وزارة التعليم العالي والبحث العلمي
الجامعة التقنية الجنوبية
المعهد التقني العمارة
قسم تقنيات المختبرات الطبية

الحقيبة التدريسية لمادة بكتريا مرضية عملي

الصف: الثاني

تدريسي المادة

م.د. رواء صادق مجيد

الفصل الدراسي الثاني

جدول مفردات مادة بكتريا مرضية عملي

Practical syllabus	
Week	Topics
1	Genus Staphylococcus General characters, Lab. diagnosis, coagulase test, catalase test.
2	Streptococcus General characters, Lab. diagnosis, sensitivity to bastracin. Treatment.
3	Genus Pneumococcus General characters, Lab. diagnosis, Optochin.
4	Corynebacterium: General characters, Lab. diagnosis, Eleck test.
5	Mycobacterium: General characters, Lab. diagnosis, Z.N. stain, petroffs method.
6	Genus Bacillus General characters, spore forming, aerobic. Lab. diagnosis.
7	Clostridium: General characters, spore forming, anaerobic. Lab. diagnosis, macntosh jar.
8	Neisseriae: General characters, oxidase test, Lab. diagnosis, growth requirements.
9	Haemophilus: General characters, X and V factors, Lab. diagnosis, satellitism phenomena.
10	Family Enterobacteriaceae General characters, G ve- Bacilli, Imvic test. Types of culture media.
11	E.coli General characters, lactose fermenter. Lab. diagnosis.
12	Klebsiella General characters. Lab. diagnosis, lactose fermenter, Imvic test.
13	Proteus General characters. Lab. diagnosis, non-lactose fermenter , Classification of species.
14	Salmonella and Shigella General characters. Lab. diagnosis.
15	Pseudomonas: General characters. Lab. diagnosis, types of pigments, oxidase test. Vibirio General characters. Lab. diagnosis.

الهدف من دراسة مادة بكتريا مرضية (الهدف العام):

تهدف دراسة مادة بكتريا مرضية للصف الثاني إلى:

التعرف على البكتريا الممرضة للإنسان وإمراضيتها وتشخيصها.

الفئة المستهدفة: طلبة الصف الثاني/قسم تقنيات المختبرات الطبية.

التقنيات التربوية المستخدمة:

1- سبورة وأقلام.

2- السبورة التفاعلية.

3- عارض شاشة Data Show.

4- جهاز حاسوب محمول Laptop.

الأسبوع الأول

الهدف التعليمي: التعرف على بكتريا المكورات العنقودية وطرق تشخيصها.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

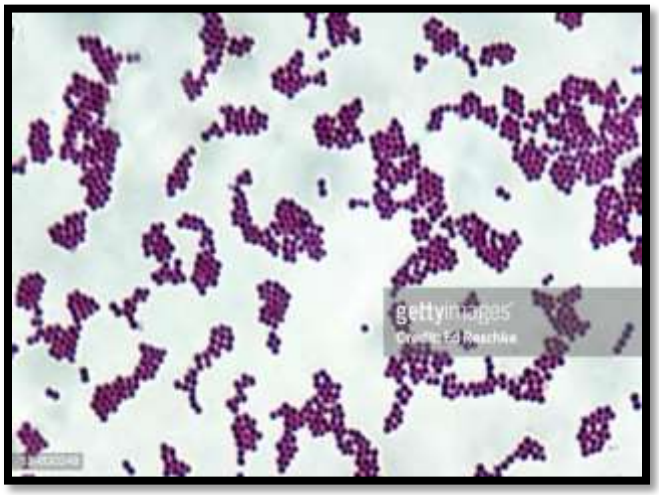
أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة:

Genus: *Staphylococcus*

General Characters:

Staphylococci, often referred to simply as "staph," are a group of Gram-positive bacteria that belong to the genus Staphylococcus. These bacteria are commonly found on the skin and mucous membranes of humans and animals. While many staphylococcal species are harmless and part of the normal human flora, some can cause various infections when they enter the body or when their numbers become imbalanced.



Laboratory Diagnosis of Staphylococci:

Laboratory diagnosis of staphylococcal infections involves a series of microbiological tests and techniques to identify and characterize staphylococcus bacteria.

1- Sample Collection:

The first step is to collect clinical samples from the patient suspected of having a staphylococcal infection. Common samples include:

- **Wound Swabs**: for skin and soft tissue infections.
- **Blood Cultures**: for suspected bloodstream infections.

2- Sample Processing: the collected samples are processed in the laboratory to isolate the bacteria. This often involves streaking the sample onto specific agar plates like blood agar or Mannitol Salt Agar (MSA).

