Diagnostic microbiology: / المنهاج المركزي المعتمد في المختبرات الطبية

Lecture-1: 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.**

5the 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.

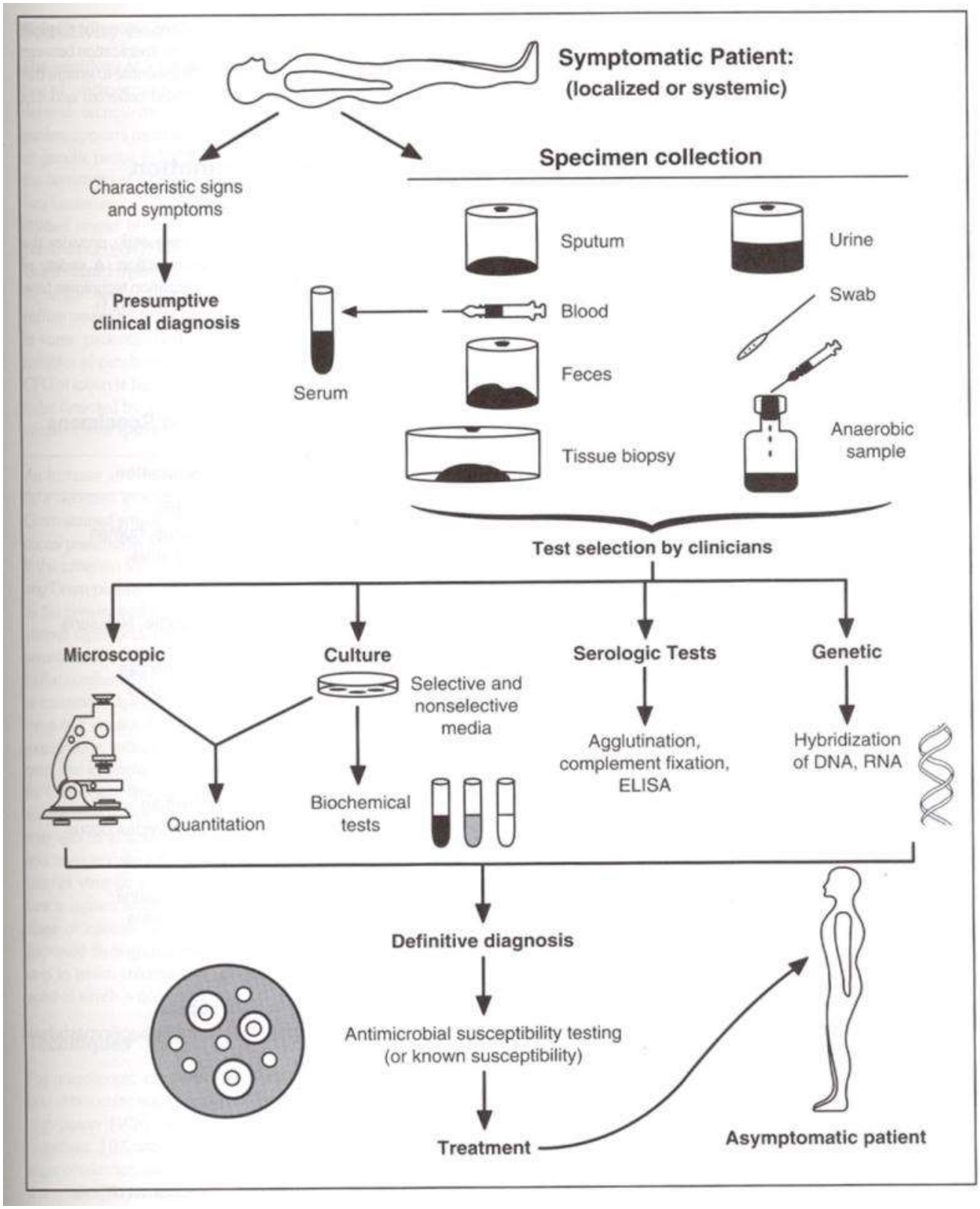


Figure (1): Represent human specimens collection and diagnoses .

Lecture-2: Laboratory safety

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 **1- organic load** (organisms and other contaminating materials such as **blood** or body **fluids**), **2- the type of organisms present, 3- the concentration and exposure time to the germicide, 4- 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:

1) **Incineration.** 2) **Moist heat.** 3) **Dry heat.** 4) **Filtration**
5) **Ionizing (gamma) radiation.**
6) **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.

Lecture- 3, 4 and 5: Selection, collection, and transport of specimens for microbiological examination

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.

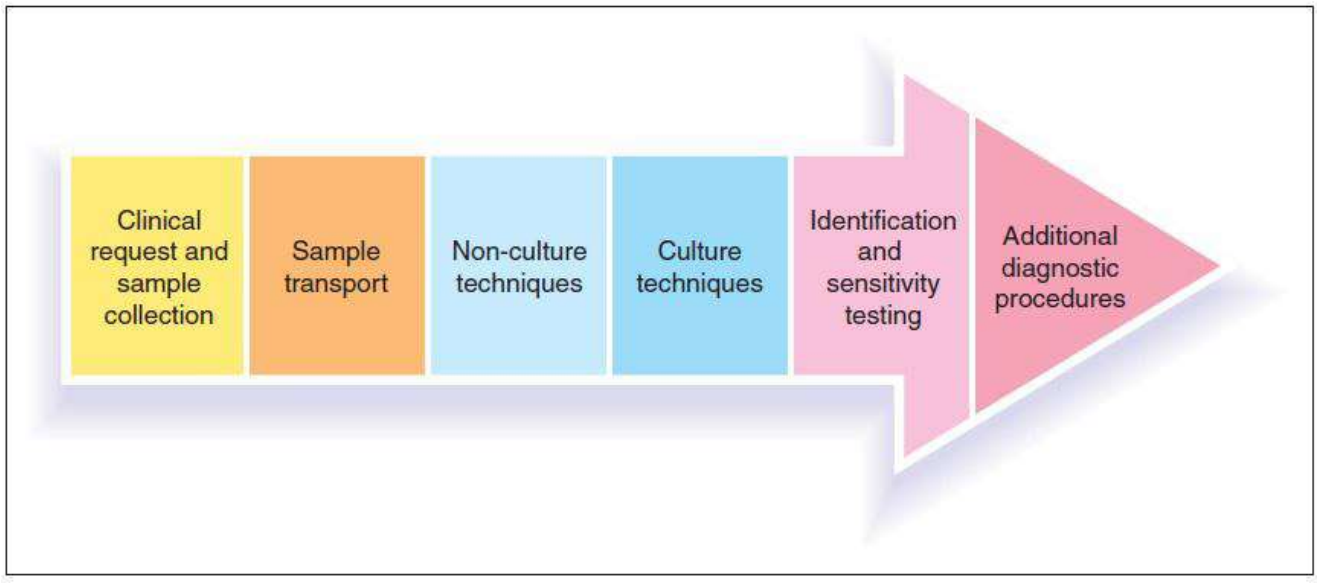


Figure (1): Laboratory diagnostic pattern.

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: 1-** use sterile, leak-proof containers; **2-** label the specimen correctly; **3-** 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. **4-** should be taken prior to 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).

……

Lecture-6: Cultivation and Isolation of Viable Pathogen

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:

1- **Water**: Distilled water or potable water with low mineral content is used for media preparation.

2- **Electrolytes**: sodium chloride or other electrolytes.

3- **Peptone**: it is a complex mixture of partially digested proteins.

• Source of peptone: 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, amino acids, 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:** It is prepared commercially from **washed cells of Baker's yeast.** It contains amino acids, inorganic salts (potassium and phosphates) and carbohydrates. (Figure-1)

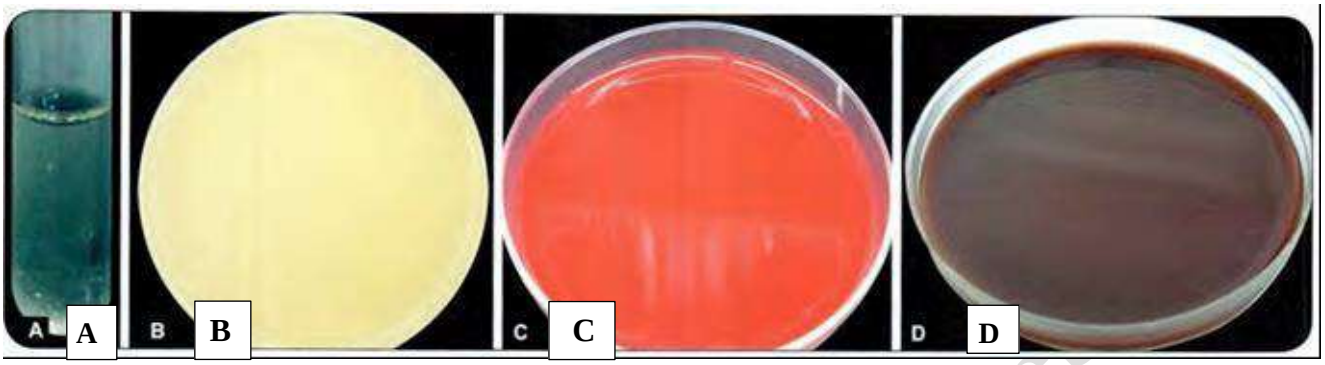


Figure (1): A. Peptone water; B. Nutrient agar; C. Blood agar; D. Chocolate agar.

- **Meat extract**: It consists of maltose (about 50%), starch, dextren, glucose and 5% of protein products.
- **Blood and serum**: The are important components of enriched media 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 <u>two ways</u>:

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**, **this type of media either 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).

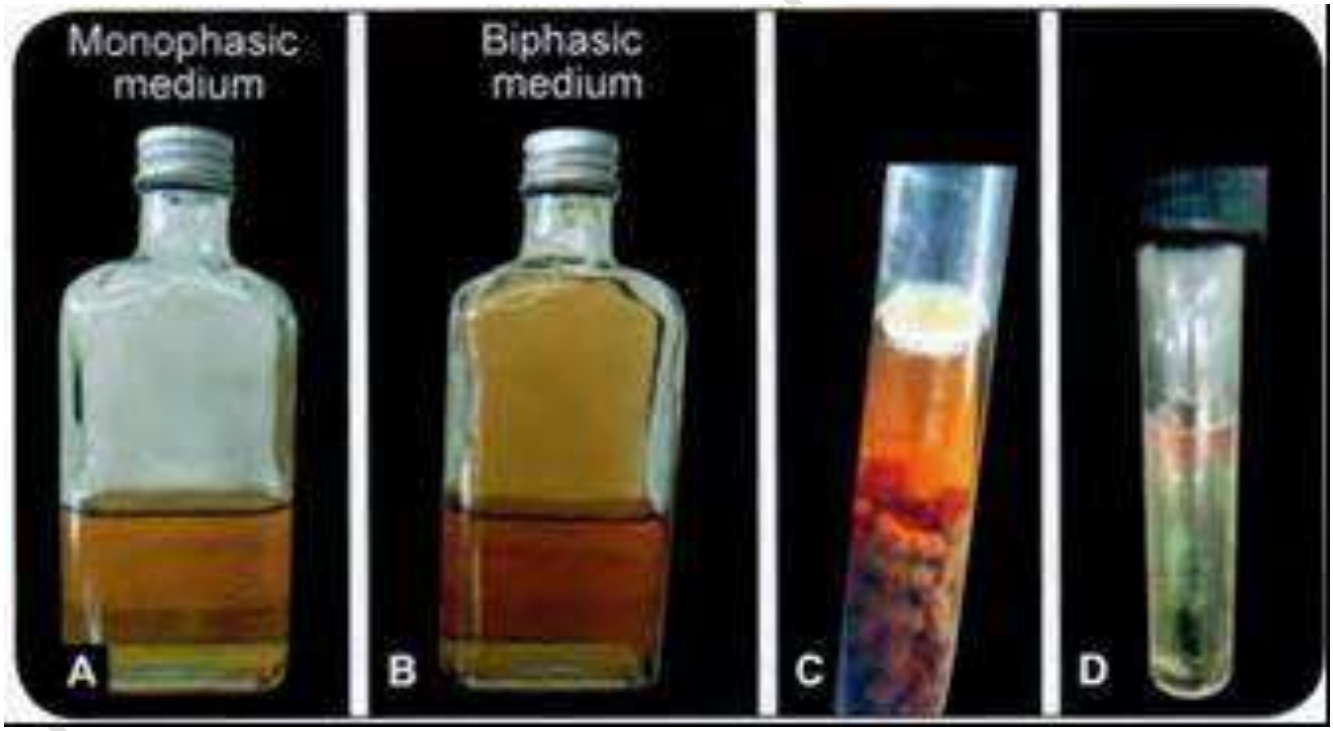


Figure (2): A. Brain-heart infusion broth; B. Biphasic medium (Brain-heart infusion broth/agar); C. Robertson's cooked meat medium; D. Thioglycollate broth.

Selective media

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

Mannitol salt agar which include increases NaCl (salt) concentration for the recovery of staphylococci.

MacConkey agar which contains bile salt and allows the growth of bile-tolerant bacteria only (such as gram negative bacteria).

Antibiotics, which are frequently added to the media to allow only some bacteria to grow while suppressing or killing others type of bacteria.

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 color change of a pH indicator, such as MacConkey agar contains lactose and a pH indicator (neutral red); lactose-fermenting bacteria (*Escherichia coli*) produces 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.

……..

Lecture-7, 8, 9: Microbiological methods for identification of microorganisms

Microbiological methods for identification of microorganisms

Various methods used to identify organisms cultivated from patient specimens, associated with bacterial identification. **The procedures for 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 provides 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 as *Treponema pallidum*, a spirochete that is smaller than 0.2 μm in diameter and therefore cannot be observed with a bright-field 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 (DIC)

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.

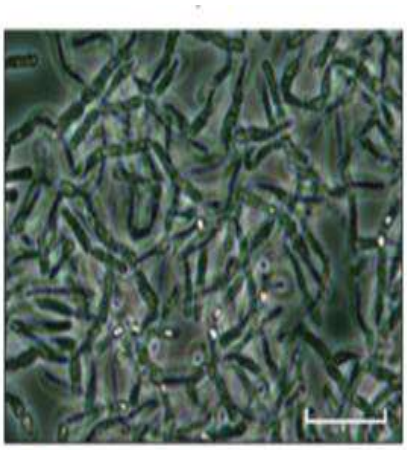
The Electron Microscope

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 **three dimensional images** of the surface of microscopic objects.

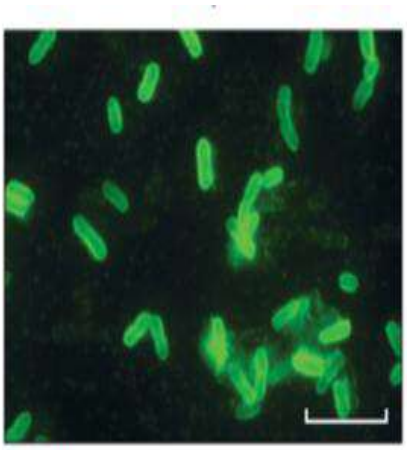
Scanning Probe Microscopes

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



(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 105 organisms per milliliter before it is likely that organisms will be seen on a smear. Liquid medium containing 105 organisms per milliliter does not appear turbid to the eye. Specimens containing **102–103 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 (H2O2 _ catalase H2O _O2); 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. **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 API test and VITEK system.

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

- ✓ **Molecular testing.**
- ✓ **Saliva based testing.**
- ✓ **Chromogenic testing.**
- ✓ **Rapid immunochromatographic testing.**

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

……

Diagnostic Microbiology 22/2023

Lecture-(11), (12-13) and (14-15)

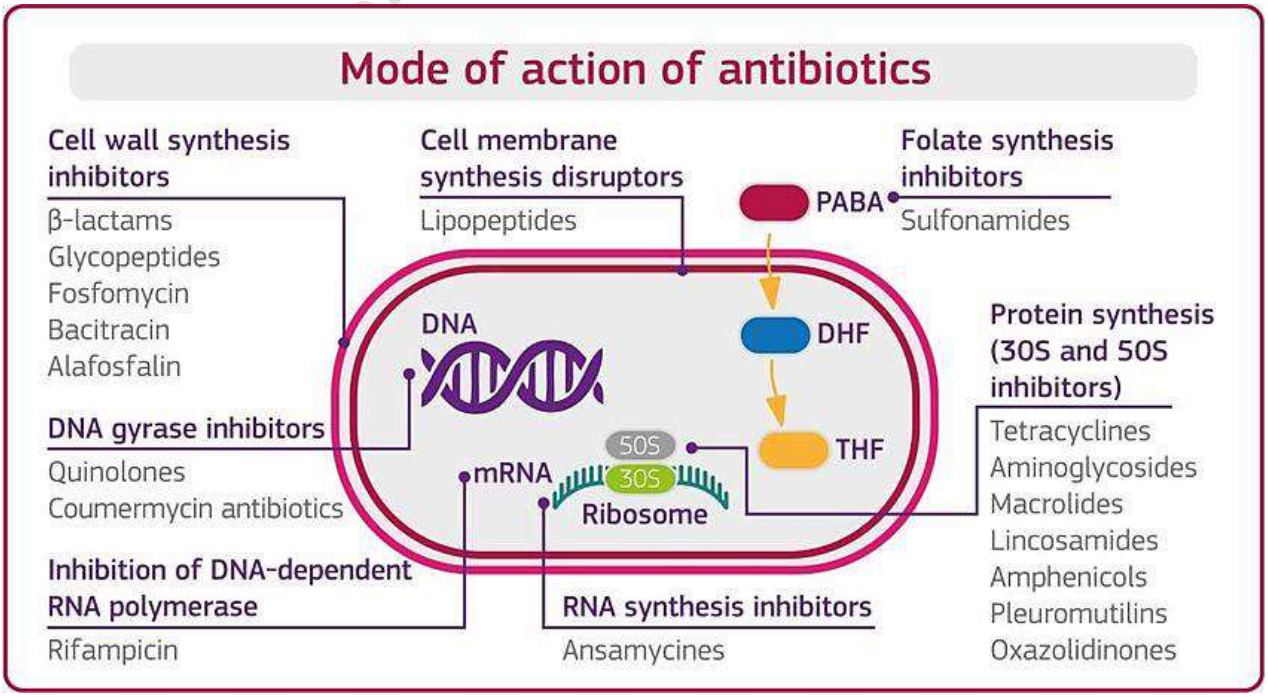
Lecture-11
Antibiotic susceptibility tests

Antimicrobial agents are nontoxic antimicrobial therapeutic agents, which include antiseptics, antibiotics, preservatives, sterilants, and disinfectants; all have the capacity to kill or suppress the 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 by **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



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**.

