# جامعة الفرات الاوسط التقنية

# كلية التقنيات الصحية والطبية /كوفة

# قسم تقنيات المختبرات الطبية

# مادة : Diagnostic Microbiology

# المرحلة الرابعة

محاضرة : ١١ -١٢ - ١٣-١٤ -١٥

**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.

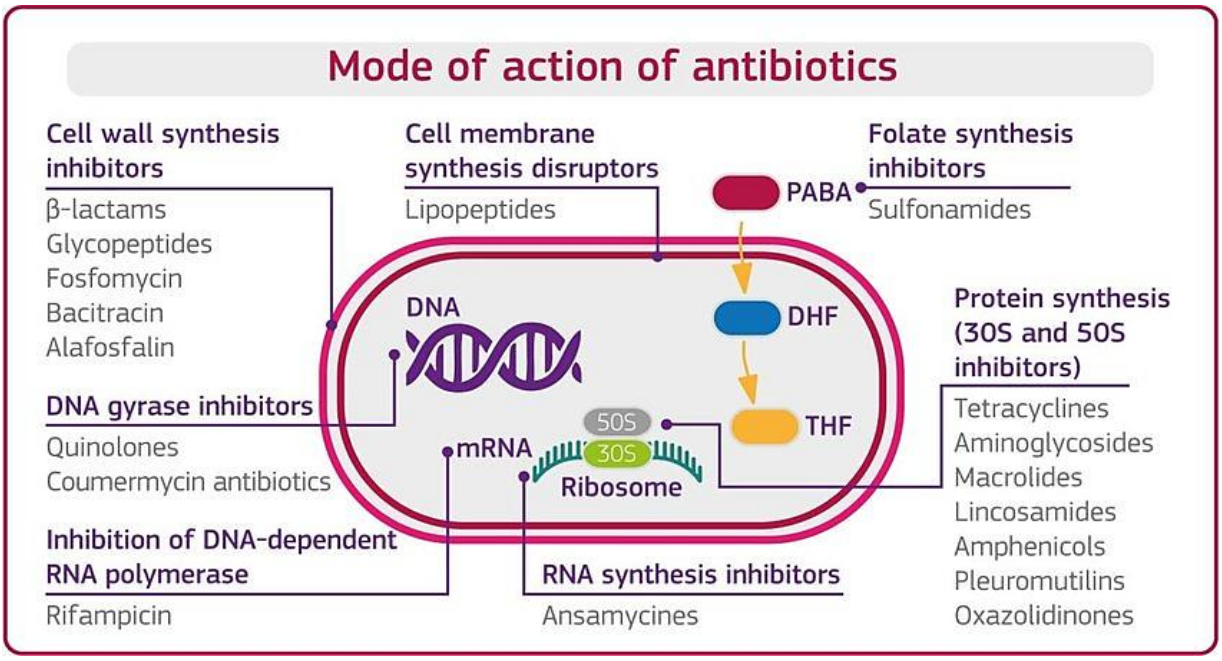
2-Cell membrane synthesis

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**.

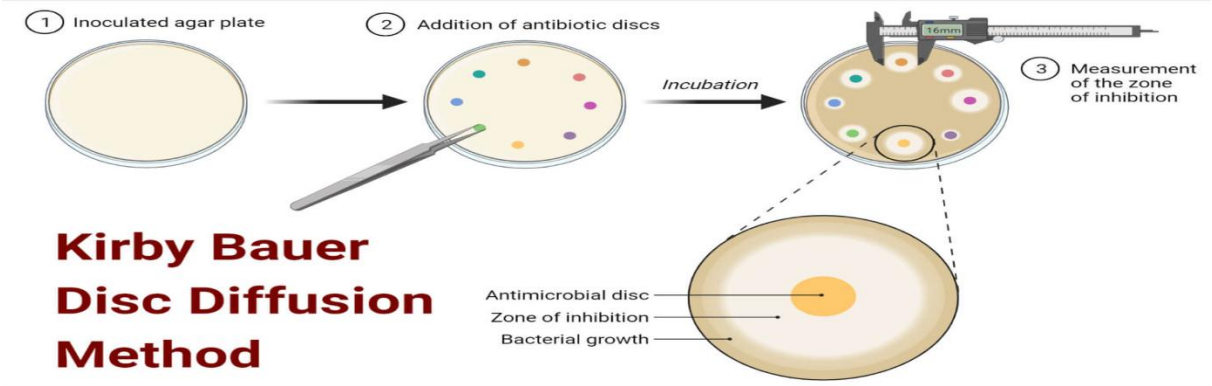
In clinical laboratories, susceptibility testing is usually performed by a disk diffusion or and minimal inhibitory concentration [MIC] methods. Standards that describe these methods are published and frequently updated by the **Clinical and Laboratory Standards Institute** (CLSI), formerly the **National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




**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).

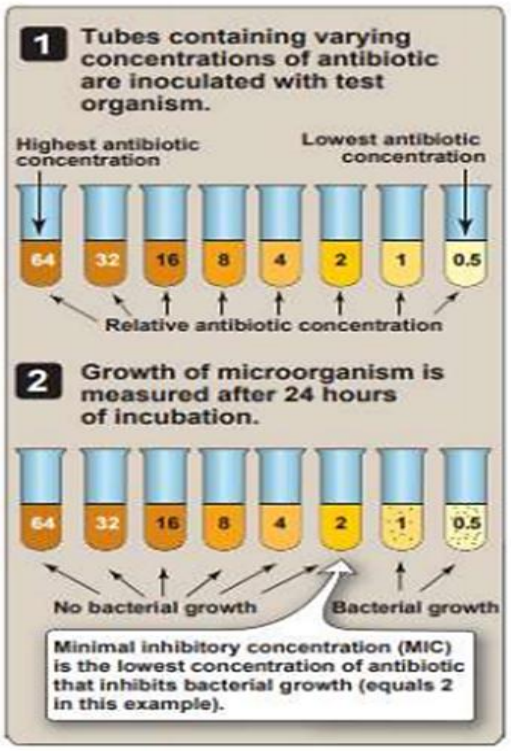



Figure-3
Determination of minimal inhibitory concentration (MIC) of an antibiotic.

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 systems.