3- Culture and Identification:

Blood Agar: staphylococci typically grow on blood agar plates and can be differentiated based on their hemolytic activity (alpha or beta hemolysis) and colony characteristics.

Mannitol Salt Agar (MSA): *Staphylococcus aureus* ferments mannitol, leading to a change in the color of the agar. This helps differentiate it from other staphylococci.

4- Gram Staining: staphylococci are Gram-positive cocci arranged in clusters or chains. Gram staining is the initial step in identifying the bacteria.

5- Biochemical Tests: additional biochemical tests may be performed to further characterize the staphylococcus species. These tests may include catalase testing (staphylococci are catalase-positive) and various sugar fermentation tests.

Coagulase Test: this test helps differentiate between *Staphylococcus aureus* (coagulase-positive) and other coagulase-negative staphylococci (CoNS). The coagulase test detects the production of coagulase enzyme by Staphylococcus aureus.

6- Antibiotic Susceptibility Testing: it is crucial to determine the susceptibility of the isolated Staphylococcus strain to antibiotics, especially if it is *Staphylococcus aureus*. This helps guide

antibiotic therapy and can identify methicillin-resistant strains (MRSA).

7- Molecular Techniques: in some cases, molecular techniques like polymerase chain reaction (PCR) may be used to detect specific genes associated with antibiotic resistance, such as the mecA gene in MRSA.

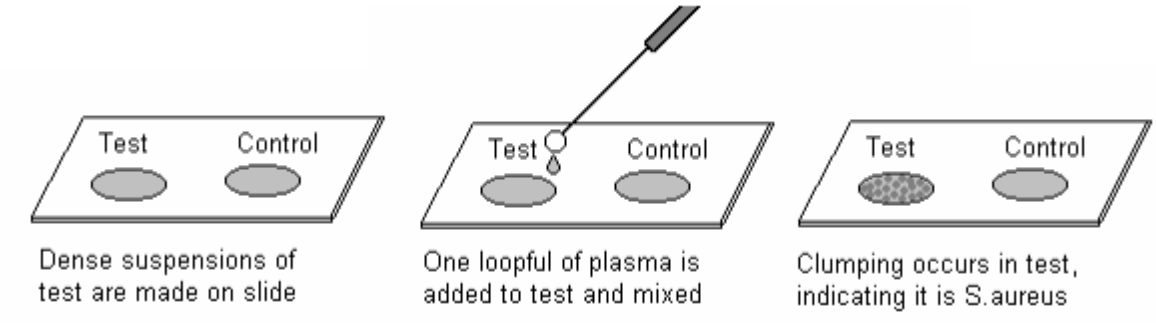
8- Serological Tests: in certain situations, serological tests may be used to detect specific antibodies against staphylococcus antigens, especially in cases of toxin-mediated diseases like toxic shock syndrome or staphylococcal scalded skin syndrome.

Coagulase Test:

Staphylococcus aureus is known to produce coagulase, which can clot plasma into gel in tube or agglutinate cocci in slide. This test is useful in differentiating *S. aureus* from other coagulase-negative staphylococci. Most strains of *S. aureus* produce two types of coagulase, free coagulase and bound coagulase. While free coagulase is an enzyme that is secreted extracellularly, bound coagulase is a cell wall associated protein. Free coagulase is detected in tube coagulase test and bound coagulase is detected in slide coagulase test. Slide coagulase test may be used to screen isolates of *S. aureus* and tube coagulase may be used for confirmation.

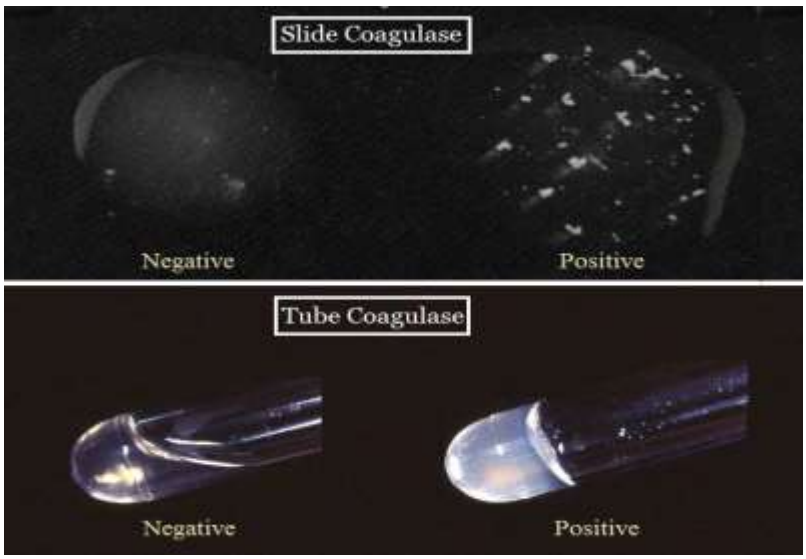
Procedure:

a. Slide method:



b. Tube method:

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



Catalase Test:

To determine the ability of bacteria that produces catalase enzyme which degrades the hydrogen peroxide . In aerobic organisms , during aerobic respiration , oxygen serves as hydrogen acceptor and hydrogen peroxide is formed in the cell. High concentration of H_2O_2 is formed which is toxic to cell . Bacteria possess the catalase enzyme converts hydrogen peroxide into oxygen and water.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Procedure:

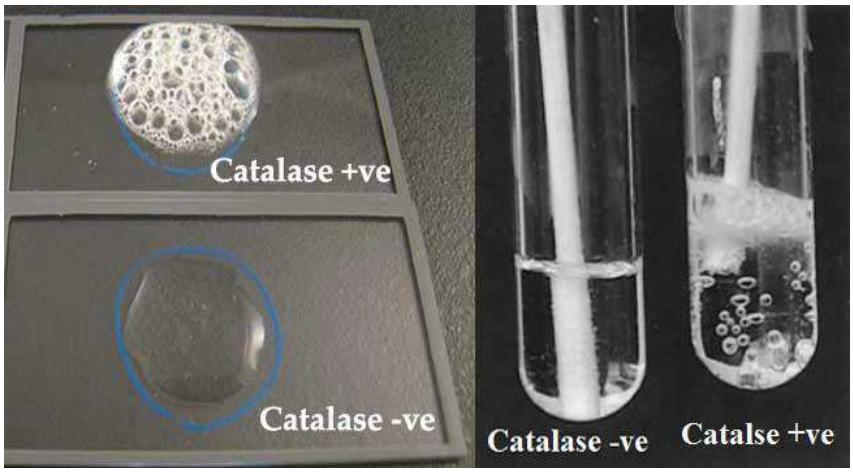
Catalase production can be determined by addition of the substrate H_2O_2 on bacterial culture.

a. Slide method:

- Pure growth of the organisms will transfer to the clean slide by using inoculation loop or glass rod.
- Immediately add a drop of 3% hydrogen peroxide on bacterial culture.
- Observe the bubble formation.

b. Tube method:

- Take one ml of 3 % hydrogen peroxide in test tube.
- Small amount of bacterial culture introduce into the solution
- Immediately observe the effervescence.



الأسبوع الثاني

الهدف التعليمي: التعرف على بكتريا المكورات المسبحية وطرق تشخيصها .

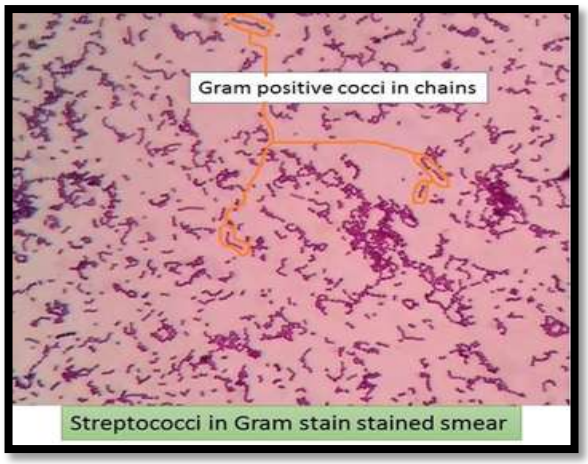
مدة المحاضرة: 4 ساعات.

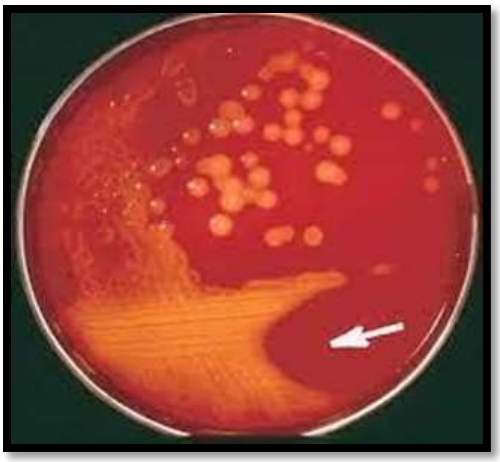
الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Genus: *Streptococcus***

They are important human pathogens causing pyogenic infection with a characteristic tendency to spread. They are also responsible for non-suppurative lesions like acute rheumatic fever and glomerulonephritis.