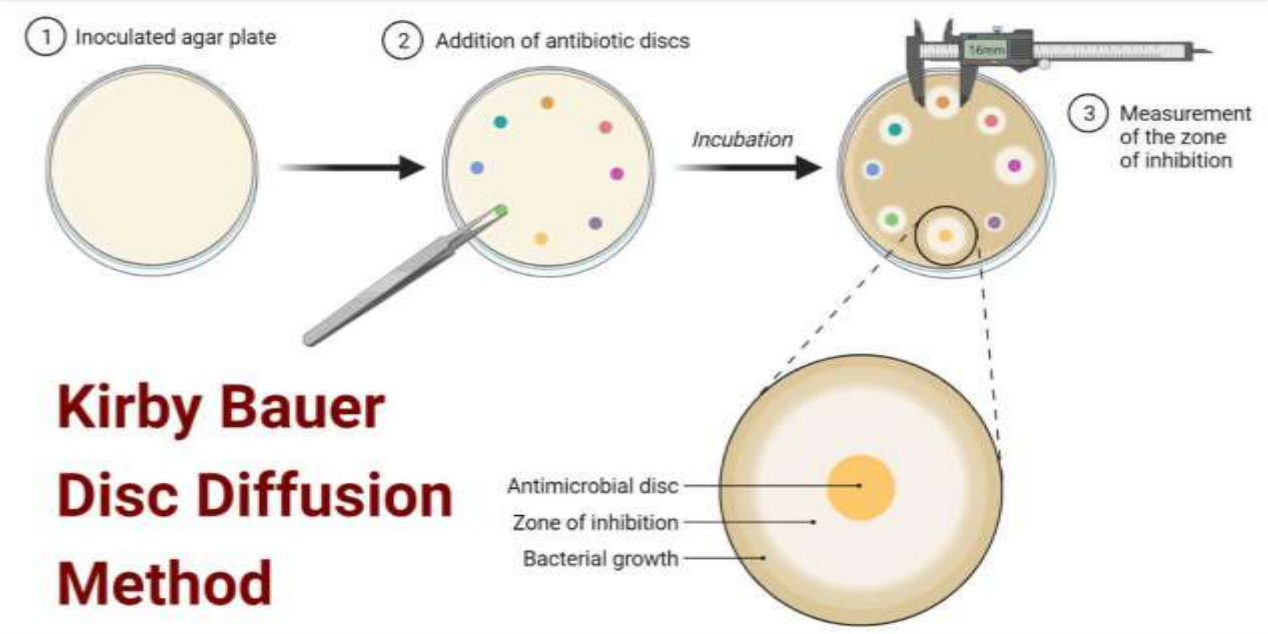
In clinical laboratories, susceptibility testing is usually performed by a disk diffusion or and minimal inhibitory concentration [MIC] methods. Standards that describe these methods are published and frequently updated by the **Clinical and Laboratory Standards Institute** (**CLSI**), formerly the **National 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 placed on culture dishes inoculated with the microorganism to be tested. The micro-organism's growth (resistance to the drug) or lack of growth



2.Minimal inhibitory concentration (MIC):

a- Broth Dilution:

In broth dilution testing method, each antimicrobial agent is tested using a range of concentrations (µg of active drug/mL. of broth). Typically, the range of concentrations examined for each antibiotic is a series of doubling dilutions (16, 8, 4, 2, 1, 0.5, 0.25 µg/mL); the lowest antimicrobial concentration that completely inhibits visible bacterial growth, as detected visually or with an automated method, is recorded as the minimal inhibitory concentration (MIC).

b- Tube dilution (quantitative or macrodilution susceptibility testing):

In this method, tubes containing serial dilutions of an antibiotic are inoculated with the tested organism. The tubes are incubated and later observed to determine the

minimal inhibitory concentration (MIC) of the antibiotic necessary to prevent bacterial growth (Figure-3).

the minimal bactericidal concentration (MBC) may need to be determined. This is the lowest concentration of antibiotic that kills 100% of the bacteria, rather than simply inhibiting growth.

3- Automated Antimicrobial Susceptibility Test Systems. The automated antimicrobial susceptibility test systems available for use include the Vitek Legacy and Vitek 2 system.

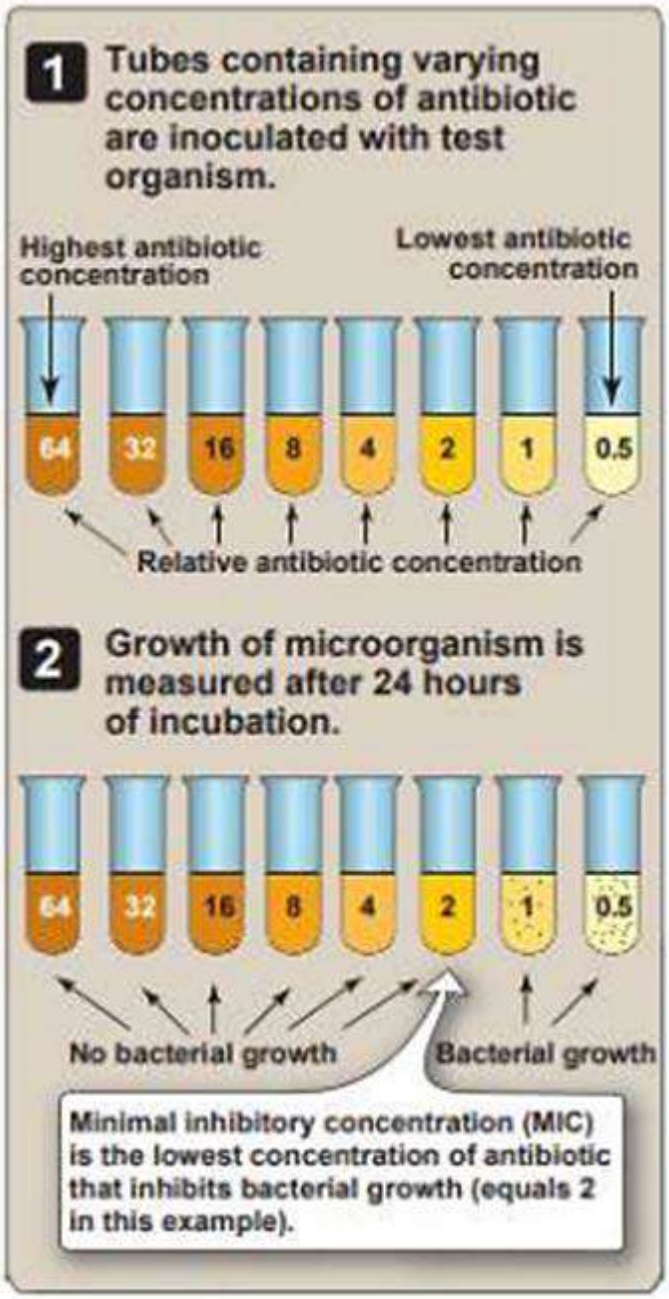


Figure-3

Determination of minimal inhibitory concentration (MIC) of an antibiotic.

Methods for identification of etiological agents of infectious disease

1- *Staphylococcus.*

Morphology: They are Gram positive, Cocci, Grapelike clusters (Cluster formation is due to cell division occurring in three planes, with daughter cells tending to remain in close, non-sporing, nonmotile and usually non- capsulate.

Cultural Characteristics: They are aerobes and facultative anaerobes, Optimum temperature for growth is 37°C, pH is 7.5, can grow readily on ordinary media.

1. On Nutrient Agar: Colonies are soft and smooth surface, entire edge, most strains produce golden-yellow pigment (*Staph. aureus*). Pigmentation is enhanced on fatty media such as Tween agar.

2. Blood Agar. Colonies may be surrounded by a zone of β-hemolysis on blood agar of sheep, rabbit or human blood.

3. Selective Salt Media. Mannitol salt agar containing 1% mannitol, 7.5% NaCl, and phenol red in nutrient agar is the selective medium for S aureus.

Laboratory Diagnosis:

1. Specimens: The specimens to be collected depend on the type of lesion, for example; Pus from suppurate lesions; sputum from respiratory infections; food remains and vomit from cases of food poisoning.

2. Direct Microscopy: Gram stained smears is useful in the case of pus, where cocci in clusters may be seen.

3. Culture: Specimens are inoculated on a blood agar plat, on selective media such Mannitol salt-agar. After incubation of blood agar, look for hemolysis around the colonies, The golden-yellow colonies on nutrient agar. The isolate is examined from the coagulase test.

4. Identification: Positive reactions for coagulase, heat-stable nuclease, alkaline phosphatase, and mannitol fermentation) can be used to differentiate *S. aureus* and the other staphylococci.

5. Coagulase Test: Coagulase test is done by two methods—slide and tube coagulase test.

6. Antibiotic Sensitivity Tests: As a guide for treatment, antibiotic sensitivity tests should be performed appropriate to the clinical situation. This is important as staphylococci readily develop resistance to drugs.

7. Bile susceptibility test (BST): This plate (Bile Esculin Agar-BEA) was inoculated with *Staphylococcus aureus*/top (negative result) and *Enterococcus faecium*/bottom (positive result). The darkening of the medium around *E. faecium* indicates a positive result.

8. Novobiocin susceptibility test (NST) is used to differentiate between *Staph. saprophyticus* (resistant/top) from other coagulase negative staphylococci.

2- *Streptococcus:*

Morphology and General characteristics:

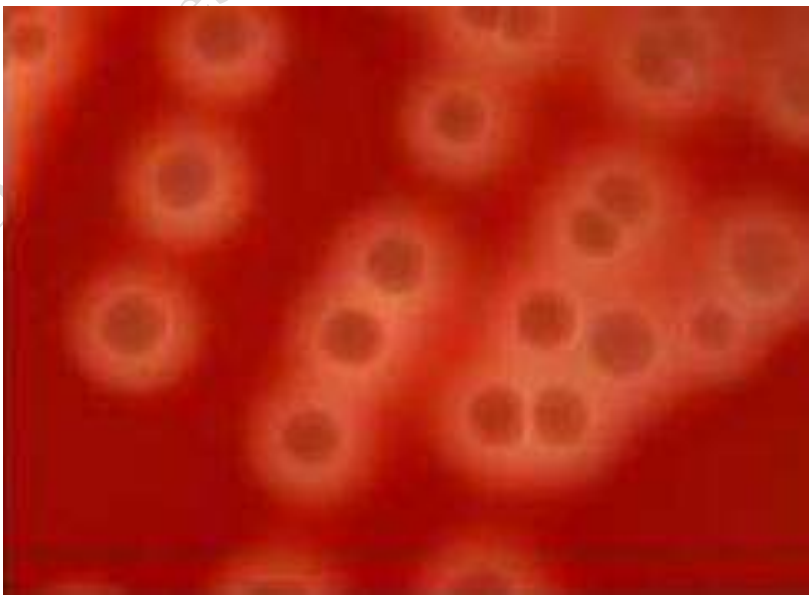
Gram positive cocci arranged in chains, non-motile and non-sporing. They require media enriched with blood for growth. They are human pathogens causing pyogenic infection. They are responsible for non-suppurative lesions (acute rheumatic fever and glomerulonephritis). Group A streptococci have a hyaluronic acid capsule.

Cultural characters:

Streptococcus pyogenes is aerobic and facultative anaerobes with optimum temperature of growth being 37°C. It grows in enriched media with whole blood or serum.

a. Fluid media: Serum broth, 24 hours after culture shows granular growth with powdery deposits.

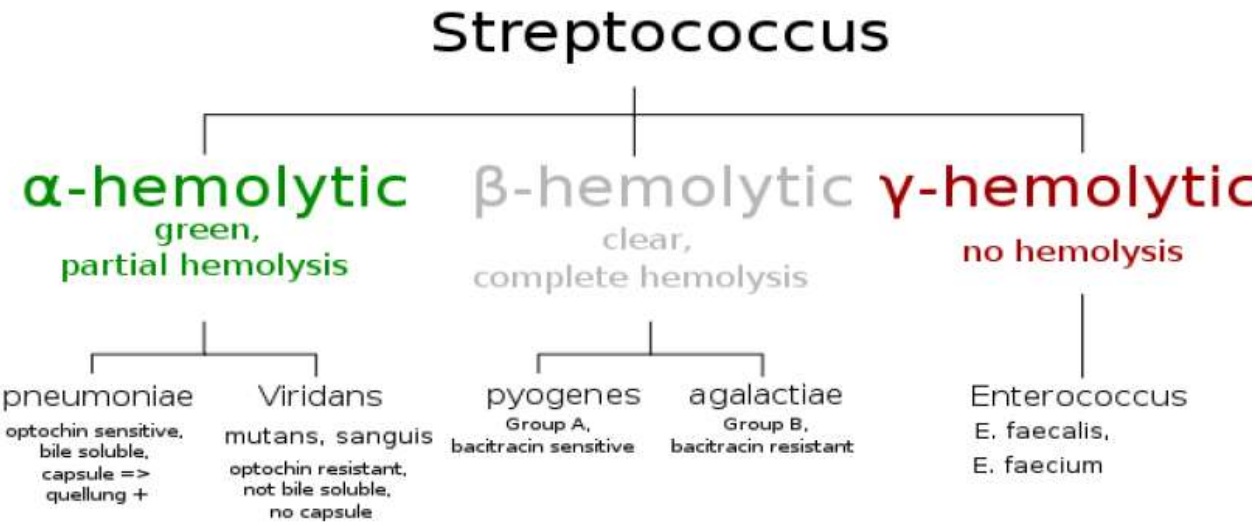
b. Blood agar: After 24 hours' incubation colony is small (pin point colonies), circular, transparent, low convex with area of hemolysis. Strains with capsules produce mucoid colonies.



***Streptococcus pyogenes* growth of blood agar medium, Beta-hemolysis.**

Columbia Agar Base with 5% Defibrinated Horse Blood. It is selective medium for the isolation of *Streptococcus spp*. from clinical samples. It is made selective by the addition of Colistin and Oxolinic Acid.

Streptococcal classification



3- *Enterococcus:*

The enterococci (enteric cocciǁ) were previously classified as group D streptococci. This group consists of gram-positive cocci, non-motile and non-capsulated, that are natural inhabitants of the intestinal tracts of humans and animals. They grow in the presence of 6.5 percent NaCl, 40% bile at 45°C. It survives heating at 60°C for 30 min, a feature distinguishing it from streptococci. On MacConkey medium they produce deep pink colonies. Enterococci are PYR test positive. They do not hydrolyze hippurate.

4- *Streptococcus pneumoniae*

Morphology:

1-gram-positive cocci in pairs (diplococci), slightly elongated cocci, with one end rounded, non-motile and non-sporing, All freshly isolated strains are capsulated and the capsule encloses each pair.

Cultural Characteristics

1-They are aerobes and facultative anaerobes.

2- It grows best in air or hydrogen with 5-10 percent CO2.

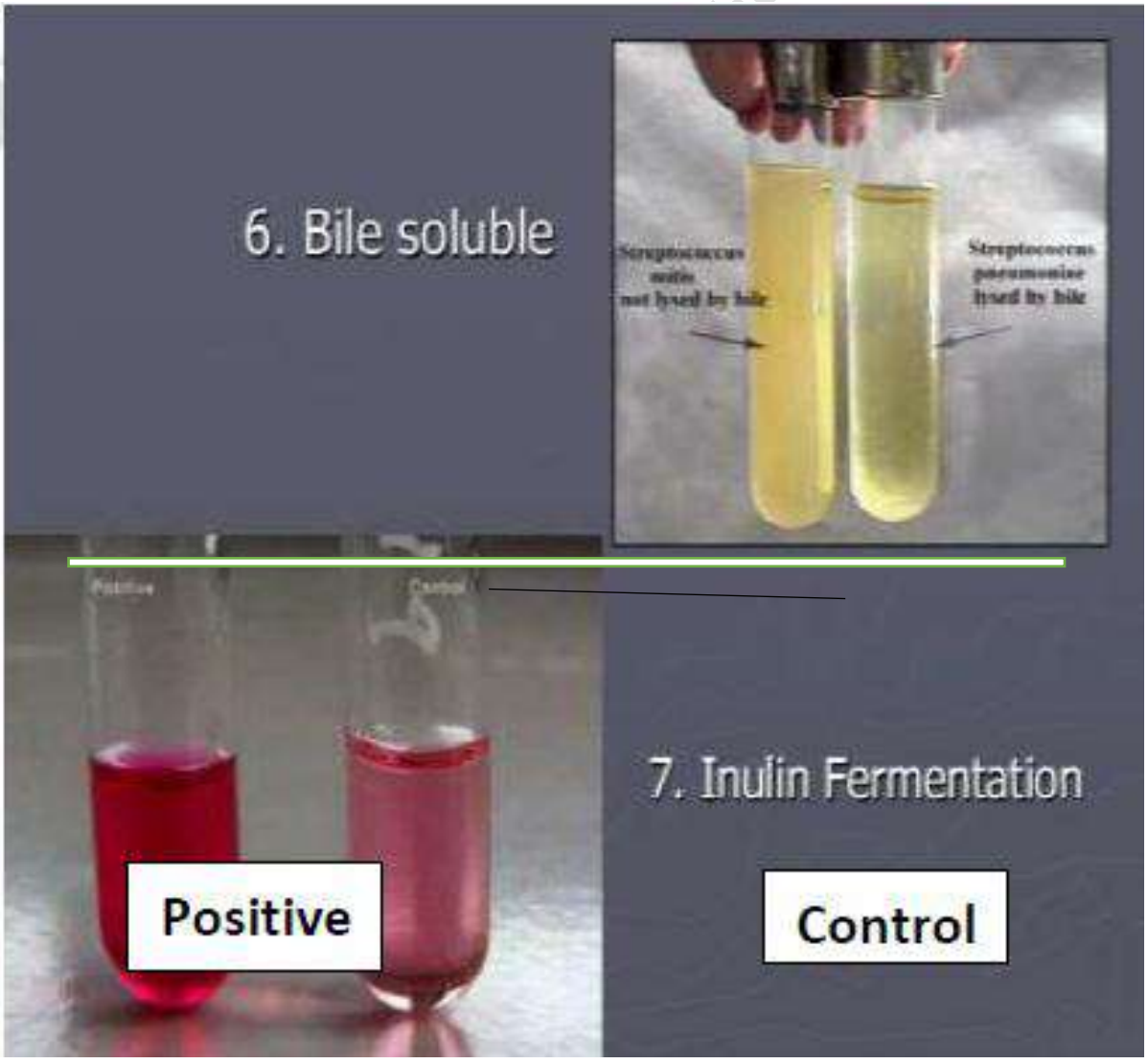
Biochemical Reactions

1. Inulin Fermentation: Pneumococci ferment inulin with the production of acid without gas. Fermentation of inulin by pneumococci is a useful test for differentiating them from streptococci as the latter do not ferment it.