## 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

## *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.

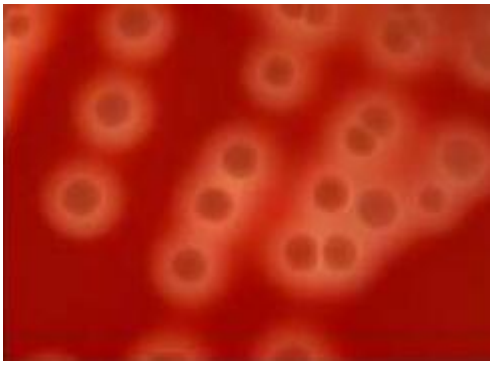
### Cultural characters:

*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.

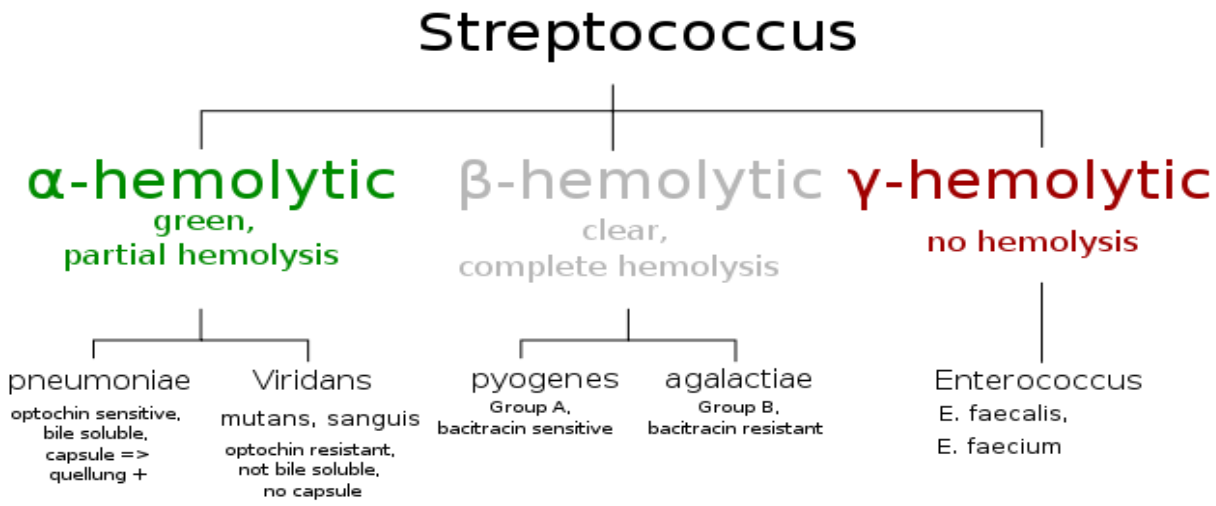
**b. Blood agar:** After 24 hours' incubation colony is small (pin point colonies), circular, transparent, low convex with area of hemolysis. Strains with capsules produce mucoid colonies.

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



**c. 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**




***Enterococcus:***

The enterococci ("enteric cocci") 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.

***Streptococcus pneumonia***

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**

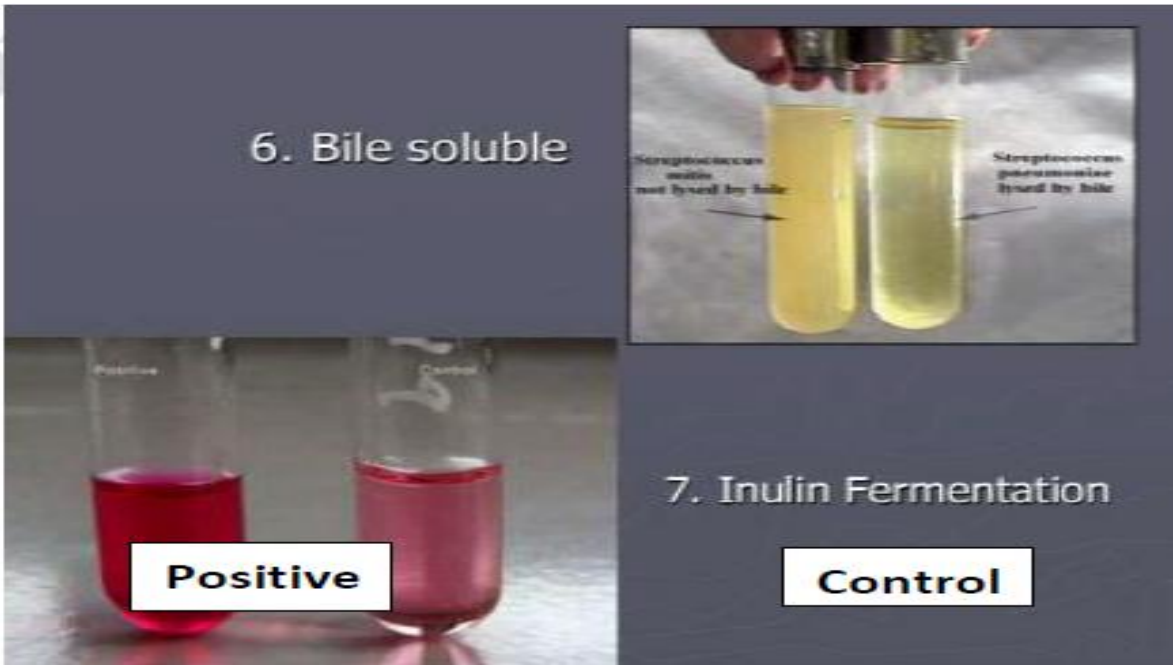
**1. Inulin Fermentation:** Pneumococci ferment inulin with the production of acid without gas. Fermentation of inulin by pneumococci is a useful test for differentiating them from streptococci as the latter do not ferment it.

**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.

3. Pneumococci are Catalase and Oxidase negative




4- **Optochin Sensitivity:**


**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.

***Pseudomonas aeruginosa***

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

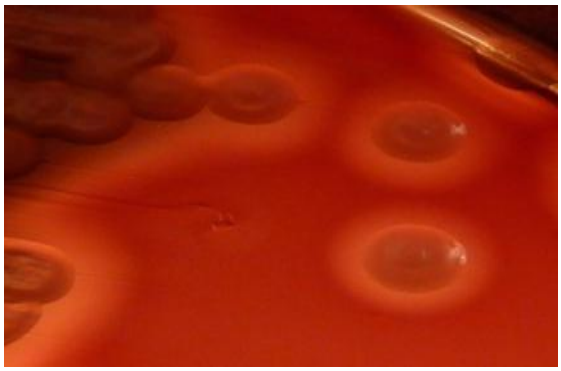
**Specimens**: Specimens depend on the site of infection including skin lesions, pus, urine, blood, spinal fluid, sputum, and other material should be obtained by different procedures .

