhea (usually non-bloody), which develop generally within **48 hours** of ingesting contaminated food or water. Fever and abdominal cramping are common. More than 95 % of cases of Salmonella infection are foodborne and salmonellosis accounts for approximately 30 % of deaths resulting from foodborne illnesses in the United States.

2. Enteric or typhoid fever: This is a severe, life-threatening systemic illness, characterized by fever and, frequently, abdominal symptoms. It has caused primarily by **serovar Typhi**. About 30 % of patients become weak and having **transient skin rose spots**. The incubation period varies from 5 to 21 days. **Untreated**, mortality is approximately 15 %. Complications can include intestinal hemorrhage. **Typhoid fever remains a global health problem**.

Laboratory identification

In patients with diarrhea, Salmonella can typically be isolated from stools on **MacConkey agar (see figure 4) or selective media**. For patients with enteric fever, appropriate specimens include **blood**, **bone marrow**, **urine, stool, and tissue** from typical rose spots (if they are present).

General biochemical tests of Salmonella are:

Short, flagellated rods, Facultative anaerobes, Ferment glucose, Do not ferment lactose, Catalase positive, Oxidase negative, Culture on MacConkey agar

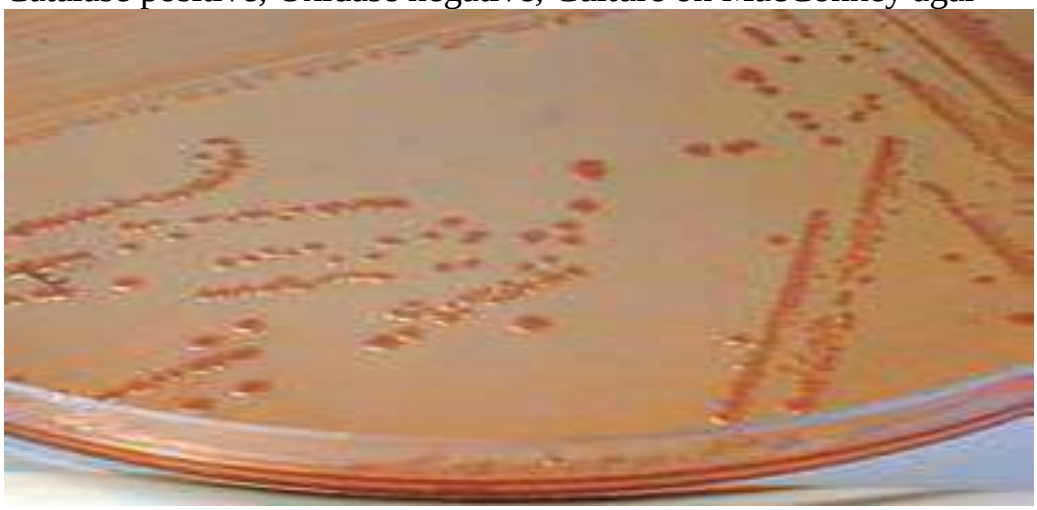


Figure (4): *Salmonella typhi* on MacConkey agar

CAMPYLOBACTER

Members of the genus Campylobacter are curved, spiral, or S-shaped organisms that microscopically resemble vibrios. A single, polar flagellum provides the organism with its characteristic darting (running) motility. Somatic, flagellar, and capsular antigens all

contribute to the numerous serotypes. Most Campylobacter are microaerophilic (that is, they require oxygen but at lower concentrations than that found in air).
Campylobacter infect the intestine and can cause ulcerative, inflammatory lesions in the jejunum, ileum, or colon. Also bacteremia may occur.
Campylobacter are **transmitted** to humans primarily via the fecal–oral route through direct contact, exposure to contaminated meat (especially poultry), or contaminated water supplies.

Laboratory identification

Campylobacter can be isolated from feces using special selective media **(blood agar containing antibiotics to inhibit growth of other fecal flora)** **(see figure 5)** and microaerophilic conditions. Members of this genus do not ferment carbohydrates.
Possible diagnosis could have based on finding curved organisms with rapid, darting motility in a wet mount of feces.

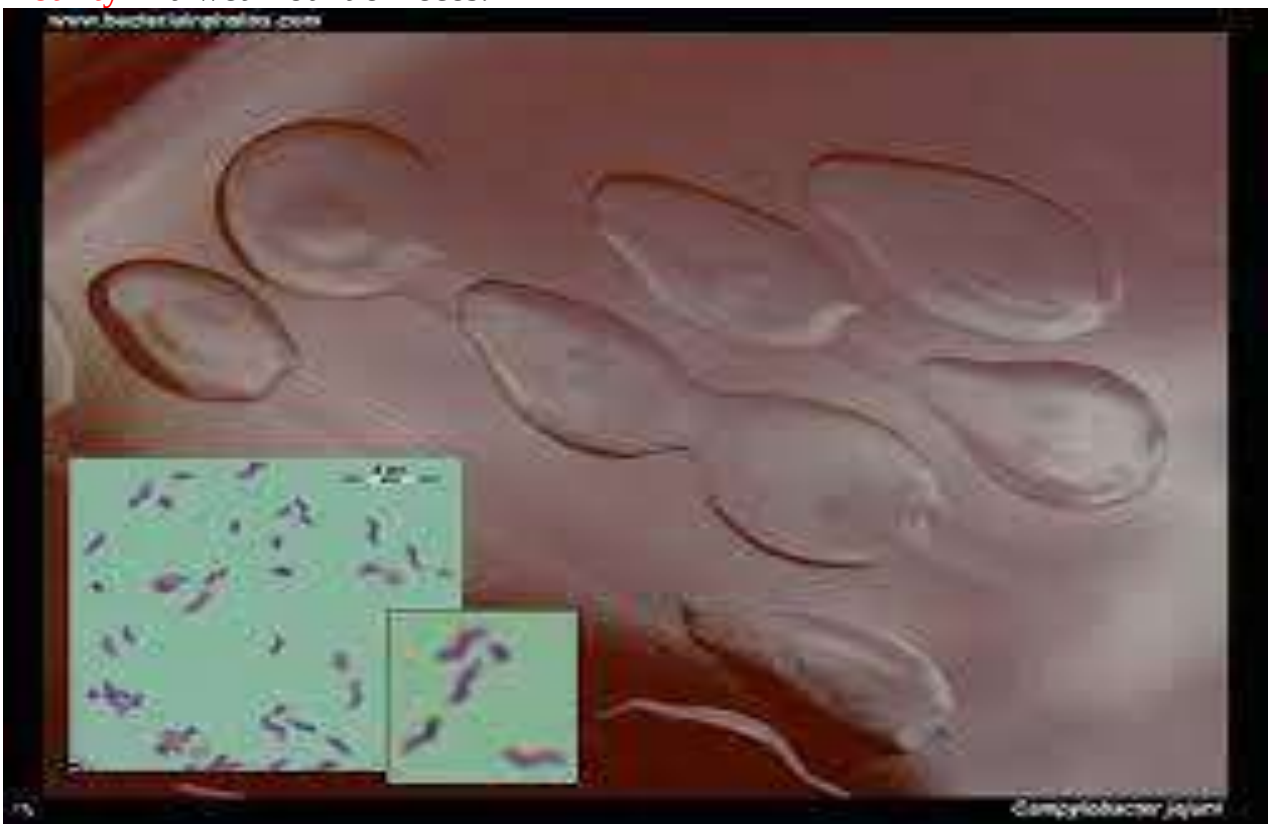


Figure (5): ***Campylobacter jejuni***

SHIGELLA (shigellosis)

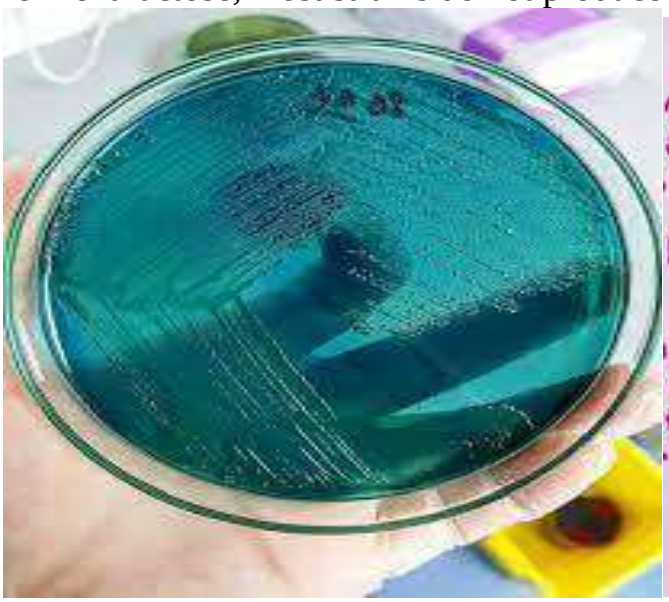
Shigella species cause shigellosis (bacillary dysentery), a human intestinal disease that occurs most commonly among young children.
Shigellae are **non-motile**, un-encapsulated, and **Lactose negative**. Most strains do not produce gas in a mixed-acid **fermentation of glucose**.

Shigella have typically spread from person to person, with contaminated stools serving as a **major source** of organisms. **Humans** are the only natural host for Shigella species. **Flies** and **contaminated food or water** can also transmit the disease. Shigellosis has a **low infectious dose**: Approximately **10–100** viable organisms are sufficient to cause disease. Therefore, secondary cases within a family are common, particularly under conditions of **crowding or poor sanitation**. *Shigella dysenteriae* causes the most serious infections, so Shigellae invade and destroy the mucosa of the large intestine.