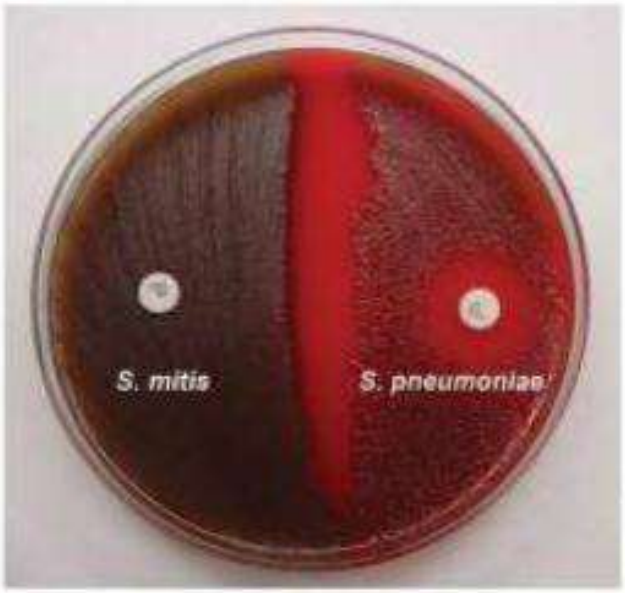
2. Bile Solubility Test:

1- Grow the isolate to be tested for 18 hours at 37°C in 5 ml serum, digest broth or infusion broth.

2- While still warm, add 0.5 ml of 10 percent, bile salt (sodium deoxycholate solution) and re-incubate at 37°C. Pneumococci are lysed within 15 minutes and the initially turbid culture becomes clear and transparent. Pneumococci are soluble in bile; viridans and other streptococci are not.



4- **Optochin Sensitivity:**



Left Side

S. mitis

Resistant to optochin

Right Side

S. pneumoniae

Susceptible to optochin

Laboratory Diagnosis

1. Specimens:

Sputum, lung aspirate, pleural fluid, cerebrospinal fluid (CSF) or blood are collected according to the site of lesion. Sputum specimens must be mucus from the lungs rather than samples of saliva.

2. Microscopy and Antigen Detection

Gram stain of sputum specimens is a rapid way to diagnose pneumococcal disease. If the smears are gram-positive lancet-shaped diplococci, a presumptive diagnosis of pneumococcal pneumonia may be made. A centrifuged deposit of the CSF should be examined immediately in a Gram film in case of meningitis and presumptive diagnosis may be made by finding gram-positive diplococci.

3. Culture:

Specimen is inoculated on plates of blood agar and heated blood agar (chocolate agar) and incubated in air with 5-10% CO2 for 18-24 hours.

5- *Pseudomonas aeruginosa*

It is gram negative, motile and rod shaped. It occurs as single bacteria, in pairs, and occasionally in short chains.

Specimens: Specimens depend on the site of infection including skin lesions, pus, urine, blood, spinal fluid, sputum, and other material should be obtained by different procedures.

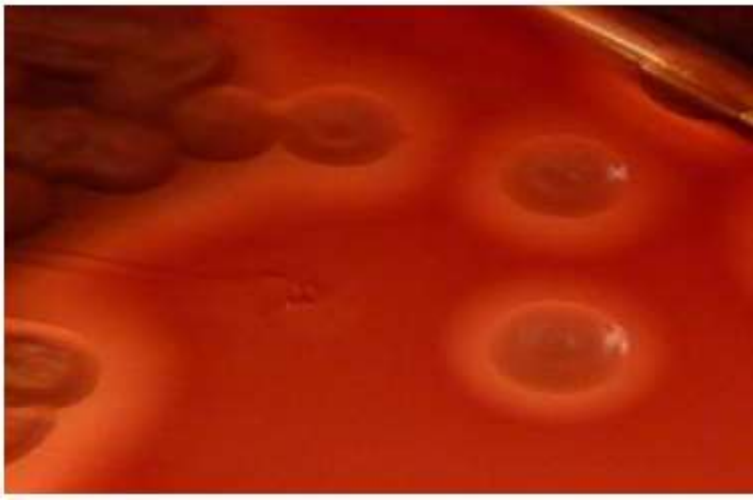
Culture: Pseudomonads grow readily on most culture media. It does not ferment lactose and is easily differentiated from the lactose- fermenting bacteria.

P. aeruginosa is an obligate aerobe but can grow anaerobically if nitrate is available, that grows readily on many types of culture media, sometimes producing **a sweet or grapelike** or corn taco–like odor. Some strains **hemolyze blood**.

P aeruginosa forms smooth round colonies with a **fluorescent greenish color**. It often produces the 1) non-fluorescent **bluish pigment pyocyanin**, which diffuses into the agar. Many strains produce the 2) **fluorescent pigment pyoverdin**, which gives a greenish color to the agar. Other strains produce the 3) **dark red pigment pyorubinor**, or the 4) **black pigment pyomelanin**.

On MacConkey agar plates (**as shown below**) it produces non-lactose fermenting colonies (**compared with *E. coli* or *Klebsiella***) and the pigments are often poorly observed.

On **blood agar** plates, it is surrounded by a zone of hemolysis (**as show below**), while in broth culture it forms a dense turbidity with a surface **pellicle**.



6- *Enterobacterceae*

Gram-negative rods related to the enteric tract include a large number of genera.

Diseases Caused by Members of the Enterobacteriaceae

Escherichia - Urinary tract infection, traveler's diarrhea, neonatal meningitis

Shigella- Dysentery

Salmonella -Typhoid fever, enterocolitis .

Klebsiella - Pneumonia, urinary tract infection.

Enterobacter- Pneumonia, urinary tract infection.

Serratia- Pneumonia, urinary tract infection.

Proteus -Urinary tract infection.

Yersinia - Plague, enterocolitis, mesenteric adenitis.

Diagnosis:

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**).

Identification of *Enterobacteriaceae* on MacConkey agar:

MacConkey agar is inoculated with tested organism using streak plate technique. Incubate the plate in incubator at 37 C for 24 hrs., then read the results as the following:

- LF organism appears as **pink colonies** (e.g. ***E. coli*** and ***Klebsiella***)
- NLF organism appears as **colorless colonies** (***Salmonella*** *and* ***Shigella***).

Key Characteristics to differentiate some group of *Enterobacteriaceae.*

Test \ Bacteria	*E. coli*	*Shigella sonnei*	*Salmonella typhi*	*Klebsiealla pneumoniae*	*Klebsiella oxytoca*	*Proteus vulgaris*	*Proteus mirabilis*	*Morganella morganii*
Indole	+	-	-	-	-	+	-	+
Methyl Red (MR)	+	+	+	v	-(v)	+	+	+
VogesProskauer (VP)	-	-	-	+	+	-	V	-
Simmons' Citrate	-	-	-	+	+	-(v)	+(v)	-
Hydrogen Sulfide (H_2S)	-	-	+w	-	-	+	+	-
Urea	-	-	-	+	+	+	+	+
Motility	v	-	+	-	-	+	+	v
Gas from D- glucose	+	-	-	+	+	+	+	+
Lactose	+	-	-	+	+	-	-	-

7- *Neisseria meningitides*: Family: *Neisseriaceae*: Genus: *Neisseria.*

N. meningitides is aerobic, gram-negative cocci typically arranged in **pairs** (**diplococci**) with adjacent sides flattened together (**resembling coffee beans**).

Specimens collection

- Nasopharyngeal swabs; body fluids (**joint** fluid or **CSF**) should be stored at 37°C because it was sensitive to cold.

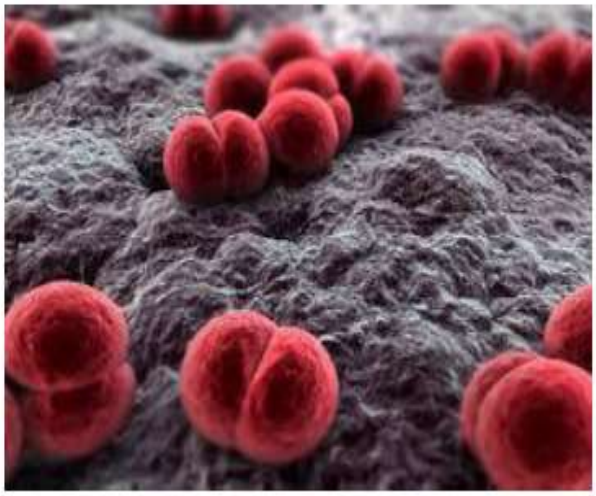
- Any volume (greater than 1 ml) of clear body fluid should be centrifuged at room temperature at 1500xg for 15 min. the sediment should be vortexed and inoculated onto appropriate media.

Diagnosis:

a) Direct detection methods

1- By Gram stain

As indicated above, *N. meningitides* is **Gram negative diplococci** with adjacent sides flattened. They are often referred to as **(Kidney bean)** shaped diplococci.



N. meningitides by electron microscope

2- Antigen detection

The detection of *Neisseria meningitids* **capsular polysaccharides antigen** in body fluids is no longer recommended.

b) Cultivation

- **5% sheep blood** agar and **chocolate agars**. Colonies of N. meningitidis are grey and unpigmented on a blood agar 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 chocolate agar.

N. gonorrhoeae, *N. meningitidis*, and *M. catarrhalis* grow best under conditions of increased CO2 (3% to 7%).

Colonial appearance (morphology)

N. meningitides colonies are **medium**, **smooth**, **round**, **moist**, **gray to white**; **encapsulated strains are mucoid**; may be greenish cast in agar underneath colonies.

Biochemical identification

Test / Organism	Growth on			Rapid fermentation sugars				Gas from Nitrate reduction
	Modifed Thayer-Martin	Nutrient Agar at 35°C	Blood or Chocolate Agar at 25°C	Glucose	Maltose	Lactose	Nitrate reductions	
N. gonorrhoeae	+	-	-	+	-	-	-	-
N. meningitides	+	-	-	+	+	-	-	-

Lecture-14-15
Diagnosis by organ system 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.

1- **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

2-**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).

3-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.

4- **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.**

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 and toxin 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 types:

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.

• **The bacteria can cross the synovial membrane of a joint to initiate septic arthritis**.

Blood collection

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

Blood Culture Media

Basic blood culture media contain a **nutrient broth and an anticoagulant**. Most blood culture bottles available commercially contain **tryptic soy broth, brain heart infusion broth, supplemented with peptone, or thioglycolate broth ,** Special media, such as **Middlebrook 7H9 broth with 0.05% SPS or BHI broth with 0.5% polysorbate 80**, enhances the recovery of *Mycobacterium spp*.

• **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 a

(**1:10 ratio)** of blood to medium was required for successful bacterial growth (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.

• Whenever **visible growth appears**, the bottle should be opened aseptically, a mall mount of broth emoved 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

Anticoagulation

1-Heparin 2-EDTA 3- citrate 4- Sodium polyanethol sulfonate(SPS, Liquoid) in concentrations of 0.025% to 0.03% is the best anticoagulant available for blood cultures.

Specimen Volume

1-collection of two sets of cultures using **10 to 20 mL** of blood per culture is strongly recommended for **adults 2**- for **infants and small children**, only **1 to 5 mL** of blood should be drawn for culture.

Lecture-16 & 17: Meningitis and other infections of the Central Nervous System (CNS)

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 fluid (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 behaviors

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 [1]**cell count,** [2]**the glucose concentration in CSF and blood and** [3]**protein content of cerebrospinal fluid,** the results of [4]**microscopic examination for microorganisms.** In addition, the results of [5]**culture, serologic tests, nucleic acid amplification tests, and other laboratory procedures.**

Common Causes of Meningitis:

- ***Neisseria meningitidis* infect all ages**
- ***Escherichia coli*** infect mainly **neonates**.
- ***Streptococcus pneumoniae*** infect all age groups; highest incidence in the young age.
- ***Haemophilus influenzae* infect children 6 months to 5 years**
- 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.**
- ***Listeria monocytogenes* also infect neonates; elderly; immunocompromised children**

Specimens

As soon as infection of the central nervous system has suspected, **blood samples** has taken for culture and **CSF** has obtained. **To obtain CSF, perform lumbar puncture with strict aseptic technique (Figure 1). Cerebrospinal fluid is usually collected in 3 to 4 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.**

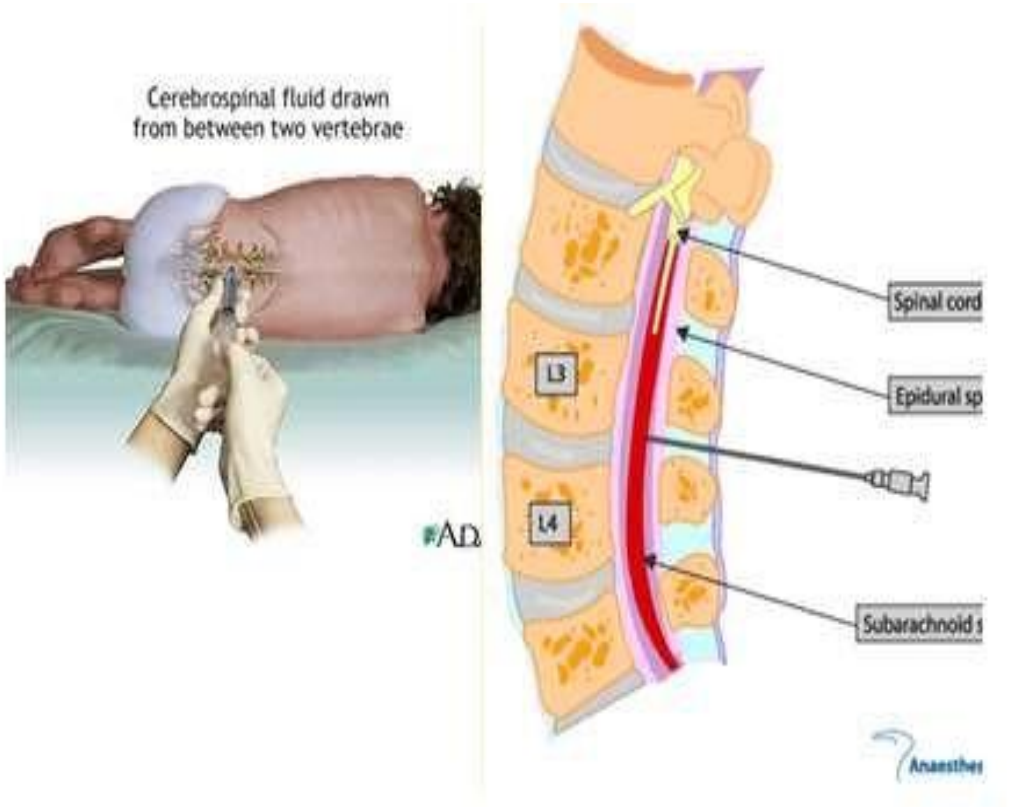


Figure (1): Collection of CSF by lumbar puncture.

Microscopic Examination of CSF

Smears have made from the sediment of centrifuged CSF. 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).**

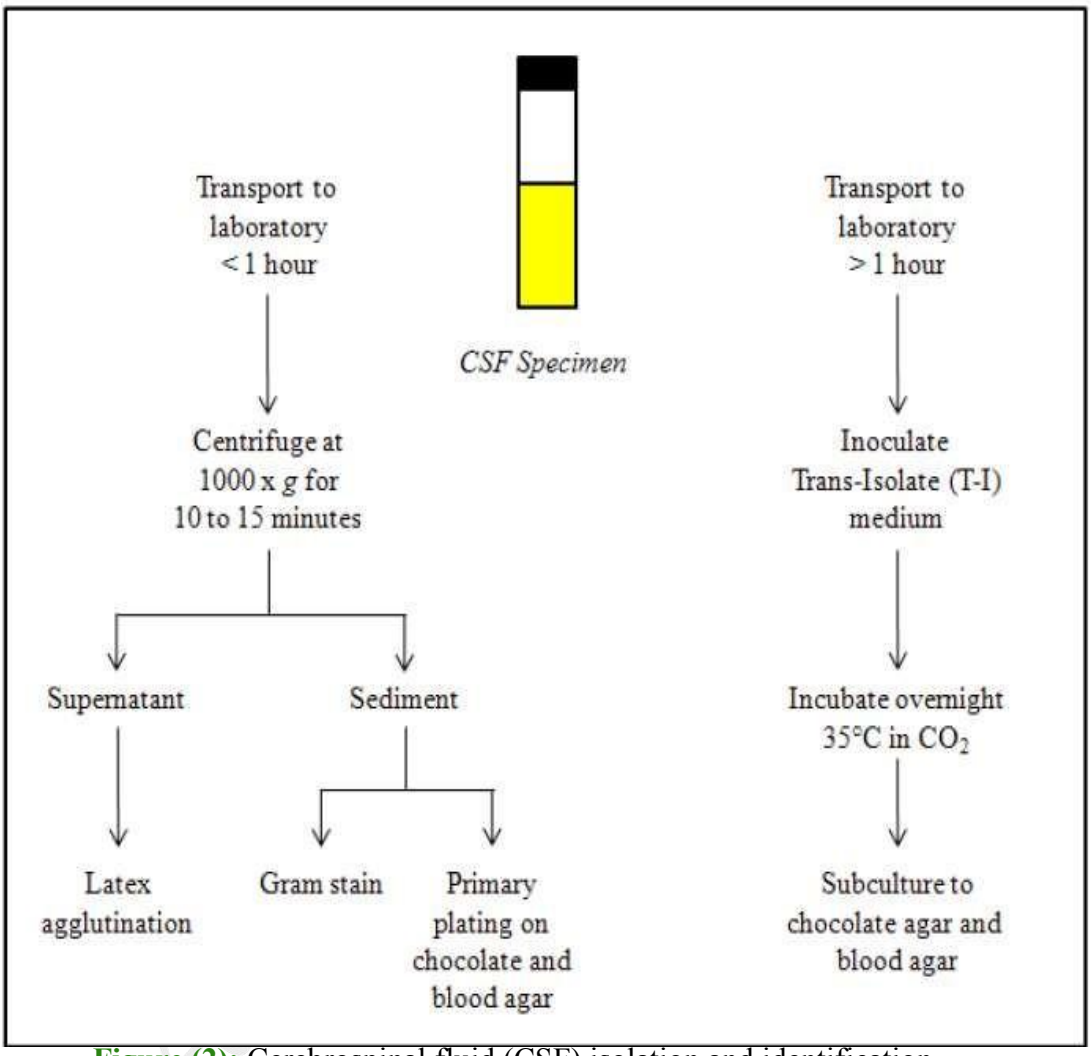


Figure (2): Cerebrospinal fluid (CSF) isolation and identification.