**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**

**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.

***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* | *Klebsiella 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** | + | - | - | + | + | - | - | - |

***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**

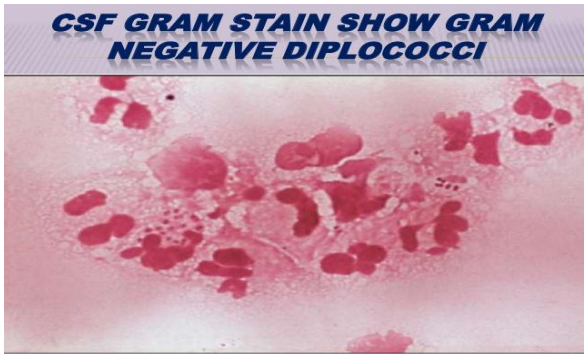
- Nasopharyngeal swabs; body fluids (**joint** fluid or **CSF**) should be stored at 37°C because it was sensitive to cold.
- Any volume (greater than 1 ml) of clear body fluid should be centrifuged at room temperature at 1500xg for 15 min. the sediment should be vortexed and inoculated onto appropriate media.

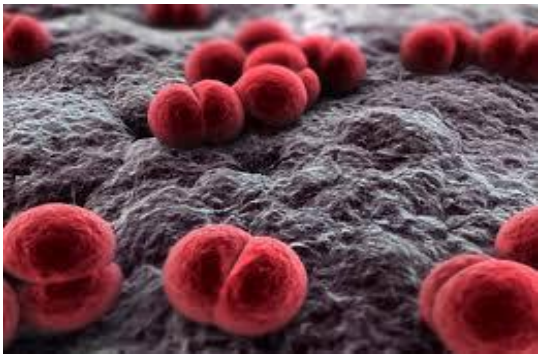
**Diagnosis:**

**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 $CO_2$(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*** | + | - | - | + | + | - | - | - |

**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 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 small amount of broth removed with a sterile loop or Pasteur pipette, and a Gram-stained smear examined for the presence of microorganisms.

Table (3): Summary of bacterial blood infections.

| Infection | Most Important Pathogens | Laboratory diagnosis |
|---|---|---|
| Endocarditis | *Streptococcus* spp. (60–80%)<br>*Staphylococcus* spp. (20–35%)<br>Gram-negative rods (2–13%)<br>Numerous other bacterial spp. (5%)<br>Fungi (2–4%)<br>Culture negative (5–25%) | **Blood culture**, three sets from three different sites, within 1–2 h, before antimicrobials if possible.<br>10–20 ml venous blood into one aerobic and one anaerobic bottle, respectively. |
| *Bacteria* | ***Staphylococcus aureus***<br>***Streptococcus pneumoniae***<br>***Enterobacteriaceae***<br>***Mycobacterium tuberculosis*** | Microscopy and culture from punctate<br>DNA test from punctate if required |
| | *Mycoplasma pneumoniae* | Serology; culture from punctate |
| | *Neisseria* spp.<br>Gram-negative anaerobes<br>*Actinomyces* spp.<br>*Nocardia* spp. | Microscopy and culture from punctate |
| | *Rickettsia* spp.<br>*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 cell count, the glucose concentration in CSF and blood and protein content of cerebrospinal fluid,** the results of **microscopic examination for microorganisms.** In addition, the results of **culture, serologic tests, nucleic acid amplification tests, and other laboratory procedures.**

## Common Causes of Meningitis:

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

**Specimens**

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

If bacterial meningitis has suspected, **CSF is the best clinical specimen** to use for isolation, identification, and characterization of the etiological agents. Suspected

agents should include ***N. meningitidis*, *Strep. pneumoniae*, and *H. influenzae*** **and other pathogens in some cases.**