S. dysenteriae type 1 produces Shiga toxin, which is structurally and genetically very similar to Shiga-like toxins 1 and 2 produced by ***E. coli.***
Shigellae cause classic bacillary dysentery, characterized by bloody diarrhea and mucus ("currant jelly" stools), with painful abdominal cramping. The disease is generally most severe in the very young; older adults; and among emaciated individuals, in whom shigellosis may lead to **severe dehydration** and, sometimes, death.

Laboratory identification

During acute illness, organisms can be cultured from stools using differential, selective Hektoen agar **(figure 6, 7)** or other media specific for intestinal pathogens.
Other **biochemical** features of Shigellae are non-motile, non-encapsulated, cannot ferment lactose, most strains do not produce gas in a mixed-acid fermentation of glucose.



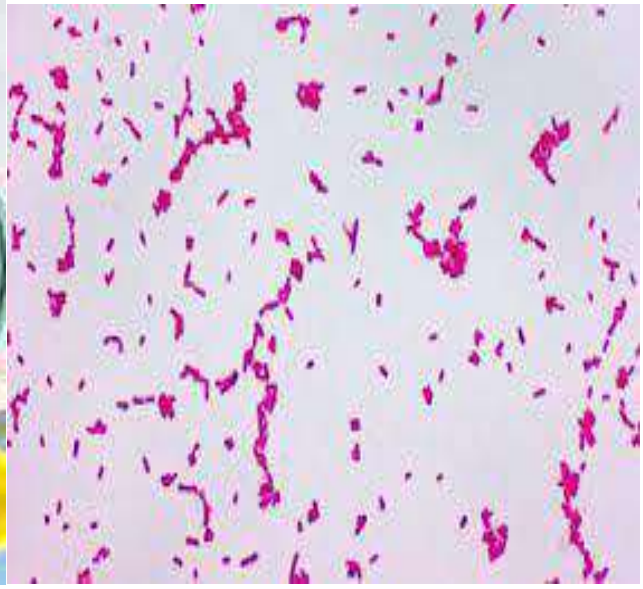


Fig. (6): Shigella species (Hektoen agar). **Fig. (7): *Shigella* on Gram stain**

VIBRIO

Members of the genus Vibrio are **short**, **curved**, **rod-shaped** organisms. Vibrios are closely related to the family Enterobacteriaceae. They are rapidly motile by means of a

single polar flagellum. This contrasts with the peritrichous flagella (distributed all over the surface) of the motile Enterobacteriaceae. O and H antigens are both present, but only **O antigens** are useful in distinguishing strains of vibrios that cause **epidemics**. Vibrios are facultative anaerobes. The growth of many Vibrio strains either requires or is **stimulated by NaCl**. Pathogenic vibrios include:

1. ***Vibrio cholerae***, serogroup O1 and O139 strains that are associated with **epidemic and pandemic cholera.**

2. Non-O1and nonO139 ***V. cholerae*** and related strains that cause **sporadic cases of choleralike** and other illnesses (extraintestinal infection).

3. ***Vibrio parahaemolyticus*** and other halophilic vibrios, which cause **gastroenteritis** and **extraintestinal** infections.

Clinical significance

Cholera is characterized by **massive loss of fluid** and **electrolytes** from the body. After an incubation period ranging from **hours to a few days**, profuse (excessive) watery diarrhea ("**rice-water" stools**) begins. Untreated, death from **severe dehydration** causing hypo - volemic shock may occur in hours to days, and the death rate may exceed 50 %. They also cause milder illness, comparable to that caused by enterotoxigenic ***E. coli***. such as gastrointestinal diseases.

Laboratory identification

V. cholerae grows on standard media such as **blood** and **Mac-Conkey** agars. **Thiosulfate-citrate-bile salts–sucrose** medium can enhance isolation. The organism is oxidase positive, but further biochemical testing is necessary for specific identification of ***V. cholerae***.

It is characterized by:

- **Short, curved, rod shaped (see figure 8).**
- **Rapidly motile as a result of single polar flagellum.**
- **Facultative anaerobes.**
- **Growth of many *Vibrio* strains requires or is stimulated by NaCl.**
- **Culture on blood or MacConkey agar.**

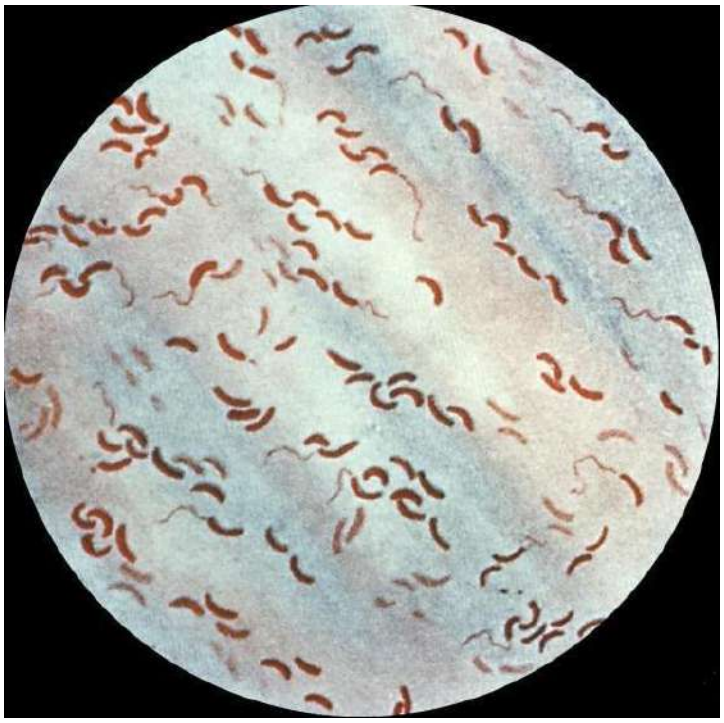


Figure (8): Gram stain of *Vibrio cholera*

Vibrio parahaemolyticus and other halophilic, non-cholera vibrios

These organisms are characterized by a requirement for higher than usual concentrations of NaCl and their ability to grow in 10 % NaCl. They are common in seawaters. ***Vibrio parahaemolyticus*** is associated with **outbreaks** of **Gastrointestinal** illness that result from ingestion of contaminated and inadequately cooked seafood, especially shellfish and crustaceans.

Other halophilic, **noncholera vibrios** are associated with soft tissue infections and septicemia resulting either from contact of **wounds** with contaminated seawater or from ingestion of **contaminated seafood**. For soft tissue infections, prompt administration of antibiotics, such as tetracycline, fluoroquinolones or cefotaxime, is important, and surgical drainage may be required.

HELICOBACTER

Members of the genus Helicobacter are curved or spiral organisms. They have a rapid, corkscrew (coiled) motility resulting from multiple polar flagella. ***Helicobacter pylori***, the species of **human significance**, is microaerophilic, and produces urease. It causes **acute gastritis** and 95% of **duodenal** and **gastric ulcers**. ***H. pylori*** are **unusual** in their ability to **colonize the stomach**, where low pH normally protects against bacterial infection. *H. pylori* infections are relatively common and worldwide in distribution.

Clinical significance

Initial infection with ***H. pylori*** causes acute gastritis, sometimes with diarrhea that lasts

about 1 week. The infection usually **becomes chronic**, with diffuse, superficial gastritis that may be associated with epigastric discomfort. ***H. pylori*** infection appears to be a risk factor for development of gastric carcinoma and gastric B-cell lymphoma.

Laboratory identification

Noninvasive diagnostic tests include serologic tests (enzyme-linked immunosorbent assay, commonly known as **ELISA**, for serum antibodies to ***H. pylori***) and breath tests for urease. [Note: Breath tests involve administering radioactively labeled urea by mouth. If *H. pylori* are present in the patient's stomach, the urease produced by the organism will split the urea to CO_2 (radioactively labeled and breathe out) and NH_3.] Invasive tests involve gastric **biopsy specimens** obtained by **endoscopy**.

It is characterized by: • **Curved or spiral rods (figure 9).**

• **Multiple polar flagella, which give organism rapid, corkscrew motility.**

• **Urease positive.**

• **Culture on selective medium containing antibiotics (figure 10).**

H. pylori can be detected in such specimens **histologically**, by **culture**, or by a test for **urease**.

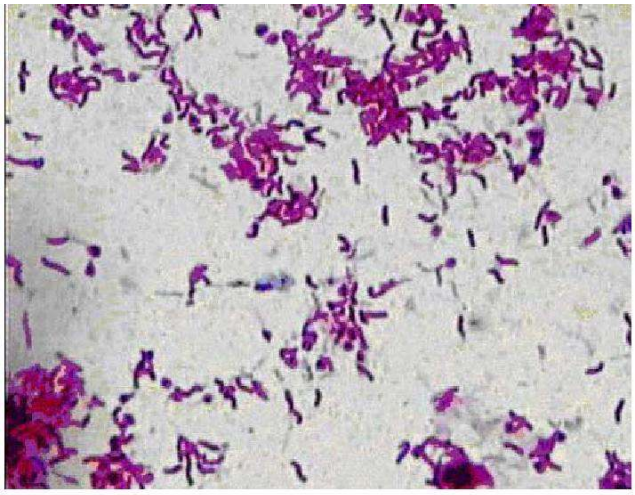


Fig. (9): Helicobacter pylori

Fig. (10): Helicobacter pylori colonies on agar plate

OTHER ENTEROBACTERIACEAE

Other genera of Enterobacteriaceae, such as **Klebsiella**, **Enterobacter, Proteus**, and **Serratia**, which can be found as normal inhabitants of the **large intestine**, include organisms that are primarily opportunistic and often nosocomial pathogens.