Culture of CSF

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 CSF

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

Neisseria meningitids

1- Gram-negative, Aerobic coffee-bean shaped diplococci

2- may occur intracellularly or extracellularly in polymorphic nuclei (PMN) leukocytes.

3- (PMNs or neutrophils are often more than 1000 WBCs/cu mm).

5- Fastidious organism, grows best at 35-37°C with ~5% CO_2 (or **in a candle-jar**).

6- It can grow on both a **blood agar** plate (BAP) and **chocolate agar** plate (CAP).

7- 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 CAP (**Fig. 3, 4**).

8- Biochemical tests such as **Oxidase test (+)** and C**arbohydrate Utilization (acid from glucose, maltose)** have recommended confirming the identity of cultures that morphologically appear to be ***N. meningitidis.*** 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*,

9-serological tests to identify the serogroup should performed. Additional methods for identification and characterization of ***N. meningitidis*** using molecular tools like

10- 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% CO2 (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 **α-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).**

Fig. 5. ***S. pneumoniae*** **colonies with a surrounding green zone of α-hemolysis (black arrow) on BAP.**

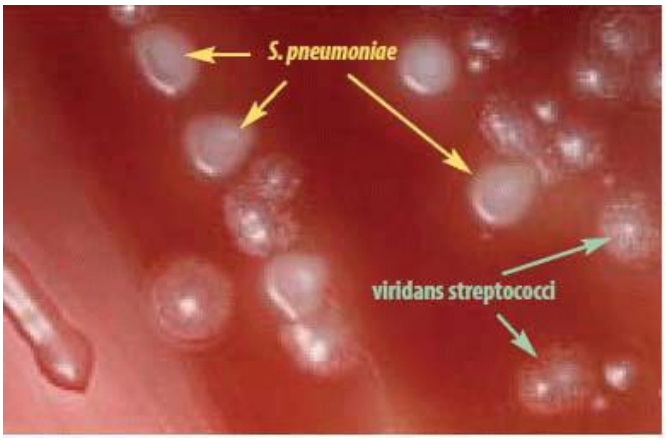


Fig. 6. ***S. pneumoniae*** **colonies have a flattened and depressed center after 24-48 hours of growth BAP, whereas the viridans streptococci retain a raised center.**

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) (<14 mm 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 (such as PCR)**.

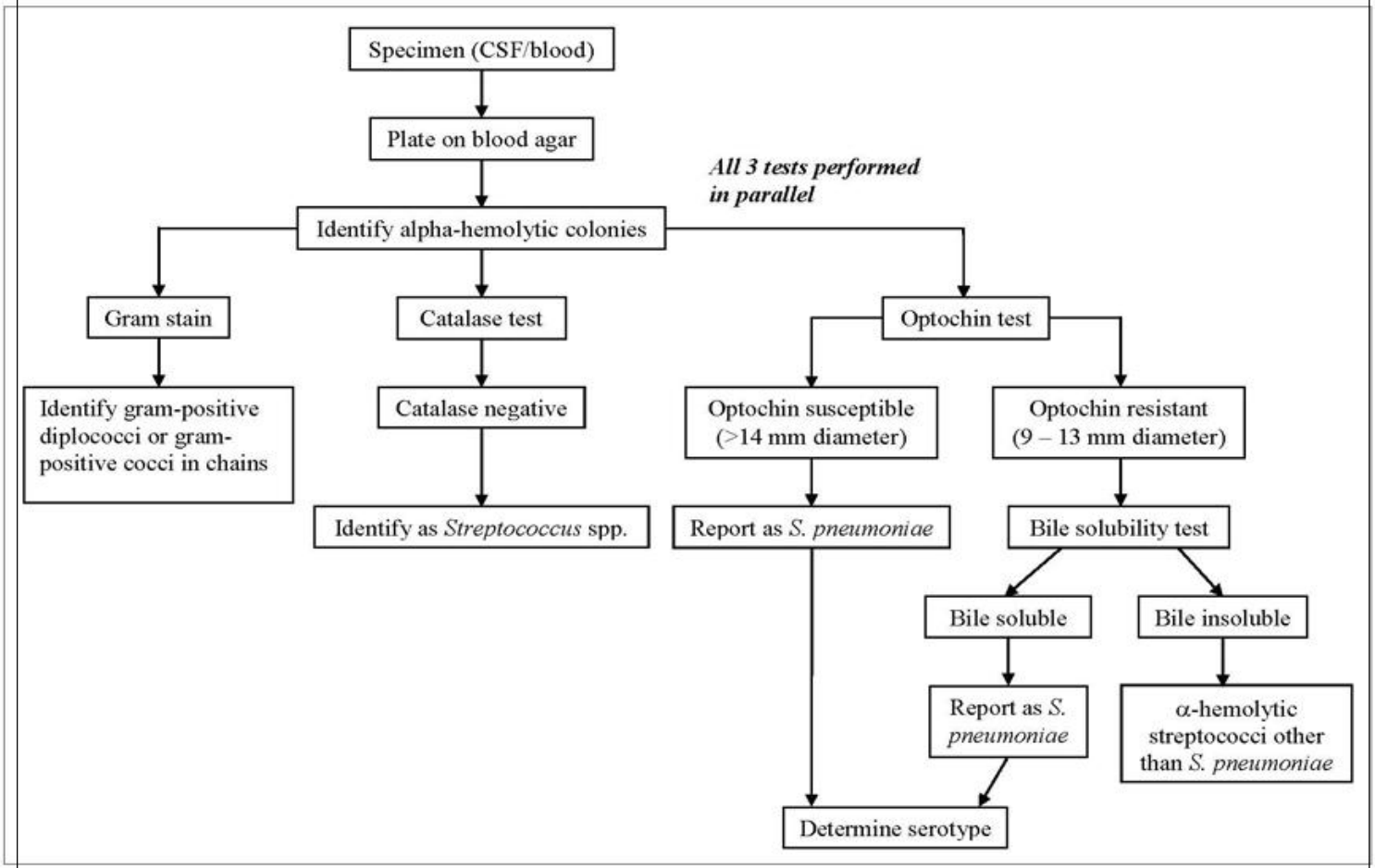


Figure-7: Flowchart for identification and characterization of S*treptococcus pneumoniae*

Figure 8: Optochin test for *S. 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 *S. 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).**

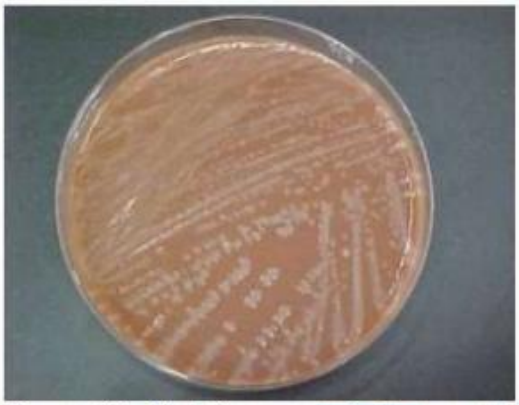
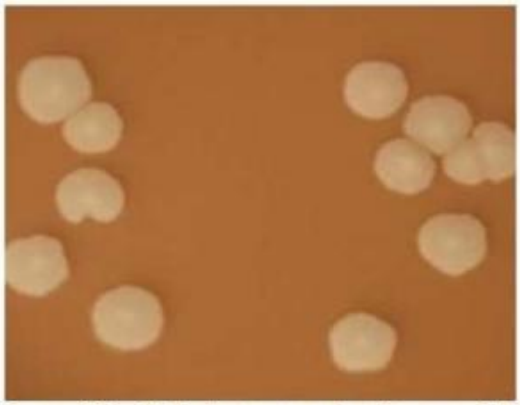


Figure (9): *H. influenzae* colonies on a CAP



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

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 of *H. influenzae* using molecular tools like PCR technique. The most common bacterial causes summarized at Table (1).

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

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

Lecture-18, 19: Diagnosis of bacterial respiratory tract infections

Bacterial infections of respiratory tract

Respiratory system has divided into two major parts:

- ✓ Upper respiratory tract 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. Accidental infection** is the infection that man acquired during daily life. **Seasonal infections** 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.

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 diseases.** ***Staphylococus, Streptococcus, Haemophilus, Corynebacterium, Neisseria, Bacteroides, Fusobacterium, and Actinomyces,*** are typical examples for these bacteria.

Nearly any type of **gram-positive** or **negative** bacteria can cause respiratory infection (**Pneumonia).** ***Mycoplasma*** and ***Chlamydia*** **spp**. 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)

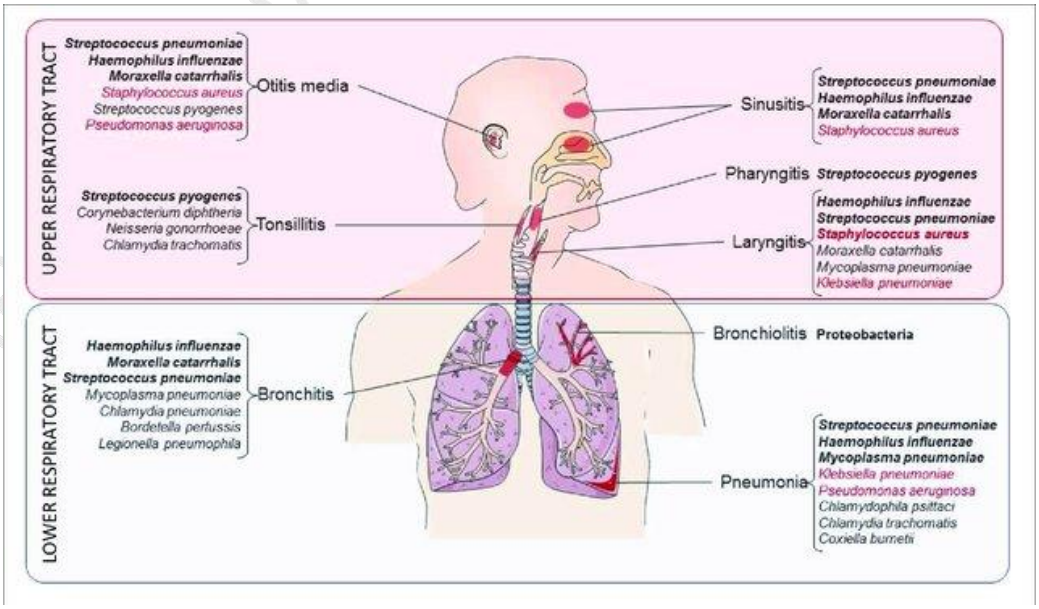


Fig. (1): Summary of bacterial respiratory tract infection

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 pneumoniae; 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 pneumoniae; Haempphilus influenzae; 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. pneumoniae, Staph. aureus, Hemophillus influenzae*** **and** ***Mycoplasma pneumoniae.***

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 secretions** which indicated by > **25 Neutrophils and < 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 be the **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 medium)**, 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.

Diphtheria disease: the causative agent of this disease is ***Corynebacterium diphtheriae***

Diphtheria is most commonly an infection of the upper respiratory tract and causes fever, sore throat, **hypoxia** due to airway obstruction by the **pseudomembrane**.and malaise. The **pseudomembrane** is a thick, gray-green fibrin membrane, forms over the site(s) of infection as a result of the combined effects of bacterial growth, toxin production, necrosis of underlying tissue, and the host immune response.

The involvement of **cervical lymph nodes may cause profound swelling of the neck** (**bull neck**) as (**Fig-2**) causing Life-threatening systemic complications as a result of the action of **diphtheria toxin**.

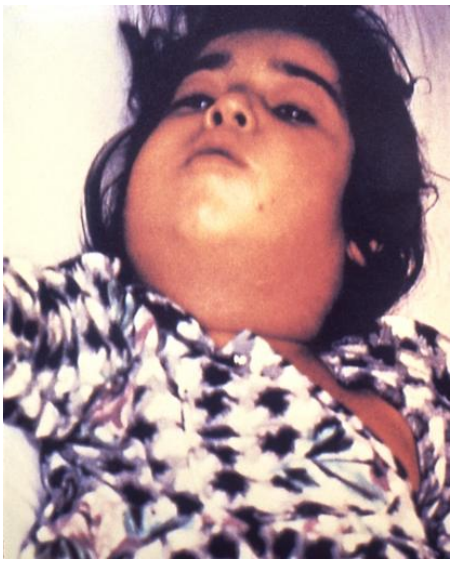


Figure-2

Corynebacterium diphtheriae is a **Gram-positive, nonmotile, club-shaped bacilli.** Older cultures often contain **metachromatic granules** (**polymetaphosphate**) which stain **bluish-purple** with methylene blue.

Diagnosis

Culture media: **Loeffler agar** or **Mueller-Miller tellurite agar**.

Specimen: **Pharyngeal tonsils** swab. The most common assay for **toxigenicity** is the **Elek immunodiffusion test** **(Figure-3)**

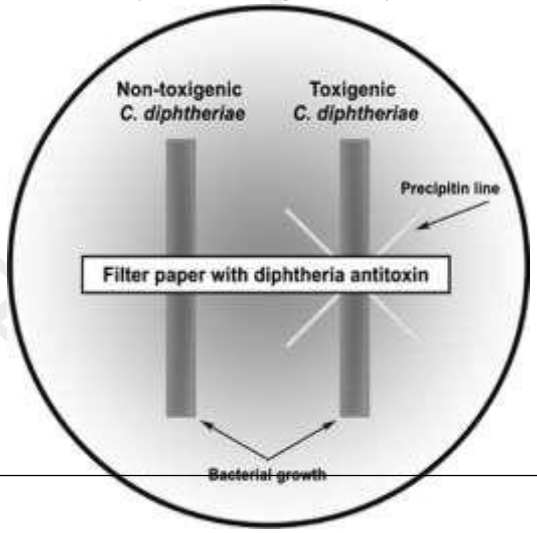


Figure-3: Procedure of Elek immunodiffusion test. A Sterile filter paper impregnated with diphtheria antitoxin is imbedded in agar culture medium. Isolates of *C. diphtheriae* are then streaked across the plate at an angle of 90° to the antitoxin strip. Toxigenic *C diphtheria*.

This test is based on the **double diffusion of diphtheria toxin and antitoxin in an agar medium.** A sterile, antitoxin-saturated filter paper strip is embedded in the culture medium, and ***C. diphtheriae*** isolates are streak-inoculated at a 90° angle to the filter paper. The production of diphtheria toxin can be detected within 18 to 48 hours by the formation of a toxin-antitoxin precipitin band in the agar.

Whooping cough: the causative agent of this disease is *Bordetella pertussis*.

***B. pertussis* is a small Gram-negative rod-shaped, encapsulated, non-motile, obligate aerobes, catalase and oxidase positive**. Numerous antigens and virulence factors are produced by *B. pertussis*.

Symptoms and signs whooping cough:

- blocked or runny nose.
- sneezing.
- raised temperature.
- uncontrolled bouts of coughing that sounds like a 'whoop' or are followed by a 'whooping' noise.

vomiting after coughing.

Diagnosis:

Specimen: nasopharyngeal secretions nasopharyngeal swabs.

These specimens should be immediately plated onto **Regan-Lowe medium** or **Bordet-Gengou agar** which is the most widely used.

B. pertussis on Bordet- Gengou Agar with blood

Bordetella pertussis usually grows after 3 to 4 days of incubation at 37° C. (Also, it can be identified by API-NE, PCR and ELISA).

Infections of the Urinary Tract

The urinary tract consists of the kidneys, ureters, bladder, and urethra. Urine is normally a sterile fluid.

Urinary tract infections (UTIs) are characterized as being either upper (U-UTI encompasses the ureters and kidneys) or lower (L-UTI encompasses the bladder and urethra) based primarily on the anatomic location of the infection.

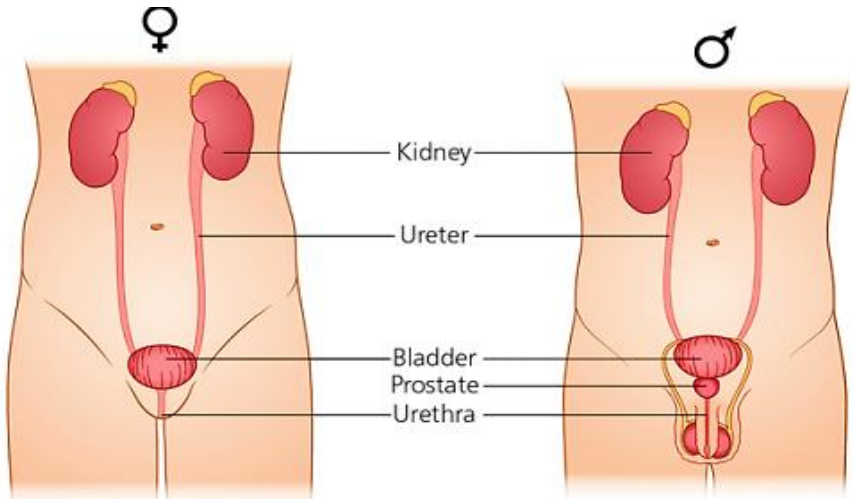


Figure (1): Male and female urinary tract system

A proper classification has employed currently: Hospital or community acquired infections.

Some terminologies that you should to know:

- **Pyelonephritis (Kidney infection):** infection of renal parenchyma, calyces, and pelvis
- **Nephrolithiasis:** stone in kidney
- **Ureterolithiasis:** stone in ureter.
- **Cystolithiasis:** stone in urinary bladder
- **Urethritis:** infection of the urethra
- **Ureteritis:** infection within the ureters.
- **Cystitis:** infection of the bladder.
- **Prostatitis:** Infection of prostate in males

Etiologic Agents

Bacterial species involved in community acquired UTI is :

1-***E. coli***; only those uropathogenic UPEC (have pili are responsible for UTIs).