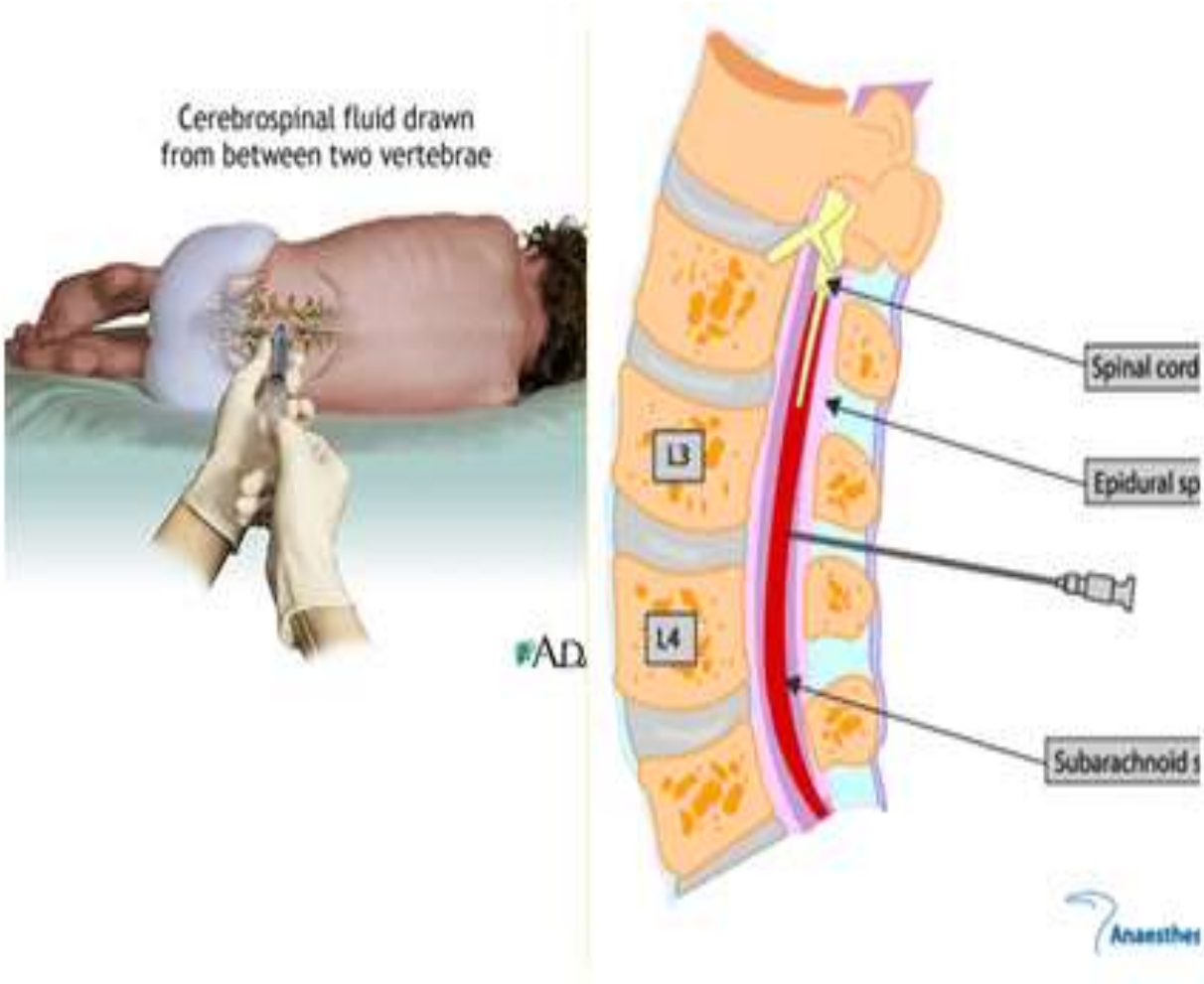



**Figure (1): Collection of cerebrospinal fluid (CSF) by lumbar puncture.**

## Microscopic Examination

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

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

**(pneumococci), or small gram-negative rods (*Hemophilus influenzae* or enteric gram-negative rods).**

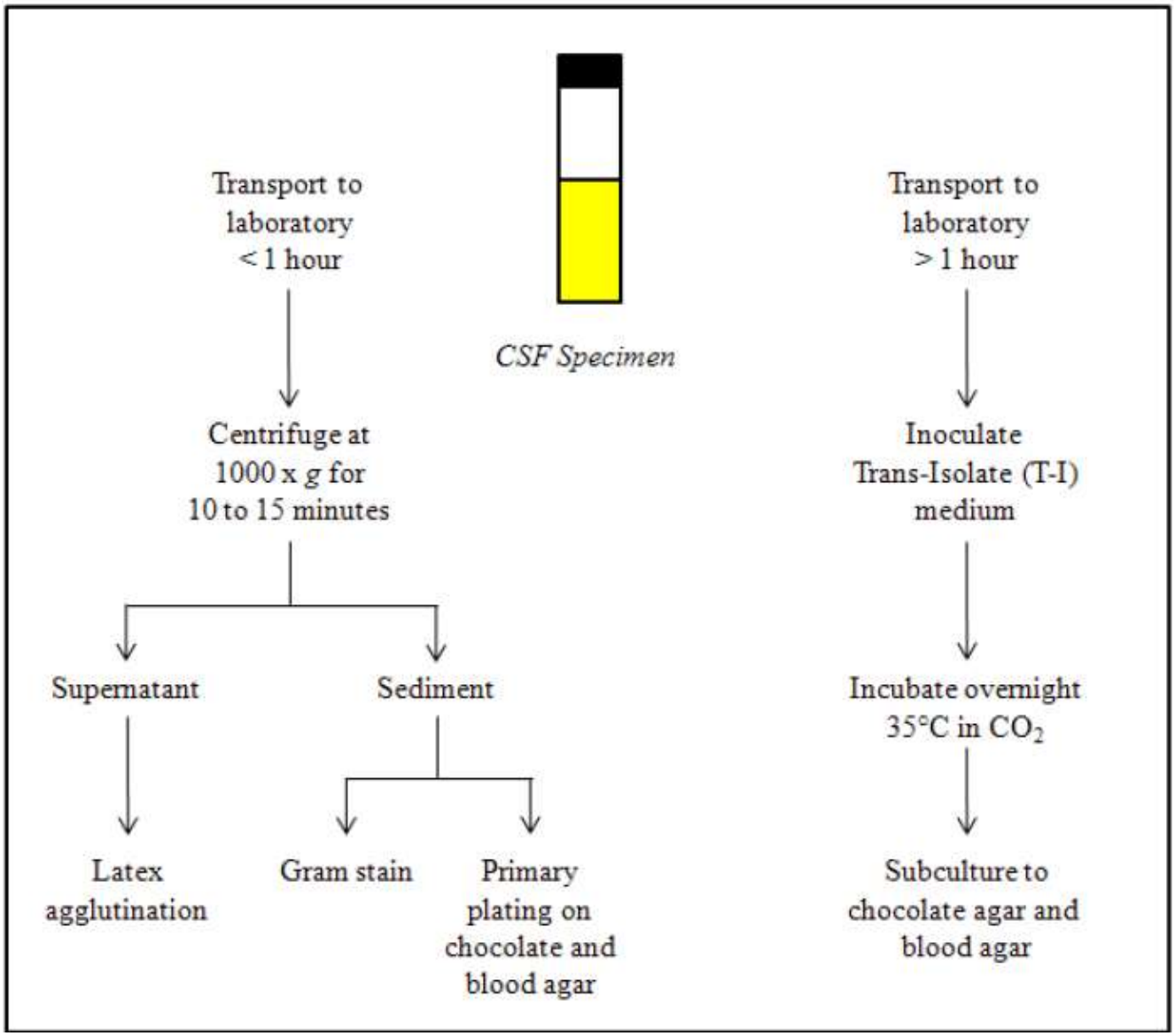



**Figure (2): Cerebrospinal fluid (CSF) isolation and identification.**

## Culture

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

**Follow-Up Examination of Cerebrospinal Fluid**

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

***Neisseria meningitids*** are **1- gram-negative, 2- coffee-bean shaped diplococci that 3- may occur intracellularly or extracellularly in polymorphic nuclei (PMN) leukocytes. 4- (PMNs or neutrophils are often more than 1000 WBCs/cu mm).**

**5-** ***Neisseria meningitidis*** is a **fastidious organism, aerobic diplococci,** which **6-** grows best at 35-37°C with ~5% CO2 (or **in a candle-jar**). **7-** It can grow on both a blood agar plate (BAP) and chocolate agar plate (CAP). **8-** Colonies of *N. meningitidis* are grey and **unpigmented** on a BAP and appear round, smooth, moist, shiny, and convex, with a clearly defined edge. ***N. meningitidis*** appear as large, colorless-to-grey, opaque colonies on a CAP (Figure 3, 4).