CLOSTRIDIA

Clostridia are the anaerobic gram-positive rods of greatest clinical importance. Clinically significant species of Clostridium include:

1. ***Clostridium perfringens***, which causes histotoxic (tissue destructive) infections (myonecrosis) and food poisoning.

2. ***Clostridium difficile***, which causes pseudomembranous colitis associated with antibiotic use.
3. ***Clostridium tetani***, which causes tetanus ("lockjaw").
4. ***Clostridium botulinum***, which causes botulism.

Clostridium perfringens

C. perfringens is a large, nonmotile, gram-positive, encapsulated bacillus. It is universal in nature, with its vegetative form as part of the normal flora of the vagina and gastrointestinal (GI) tract. Its spores are found in soil. [Note: Spores are rarely seen in the body or following *in vitro* cultivation.] When introduced into tissue.
Clostridium Gram stain. Individual gram-positive bacilli are present. Many are in chains. Some of the bacilli have spores, which are the unstained or clear ovoid shapes as shown in figure (11).

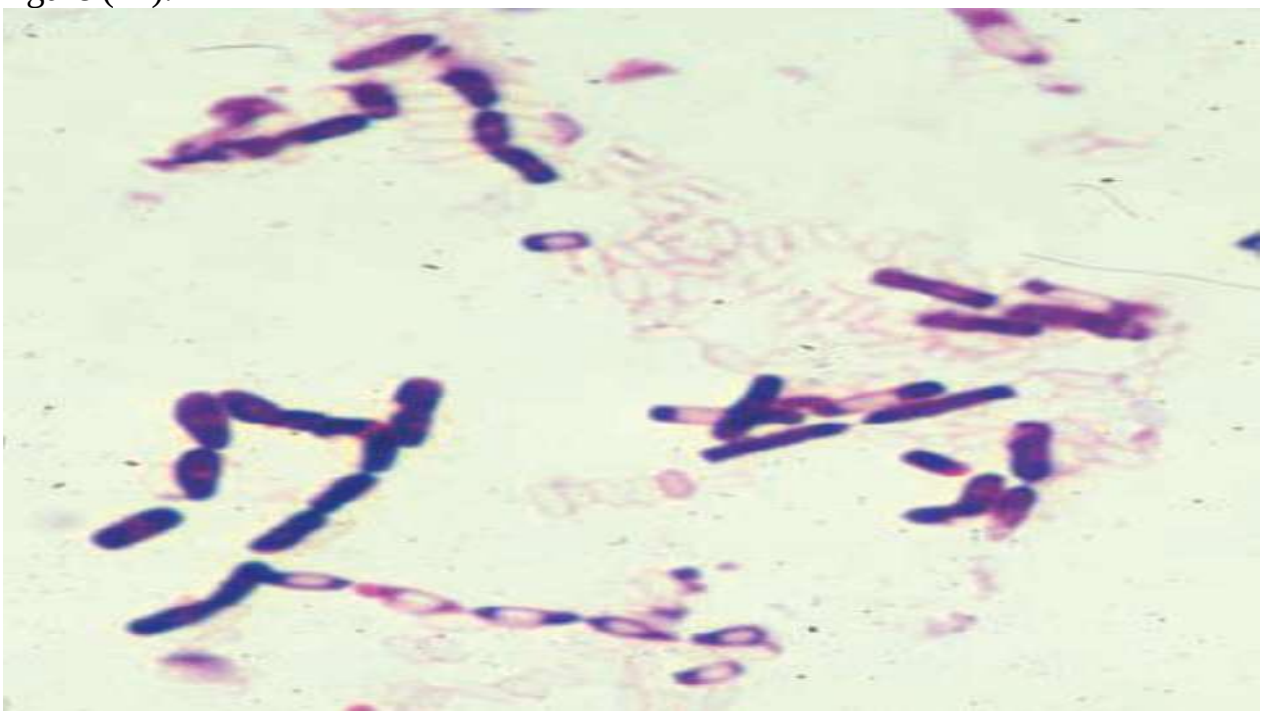


Figure (11): Gram stain of *Clostridium perfringens*

However, *C. perfringens* can cause anaerobic cellulitis and myonecrosis (gas gangrene). Some strains of *C. perfringens* also cause a common form of food poisoning.

Pathogenesis of *C. perfringens* secretes a variety of exotoxins, enterotoxins, and hydrolytic enzymes that facilitate the disease process.

Exotoxins: *C. perfringens* elaborates at least 12 exotoxins. The most important of these, and the one that seems to be required for virulence in tissue, is α toxin. α Toxin is a lecithinase (phospholipase C) that degrades lecithin in mammalian cell membranes, causing lysis of endothelial cells as well as erythrocytes, leukocytes, and platelets.

Perfringolysin O, or theta (θ) toxin, is a cholesterol-dependent hemolysin and an

important **virulence factor**. C. perfringens strains are grouped A through E on the basis of their spectrum of exotoxins.
Enterotoxin: *C. perfringens* enterotoxin, a small, heat-labile protein, acts in the lower portion of the small intestine.

Clinical significance: The disease processes initiated by *C. perfringens* result from a combination of infection and the **production of exotoxins and/or enterotoxins and degradative enzymes.**
Foodborne infection: *C. perfringens* is a common cause of **foodborne infection** in many situations. Typically, the onset of **nausea**, **abdominal cramps**, and **diarrhea** occurs 8 to 18 hours after eating contaminated food. Fever is absent and vomiting rare. The attack is usually self-limited, with **recovery** within 1 to 2 days. The occurrence of clinical symptoms requires a large inoculum of 10^8 organisms or greater. Therefore, a typical episode of clostridial enterotoxin food poisoning involves cooking that fails to inactivate spores, followed by holding the food for several hours under conditions that allow bacterial germination and several cycles of growth. Vegetative cells are consumed in the contaminated product, and *C. perfringens* then reproduces following ingestion (food infection) and produces toxin in vivo. **Meats, meat products**, are the most commonly implicated foods in *C. perfringens* foodborne illness.

Laboratory identification
Stool or diseased tissue specimens cultured anaerobically on **blood agar**, *C. perfringens* grows rapidly, producing colonies with a **unique double zone** of hemolysis due to production of α toxin (partial hemolysis) and perfringolysin O (complete hemolysis) as shown in Figure (12). In food infection, the organism can be **required** in suspected food and the patient's feces. Gram stain and other laboratory findings greatly help planning of antibiotic therapy in patients (figure 12).

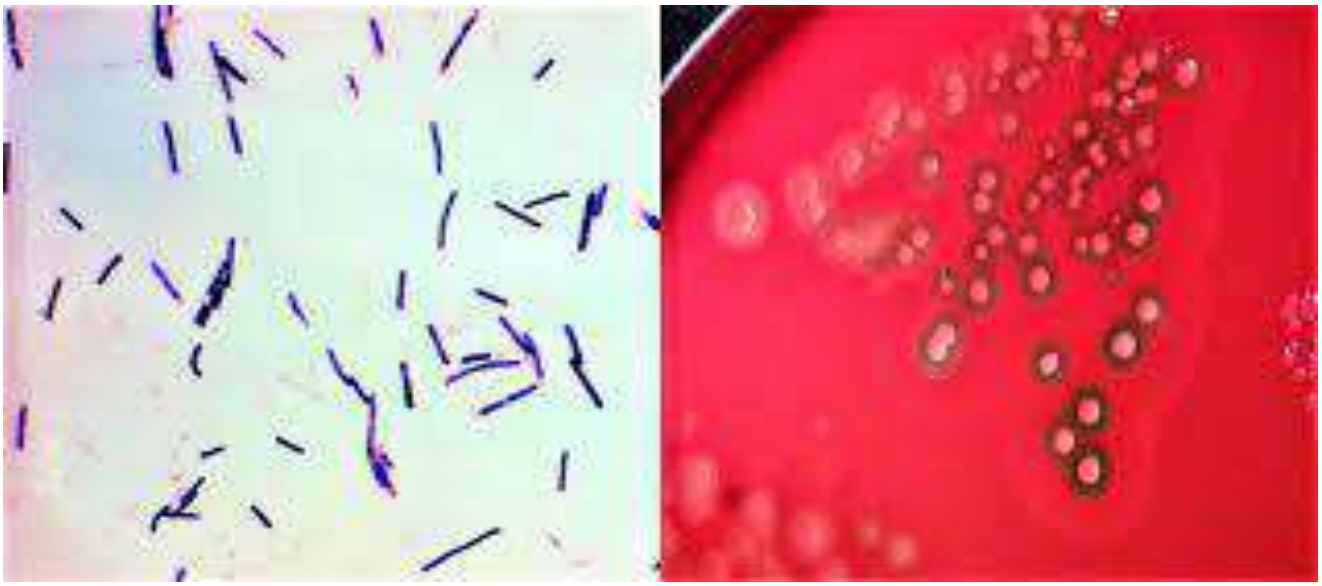


Fig (12):Morphology & Culture Characteristics of *Clostridium perfringens*

Clostridium botulinum

C. botulinum causes **botulism**, which occurs in several clinical forms. Botulism is caused by the action of a neurotoxin that is one of the most **potent poisons** known and causes a limp paralysis. **Contact with the organism itself is not required**, and the the disease can be specially due to ingestion of toxin-contaminated food.

Epidemiology of *C. botulinum* is found worldwide in soil and aquatic sediments, and the spores frequently contaminate vegetables and meat or fish. Under appropriate conditions, including a strictly anaerobic environment at neutral or alkaline pH, the organism **germinates**, and **toxin is produced** during vegetative growth. Because the toxin is often elaborated in food, outbreaks frequently occur in families or other eating groups.