2-Other microorganisms are ***Proteus spp.*, *Klebseilla sp.*, *Enterobacter sp.*** and ***Acinetobacter sp***.

Note:*Proteus spp.* produce urease that turns the environment alkaline which causing damage to tissues leading to renal stone (normal vaginal pH level is between 3.8-4.5).

3-On the other hand, ***Staph. saprophyticus*** is more efficient in attaching to UT epithelial cells than coagulase positive ***Staphylococcus*** or ***Staph. epidermidis*.**

Predisposing factors of UTIs

1. Sex (male or female). Female usually gets infection, because she has shorter urethra and its closer to vaginal & anal opening also due to the way of wiping & cleaning while male rarely gets infection due to longer urethra.
2. Obstruction of urethra.
3. Any obstructions (Tumor and Stones).
4. Pregnancy
5. Diabetes mellitus
6. Immunosuppression and immunodeficiency
7. Catheterization

Routes of Infection

There are three routes for bacteria to gain excess to UT.:

1. **Ascending route** (passage of bacteria from urethra to bladder and kidney).

2. **Haematogenous route** (Bloodstream).

3- **lymphatic route**

- ✓ Although the ascending route is the **most common** course of infection in females, it is association with instrumentation (e.g., urinary catheterization, cystoscopy) is the most common cause of hospital acquired UTIs in both sexes.

Note: The only part of UT has a limited number of resident bacteria is urethra, these microflorae colonize the epithelium in the distal portion.

The Host-Parasite Relationship

In most cases, the host defense mechanisms are able to eliminate the organisms through the following:

1. **Inhibitory effect of urine** (urethral flora).
2. **Urine properties**
3. The constant **flushing** of contaminated urine from the body
4. The **bladder mucosal surface** has antibacterial properties.
5. **Valve-like mechanism** at the junction of ureter and bladder prevents the reflux (backward flow) of urine from the bladder to the upper urinary tract.
6. Activation of the host **immune response**
7. **Anti-adherence factor** synthesized exclusively by epithelial cells in kidney .
8. **Defensins**, a group of small antimicrobial peptides.

Type of UT infections

Urethritis

Symptoms associated with urethritis are, dysuria (painful or difficult urination), and frequency are similar to those associated with

lower UTIs. Urethritis is a common infection. Because ***Chlamydia trachomatis***, ***Neisseria gonorrhoeae***, and ***Trichomonas vaginalis*** are common causes of urethritis and considered to be sexually transmitted.

Ureteritis

Inflammation or infection within the ureters is considered in combination with kidney infections. UTI within the ureters indicates that organisms are in the process of ascending into the kidneys and should be treated to prevent further infection.

Cystitis

Patients with cystitis (infection of the bladder) complain of dysuria, frequency, and urgency (compelling need to urinate). These symptoms are due not only to inflammation of the bladder but also to multiplication of bacteria in the urine and urethra, here is pain and urine is bloody cloud and a bad odor. Because cystitis is a localized infection, fever and other signs of a systemic illness are usually not present.

Laboratory Diagnosis of Urinary Tract Infections

The diagnosis of UTI include :

1- general examination of urine
2- culture has done depending on findings of general examination.
3- Other parameters of diagnosis might aid the diagnosis of UTI :

A-biochemical parameters

B-hematological parameters aid the diagnosis by showing of elevation (raise) in number of leucocytes in general and neutrophils in specific.

Note : Culture, is on the **top 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. **Vitek system**, **PCR**, or other techniques come to confirm the diagnosis.

1-Specimen Collection and Transport

Prevention of contamination by normal flora is the most important consideration for collection of a clinically relevant urine specimen.

a) Clean-Catch Midstream Urine

b) Straight Catheterized Urine: collection of uncontaminated urine from bladder.

c) Suprapubic Bladder Aspiration: contamination-free urine specimen is withdrawn directly into a syringe through inserted needle.

d) Indwelling Catheter.

Bacterial counts remain constant for as long as **24 hours** by: Refrigeration at (**4°C**) or use Urine transport tubes.

2-Screening procedures

as many as 60% to 80% of all urine specimens will be negative on culture or contain contaminants, so use the following procedure:

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.

Gram stain:

If a Gram stain of an un-centrifuged, clean-catch, midstream urine specimen reveals the presence of 1 bacterium per oil-immersion field, it represents 10,000 bacteria/mL of urine. This confirms urinary tract infection.

Indirect Indices

Frequently, screening tests detect bacteriuria or pyuria by examining for the presence of bacterial enzymes or PMN enzymes rather than the organisms or PMNs themselves.

1-Nitrate Reductase Test

2-Leukocyte Esterase Test

3-Catalase

Automated and Semiautomated Systems

There are an instrument analyzes both the microscopic components (bacteria and leukocytes) and the chemistries of urine and body system

3-Urine culture

Most often, microbiologists use a calibrated loop designed to deliver a known volume, either 0.01 or 0.001 mL of urine. The (0.01 mL) loop is recommended to detect lower numbers of organisms in certain specimens.

Culture media:

- ❖ blood agar and MacConkey agar for general isolates
- ❖ chromogenic media for special isolates

Interpretation of Urine Cultures

dependent on:

- The type of urine submitted (e.g., voided, straight catheterization)
- The clinical history of the patient (e.g., age, sex, symptoms, antibiotic therapy).

Contaminated with normal flora, including *Enterobacteriaceae*

Lab diagnosis for Urethritis & Cervicitis / Vaginitis

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*e

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 period about 2-3 weeks.

Clinical findings: White mucoid discharge.

-**Specimen for Urethritis:** Urethral discharge or swab (Before antibiotics)

-**Gram stain**: Gram-negative intracellular diplococci

-**Culture**: Modified Thayer-Martin medium

-**Biochemical and serology**: Species identification

2. Cervicitis / Vaginitis

It manifests with vaginal discharge.

Causative agents: *N. gonorrheae*

(Mucopurulent vaginal discharge).

Non-specific vaginitis (Yellowish homogenous vaginal discharge). It is caused by anaerobes and *Gardnerella vaginalis*

-**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.

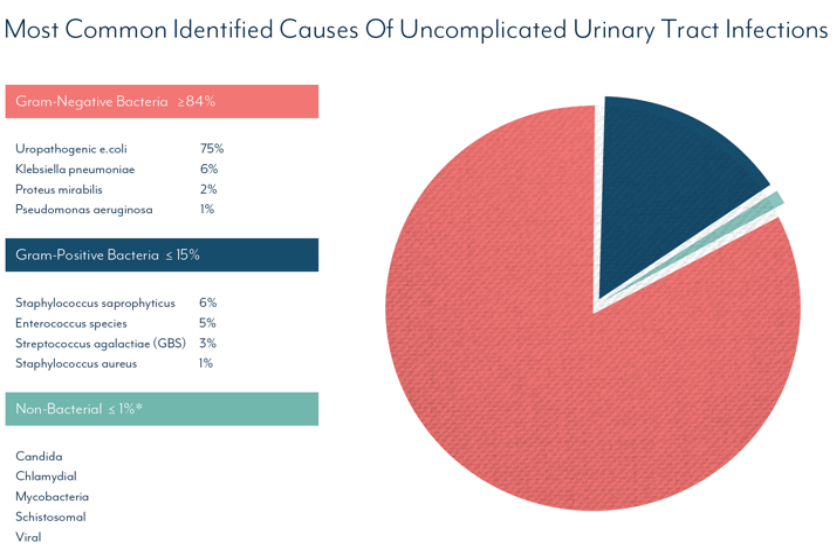


Figure. 2: % of UTIs bacterial causes

Genital tract infections

Anatomy :

The male reproductive system is located in close proximity to the urinary system, and the urethra is part of both systems. The testes are responsible for the production of sperm. The epididymis is a coiled tube that collects sperm from the testes and passes it to vas deferens The epididymis is also the site of sperm maturation after they leave the testes. The seminal vesicles and prostate are accessory glands that produce fluid that supports sperm.

The female reproductive system is located near the urinary system .The external genitalia (vulva) in females open to the vagina, a muscular passage way that connects to the cervix. The cervix is the lower part of the uterus .The cervix is a common site of infection, especially for viruses that may lead to cervical cancer. The uterus leads to the fallopian tubes and eventually to the ovaries. Ovaries are the site of ova (egg) production, as well as the site of estrogen and progesterone production .

Resident microbial flora

The colonization of the surface by resident microbiota produces a biologic barrier preventing the adherence of pathogenic organisms. **Normal urethral microbiota** include coagulase-negative *Staphylococci* and *Corynebacteria*, as well as various anaerobes. The microbiota of the female genital tract varies with the pH and estrogen concentration of the mucosa, which depend on the host's age. The **females** of reproductive age may harbor large numbers of facultative bacteria such as ***Enterobacteriaceae, Streptococci***, and ***Staphylococci***, as

well as anaerobes such as ***Lactobacilli***, anaerobic non–spore-forming **bacilli** and **cocci**, and ***Clostridia***.

Note: The lactobacilli present in vaginal secretions metabolize glucose to lactic acid, resulting in a pH of approximately 4.0. The acidic pH coupled with the organism's ability to produce hydrogen peroxide prevents infection by exogenous sexually transmitted pathogens.

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**.

Bacterial STIs include:

1. Chlamydia or Chlamydial urethritis (*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.

Routes of Transmission

1- endogenous infections (genital microbiota)

2- exogenous infections

Clinical Manifestations

1- Asymptomatic

Although symptoms of genital tract infections generally cause the patient to seek medical attention, a patient with an STD, especially a female, may be free of symptoms (i.e., asymptomatic). For example, gonorrhea (*N. gonorrhoeae*) or chlamydia (*C. trachomatis*) infection is usually obvious in males because of a urethral discharge, yet females with either or both of these infections may have either minimal symptoms or no symptoms at all.

2-Dysuria

Although a common presenting symptom associated with urinary tract infection, dysuria (painful urination) can also result from an STD caused by organisms such as *N. gonorrhoeae, C. trachomatis*, and HSV.

3-Urethral Discharge

The presence of an inflammatory exudate at the tip of the urethral meatus is generally observed in males; the symptoms of urethral infection in females are not commonly localized. Most males complain of discomfort at the penile tip as well as dysuria. Urethritis (swelling and irritation of the urethra) may be gonococcal, caused by *N. gonorrhoeae*, or nongonococcal.

4-Lesions of the Skin and Mucous Membranes

Numerous organisms can cause genital lesions that are diverse in both their appearance and their associated symptoms but are most often associated with sexually transmitted diseases.The characteristics of the lesions may vary from one type of infectious process to another for the same organisms. For example, specific HPV genotypes infect mucosal cells in the cervix and anus.

5- Vaginitis

Inflammation of the vaginal mucosa, called vaginitis. Females who present with vaginal symptoms often complain of an abnormal discharge and additional symptoms such as an offensive odor or itching.

6- Cervicitis

Polymorphonuclear neutrophils (PMNs) are normally present in the endocervix; however, an abnormally increased number of PMNs may be associated with cervicitis (inflammation of the cervix). Therefore a purulent discharge from the endocervix can be observed in some cases of cervicitis. The endocervix is the site from which *N. gonorrhoeae* is most frequently isolated in females with gonococcal infections.

Lower Genital Tract Infections: Urethritis, Cervicitis, and Vaginitis

1-Urethritis

Urethral discharge may occur in both males and females infected with pathogens such as *N. gonorrhoeae* and *T. vaginalis*. The presence of infection is more likely to be asymptomatic in females, because the discharge is usually less profuse and may be masked by normal vaginal secretions. to obtain a urethral specimen, a swab is inserted approximately 2 cm into the urethra and rotated gently before withdrawing. Because *Chlamydiae* are intracellular pathogens, it is important to remove epithelial cells (with the swab) from the urethral mucosa. When profuse urethral discharge is present, particularly in males, the discharge may be collected externally without inserting a sampling device into the urethra.

2. Cervicitis and Vaginitis

Organisms that cause purulent vaginal discharge (vaginitis) include *T. vaginalis*, *gonococci*, and, rarely, beta-hemolytic *Streptococci*.

The same organisms that cause purulent infections in the urethra may also infect the epithelial cells in the cervical opening, as can HSV. Mucous is removed by gently rubbing the area with a cotton ball. The urethral swab is inserted into the cervical canal and rotated and moved from side to side for 30 seconds before removal.

Direct Microscopic Examination

In addition to culture, urethral discharge may be examined by Gram stain for the presence of gram-negative intracellular diplococci, usually indicative of gonorrhae in males. After inoculation to culture media, the swab is rolled over the surface of a glass slide, covering an area of at least 1 cm^2. Specimens collected from within the urethra may contain small cuboidal epithelial cells with a large nucleus. cultures of urethral discharge need not be performed.

Culture. Modified Thayer-Martin medium is most often used, although New York City (NYC) medium has the added advantage of supporting the growth of mycoplasmas and gonococci.Specimens must be inoculated to additional media for isolation of yeast, streptococci, and mycoplasmas. Yeast grows well on Columbia agar base with 5% sheep blood and colistin and nalidixic acid (CNA), although more selective media are available.

Example on bacterial STIs include:

***1-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).

***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. Chlamydial pharyngitis or inflammation of the anus (proctitis) is possible through anal intercourse.

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.

2-*Neisseria gonorrhoeae*

One of the most common STIs in men and women is gonorrhea caused by *Neisseria gonorrhoeae*. The organism, commonly known as the gonococcus. The great majority of cases of gonorrhea are transmitted during sexual intercourse.

Gonorrhea is the second most frequently reported nationally.

Virulence Factors of *Neisseria gonorrhoeae*

1. Receptors for human transferrin
2. Capsule (*N. meningitidis*)
3. Pili (fimbriae)
4. Cell membrane proteins
5. 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 day. Patients often report abdominal pain and a burning sensation on urination, and the normal menstrual cycle might be interrupted.

Symptoms of gonorrhea tend to be more acute in males than in females, and males thus tend to seek diagnosis and treatment more readily. 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.

1. Growth conditions for culture: *N. gonorrhoeae* grows best under aerobic conditions, and most strains require enhanced CO2. *N. gonorrhoeae* utilizes glucose as a carbon and energy source but not maltose, lactose, or sucrose .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.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. Culture of *N. gonorrhoeae* on Thayer-Martin agar remains the “gold standard” for diagnosis.

3-*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.

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.

_ **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:

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.

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.

Lecture-23-24: Bacterial Infections of the Gastrointestinal (GI) Tract (Gastroenteritis)

Diarrheal diseases are the second leading cause of death; about 48 million enteric infections occur each year. Most of these infections cause morbidity and death, particularly in elderly people and children younger than 5 years of age.

Pathogenesis:

Host factors: The human host has numerous defenses factors that prevent the disease produced by enteric pathogens such as 1- the acidity of the stomach, 2- the normal peristalsis, 3- the mucous layer coating the epithelium, 4- Normal flora prevents colonization by potential pathogens.

Microorganisms cause GI Infection for Each Primary Mechanism:

Mechanism	Examples of Microorganisms
Toxin Production:	***Vibrio cholerae***
Enterotoxin	**Non-cholera vibrios**
	***Shigella dysenteriae* type 1**
	Enterotoxigenic *E. coli* (ETEC)
	***Salmonella* spp.**
	***Clostridium difficile* (toxin A)**
	Aeromonas
	Campylobacter jejuni
	Staphylococcus aureus
	Bacillus cereus
Cytotoxin	***Shigella* spp.**
	***Clostridium difficile* (toxin B)**
	Enterohemorrhagic *E. coli* (EHEC)
Neurotoxin	***Clostridium botulinum***
Attachment/Adherence	**Enteropathogenic *E. coli* (EPEC)**
	Enterohemorrhagic *E. coli* (EHEC)
Invasion	**Shigella spp.**
	Enteroinvasive *E. coli* (EIEC)
	Campylobacter jejuni
	Yersinia enterocolitica

Laboratory Diagnosis of Gastrointestinal Tract Infections

Specimen Collection

Stool Specimens for Bacterial Culture

Feces and rectal swabs are the most readily available specimens. The presence of blood, mucus must be noted.

Direct detection of agents

Wet Mounts: A direct wet mount of fecal material is the fastest method for detecting motile trophozoites of intestinal parasites.

Stains

Feces may be Gram stained for detection of certain etiologic agents.

Culture for isolation of bacteria

Stools should be suspended in broth and cultured on ordinary media as well as on selective/differential media **(MacConkey agar, EMB agar).**

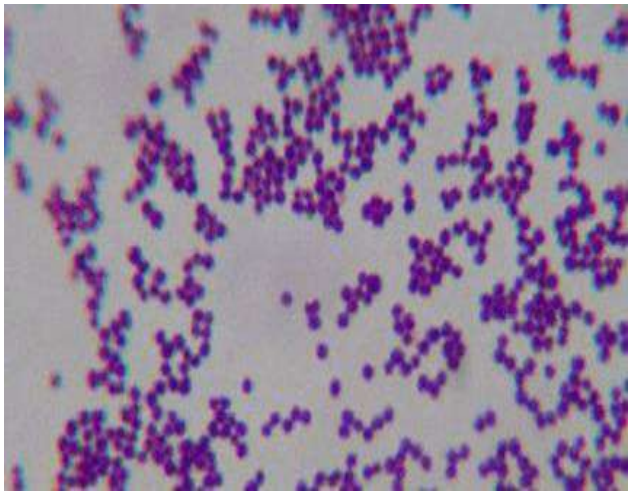
If **Salmonella** infection has suspected, the specimen is inoculated in **Selenite broth** for 18 hours before it has placed in differential media (**Hektoen enteric or S-S agar**.

For ***Vibrio cholerae*** suspected infection, **TCBS agar** is used.

Staphylococcal Food Poisoning:

When ***Staphylococcus aureus*** grows in food, it may produce enterotoxins that, cause symptoms such as nausea, diarrhea, cramping, and vomiting within one to six hours. The enterotoxins are **proteins** that are resistant to **low pH**, allowing them to pass through the stomach. They are **heat stable** and are not destroyed by boiling at 100 °C. Even though the bacterium itself may be killed, the **enterotoxins** alone can cause vomiting and diarrhea (**Intoxification**).

Staph. aureus is diagnosed by staining and culturing on Mannitol agar (mannitol fermenting) and **confirmed by identifying the toxin in a food sample or** in biological specimens **(feces or vomitus) from the patient.**
Serological techniques, including ELISA, can also be used to identify the toxin in food samples.