Biochemical tests have recommended confirming the identity of cultures that morphologically appear to be ***N. meningitidis*** such as **oxidase test (+)** and **carbohydrate utilization (acid production from glucose, maltose).** If the oxidase test is positive, carbohydrate utilization testing should have performed. If the carbohydrate utilization test **indicates** that the isolate may be *N. meningitidis*, **10- serological tests** to identify the serogroup should performed. Additional methods for identification and characterization of ***N. meningitidis*** using molecular tools like **11-** PCR technique.

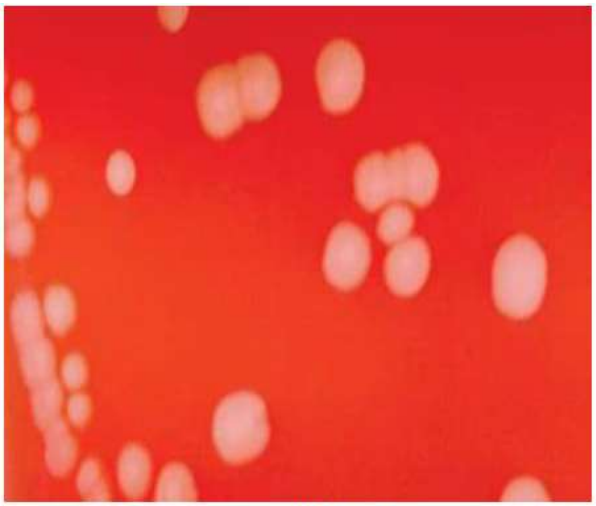


**Figure (3): *N. meningitidis* colonies on a BAP**

**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 **alphahemolysis** (green) (**Figure 5**). The alpha-**hemolytic property differentiates** this organism from many species, but not from the commensal **alpha-hemolytic (viridans)** streptococci. Differentiating pneumococci from viridans streptococci is **difficult** as young pneumococcal colonies appear raised, similar to viridans streptococci. However, once the pneumococcal **culture ages 24-48 hours, the colonies become flatten**, **and the central portion becomes depressed**, which **does not occur with viridans streptococci (Figure 6).** 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% CO2 (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 susceptible to optochin tests (see figure 8) (<14mm diameter) at the same time, with bile solubility (+) as a confirmatory test.** If these tests indicate that, the isolate is *Strep. pneumoniae*, then **serological tests used** to identify the serotype caught performed. This sequence of testing is an efficient way to save costly serotyping reagents and time. Additional methods for identification and characterization of ***Strep. pneumoniae*** using **molecular tools**.

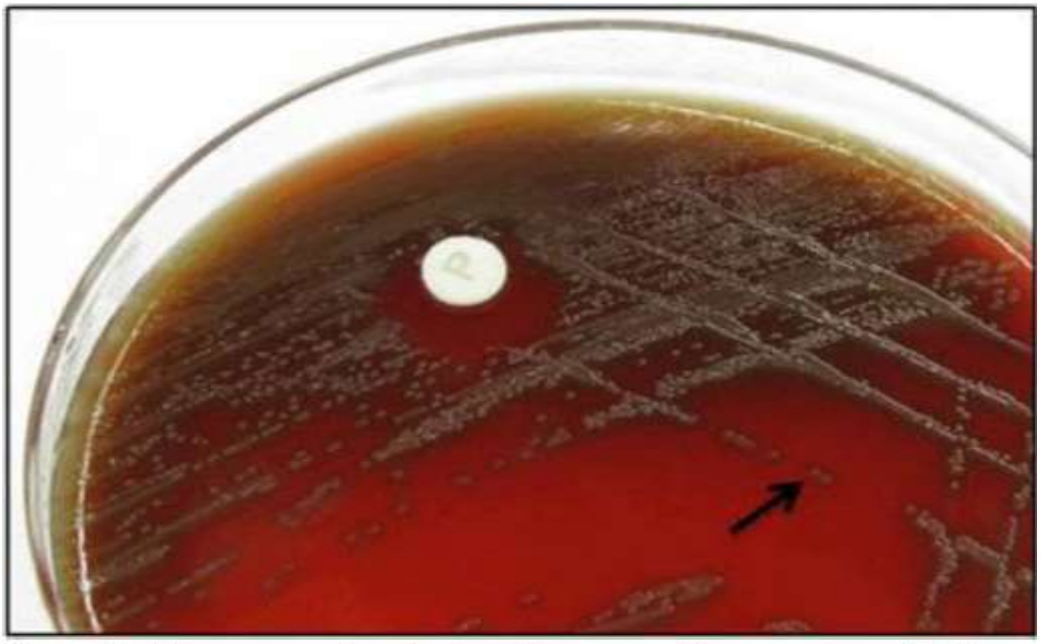


Figure (5): *Strep. pneumoniae* colonies with a surrounding green zone of alpha-hemolysis (black arrow) on a Blood Agar Plate.

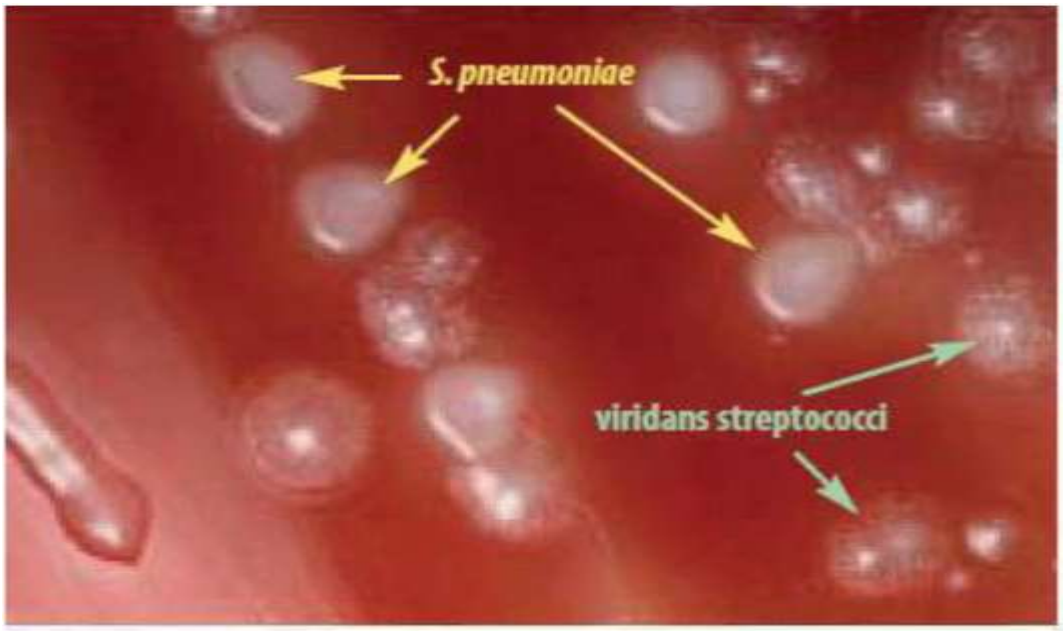



Figure (6): *Strep. pneumoniae* colonies have a flattened and depressed center afte