Pathogenesis: There are several types of botulinum toxin, but human disease is almost always caused by types A, B, or E toxins. The botulinum and tetanus toxins constitute a homologous set of proteins whose neurotoxicity, causing subsequent failure of **neurotransmission**. In contrast to **tetanus toxin**, which causes constant contraction or spasms.

botulinum toxins affect peripheral cholinergic synapses by blocking the neuromuscular junction and inhibiting release of the neurotransmitter acetylcholine, preventing contraction and causing flaccid paralysis.

Clinical significance:

Classic botulism at food poisoning in which a patient first begins to experience **difficulties** in focusing vision, swallowing, and other **cranial nerve functions**, 12 to 36 hours after ingesting toxin-containing food but not essentially viable organisms.

There is **no fever** or sign of sepsis. A progressive paralysis of striated muscle groups

develops, and mortality rate is about 15 %, with the patient usually yielding to respiratory paralysis.

Laboratory identification

The organism can be cultured and identified by standard anaerobic methods (Isolation of a bacterium is usually performed on solid medium. Liquid medium is used to grow larger quantities of a culture of bacteria that have already been isolated as a pure culture on **Enriched media (**blood agar, yeast extracts, or brain or heart infusions are useful in growing this fastidious organisms**)**. Toxin is also identifiable in serum, stool, and food. Also MacConkey agar used as **Selective media.**

Clostridium difficile

Diarrhea, a common complication of antimicrobial drug treatment, can range from loose stools to life-threatening pseudomembranous colitis (PMC). ***C. difficile*** is estimated to be responsible for at least one fourth of antibiotic-associated diarrheas (AADs) in hospitalized patients and almost all cases of PMC. After its introduction to a site, the environment (that is, dust, bedding, toilets, etc.) becomes persistently **contaminated with spores**. They are then at higher risk for developing the adverse intestinal effect of antibiotic treatments.

Pathogenesis of *C. difficile* is a minor component of the **normal flora** of the large intestine. When antimicrobial treatment suppresses more predominant species in this community, *C. difficile* proliferates.

Pathogenic strains produce two toxic polypeptides, designated toxins A and B. Toxin A is an enterotoxin that causes **excessive fluid secretion**, but also stimulates an inflammatory response, and has some cytopathic effect in tissue culture. Toxin B is a cytotoxin.

Clinical significance

Fundamentally **all antimicrobial** drugs have been reported as predisposing to clostridial AAD (antibiotic-associated diarrhea) and colitis. The three drugs most commonly implicated are clindamycin, ampicillin, and the cephalosporins. The pseudo membranous exudate, composed of mucus, fibrin, inflammatory cells, and cell debris overlying an **ulcerated epithelium**, is best demonstrated by endoscopy.

Laboratory identification

C. difficile can be cultured from stools and identified by routine anaerobic procedures, but the more rapid and useful tests are directed at **demonstrating toxin** production in **stool extracts**. Enzyme immunoassays (ELISA) for exotoxins A and B have replaced earlier immunologic or tissue culture cytotoxicity assays. Polymerase chain reaction–based detection strategies are also widely available.

BACILLUS SPECIES

Species of the genus Bacillus are **gram-positive**, form **endospores**, and are either strict aerobes or aerotolerant anaerobes (that is, they can grow in the presence of oxygen, **but do not require it**). Most of the 70 species of Bacillus are found in soil and water and are

usually essential in the medical laboratory as **airborne contaminants**. ***B. anthracis***, the cause of the disease anthrax, is clinically the most important member of this genus.
Bacillus cereus

Bacillus cereus is a **soil** organism which commonly contaminates **rice** and produce a **tissue-destructive exotoxin**. When large amounts of rice are cooked and allowed to cool slowly, the ***Bacillus cereus*** spores germinate, and the vegetative cells **produce** the **toxin** during log-phase growth or during sporulation.
Food poisoning caused by ***Bacillus cereus*** has two separate forms, the **emetic type**, which is associated with **cooked** rice, and the diarrheal type, which is associated with **meat dishes and sauces**. The **emetic form** is manifested by **nausea, vomiting, abdominal cramps, and occasionally diarrhea** and is self-limiting, with recovery occurring within24 hours. The **diarrheal form** has an incubation period of 1–24 hours and is manifested by **abundant diarrhea** with **abdominal pain** and **cramps**; fever and vomiting are uncommon.

Bacillus cereus is a gram-positive, rod-shaped, aerobic, motile, beta hemolytic bacterium commonly found in soil (on vegetables) and food (raw and processed). ***B. cereus*** bacteria are facultative anaerobes, and like other members of the genus *Bacillus*, can **produce** protective **endospores**. Its **virulence factors** include cereolysin and phospholipase C.
The **presence** of *Bacillus cereus* in a patient's **stool is not sufficient** to make a diagnosis of *Bacillus cereus* disease because the bacteria may be **present in normal** stool specimens; a **concentration of 10_5 bacteria** or more per gram of food is considered diagnostic. Some strains of *B. cereus* produce cereins, bacteriocins active against different *B. cereus* strains or other Gram-positive bacteria

Pretest: **الاختبار القبلي :**

1- what are the Microbial causes of GIT infections?

Scientific Content: المحتوى العلمي:

- Diagnosis Gastrointestinal Tract Specimens
- Gastrointestinal Gram-negative rods
- OTHER ENTEROBACTERIACEAE

Posttest الاختبار البعدي:

1- Mention the characteristics ofv *V.cholerae* with Lab.diagnosis?

3. *Salomnella typhi* cause----------,----------?

3-diagnosis of peptic ulcer done by the followings/

1-

2-

3-

المصادر :

References:

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2-Alfred E. Brown; Heidi R. Smith. Benson's microbiological applications: laboratory manual in general microbiology, concise version, Fourteenth edition. Published by McGraw-Hill Education, 2 Penn Plaza, New York, 2017
3-Apurba S. S; Sandhya Bhat K. Review of Microbiology and Immunology. 4th Edition. The Health Sciences Publisher. 2015
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5-Cornelissen C. N.; Fisher B. D.; Harvey R. A. Lippincott's illustrated reviews: Microbiology. 3rd Ed. Lippincott Williams & Wilkins, 2013
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الجامعة التقنية الوسطى كلية التقنيات الصحية والطبية/ بغداد قسم تقنيات المختبرات الطبية المادة: الاحياء المجهرية التشخيصي المرحلة:

الرابعة

Infections of the Eyes, Ears and Sinuses

Title: **Lecture 25**

العنوان:

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

م.د.دنيا عبد الرزاق محمود

Target population: **الفئة المستهدفة:**

طلبة المرحلة الرابعة

Introduction:

المقدمة:

Bacterial infections of eyes, ears and sinuses

Ear infections:

The middle and inner ear are normally sterile, while outer ear and auditory canal contain the normal flora of mouth, nose and skin. When a person coughs, sneezes or blow his nose these microorganisms may reach middle or inner ear and causing infection.

The most common bacteria that cause ear infections are coagulase positive **Staphylococci,** beta hemolytic **Streptococci**, alpha hemolytic Streptococci (**Strep. pneumonia) (figure 36), Proteus spp., Pseudomonas aeruginosa** and **E. coli**.

While **Klebsiella pneumonia** (less common) and **anaerobic** bacteria are **rare**.

Ear infection, may be the way for enteric bacteria to reach to un limited area in respiratory tract or nervous systems, E. coli meningitis is one example among many of such cases.

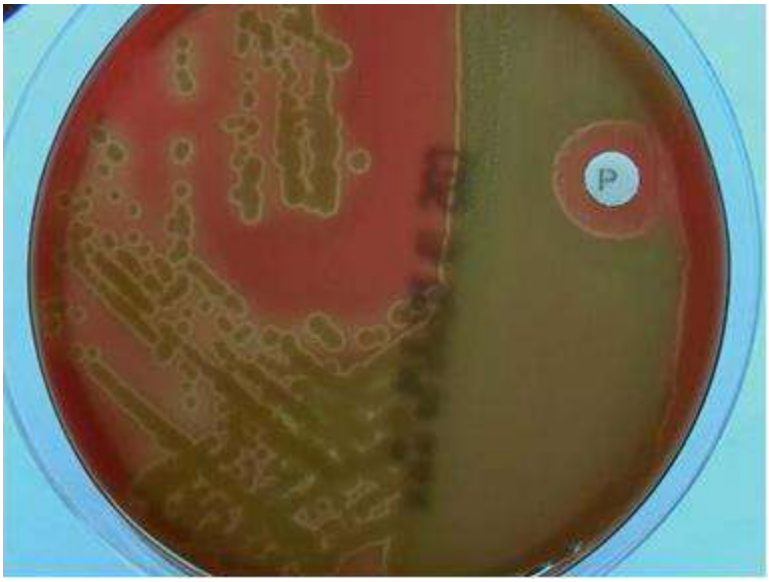

Figure (36): Alpha hemolytic streptococci grown on a blood agar plate. Alpha hemolysis presents with a dark green color.

Outer Ear

P. aeruginosa is a Gram-negative rod-shaped bacterium with a flagellum at one pole. P. aeruginosa is a facultative anaerobe, but prefers aerobic respiration. This makes it well

Figure (36): Alpha hemolytic streptococci grown on a blood agar plate.
Alpha hemolysis presents with a dark green color.

suited for life on the skin and the outer ear which is exposed to the oxygen-filled atmosphere.

P. aeruginosa is able to utilize a wide variety of metabolites. P. aeruginosa is also an opportunistic pathogen that causes multiple different diseases such as pneumonia, UTIs, and other skin diseases including **acute** diffuse **otitis externa** **(figure 37).**

S. epidermidis is found in the outer ear due to the similarity in environments to human skin, that may cause ear infection.