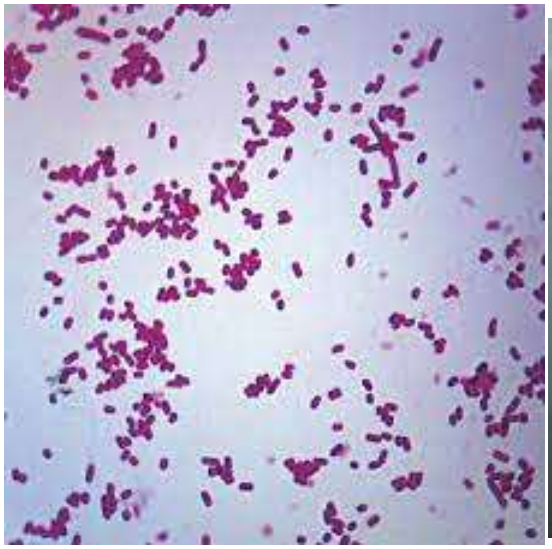
***Staph. aureus*- gram stain**

Shigellosis (Bacillary Dysentery)

When gastrointestinal illness is associated with *Shigella*, it is called **bacillary dysentery, or shigellosis.** Infections can be caused by *S. dysenteriae*, *S. flexneri*, *S. boydii*, and/or *S. sonnei* that colonize the GI tract. *Shigella* is gram negative non-motile rod shaped. It invades intestinal epithelial cells. *Shigella* can escape from the immune system and then live within the cytoplasm of the cell or move to adjacent cells. More severe cases may result in ulceration of the mucosa, dehydration, and rectal bleeding. Patients may develop **hemolytic uremic syndrome** (**HUS**), a serious condition which may cause **kidney failure**. *S. dysenteriae* is able to produce **Shiga**

toxin, which targets the endothelial cells of small blood vessels in the small and large intestine.

Stool samples are analyzed using serological or molecular techniques. The **presence of WBCs and blood in fecal samples** occurs in about 70% of patients.



Shigella- gram stain

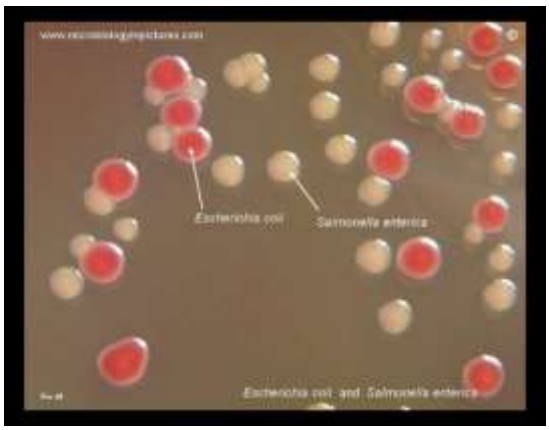
Shigella-on MacConkey agar

Salmonellosis

Salmonella **gastroenteritis** (salmonellosis), is caused by the rod-shaped, motile, gram-negative bacterium *Salmonella*. Infection is caused by ingestion of contaminated food, raw eggs and raw poultry. *Salmonella* can cross the epithelial cell membrane and enter the bloodstream and lymphatic system. Infected individuals develop fever, nausea, abdominal cramps, vomiting, headache, and diarrhea. These signs and symptoms generally last a few days to a week.

Colonies of *E. coli* & *Salmonella enterica* on MacKonkey agar:

Red, lactose positive colonies of *E. coli* ; and coloeless, colonies of lactose negative *S. enterica.*



Typhoid Fever:

S. typhi and *S. Paratyphi*, cause severe type of salmonellosis called **typhoid fever. *S. typhi*** penetrate the intestinal mucosa, grow within the macrophages, and are transported through the body, most notably to the liver and gallbladder. The macrophages lyse, releasing *S. typhi* into the bloodstream and lymphatic system.

The bacteria can be cultured from feces, urine, blood, or bone marrow. Serology, including ELISA, is used to identify *Salmonella* but confirmation with PCR test.

E. coli Infections

There are five pathogenic groups of *E. coli*, but we will **focus here on four**, the most commonly transmitted through food and water.

1- Enterotoxigenic *E. coli* (ETEC), also known as **traveler's diarrhea**, causes diarrheal illness. The patients develop a **watery diarrhea, abdominal cramps, malaise** (a feeling of being unwell), **and a low fever**. ETEC produces a heat-stable enterotoxin and adhesins called colonization factors that help the bacteria to attach to the intestinal wall. Diagnosis involves staining, culturing and PCR.

2- Enteroinvasive *E. coli* (EIEC) it carries a large plasmid that is involved in epithelial cell penetration. The signs and symptoms include **watery diarrhea, chills, cramps, malaise, fever, and dysentery.**

3- Enteropathogenic *E. coli* (EPEC) can cause a potentially fatal diarrhea, especially in infants. Fever, vomiting, and diarrhea can lead to severe dehydration. This *E. coli* produces a protein (Tir) that attaches to the surface of the intestinal epithelial cells. Diagnosis involves staining, culturing and PCR.

4- Enterohemorrhagic *E. coli* (EHEC), the strains capable of causing epidemics. EHEC can cause disease ranging from relatively mild to life-threatening. **Symptoms** include **bloody diarrhea with severe cramping, but no fever.**

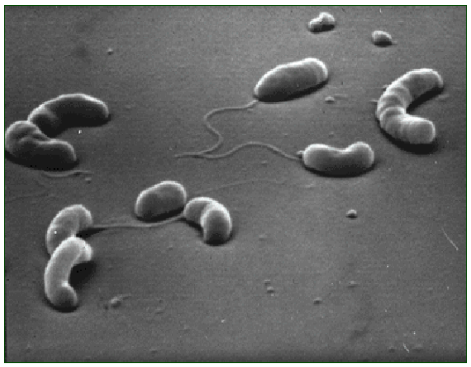
Diagnosis involves culture**,** often using MacConkey**.**

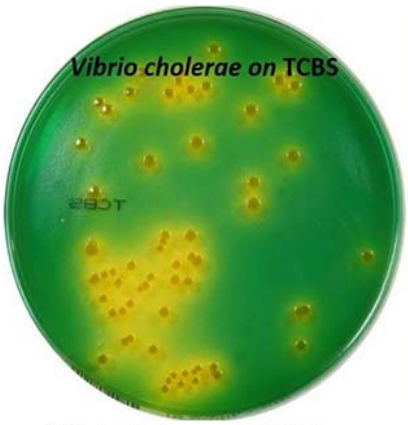
The Primary Groups of *E. coli* That Cause Diarrhea in Humans

Type	Primary Mode of Pathogenesis	Other Comments
Enterotoxigenic (ETEC)	Produces heat-labile (LT) or heat stable (ST) enterotoxins; genes of both toxins reside on a plasmid; LTs are closely related in structure and function to cholera toxin; STs result in net intestinal fluid secretion by stimulating guanylate cyclase	Common cause of traveler's diarrhea; infects all ages
Enteroaggregative (EAEC)	Binds to small intestine cells via fimbriae encoded by a large molecular weight plasmid, forming small clumps of bacteria on the cell surface; other plasmid-borne virulence factors include structured pilin, a heat-stable enterotoxin, novel anti-aggregative protein, and a heat-labile enterotoxin, all believed to be the cause of the associated diarrhea	Infects primarily young children
Enteroinvasive (EIEC)	Pathogenesis has yet to be totally elucidated; studies suggest that mechanisms by which diarrhea results are virtually identical to those of *Shigella* spp.	Very difficult to distinguish from *Shigella* spp. and other *E. coli* strains
Enteropathogenic (EPEC)	Initially attaches in the colon and small intestine and then becomes intimately adhered to intestinal epithelial cells, subsequently causing the loss of enterocyte microvilli (effacement); genes for attachment/effacement reside in a cluster on the bacterial chromosome (i.e., pathogenicity island)	Diarrhea in infants, particularly in large urban hospitals
Enterohemorrhagic (EHEC) OR	Attaches to and effaces gut epithelial cells in a similar manner as EPEC; in addition, EHEC elaborates shiga toxins	Although many outbreaks are caused by *E. coli* 0157:H7, other serotypes have been implicated in outbreaks and sporadic cases Gene recombination among strains makes classification difficult

Cholera

Cholera is a serious infection often associated with **poor sanitation**, It is caused by ***Vibrio cholerae*** **serotype O1,** a gram-negative, motile **(darting movement)** by single polar flagellum, **curved rod (comma shaped**. Because *V. cholerae* is killed by stomach acid, therefore, relatively large doses are needed to reach the intestines and cause infection. They attach to epithelial cells and release cholera **enterotoxin**. Within the intestinal cell, **cyclic AMP (cAMP)** levels increase, which activates a chloride channel and results in the release of ions into the intestinal lumen. It causes rapid dehydration and electrolyte imbalance. Diarrhea is so profuse that it is called "**rice water stool,**". Cholera is diagnosed by taking a stool sample and culturing for *Vibrio*. The bacteria are **oxidase positive** and non-lactose fermentation on MacConkey agar. ***V. cholerae*** **m**ay also be **cultured on thiosulfate citrate bile salts sucrose (TCBS) agar,** which is **selective and differential medium** for ***Vibrio*** **spp.,** which **produce** a distinct **yellow colony**.





Vibrio cholerae on TCBS Agar

Vibrio parahaemolyticus on TCBS Agar

Helicobacter pylori and "Peptic Ulcers"

H. pylori is gram negative, motile, **spiral** (helical) **shaped**. It is able to tolerate the acidic environment of the human stomach and has been shown to be a major cause of **peptic ulcers**, which are ulcers of the stomach or duodenum. The bacterium is also associated with increased risk of stomach cancer.

H. pylori colonizes epithelial cells in the stomach using pili for adhesion. These bacteria produce **urease**, which stimulates an immune response and creates ammonia that neutralizes stomach acids. The infection damages the cells of the stomach lining. As a result, inflammation (gastritis) occurs. **Signs and symptoms** include **nausea, lack of appetite, bloating, burping, and weight loss.** Bleeding ulcers may produce **dark stools.** If no treatment is provided, the ulcers can become deeper, more tissues can be involved.

To diagnose *H. pylori* infection, multiple methods are available:

1) In a **breath test,** the patient swallows radiolabeled urea. If *H. pylori* is present, the bacteria will produce urease to break down the urea. This reaction produces **radiolabeled carbon dioxide** that can be detected in the patient's breath.

2) **Blood testing** can also be used to detect antibodies to *H. pylori*.

3) **The bacteria can be detected using either a stool test or a stomach biopsy.**

Clostridium difficile

Clostridium difficile is a **gram-positive rod** that can be a commensal bacterium as part of the normal microbiota of healthy individuals. When the **normal microbiota is disrupted by long-term antibiotic use,** it can allow the overgrowth of this bacterium, resulting in **antibiotic-associated diarrhea** caused by *C. difficile.*

Patients at the greatest risk of *C. difficile* infection are those who are immunocompromised, have been in health-care settings for extended periods. Because this species can form **endospores**, it can survive for extended periods of time in the environment under harsh conditions.

This bacterium produces two toxins, *C. difficile* toxin A (TcdA) and *C. difficile* toxin B (TcdB). These toxins inactivate small GTP-binding proteins, resulting in cell death. Infections begin with focal necrosis, then ulceration with exudate, and can progress to **pseudomembranous colitis**, which involves inflammation of the colon and the development of a pseudomembrane of fibrin containing dead epithelial cells and leukocytes. The disease is characterized by watery diarrhea, dehydration, fever, loss of appetite, and abdominal pain can result.

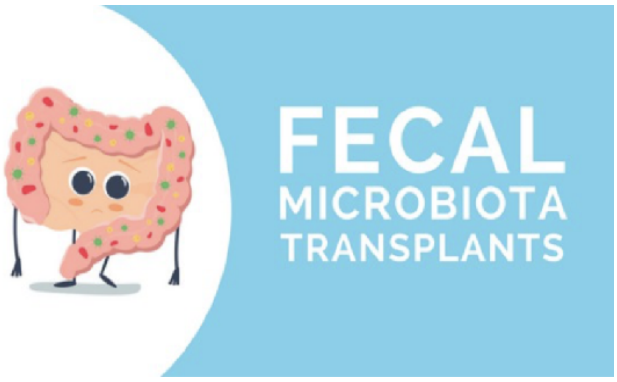
Perforation of the colon can occur, leading to septicemia, shock, and death

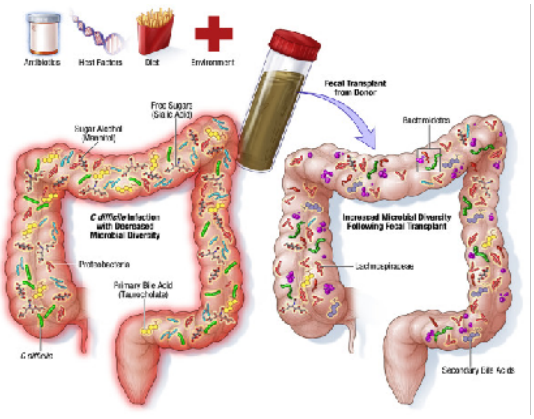
Diagnosis of *C. difficile* infection:

Stool Test: The simplest way to detect *C. difficile* is through a stool test.

Blood Test: A blood test can reveal high levels of white blood cells, a sign of infection. Very high levels can signify a more severe *C. difficile* infection, in which a person may have watery diarrhea, intense stomach cramps, and dehydration.

Colonoscopy: A colonoscopy enables a doctor to examine the entire colon and rectum.The test can indicate whether inflammation is present, indicating a *C. difficile* infection.





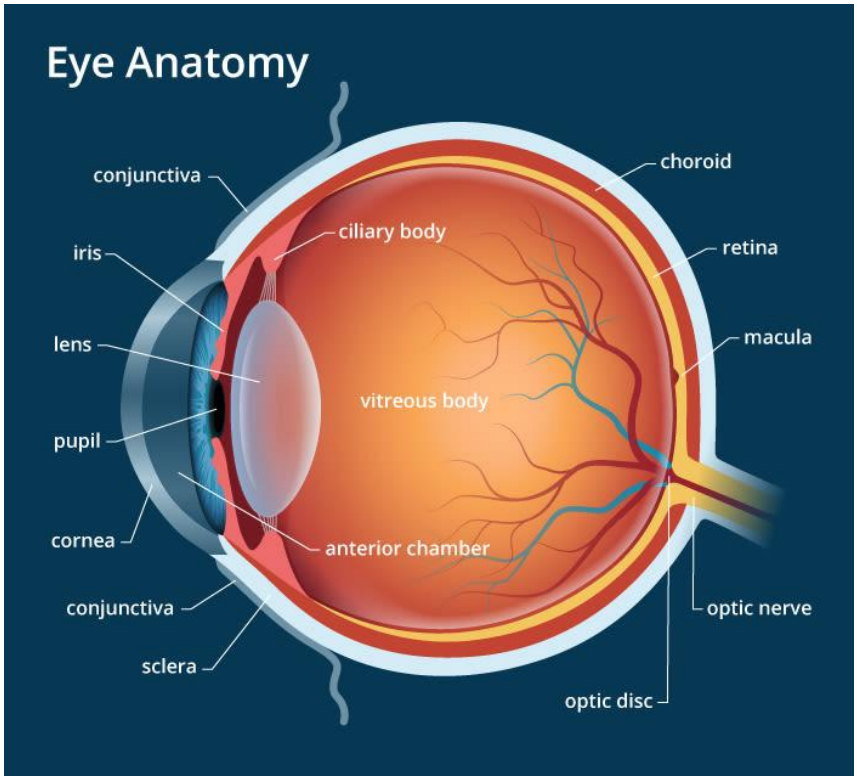
Diagnostic Microbiology

Lecture-25: Bacterial infections of eyes, ears, & sinuses

Eye (ocular) Infections

Eye infections can be divided based on the area of the eye into external and internal sites of the eye.

The external structures of the eye include eyelids, conjunctiva, sclera, and cornea**. The eyeball** comprises three layers**;** from the outside in, these tissues are **the sclera, choroid, and retina.** large interior space of the eyeball is divided into two sections: the anterior and posterior cavities. The anterior cavity is filled with a clear and watery substance called **aqueous humor;** the posterior cavity is filled with a soft, gelatin-like substance called **vitreous humor.**



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.

Resident Microbial Flora: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Lactobacillus* spp. *Propionibacterium acnes*, *Haemophilus influenzae* and *Enterobacteriaceae*.

Pseudomonas aeruginosa and *S. aureus* are most common causes of eye infections, while *Streptococcus* spp. are less common.

Eye infection and etiology

1- Blepharitis

Blepharitis is a bump that appears on the eyelid that is red, swollen, and resembles a pimple. Most bumps on the eyelid are caused by an inflamed oil gland on the edge of the eyelid commonly referred to as a **Sty**. ***S. aureus* and *S. epidermidis*** are the most common infectious agents associated with **blepharitis.** Symptoms include burning, itching, the sensation of the presence of a foreign body, and crusting of the eyelids. Herpes simplex virus (HSV) produces vesicles on the eyelids that typically crust and heal with scarring within 2 weeks.

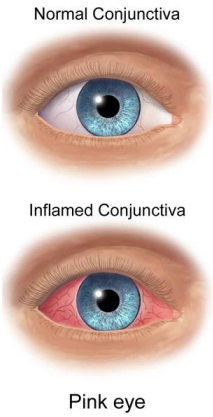
Stye (sty): is a red, painful lump (mass) near the edge of the eyelid that may look like a boil.

2- Conjunctivitis: commonly referred to as **"pink eye"** is the most common type of ocular infection and may be caused by allergies or bacterial or viral infection.

- Bacterial conjunctivitis: In children the most common causes of **bacterial conjunctivitis** include *Haemophilus influenzae*, *S. pneumoniae*, *S. aureus,* and *H. aegyptius.*

- Neonatal conjunctivitis: In neonates, Neisseria and chlamydia infections are frequent and are acquired during passage through an infected vaginal canal.

- Trachoma: ***Chlamydia trachomatis*** remains responsible for one of the most important types of conjunctivitis, **trachoma** is one of the leading causes of **blindness** in the world.



3- Keratitis: Keratitis (corneal infection) may be caused by a variety of infectious agents such as ***Pseudomonas aeruginosa* and *S. aureus*.** With *P. aeruginosa* and *Neisseria gonorrhoeae* are responsible for the corneal destruction. The principal causes of eye infections are listed in the following (Table-1).