**24-48 hours of growth on BAP, whereas the viridans streptococci retain a raised center.**

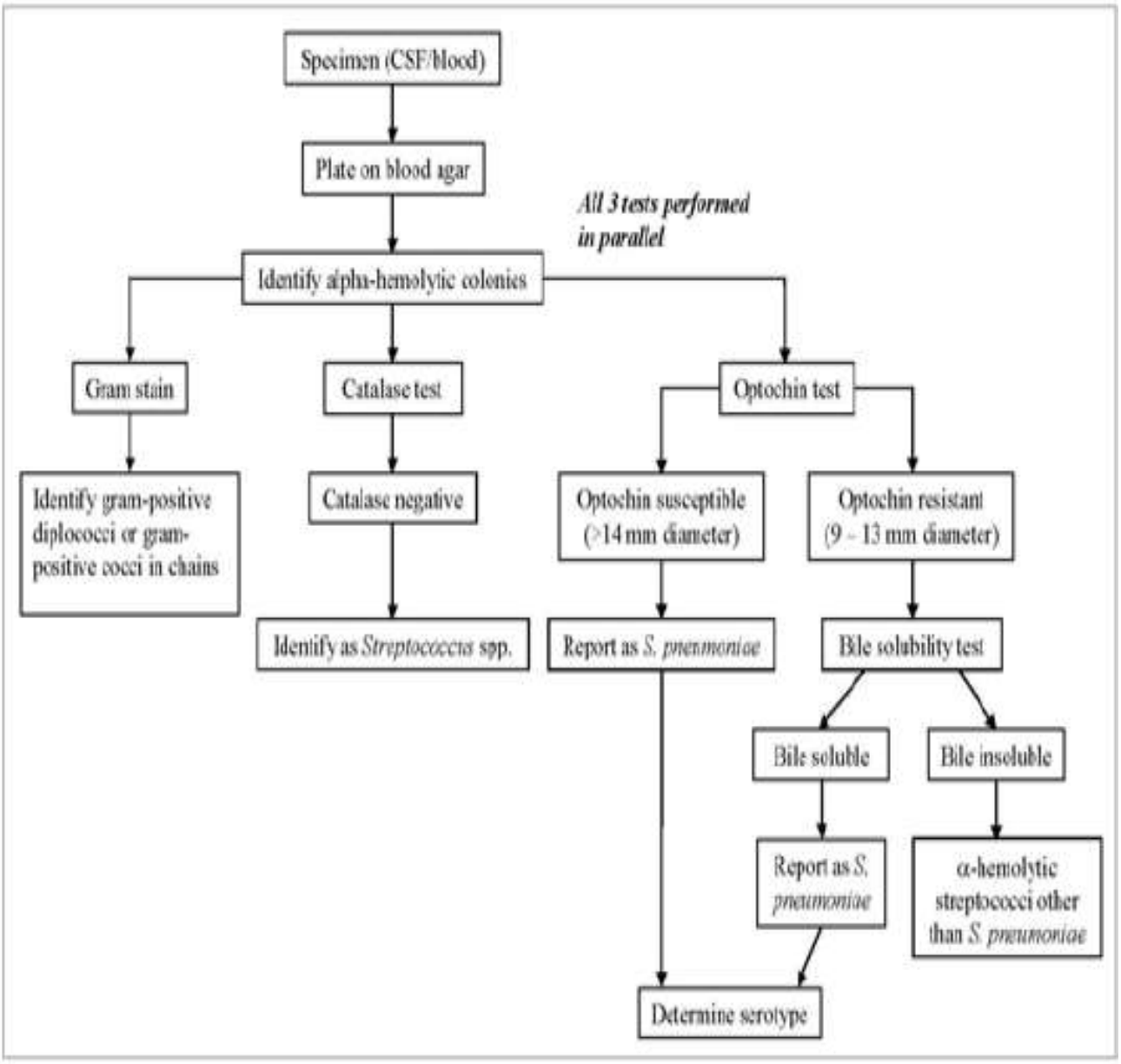


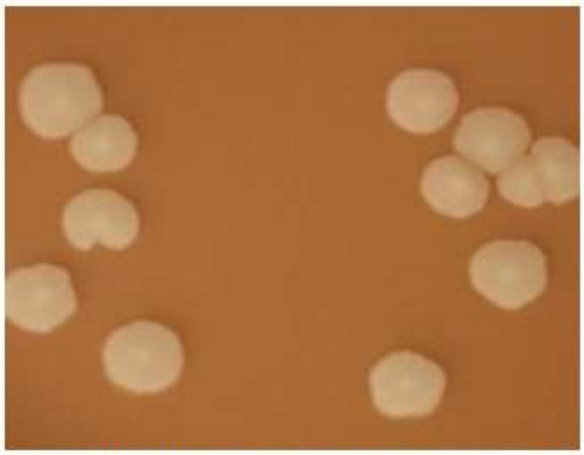

**Figure (7): Flow chart for identification and characterization of a *Strep. pneumo* isolate.**

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

***Haemophilus Influenzae*** are small, pleomorphic, **gram-negative bacilli** or coccobacilli with random arrangements. ***H. influenzae*** is a fastidious organism, which grows best at 35-37°C with ~5% $CO_2$ (or in a candle-jar) and requires **hemin** (X factor) and **nicotinamide-adenine-dinucleotide** (NAD, also known as V factor) for growth. The standard medium used for growth of ***H. influenzae*** is a **chocolate agar plate (CAP),** which can be prepared with heat-lysed horse blood, a good source of both hemin and NAD, although sheep blood can also be used. Growth occurs on a CAP because NAD has released from the blood during the heating process of chocolate agar preparation and hemin is available from non-hemolyzed as well as hemolyzed blood cells. ***H. influenzae*** appear as **large, round, smooth, convex, colorless-to-grey, cloudy colonies on a CAP (Figure 9).** ***H. influenzae*** **produce a sharp indol smell,** plates should not be opened in order

to smell the cultures. ***H. influenzae*** cannot grow on an unsupplemented Blood Agar Plate. **(Figure 10). Biochemical tests** have recommended confirming the identity of cultures that morphologically appear to be ***H. influenzae***. ***H. influenzae*** caught identified using **Kovac's oxidase** test and determining the necessity of hemin and **NAD as growth** requirements. If the **oxidase test is positive**, hemin and **NAD growth factor** requirement testing should **have performed.** If the growth factor requirement test indicates that the isolate may be ***H. influenzae*****, serological tests** to identify the serotype should have performed. This sequence of testing is an efficient way to save costly antisera and time. **Additional methods** for identification and characterization of ***H. influenzae*** using molecular tools like PCR technique. Some of most common bacterial causes summarized at table (1).