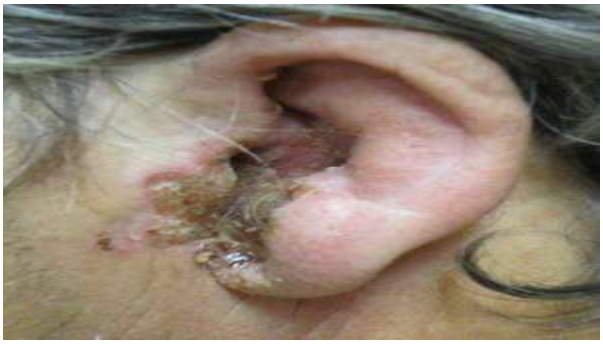

Figure (37): Acute diffuse otitis externa.

Streptococcus pneumoniae; Haemophilus influenzae & Moraxella catarrhalis these microbes are the most common cause for acute otitis media. Strep. pneumoniae, H. influenzae and M. catarrhalis are all associated with the **upper respiratory tract** of humans. (figure 38).

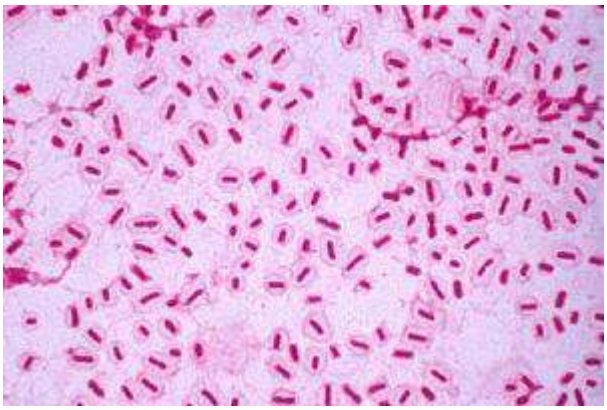

Figure (38): Haemophilus influenzae Gram stain under light microscopy.

Beneficial Ear Microbiota:

More and more scientists are beginning to realize that the interplay between the human body and naturally occurring biological flora can be beneficial for both the host and the microbe.In order to restore proper gut health fecal transplants were performed in order to replace the **beneficial bacteria**. This logic has been extended to the treatment of ear infections. Alpha hemolytic streptococci are **present in the middle ear naturally** and evidence suggests that these bacteria effectively interfere with the activity of Strep. pneumonia, H. influenza, and M. catarrhalis and essentially crowd out the growth of these pathogens. In this way alpha hemolytic streptococci and other **probiotics** can be utilized to combat irritating ear infections by essentially replacing the naturally occurring bacterial flora in the middle ear in this case

Infection of middle ear and sinuses

1. **Acute infection**

a. **Acute otitis media**: Causative agent: Hemophillus influenzae,
Strep. pneumoniae, Morexella catarrhalis
Source: Endogenous; normal flora of the oropharynx

Lab. diagnosis:
Specimen: Ear discharge (pus)
Procedures: Gram staining, culture, biochemical testing, serological testing, sensitivity testing

b**. Acute sinusitis:**
Acute infections of middle ear and sinuses are often due to secondary bacterial invasion

following a viral infection of respiratory tract.
Causative agent: Hemophillus influenza; Strep.pneumoniae; Strep. pyogenes
Source: Endogenous: normal flora of the nasopharynx
Clinical features: Discomfort over the frontal or maxillary sinuses, Pain and tenderness of sinuses with purulent nasal discharge.
Lab. Diagnosis:
Specimen: Lavage/drainage of sinuses
Procedure: Gram staining, culture, biochemical testing for bacterial isolation, serological testing and sensitivity testing.
2. **Chronic infection**
a. Chronic suppurative otitis media
Long standing ear disease characterized by periods of exacerbation with profuse ear discharge and pain; and remission with relatively dry ear.
Risk factors: History of acute or chronic otitis media; Parental (source) history of otitis media; Crowding.
Causative agent: Pseudomonas aeruginosa, Strep. pneumoniae
Laboratory diagnosis:
Specimen: Swabs of pus from the infected ear.
Procedure: Gram staining, culture, biochemical and serological test for microbe identification.
b. Chronic sinusitis
Painful sinuses and head ache are prominent symptoms; often associated with mucoid or purulent nasal discharge and nasal obstruction. Causal organisms are same as those implicated in acute sinusitis.
Laboratory diagnosis:
Specimen: Saline washings from the affected sinus
Procedure: Gram staining, culture, biochemical and serological test for microbe identification.

Diagnosis of bacterial eye infections
Ear, eye and nose are all share common canal, so any infection of one of these parts may cause infection to others.
Eye Infections:
Normally eyes are **quite sterile** sites of infections because of many defense mechanisms such as tear through lacrimation. **Tears** in eyes **decreases** the number of microorganisms that may find its way to eye because its content of **lysozyme** that destroys bacterial cells.
External part of eye considers as a part of skin, so any bacterial infections of skin is

expected to cause infection to the outside part of eye.
Pseudomonas aeruginosa and **Staphylococcus aureus** are the most common pathogenic causes of eye infections, while **Streptococcus spp.** are less common.
Common cases of eye infections:
A. Conjunctivitis (pinkeye)
1. Infection of the conjunctiva.
2. Bacterial conjunctivitis is often caused by **Pseudomonas aeruginosa**, **Staphylococcus aureus**, **Haemophilus influenzae**, **Streptococcus pneumoniae, Streptococcus pyogenes, and Neisseria gonorrhoeae (infant)**.
3. Symptoms include a sensitivity to bright lights, swelling of the eyelids, increased tears, redness, and large amounts of pus (Bacterial infections – **milky discharge**).75
B. Keratitis
1. More serious infection than conjunctivitis.
2. Invasion of **deeper eye tissues** occurs, can lead to **complete corneal destruction**.
3. Also cause conjunctivitis, sharp pain, and sensitivity to light.
4. Can lead to blindness.

Diagnosis of bacterial Nose infections
Nose infections:
Nasal cavity considers as a reservoir for Genus **Staphylococcus** along with other Gram positive bacteria such as alpha and beta **hemolytic Streptococci**. Nasal cavity is the pathway for deeper parts of respiratory tract, so that resident bacteria of nasal cavity may and will find its way to the system causing problems here and there in respiratory tract or from this location to nervous system such as meningitis, which could happen due to infection with bacteria resident in nasal cavity.
Enteric bacteria such as **E. coli, Klebsiella spp.** and **Proteus spp.** are either transit or resident which depend on the hygienic and immunologic status of individual.
Pseudomonas aeruginosa is less common because it is less competitive.
Laboratory diagnosis
Bacterial parameter could be enough for diagnosis of classical infections with the aid of **CBC** looking for **raising in number of leucocytes** in general, and neutrophils in particular, biochemical exams may be of little assistant in this regard. Mycoplasmal and chlamydial infection cannot be diagnose using bacterial parameter since there is no suitable media to isolate them routinely, for that, serological or immunological parameters are prime diagnostic tool in this aspect, again CBC, and differential blood count could aid the diagnosis.

Table (5): Summary for bacterial diagnosis of Ear, Eye and Nose infections:

Infection	**Most important pathogens**	**Laboratory diagnosis**
Otitis externa (involved in this aspect will just that of skin infections)	**Pseudomonas aeruginosa** **Staphylococcus aureus** **Streptococcus pyogenes**	Culture and Microscopy for bacteria of swab material
Otitis media	***Streptococcus pneumonia*** ***Haemophilus influenzae*** ***Streptococcus pyogenes*** ***Staphylococcus aureus***	Culture and Microscopy for bacteria of middle ear
Keratitis	***Staphylococcus spp.*** ***Streptococcus spp.*** ***Neisseria gonorrheae*** ***Pseudomonas spp.*** ***Bacillus spp.*** ***Mycobacterium spp.*** ***Moraxella lacunata*** ***Actinomyces spp.*** ***Nocardia spp*** ***Treponema pallidum*** ***Chlamydia trachomatis***	Culture and Microscopy for bacteria swab or corneal scrapings Diagnostic procedures with corneal swab or scrapings Serology Diagnostic procedures with corneal swab or scrapings see at "trachoma"
Trachoma	***Chlamydia trachomatis*, serovars A, B, Ba, C**	Microscopical detection of inclusions in conjunctival cells (Giemsa

		stain); direct immunofluorescence; cell culture; PCR. Serology: recombinant immunoassay for antibodies to genus-specific antigen (LPS).77 Conjunctivitis **Neisseria spp.** **Streptococcus spp.** **Staphylococcus aureus** **Haemophilus spp.** **Pseudomonas spp.** **Mycobacterium spp.** **Moraxella lacunata** **Treponema pallidum** **Chlamydia trachomatis**
Conjunctivitis	***Neisseria spp.*** ***Streptococcus spp.*** ***Staphylococcus aureus*** ***Haemophilus spp.*** ***Pseudomonas spp.*** ***Mycobacterium spp.*** ***Moraxella lacunata*** ***Treponema pallidum*** ***Chlamydia trachomatis***	Microscopy and culture for bacteria in conjunctival secretion or in scrapings Serology (basic diagnostics) See at “trachoma
Endophthalmitis	***Staphylococcus spp.*** ***Streptococcus spp.*** ***Neisseria gonorrhoeae*** ***Pseudomonas spp.*** ***Bacillus spp.*** ***Mycobacterium spp.***	Microscopy (Gram) and culture for aerobic and anaerobic bacteria and Mycobacterium.

	Moraxella lacunata *Nocardia spp.* *Chlamydia trachomatis* *Treponema pallidum*	

(

Pretest: الاختبار القبلي:

- What are the most common bacteria which cause ear infections?
-Enumerate the common cases of eye infections.
-how can diagnosis chlamydial infection .