Table-1: Infection of the eye and the causative agents

Infection	Description	Bacteria	Viruses	Fungi	Parasites
Blepharitis	Inflammation of the margins (edges) of the eyelids; (eyelids, eye lashes or associated pilosebaceous glands or meibomiam glawds) symptoms include irritation, redness, burning sensation, and occasional itching. Condition is typically bilateral	*Staphylococcus aureus*	Herpes simplex virus	Staphylococcues epidermidrs Malassezia furfar	Phthirus pulis
Conjunctivitis	Inflammation of the conjunctiva; symptoms vary according to the etiologic agent, but most patients have swelling of the conjunctiva, inflammatory exudates, and burning and itching	*Streptococcus pneumoniae, Haemophilus influenzae, S. aureus, Haemophilus* spp., *Chlamydia trachomatis, Neisseria gonorrhoeae, Streptococcus pyogenes, Moraxella* spp., *Corynebacterium* spp.	Adenoviruses, herpes simplex (HSV), varicella zoster. Epstein-Barr virus (EBV) influeza vius, pararryxovirus, rubella, HIV enterovirus, coxscukie A		
Keratitis	Inflammation of the cornea; although there are no specific clinical signs to confirm infection, most patients complain of pain and usually some decrease in vision, with or without discharge from the eye	*S. aureus S. pneumoniae, Pseudomonas, aeruginosa Moraxella lacunata, Bacillus* spp.	HSV, adenoviruses, varicella zoster	*Fusarium solani, Aspergillus* spp., *Candida* spp., *Acremonium, Curvularia*	*Acanthamoeba* spp.

Laboratory diagnosis

Specimen Collection: Purulent material from the surface of the lower conjunctiva sac and inner canthus (angle) of the eye is collected on a sterile swab for Gram stain and cultures. Both eyes should be cultured separately.

Chlamydial cultures are taken with a dry calcium alginate swab and placed in 2-SP (2-sucrose phosphate) transport medium. An additional swab may be rolled across the surface of a slide, fixed with methanol, and examined by **direct fluorescent antibody (DFA). chlamydia stains are used for detection**.
Multiple inoculations with the spatula are made to blood agar, chocolate agar, an agar for the isolation of fungi, thioglycollate broth, and an anaerobic blood agar plate.

Direct Visual Examination

All material submitted for culture should be smeared and examined directly by Gram stain or other appropriate microscopic techniques. In bacterial conjunctivitis, polymorphonuclear leukocytes predominate; in viral infection, the host cells are primarily lymphocytes and monocytes.

Culture

Because of the constant washing action of the tears, the number of organisms recovered from cultures of eye infections may be relatively low. Unless the clinical specimen is obviously purulent, using a relatively large inoculum and a variety of media is recommended to ensure recovery of the etiologic agent. **Conjunctival scrapings** placed directly onto media yield the best results. At a minimum, **blood and chocolate agar** plates should be used for isolation and identification of fastidious bacteria. PCR and ELISA techniques may be used also.

EAR Infections

Anatomy

The ear is divided into three anatomic parts: **the external**, **middle**, and **inner ear.** The middle ear is part of a continuous system including the nares, nasopharynx, auditory tube, and the mastoid air spaces. cells, mucus-secreting goblet cells.

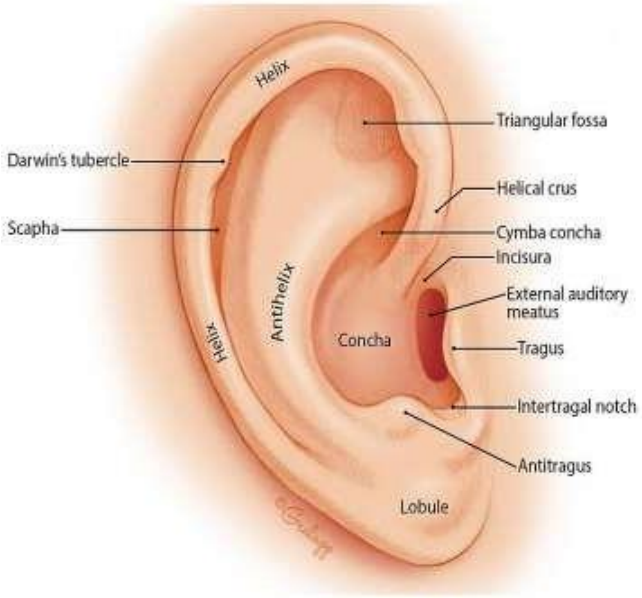


Fig.2: Anatomy of ear

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.

Resident Microbial Flora:

- such as pneumococci, *Streptococcus pneumoniae, Propionibacterium acnes, S. aureus, Pseudomonas aeruginosa and Enterobacteriaceae***.**

- The most common bacteria that cause ear infections are: **coagulase positive staphylococci, alpha & beta hemolytic Streptococci, *Proteus* spp. *Pseudomonas aeruginosa* and *E. coli***.

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.

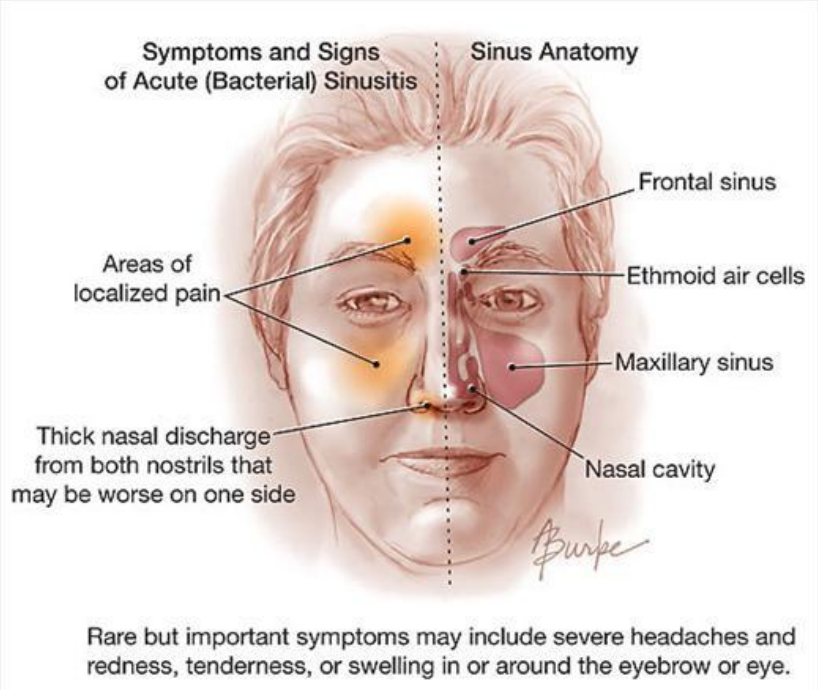
Causative agent: *Hemophillus influenzae; Strep. pneumoniae; Strep. pyogenes.*

Source: Endogenous: normal flora of the nasopharynx.

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.

Risk factors: History of acute or chronic otitis media; Parental (source) history of otitis media.

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, nonculture methods include conventional and real-time PCR.

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, nonculture methods include conventional and real-time PCR.

Note: The external ear should be cleansed with a mild germicide to reduce the numbers of contaminating skin flora before obtaining the specimen (Specimens should be transported anaerobically).

Diagnostic Microbiology

Lecture-26: Skin, Soft tissue and wound infection

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.**

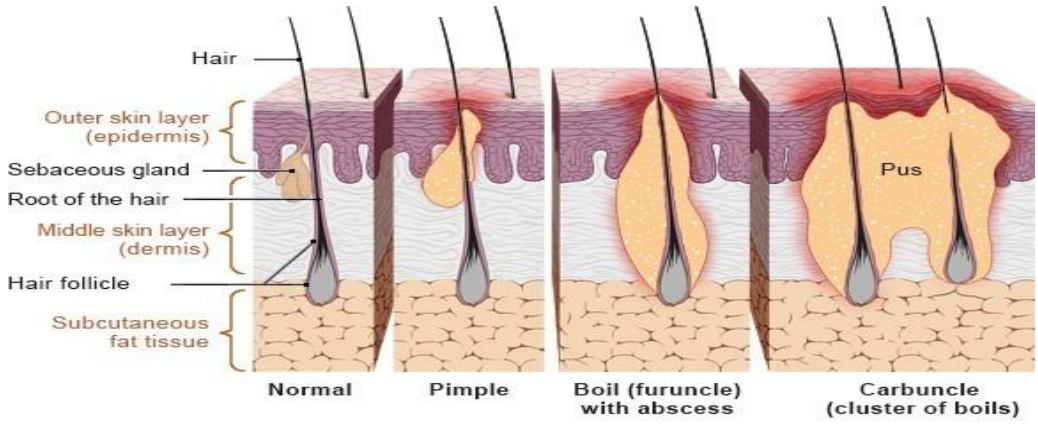
Normal skin has **numerous mechanisms** to prevent infection and protect the underlying tissue from invasion by potential pathogens. These mechanisms include:

1. physical separation of microorganisms from the tissues.
2. The normal flora that prevent most pathogens from colonizing skin
3. presence of fatty acids that inhibit many microorganisms
4. 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.



S. 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 and swimming pools.

- 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 dermal tissues.
- 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** may manifest as abscesses, ulcers, or boils. The most common etiologic agent **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*.

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 **Staphylococcal scalded skin syndrome (SSSS)** and **toxic shock syndrome (TSS).**

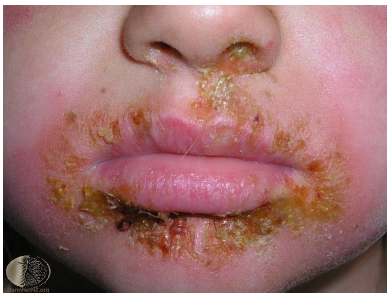
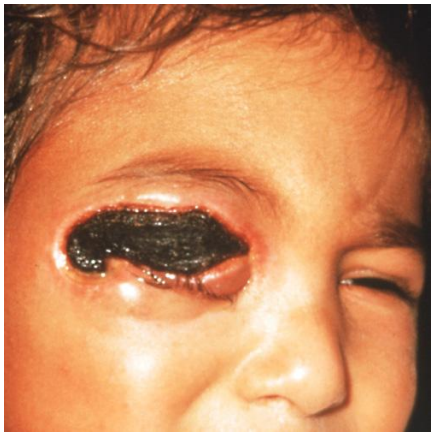


Fig-1: **Impetigo**

Bacillus anthracis, a spore forming, aerobic, gram-positive rod caused **Anthrax**. Endospores can survive for years in soil. 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.



Cutaneous anthrax

Pulmonary anthrax

Diagnostic Lab Tests of Anthrax

Specimens to be examined are depend on the type of the anthrax:

For **cutaneous anthrax**: fluid or pus from a local lesion

For **Inhalational** (pulmonary) **anthrax**: specimens include blood and sputum.

For **gastrointestinal anthrax**: stool or other intestinal contents.

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. gram stain shows large Gram positive rods.

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 of Tetanus

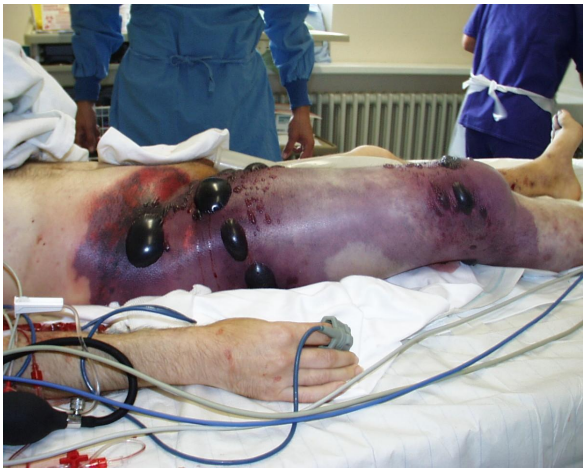
Spores enter the body through a deep puncture wound resulting from a fracture, animal bite, a piece of glass, a rusty nail contaminated with soil. Like anthrax, even illicit drugs can contain spores. In dead, oxygen-free tissue of the wound, spores germinate into vegetative bacilli that produce several toxins. The most important of these is the tetanus toxin (tetanospasmin).

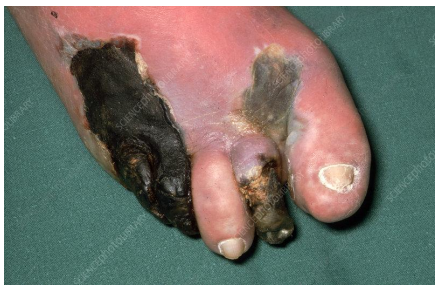
At the nerve-muscle synapse of the spinal cord or peripheral nerves, the neurotoxin prevents the release of neurotransmitters needed to inhibit muscle contraction causing uncontrolled, continuous muscle contraction (spasms).

Tetanus

Clostridium perfringens

Gas gangrene, or myonecrosis (myo = "muscle"; necros = "death") is caused primarily by *Cl. perfringens*, an anaerobic, sporeforming, 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. 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.

Lab diagnosis of *C. perfringens*

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-pak container (**Fig**.)

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.

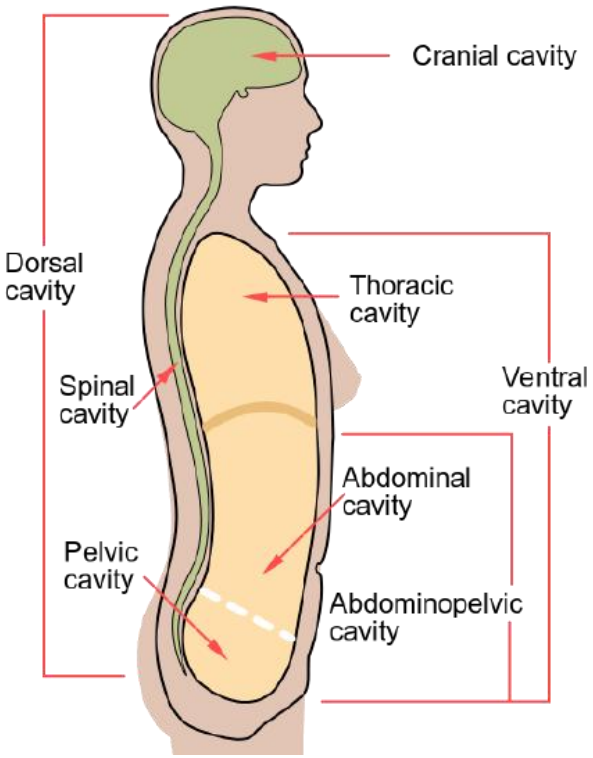
3. **Colony Forms:** Some clostridia produce large raised colonies (eg, *C. perfringens*); others produce smaller colonies (eg, *C. tetani*). Many clostridia produce a zone of β-hemolysis on blood agar.

4. **Growth Characteristics**: The 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.

Diagnostic Microbiology

Lecture-27: Normally Sterile Body Fluids, Bone and Bone Marrow, and Solid Tissues

The human body is divided into five main **body cavities**: cranial, spinal, thoracic, abdominal, and pelvic. Each cavity is lined with membranes, and within the body wall and these membranes, or between the membranes and organs, are small spaces filled with minute amounts of fluid. The purpose of this fluid is to bathe the organs and membranes, reducing friction between organs. Bacteria, fungi, viruses, or parasites can invade any body tissue or sterile body fluid site.



Specimens from Sterile Body Sites

Fluids: In response to infection, fluid may accumulate in any body cavity. Infected solid tissue often presents as cellulitis or with abscess formation. Areas of the body from which fluids are typically sent for microbiologic studies (in addition to blood and CSF).

Table -1: Microbiology Laboratory Body Fluid Collection Sites

Body Area	Fluid Name(s)
Thorax	Thoracentesis or pleural or empyema fluid
Abdominal cavity	Paracentesis or ascitic or peritoneal fluid
Joint	Synovial fluid
Pericardium	Pericardial fluid

A- Pleural Fluid:

1. Lining the entire thoracic cavity of the body is a serous membrane called the parietal pleura.

2. Covering the outer surface of the lung is another membrane called the visceral pleura.

3. Within the pleural space between the lung and chest wall is a small amount of fluid called pleural fluid that lubricates the surfaces of the pleura (the membranes surrounding the lungs and lining of the chest cavity).

4. The fluid can be collected for testing by **Thoracentesis** (the insertion of a needle into the thoracic cavity and removal of fluid).

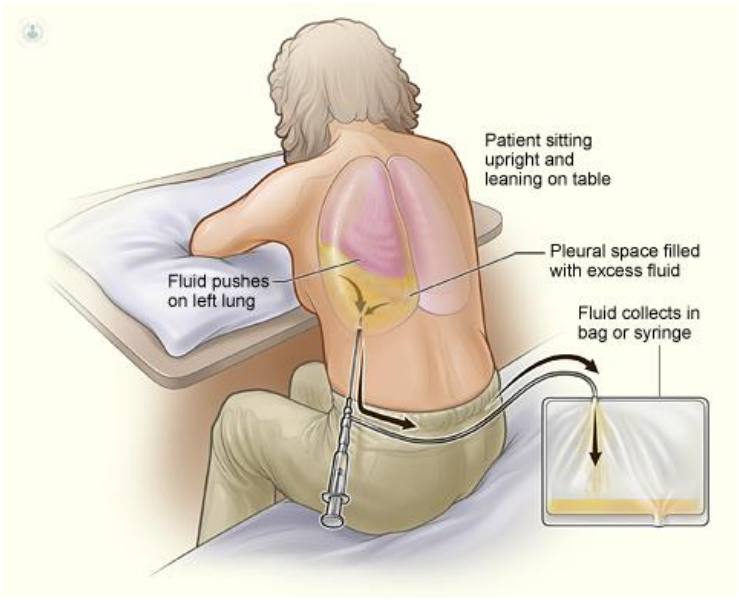


Figure-1: Thoracentesis

B- Peritoneal Fluid

The peritoneum is a large, moist, continuous sheet of serous membrane lining the walls of the abdominal-pelvic cavity and the outer coat of the organs contained within the cavity.

- Normal peritoneal fluid contains as many as 300 white blood cells per milliliter, but the protein content and specific gravity of the fluid are low.
- During an infectious or inflammatory process, increased amounts of fluid accumulate in the peritoneal cavity, a condition called **ascites**. Most cases of ascites are caused by liver disease, and in severe cases, the abdomen is often distended.
- The fluid can be collected for testing by **paracentesis** (the insertion of a needle into the abdomen and removal of fluid).