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



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

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

| 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. The latter has associated with possible changes in the weather, from winter to summer and vice versa, bacterial infection may come second to viral infection in this aspect. Accidental infection is the infection that man acquired during daily life.**

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

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

**<u>Sore throat</u>** 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)

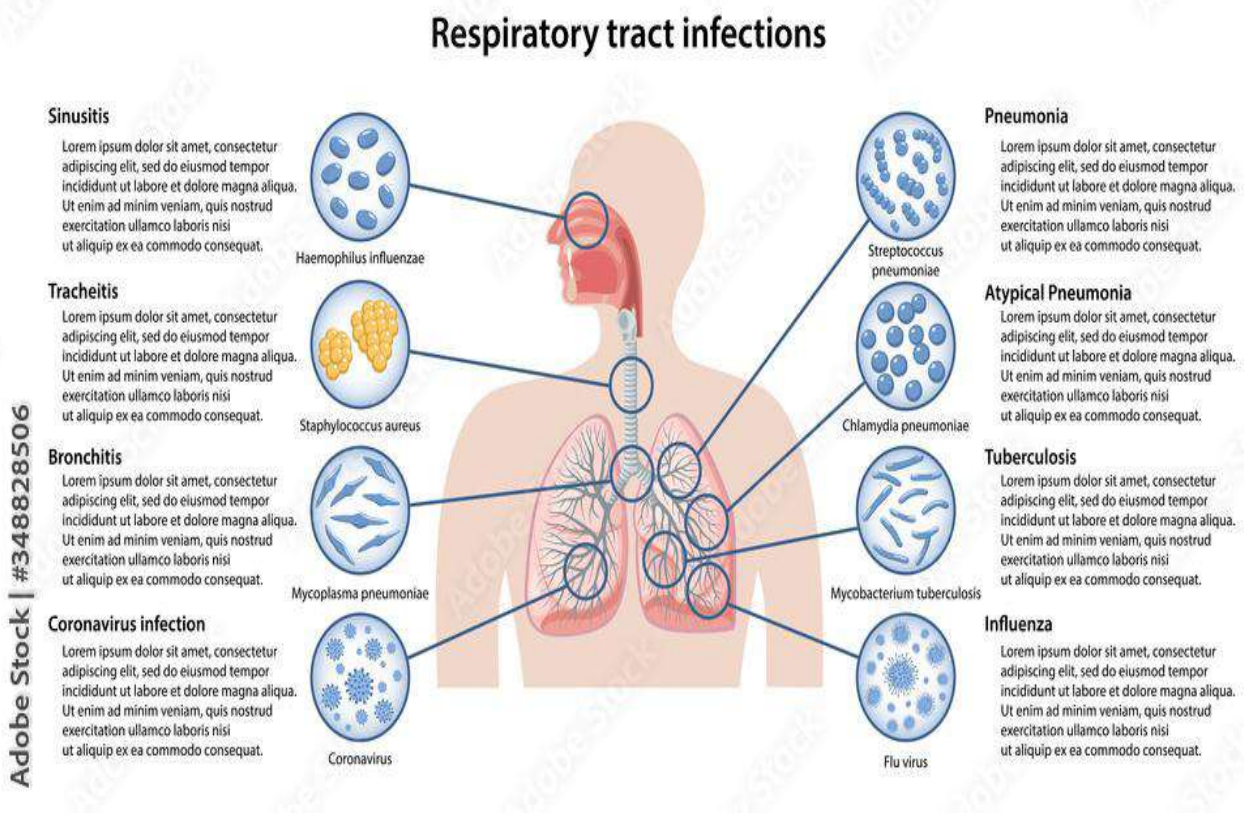



Fig. (1): Summary of bacterial respiratory tract infection

# 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 pneumonia; Haempphilus influenza; Mycoplasma pneumoniae Branhamella catarrhalis.***

**Laboratory diagnosis:**

Specimen: **Sputum**

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

**Pneumonia:** It is infection of the lung parenchyma.

**Causative agents**: ***Strep. 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 secretion** which indicated by **greater than 25 Neutrophils and less than 10 squamous epithelial cells per high power field.**

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

## Bacterial Diagnosis of TB infection

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

The main problem of these bacteria is:

1. Their high resistance to environmental stress such as dryness.
2. Survive in dry sputum for months.

3. Members of genus mycobacterium are very resistant to chemical and antibiotic treatment.

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

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

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

## Lab. diagnosis:

Mycobacterium may come from a wide range of samples, these include; **sputum, lung wash, urine, wound, CSF, lymph secretion, bone, gastro-intestinal material.** The prime diagnostic parameter is **culturing of materials** (regardless the origin of it) on suitable culture medium, the medium commonly used is (**L-J M**), enriched media with **high contents** of **nutrition** to aid the **long period of incubation**. TB bacilli

appear as **hydrophobic colonies with wrinkled (crumpled) surface**. Because of long time of incubation,