Scientific Content: المحتوى العلمي:

-chronic suppurative otitis media
-conjunctivitis
-complete corneal destruction
- *Treponema pallidum , Moraxella lacunata, Nocardia spp*

Posttest الاختبار البعدي:

Fill in the blank :

-The most common microbes cause acute otitis media are ------- ,-------- and ---------.

-The prominent symptoms of chronic sinusitis are ----------and ---------.

-Tears in eyes decrease the number of microorganisms because it contain of ---------which responsible destroys bacterial cells.

References: المصادر :

1- Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
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الجامعة التقنية الوسطى كلية التقنيات الصحية والطبية/ بغداد قسم المختبرات الطبية

المادة Diagnostic Microbiology المرحلة: الرابعة

Title: **Lecture 26**

العنوان:

Bacterial infections of skin, soft tissues and wounds

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

أ.م.د.امال عزيز كريم

Target population: طلبة المرحلة الرابعة **الفئة المستهدفة:**

طلبة المرحلة الرابعة

Introduction: المقدمة:

Bacterial infections of skin, soft tissues and wounds:

Wound infections occur primarily from breaks in the skin as a result of complications associated with surgery, trauma, and bites or from diseases that interrupt the mucosal or skin surface.

Sources of wound infections can include the patient's normal microbiota or organisms present in soil or the hospital environment.

Normal skin has numerous mechanisms to prevent infection and protect the underlying tissue from invasion by potential pathogens.These mechanisms include:

- physical separation of microorganisms from the tissues
- presence of fatty acids that inhibit many microorganisms,
- excretion of lysozyme by sweat glands, and desquamation of the epithelium.

The skin contains a wide variety of microorganisms, most of which are found on the most superficial layers of cells and the upper parts of hair follicles. Scrubbing and washing may reduce the number of bacteria present on the skin by about 90% but do not completely eliminate the organisms present, and their numbers return to normal within a few hours.

Infections in or Around Hair Follicles

Folliculitis, furuncles, and carbuncles are localized abscesses either in or around hair follicles. For the most part, these infections are precipitated by blockage of the hair follicle with skin oils (sebum) or because of minor trauma resulting from friction such as that caused by clothes rubbing the skin. ***Staphylococcus aureus*** is the most common etiologic agent for all three infections. Members of the Enterobacteriaceae family may also cause folliculitis. *Pseudomonas aeruginosa* have been reported to be associated with the use of whirlpools, swimming pools, and hot tubs

- Most infections in the deeper layers of the epidermis and dermis result from the inoculation of microorganisms by traumatic breaks in the skin.**Cutaneous ulcers** usually involve a loss of epidermal and part of the dermal tissues. In contrast, **nodules** are inflammatory foci in which the epidermal and dermal layers remain largely intact.
- Various bacteria and fungi can cause ulcerative or nodular skin lesions after direct traumatic inoculation. causative agents include *Bacillus anthracis, Corynebacterium diphtheriae, Mycobacterium marinum*, *Nocardia* spp., and *Sporothrix schenckii.*
- Infections of the Subcutaneous Tissues Infections of the subcutaneous tissues may manifest as **abscesses, ulcers,** or **boils.**The most common etiologic agent of subcutaneous abscesses in healthy individuals is *S. aureus.*

Necrotizing fasciitis is infection of the fascia overlying the muscles, often with involvement of the overlying soft tissue.At the fascial level, no barrier exists to prevent the spread of infection.This process typically involves group A streptococci or *S. aureus*. Necrotizing fasciitis commonly involves anaerobic bacteria, especially *Bacteroides* and *Clostridium* species.

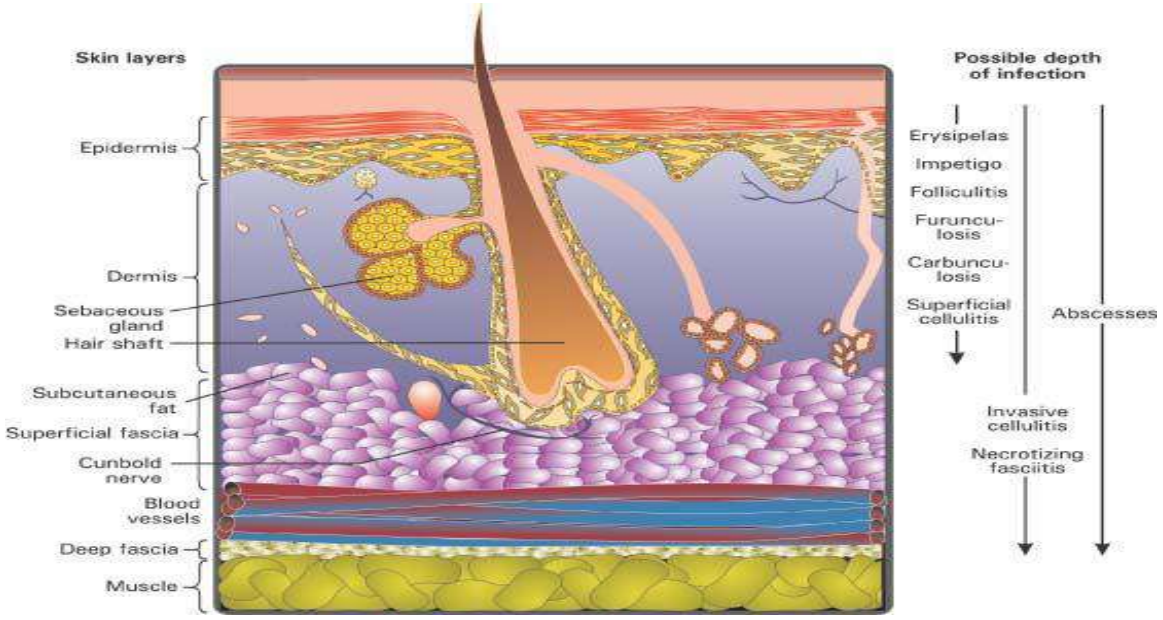


Figure (14-1): Bacterial infections of skin

S. aureus is the most clinically significant species. It causes various cutaneous infections and purulent abscesses. These skin and soft tissue infections can be superficial, such as impetigo or cellulitis. Cutaneous infections can progress to deeper abscesses, such as **carbuncles**, and involve other organ systems and produce bacteremia and septicemia. *S. aureus* is a common cause of infective endocarditis and toxin-induced diseases, such as food poisoning, and is associated with **scalded skin syndrome** (SSS) and **toxic shock syndrome** (TSS).

Bacillus anthracis

Bacillus anthracis, a sporeforming,aerobic, gram-positive rod caused Anthrax . Endospores can survive for years in soil rich. When transmitted to animal tissues, the spores germinate rapidly to produce vegetative cells. The thick capsule of the cells impedes phagocytosis by immune cells, and the bacilli produce three toxins that work together to cause disease. Humans acquire anthrax from infected animal products, contaminated dust, or directly

from the soil.

Diagnostic Laboratory Tests

Specimens to be examined are fluid or pus from a local lesion, blood, pleural fluid, and cerebrospinal fluid in inhalational anthrax associated with sepsis and stool or other intestinal contents in the case of gastrointestinal anthrax. Stained smears from the local lesion or of blood from dead animals often show chains of large Gram-positive rods.

Anthrax can be identified in dried smears by immunofluorescence staining techniques. When grown on blood agar plates, the organisms produce nonhemolytic gray to white, tenacious colonies with a rough texture and a ground-glass appearance. Commashaped outgrowths (Medusa head, "curled hair") may project from the colony. Demonstration of capsule requires growth on bicarbonate-containing medium in 5-7% carbon dioxide. Gram-stain shows large Gram-positive rods.

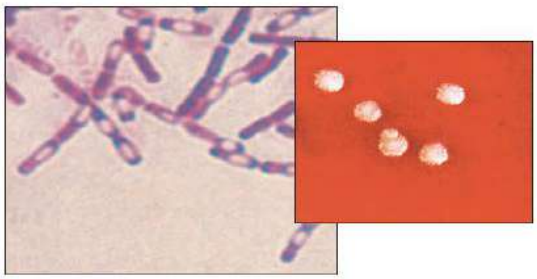


Figure (14-2): ***Bacillus anthracis***

Clostridium tetani is an anaerobic, gram-positive rod that forms endospores. Globally, neonatal tetanus accounts for the majority of cases and deaths, often the result of the umbilical stump becoming infected from nonsterile instruments or dressings.

Clinical Presentation

Spores enter the body through a deep puncture wound resulting from a fracture, gunshot, animal bite, a piece of glass, a thorn, or a rusty nail contaminated with soil. Like anthrax, even illicit drugs can contain spores. In dead, oxygen-free tissue of the wound, spores germinate into vegetative bacilli that produce several toxins. The most important of these is the tetanus toxin (tetanospasmin). At the nerve-muscle synapse of the spinal cord or peripheral nerves, the neurotoxin prevents the release of neurotransmitters needed to inhibit muscle contraction.Without any inhibiting influence, volleys of spontaneous impulses arise in the motor neurons, causing uncontrolled, continuous muscle contraction (spasms).

Symptoms of tetanus intoxication develop rapidly, often within hours of exposure. Person first experiences generalized muscle stiffness, especially in the facial and swallowing muscles. Spasms of the jaw muscles are usually the first affected, causing the teeth to clench and bringing on a condition called trismus, or lockjaw. Severe cases are characterized by a "fixed smile" (risus sardonicus), and muscle spasms cause an arching of the back Spasmodic inhalation and seizures in the diaphragm and rib muscles lead to reduced ventilation, and patients often experience violent deaths through asphyxiation.