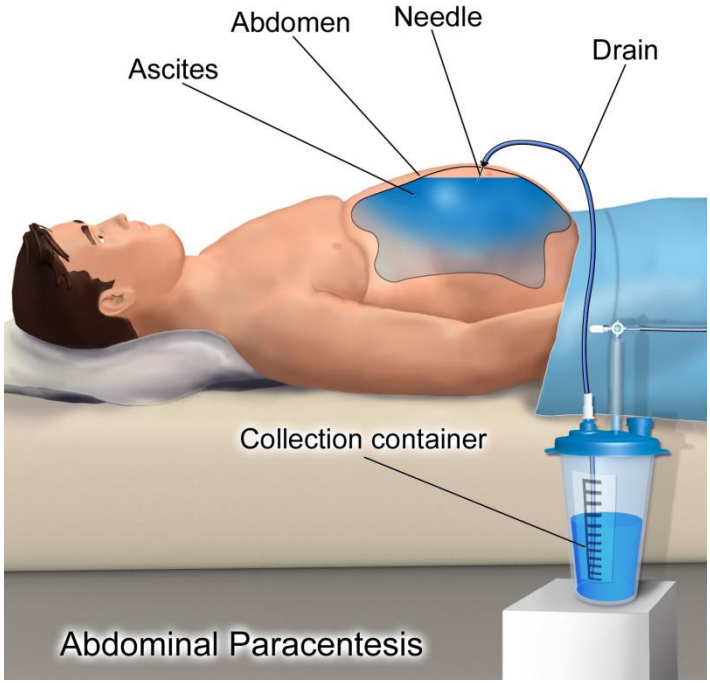


Figure-2: Abdomibal Paracentesis

1. **Primary Peritonitis** results when the peritoneal membrane becomes inflamed and can be either primary or secondary. The most common etiologic agents in children are *Streptococcus pneumoniae* and group –A streptococci, *Enterobacteriaceae*, other gram-negative bacilli, and staphylococci.

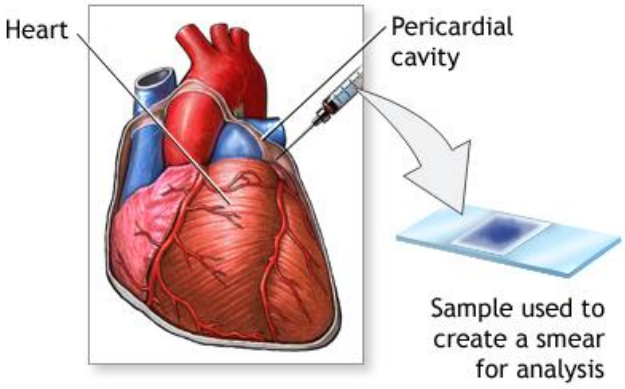
❖ In adults, *E.coli* is the most common bacterium, followed by *S. pneumoniae* and group A streptococci.

❖ Among sexually active young women, ***Neisseria gonorrhoeae*** and ***Chlamydia trachomatis*** are common etiologic agents of peritoneal infection, often in the form of a perihepatitis (inflammation of the surface of the liver, called Fitz-Hugh–Curtis syndrome).

2- Secondary peritonitis is a complication of a perforated viscus (organ), surgery, traumatic injury, loss of bowel wall integrity after a destructive disease (e.g**., ulcerative colitis, ruptured appendix, carcinoma**), obstruction, or a preceding infection (liver abscess, **salpingitis**, **septicemia**).

Salpingitis is inflammation of the fallopian tubes. Common causes include sexually transmitted diseases such as gonorrhoea and chlamydia. Salpingitis is a common cause of female infertility.

C- Pericardial Fluid: The heart and contiguous (adjacent) major blood vessels are surrounded by the pericardium, a protective tissue.



1- The area between the epicardium, which is the membrane surrounding the heart muscle, and the pericardium is called the pericardial space (cavity) and normally contains 15 to 20 mL of clear fluid.

2- Agents of pericarditis (inflammation of the pericardium) are usually viruses, especially coxsackie virus. Parasites, bacteria, certain fungi.

Myocarditis (inflammation of the heart muscle itself) may accompany or follow pericarditis. The pathogenesis of disease involves the host inflammatory response contributing to fluid buildup as well as cell and tissue damage. Common causes of **myocarditis** include viral infections with coxsackie virus, echoviruses, or adenovirus.

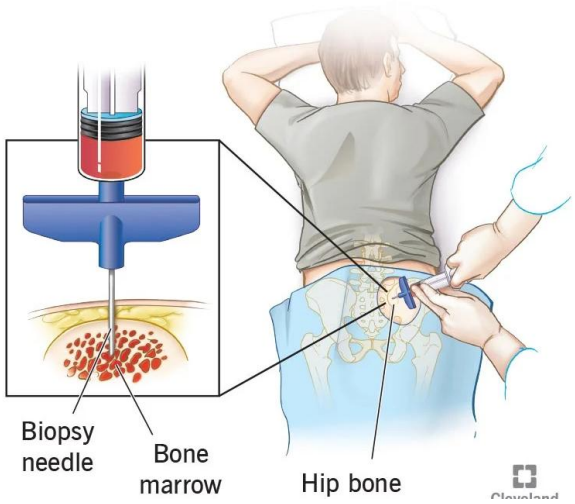
D- Bone Marrow Aspiration or Biopsy:

1- **Diagnosis of diseases**, including brucellosis, histoplasmosis, blastomycosis, tuberculosis, and leishmaniasis, can sometimes be made by detection of the organisms in bone marrow.

2- ***Brucella* spp**. can be isolated on culture media, as can fungi, but parasitic agents must be visualized in smears or sections made from bone marrow.

3- Many of the etiologic agents associated with disseminated infections in patients with human immunodeficiency virus (HIV) may be visualized or isolated from bone marrow.

Some of these organisms include cytomegalovirus, *Cryptococcus neoformans*, and *Mycobacterium avium* complex.



E- Bone Biopsy

A small piece of infected bone is occasionally sent to the microbiology laboratory to identify the etiologic agent of osteomyelitis (infection of bone).

- ✓ Patients develop **bone infection (osteomyelitis)** from hematogenous spread of an infectious agent, invasion of bone tissue from an adjacent site (e.g., joint infection, dental infection), breakdown of tissue caused by trauma or surgery, or lack of adequate circulation followed by colonization of a skin ulceration with microorganisms.
- ✓ ***S. aureus,*** seeded during **bacteremia**, is the most common etiologic agent of osteomyelitis among patients of all age groups.
- ✓ Other organisms recovered from **hematogenously acquired osteomyelitis** include ***Salmonella*** spp., ***Haemophilus*** spp., ***Enterobacteriaceae***.
- ✓ **Nucleic acid–based testing**, such as **PCR**, may be useful in determining the infectious organism.

Diagnostic Microbiology (Lectures-28, 29, and 30)

Lectures-28: Laboratory Methods for diagnosis of Parasitic Infections

Many kinds of laboratory tests to diagnose parasitic infections **based on patient's signs** and **symptoms** and **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 prevalent infections.

This group of pathogens includes **protozoans** such as, *Trypanosoma* (**Chagas disease** and African sleeping sickness), *Leishmania* (**leishmaniasis**), *Plasmodium* (**malaria**), and the **helminths** such as *Schistosoma* (**schistosomiasis**), *Wuchereria* (**filariasis**), and *Echinococcus* (**echinococcosis**). Many of these infections have always been described as being tropical or subtropical.

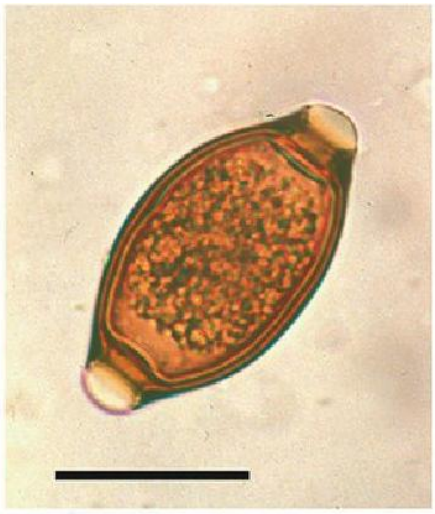
Currently, diagnostic laboratories use several techniques, including **microscopy, molecular assays**, and **serological assays**. Real-time polymerase chain reaction (q**PCR**) procedures for the detection of various parasites are continuously being optimized. These assays have the ability to detect mixed infections simultaneously. These assays rely on antigen or antibody detection from the provided patient samples.

Common laboratory tests used for diagnosis parasitic infections are:

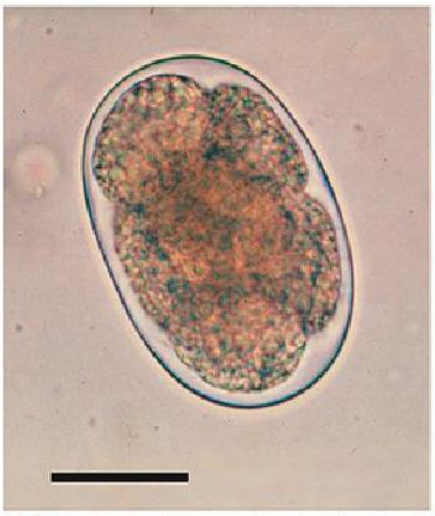
1. A fecal (stool) examination, also called an ova and parasite test (O & P):

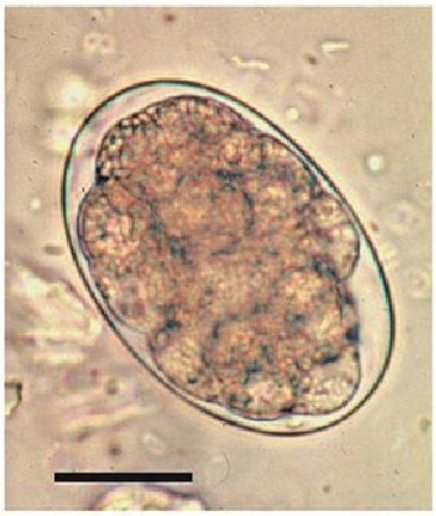
This test is used to find parasites that cause diarrhea or watery stools, cramping, flatulence (gas) and other abdominal illness. This test **looks for ova (eggs) or the parasite**. Specimens not collected in a preservative fluid should be refrigerated, but not frozen, until delivered to the lab.

Normal fertile ***Ascaris lumbricoides*** eggs measure 55–75 µm by 35–50 µm, are golden yellow to brown in colour and are in the single cell stage when passed in the faeces. The egg has conspicuous mamillations on its surface.



Typical ***Trichuris trichiura*** eggs measure 50–55 µm by 22–24 µm, have a brown, smooth shell, bipolar prominences (plugs) and contain a single cell ovum.





Hookworm eggs found in faeces are characteristically barrel-shaped with a thin, hyaline shell; they measure 60–75 µm by 36–40 µm. They are usually in the 4- or 8-cell stage in fresh faeces or in a more advanced stage of cleavage in faeces that have been kept at room temperature for even a few hours.

Strongyloides stercoralis infection is routinely diagnosed by the presence in faeces of first-stage rhabditoid larvae of 180–380 µm by 14–20 µm. Larvae have a short buccal capsule, an attenuated tail and a prominent genital primordium (arrow).

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:

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

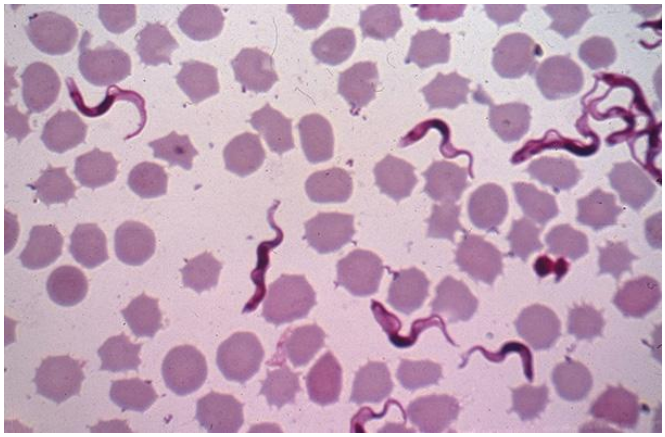
4. Blood tests:

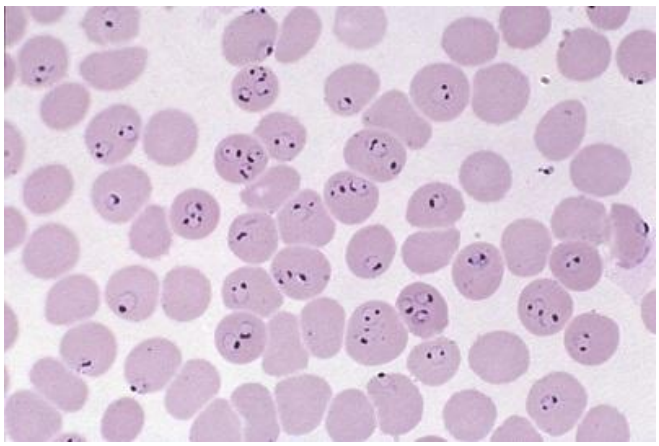
Some, but not all, parasitic infections can be detected by testing the blood. **Blood tests look for a specific parasite infection (such as Chagas disease, leishmaniasis, malaria); there** is no blood test that will look for all parasitic infections.

There are two general kinds of blood tests that used for diagnosis of parasitic infections:

A- 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.

B- 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.



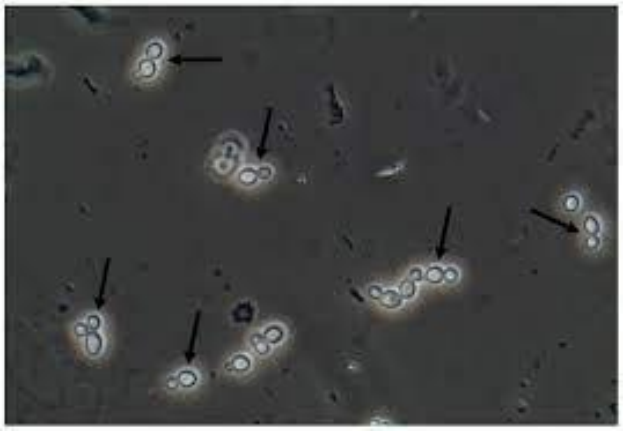


5. X-ray, Magnetic Resonance Imaging (MRI), Computerized Axial Tomography scan (CAT):

These tests are used to look for some parasitic diseases that may cause lesions in the organs.

Lectures-29: Lab Methods in basic Mycology

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



Candida albicans yeasts in urine sediment

***Aspergillus* spp. showing hyphae, conidiophore, conidia**

Early and accurate detection (lab diagnosis) is important for appropriate application of antifungal therapy and decreasing the unnecessary use of toxic antifungal agents. In addition, the accurate and timely diagnosis could reduce the use of experiential anti-fungal therapy.

Standard approaches to the lab diagnosis of fungal Infections:

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. The recent alternative techniques especially elaborating antigen detection and molecular techniques which are also available for diagnosis.

Diagnostic methods for fungal infections

1- Direct examination: wet mount, with fluorescent staining fungal culture.
2- Radiology.
3-Non culture methods:

A. Serological methods- antigen detection, antibody detection.

B. Tests for detection of metabolites.

C. Tests for detection of CMI (cell mediated immunity).

D. Molecular methods.

E. Others (Matrix-assisted laser desorption/ionization - time of flight mass spectrometry (**MALDI-TOF MS**).

Lectures-30: Lab Methods in basic Virology

There are several laboratory steps used for viral diagnosis:

1. Sampling (specimen).
2. Virus isolation.
3. Nucleic acid based methods such as PCR and gene sequencing.
4. Microscopy based methods like immunofluorescence or immune-peroxidase 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 specimen 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.

Types of samples include:

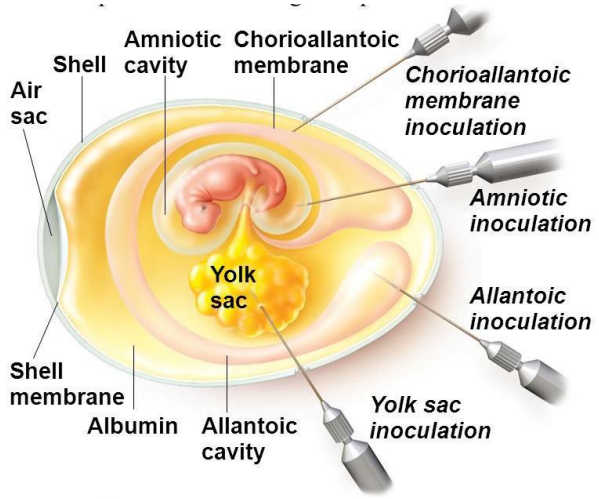
- **Blood • Skin • Sputum (**and or gargles and bronchial washings**)**
- **Urine • Semen • Faeces • CSF • Tissues (**biopsies or post-mortem**)**
- **Dried blood spots.**

2. Virus isolation

Viruses are often isolated from the initial patient sample. This allows the virus to be grown into larger quantities and allows a larger number to tests to be run on them. 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.

There are different **cells lines** 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 (MRC5), 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 (**cytopathic effects**) 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** (**lyssa viruses**).



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.

- **PCR:** Detection of viral **RNA and DNA genomes can be performed** using PCR. Variations of PCR such as nested reverse transcriptase PCR and real time PCR can also be used to determine viral loads in patient serum.

- **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. Sequencing is useful when specific mutations in the patient are tested for in order to determine antiviral therapy and susceptibility to infection.

4. Microscopy based methods

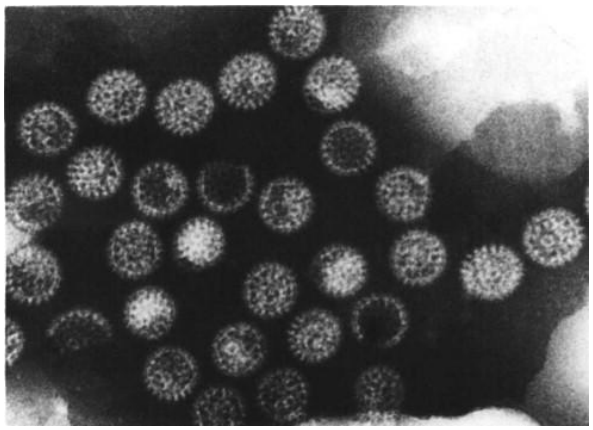
➢ Immunofluorescence or immunoperoxidase

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**. They are also conjugated to a special kind of tag that allows the antibody to be visualized in the lab, so that it will **produce 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 multipurpose 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 tests.



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.**

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.