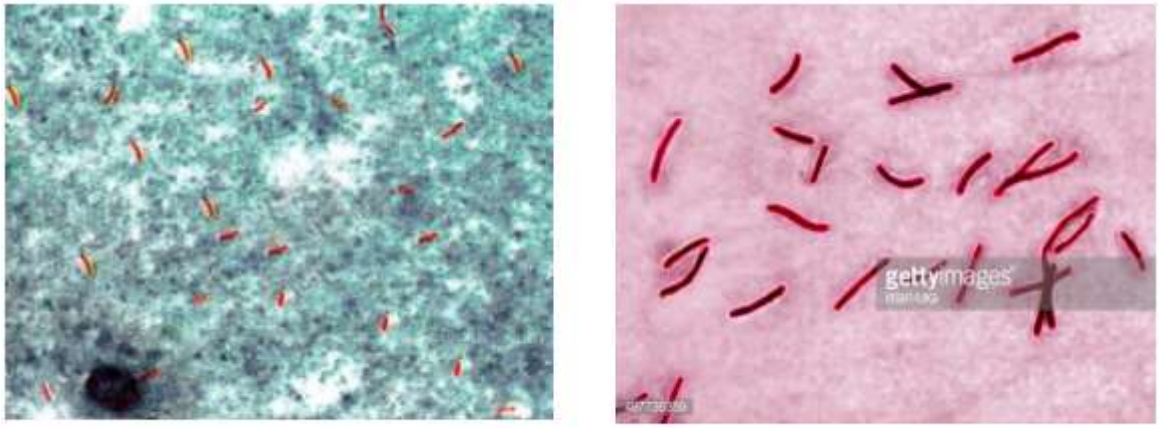


**Fig.2 :*Mycobacterium tuberculosis* in Ziehl-Neelsen stained smear**

**An 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 diphtheria*e**

**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 diphtheria**) as shown in **figure-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)**

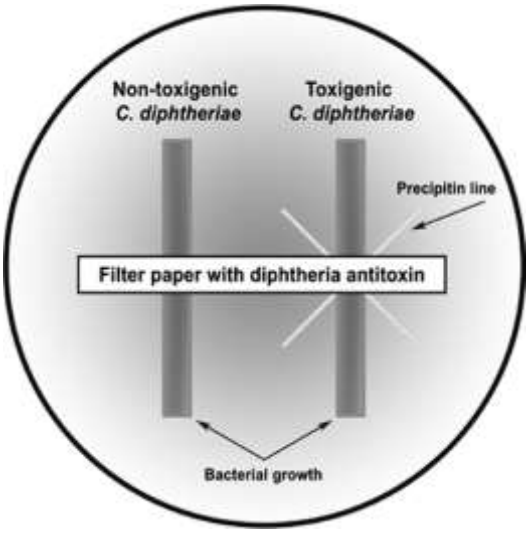



**Figure-3: Procedure of Elek immunodiffusion test. A Sterile filter paper impregnated with diphtheria antitoxin is imbedded in agar culture medium. Isolates of *C. diphtheriae* are then streaked across the plate at an angle of 90° to the antitoxin strip. Toxigenic *C diphtheria*.**

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 disease: 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).

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# Infection 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.

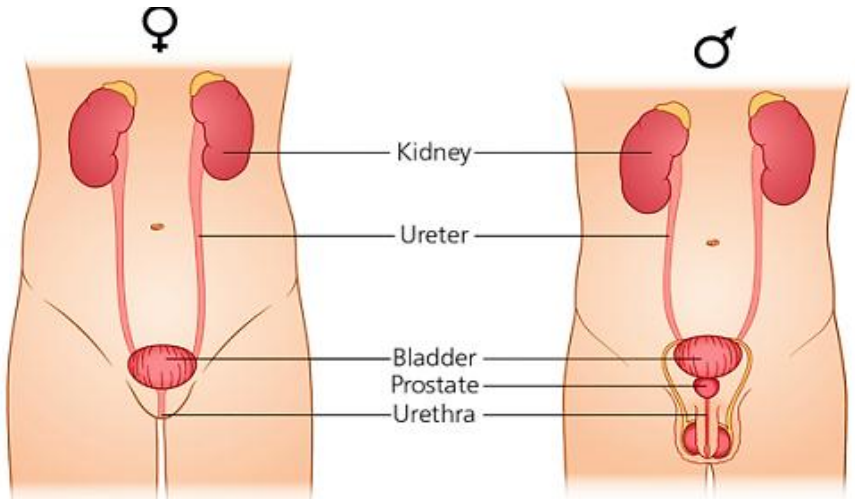


**Figure (1): Male and female urinary tract system**

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

**Some terminologies that you should to know:**

- **Pyelonephritis:** infection of the 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*.** that produce urease 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**

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** ( hematogenous).

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. **Valvelike mechanism** at the junction of the 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 infection

### 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:**

The presence of one bacterium in un-centrifuged gram stained urine confirms urinary tract infections.

**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*

## Laboratory 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*

**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**: Urethral discharge or swab (Before urination or 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**: *Neisseria gonorrhea* (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.

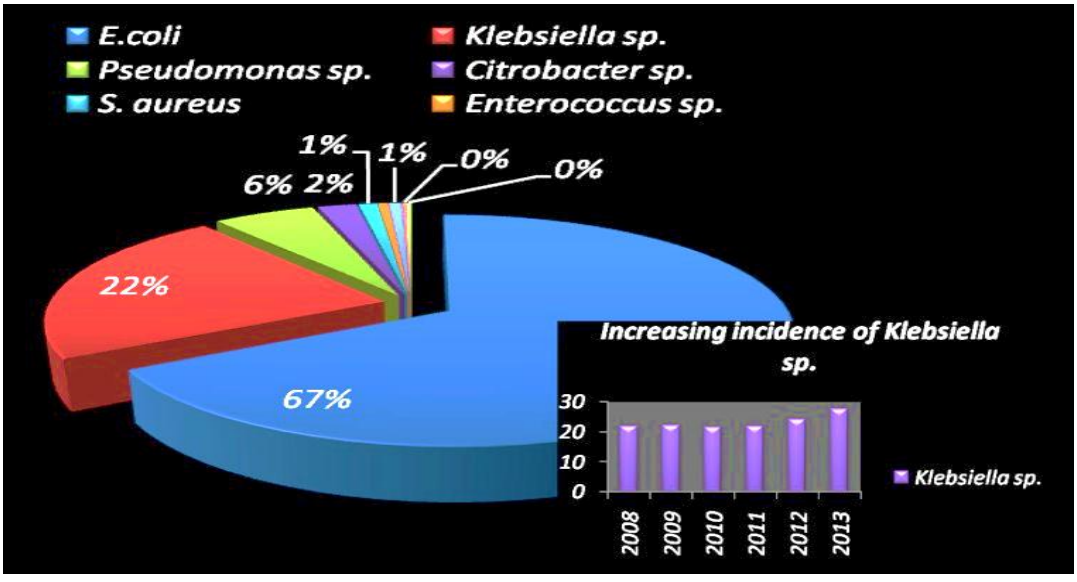


**Figure (2): Percentages of UTIs bacterial causes**