Clostridium perfringens

Gas gangrene, or myonecrosis (*myo* = "muscle"; *necros* = "death") is caused primarily by *Clostridium perfringens*, an anaerobic, spore-forming, gram positive rod typically found in soil. After endospores in contaminated soil are introduced through a severe, open wound, the spores germinate, and the vegetative cells multiply rapidly in the anaerobic environment. As they grow, they ferment muscle carbohydrates and decompose the muscle proteins "myonecrosis".

Large amounts of gas can result from this metabolism, causing a crackling sound as the gas accumulates under the skin. The gas also presses against blood vessels, thereby blocking the flow and forcing cells away from their blood supply. In the infection process, the organisms secrete at least 12 toxins.

The most important is α-toxin, is responsible for toxemia typically observed during gas gangrene which damages membranes and disrupts tissues, facilitating the passage of bacterial cells into the blood (sepsis).

The symptoms of gas gangrene include a foul odor and intense pain and swelling at the wound site. Initially the body site turns dull red, then green, and finally blue black .without treatment, the disease spreads rapidly, and death frequently occurs within days of gangrene initiation.

Laboratory diagnosis:

1.Sample collection

Sampling is the essential step in the process of diagnosis, since **sterile pus** is common in inflamed wounds, **pus should be cleaned** of the wound prior to sampling. Cotton swab should be cultured as soon as possible to avoid dryness and contamination of the swab. Wound swab, or any material related to wound should be inoculated onto **blood agar**, **chocolate agar**, and **MacConkey's agar**.

Samples of **deep wound** should be cultured under **anaerobic conditions** using Gas bag. If sampling is correct, then any bacterial growth of any type (opportunistic or potential pathogens) are counted and diagnosis process is forwarded.

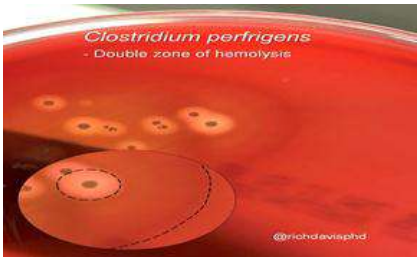
2. Culture

Clostridia are anaerobes and grow under anaerobic conditions; a few species are aerotolerant and also grow in ambient air, grow well on the blood-enriched media or other media used to grow anaerobes.

3. Colony Forms

Some clostridia produce large raised colonies (eg, *C. perfringens*); others produce smaller colonies (eg, *C. tetani*). Many clostridia produce a zone of β-hemolysis on blood agar. *C. perfringens* characteristically produces a double

zone of β -hemolysis around colonies.as show in figure below.



4. Growth Characteristics

Clostridia can ferment a variety of sugars (saccharolytic), and many can digest proteins (proteolytic); some species do both. These metabolic characteristics are used to divide the clostridia into groups. Milk is turned acid by some and digested by others and undergoes "stormy fermentation" (ie, clot torn by gas) with a third group (eg, *C. perfringens*). Various enzymes are produced by different species. *Clostridium* species produce more toxins than any other group of bacteria .

Other bacteriological methods such as **biochemical tests**, **API** technique and **Vitek** can used for complete diagnosis. Also **PCR** or any other method using to diagnose bacteria should be followed in order to give a complete scientific results.Complete blood picture is recommended in special cases; biochemical parameter may be involved in this respect.

Pretest: **الاختبار القبلي:**

Define the followimg? 1- Folliculitis, 2-furuncles, and 3-carbuncles

Scientific Content: المحتوى العلمي:

-effective factors of wound infections
- **Pseudomonas aeruginosa**
- **Clostridium tetani**
- **Clostridial myonecrosis (Gas gangrene)**
- **Bacillus anthracis**

Posttest الاختبار البعدي:

***1-B.anthracis* is of public health importance in skin infections.Explain the diagnostic methods of this bacteria?**

2-Numerate the effective factors of wound infections?

3-Which are bacteria more dangerous Pseudomonas aeruginosa or Clostridium tetani?compaire between them by microscopically?

References: **المصادر :**

1. Jawetz, Melnick, & Adelberg's.(2019):Medical Microbiology.Twenty-Eighth Edition.
2. Connie R. Mahon, Donald C. Lehman (2019): Textbook of Diagnostic Microbiology, Sixth Edition.
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4. Jeffrey C. Pommerville. (2018): Fundamentals of microbiology, Eleventh edition.
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Title: **Lecture 28**

العنوان :

Laboratory methods for diagnosis parasitic infections
Laboratory methods in basic Mycology
Laboratory methods in basic Virology

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

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Target population: **الفئة المستهدفة :**

طلبة المرحلة الرابعة

Introduction: **المقدمة:**

Diagnosis of Parasitic Diseases

Many kinds of lab tests are available to diagnose parasitic infections. These tests based on patient's signs and symptoms, any other medical conditions may have, also his travel history. Diagnosis may be difficult, so that many laboratory tests must be done.

Parasitic organisms are the causative agents of some of the world's most devastating and prevalent infections. This group of pathogens includes members such as the protozoans *Trypanosoma* (Chagas disease and African

sleeping sickness), *Leishmania* (leishmaniasis), *Plasmodium* (malaria), and the helminths such as *Schistosoma* (schistosomiasis), *Wuchereria* (filariasis), and *Echinococcus* (echinococcosis), among others. Many of these infections have always been described as **being tropical or subtropical**. However, the increase in international travel as well as the arrival of new immigrants has made some of these tropical diseases realities in developed countries as well. In addition, a new trend arose; individuals, who never traveled to **endemic areas** were being infected by "tropical" blood-borne protozoans.

This unusual observation could be traced back to blood transfusions and organ transplants. Asymptomatic carriers migrate and become part of the blood bank donor and transplant donor populations. Quick diagnosis has always been a priority to determine the appropriate treatment and prevent fatalities. In addition, now more than ever, advances in diagnostics can help prevent transmission and provide active surveillance. Unfortunately, there have been few major advances in diagnostic methods for parasitic infections. Efforts have stagnated, and the majority of definitive diagnoses still rely on labor-intensive and time-consuming methods such as microscopy. To have the most significant diagnostic impact, new techniques and assays should be simple and yield rapid results. Such characteristics could be achieved by **reducing** the **number of steps** to be performed in a procedure and making result interpretation obvious enough to avoid significant operator-dependent biases. An optimal diagnosis method would possess these favorable features while still maintaining a high level of sensitivity and specificity. Moreover, many of the tests that are used today **cannot differentiate** between current and past infections. Assays that are capable of making this distinction are necessary to properly determine **disease prevalence**, choose the appropriate treatment, and assess the effect of treatment.

Currently, diagnostic and reference laboratories use several techniques, including microscopy, molecular assays, and serological assays. Each method has its advantages as well as disadvantages. Furthermore, many research laboratories are focusing on the development of new diagnostic methods as well as the improvement of old ones. There has especially been a focus on the development of **molecular diagnostic techniques**. Real-time polymerase chain reaction **(PCR)** procedures for the detection of various parasites are continuously being optimized. Recently, loop-mediated isothermal amplification **(LAMP)** has attracted much attention and seems to be the molecular tool of the future.

The development of multiplex real-time PCR protocols has also been

emphasized. These assays have the ability to detect **mixed infections simultaneously**. Serology-based techniques have been complementing microscopy for many years; however, for many infections, they still cannot fully replace microscopic diagnosis. These assays rely on antigen or antibody detection from the provided patient samples. Rapid diagnostic tests have become some of the most popular serology-based assays.

Common laboratory tests used for diagnosis parasitic infections are:

1. A fecal (stool) exam, also called an ova and parasite test (O&P): This test is used to find parasites that cause diarrhea or watery stools, cramping, flatulence (gas) and other abdominal illness. This test looks for ova (eggs) or the parasite. Specimens not collected in a preservative fluid should be refrigerated, but not frozen, until delivered to the lab. In addition, it may request that the lab use special stains or that special tests be performed to look for parasites not routinely screened for. (Figure 1)

2. Endoscopy/Colonoscopy: Endoscopy is used when stool exams do **not reveal** the parasitic causes of illness. This test is a procedure in which a tube is inserted into the mouth (endoscopy) or rectum (colonoscopy). This test looks for the parasite or other abnormalities that may be causing patient's signs and symptoms.

3. Culture Methods:

Unlike Bacteriology, culture methods are rarely used as a diagnostic tool in parasitology. Culture methods are available for some of the protozoan parasites (*Entamoeba histolytica, Balantidium coli*), and Helminths e.g. Harada-Mori
culture for recovering larvae of nematodes such as Hookworms, *Strongyloides stercoralis*.

4. Blood tests

Some, but not all, parasitic infections can be detected by testing your blood. Blood tests look for a specific parasite infection; there is no blood test that will look for all parasitic infections. There are two general kinds of blood tests that used for diagnosis of parasitic infections:

- **Serology**: This test is used to look for antibodies or for parasite antigens produced when the body is infected with a parasite and the immune system is trying to fight off the invader. This test is done by examination of blood sample at specific lab.
- **Blood smear**: This test is used to look for parasites that are found in the blood. By looking at a blood smear under a microscope, parasitic diseases such as filariasis, malaria, or babesiosis, can be diagnosed. This test is done by placing a drop of blood on a microscope slide. The slide is then stained and

examined under a microscope.

5. X-ray, Magnetic Resonance Imaging (MRI) scan, Computerized Axial Tomography scan (CAT): These tests are used to look for some parasitic diseases that may cause lesions in the organs.

Figure 1): Microscopic appearance of some parasitic ova (eggs).

Laboratory methods in basic virology

In the diagnostic laboratory virus infections can be confirmed by a multitude of methods. Diagnostic virology has changed rapidly due to the advent of molecular techniques and increased clinical sensitivity of serological assays. There are several laboratory steps used for viral diagnosis:

1. Sampling.
2. Virus isolation.
3. Nucleic acid based methods such as polymerase chain reaction(PCR) and gene sequencing.
4. Microscopy based methods like immunofluorescence or immunoperoxidase and electron microscopy.
5. Host antibody detection.
6. Hemagglutination assay.

1. Sampling

A wide variety of samples can be used for viral diagnosis. The type of sample sent to the laboratory often depends on the type of viral infections being diagnosed and the test required. Proper sampling technique is essential to

avoid potential pre-analytical errors. Different types of samples must be collected in appropriate tubes to maintain the integrity of the sample and stored at appropriate temperatures (usually 4°C) to preserve the virus and prevent bacterial or fungal growth. Sometimes multiple sites may also be sampled. Types of samples include:

- Blood
- Skin
- Sputum, gargles and bronchial washings
- Urine
- Semen
- Faeces
- Cerebrospinal fluid (CSF)
- Tissues (biopsies or post-mortem)
- Dried blood spots

2. Virus isolation

Viruses are often isolated from the initial patient sample. This allows the virus sample to be grown into larger quantities and allows a larger number to tests to be run on them. This is particularly important for samples that contain new or rare viruses for which diagnostic tests are not yet developed.

Many viruses can be grown in **cell culture** in the lab. To do this, the virus sample is mixed with cells, a process called adsorption, after which the cells become infected and produce more copies of the virus. Although different viruses often only grow in certain types of cells, there are cells that support growth of a large variety of viruses and are a good starting point such as African monkey kidney cell line (Vero cells), human lung fibroblasts (MRC-5), and human epidermoid carcinoma cells (HEp-2). One sign of knowing whether the cells are successfully replicating the virus is to **check for a change** in cell morphology or for the presence of cell death using a microscope.

Other viruses may require alternative methods for growth such as the inoculation of embryonated **chicken eggs** (avian influenza viruses) or the intracranial inoculation of virus using **newborn mice** (lyssaviruses).

3. Nucleic acid based methods

Molecular techniques are the most specific and sensitive diagnostic tests. They are capable of detecting either the whole viral genome or parts of the viral genome. In the past nucleic acid tests have mainly been used as a secondary test to **confirm positive** serological results. However, as they become cheaper and more automated, they are increasingly becoming the **primary tool for diagnostics**.

❖ Polymerase chain reaction

Detection of viral RNA and DNA genomes can be performed using polymerase chain reaction. This technique makes many copies of the virus genome using virus-specific probes. Variations of PCR such as nested reverse transcriptase PCR and real time PCR can also be used to determine viral loads in patient serum. This is often used to monitor treatment success in HIV cases.

❖ Sequencing

Sequencing is the only diagnostic method that will provide the full sequence of a virus genome. Hence, it provides the most information about very small differences between two viruses that would look the same using other diagnostic tests. Currently it is only used when this depth of information is required. Sequencing is useful when specific mutations in the patient are tested for in order to **determine antiviral therapy** and **susceptibility to infection**. However, as the tests are getting cheaper, faster and more automated, sequencing will likely become the **primary diagnostic tool in the future**.

4. Microscopy based methods

❖ **Immunofluorescence or immunoperoxidase**

Immunofluorescence or immunoperoxidase assays are commonly used to detect whether a virus is present in a tissue sample. These tests are based on the principle that if the tissue is infected with a virus, an antibody specific to that virus will be able to bind to it. To do this, antibodies that are specific to different types of viruses are mixed with the tissue sample. After the tissue is exposed to a specific wavelength of light or a chemical that allows the antibody to be visualized.

These tests require **specialized antibodies** that are produced and purchased from commercial companies. These commercial antibodies are usually well characterized and are known to bind to only one specific type of virus. They are also conjugated to a special kind of tag that allows the **antibody to be visualized in the lab**, so that it will emit fluorescence or a color. Hence, immunofluorescence refers to the detection of a fluorescent antibody (immuno) and immunoperoxidase refers to the detection of a colored antibody (peroxidase produces a dark brown color).

❖ **Electron microscopy**

Electron microscopy is a method that can take a picture of a whole virus and can reveal its shape and structure. It is **not typically used** as a routine diagnostic test as it requires a **highly specialized type of sample** preparation, microscope and **technical expertise**. However, electron microscopy is highly versatile due to its ability to analyze any type of sample and identify any type

of virus. Therefore, it remains the gold standard for identifying viruses that do not show up on routine diagnostic tests or for which routine tests present conflicting results (Figure 2).

5**.** Host antibody detection

A person who has recently been infected by a virus will produce antibodies in their bloodstream that specifically recognize that virus. This is called humoral immunity. Two types of antibodies are important. The first called IgM is highly effective at neutralizing viruses but is only produced by the cells of the immune system for a few weeks. The second, called, IgG is produced indefinitely. Therefore, the presence of IgM in the blood of the host is used to test for acute infection, whereas IgG indicates an infection sometime in the past. Both types of antibodies are measured when tests for immunity are carried out. Antibody testing has become widely available. It can be done for individual viruses (by using an ELISA assay) but inautomated panels that can screen for **many viruses at once are becoming increasingly common**.

6. Haemagglutination assay

Some viruses attach to molecules present on the surface of red blood cells, such as influenza virus. A consequence of this is that at certain concentrations a viral suspension may bind together (agglutinate) the red blood cells thus preventing them from settling out of suspension.

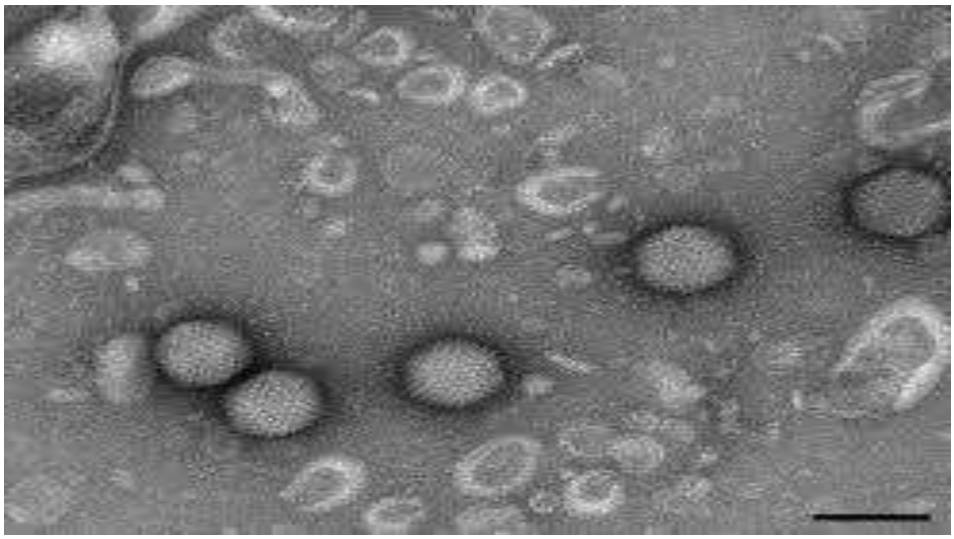

Figure (2): Virus appearance under electron Microscope.

Laboratory methods in basic mycology

Mycological agents are saprophytic and parasitic eukaryotic organisms. Historically, the fungi were regarded as relatively insignificant causes of

infection. However, the frequency of fungal infections, especially of Invasive Fungal Infections (IFIs), has risen dramatically in recent years due to their prolonged antibiotic therapy like *Candida spp* and Aspergillus. These pathogenic causes remain an important cause of morbidity and mortality.

Early and accurate detection (laboratory diagnosis) is important for timely implementation of antifungal therapy and decreasing the unnecessary use of **toxic antifungal** agents. In addition the availability of accurate and timely diagnosis could **reduce** the use of empirical anti-fungal therapy, thereby decreasing antifungal selection pressure and the emergence of **antifungal resistance**.

Standard approaches to the laboratory diagnosis of Fungal Infections include:
1. Direct microscopic examination in freshly obtained samples.
2. Histopathological demonstration of fungi within tissue sections.
3. Culture of the causative fungus yeasts and its further identification.
However, these approaches are often not sufficiently sensitive and/or specific to diagnose molds or yeasts, and they sometimes **require invasive procedures** to obtain the necessary specimens and culture takes 2-4 weeks to become positive. So there is need for rapid methods which are sensitive and specific for diagnosis of the mycological pathogenic agents. This is a brief review of all the microbiological techniques- conventional and recent alternative techniques especially elaborating antigen detection and molecular techniques which are also available for diagnosis.

Diagnostic Methods for Fungal Infections

Direct examination – wet mount, with flourescent staining
Fungal culture
Radiology
Non culture methods:
1. Serological methods- antigen detection, antibody detection.
2. Tests for detection of metabolites.
3. Tests for detection of CMI (cell mediated immunity).
4. Molecular methods
5. Others- MALDI-TOF MS (Matrix-assisted laser desorption/ionization - time of flight mass spectrometry (MALDI-TOF MS), a Noble prize winning technology is making a revolutionary entry in clinical microbiology laboratory laboratories all over the world).

Pretest: الاختبار القبلي :

1-Parasitic organisms are the causative agents of some of the world's most infections. This group of pathogens includes members such as the protozoan----------,----------,----------

2- Specimens for parasitic infections include -----,------,-----,------,------.

Scientific Content: المحتوى العلمي:

- Diagnosis of Parasitic Diseases
- Laboratory methods in basic virology
- Laboratory methods in basic mycology

Posttest الاختبار البعدي:

1- Mention Laboratory diagnosis of parasitic infection?
2. Mention Laboratory diagnosis of viral infection?
3- Mention Laboratory diagnosis of fungal infection

المصادر :

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