Laboratory Diagnosis of Streptococci:

The laboratory diagnosis of streptococcal infections involves a series of microbiological tests and techniques to identify and characterize Streptococcus bacteria. Accurate diagnosis is crucial for determining the type of Streptococcus involved (e.g., Group A Streptococcus or Group B Streptococcus) and for selecting appropriate treatment options. Here are the typical steps involved in the laboratory diagnosis of streptococci:

1. Sample Collection: clinical samples are collected from the patient suspected of having a streptococcal infection. Common samples include:

- Throat Swabs: for diagnosing strep throat caused by Group A Streptococcus (*Streptococcus pyogenes*).

- Blood Cultures: for suspected bloodstream infections.

- Sputum or Respiratory Secretions: for respiratory infections.

- Skin Lesions or Wound Swabs: for skin and soft tissue infections.

2. Sample Processing: the collected samples are processed in the laboratory to isolate the Streptococcus bacteria. This may involve streaking the sample onto specific agar plates like blood agar.

3. Culture and Identification:

Blood Agar: streptococci typically grow on blood agar plates. They can be differentiated based on hemolytic activity, including alpha-hemolysis (incomplete hemolysis), beta-hemolysis (complete hemolysis), or gamma-hemolysis (no hemolysis). Group A Streptococcus (*Streptococcus pyogenes*) typically exhibits beta-hemolysis.

4. Gram Staining: streptococci are Gram-positive cocci arranged in chains or pairs. Gram staining is the initial step in identifying the bacteria.

5. Biochemical Tests: additional biochemical tests may be performed to further characterize the Streptococcus species. These tests may include catalase testing (streptococci are catalase-negative), bile solubility testing, and specific sugar fermentation tests.

6. Antibiotic Susceptibility Testing: determining the susceptibility of the isolated Streptococcus strain to antibiotics is important, especially for cases involving beta- hemolytic streptococci like Group A Streptococcus.

7. Antigen Detection Tests: rapid antigen detection tests, such as the rapid strep test, can be used to quickly identify Group A Streptococcus in throat swab samples. These tests detect

specific antigens associated with the bacterium.

8. Molecular Techniques: in some cases, molecular techniques like polymerase chain reaction (PCR) may be used to detect specific genes associated with streptococcal species or antibiotic resistance.

9. Serological Tests: in certain situations, serological tests may be used to detect specific antibodies against Streptococcus antigens, particularly in cases of post-streptococcal complications like rheumatic fever or post-streptococcal glomerulonephritis.

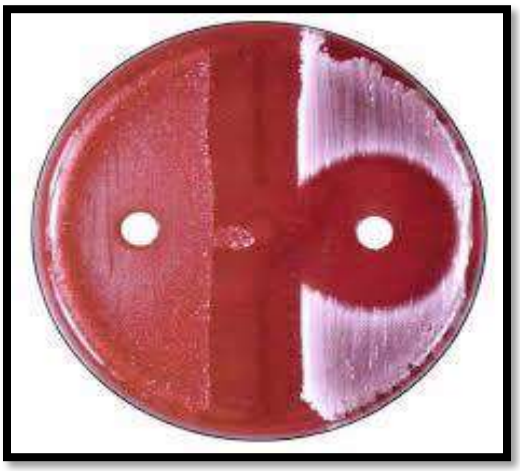
Bacitracin Sensitivity Test:

Bacitracin sensitivity test is commonly used to distinguish between the b-hemolytic streptococci: *Streptococcus agalactiae* (bacitracin resistant) and *Streptococcus pyogenes* (bacitracin sensitive).

Procedure:

A disk saturated with a small amount of bacitracin (0.04 units) is placed on an agar plate, allowing the antibiotic to diffuse into the medium and inhibit the growth of susceptible organisms. After incubation, the inoculated plates are examined for zones of inhibition surrounding the disks.

If the organism grows up to the edge of the disk, it is resistant to the antimicrobial compound infusing the disk. If there is a zone around the edge of the disk where the organism has not grown, the organism is susceptible to the antimicrobial in the disk.



Treatment: streptococcal infections are treated with antibiotics, and the choice of antibiotic depends on the specific species and its susceptibility to drugs. For example, penicillin is often effective against Group A Streptococcus, but antibiotic resistance can be a concern.

الأسبوع الثاني

الهدف التعليمي: التعرف على بكتريا المكورات المسبحية الرئوية وطرق تشخيصها .

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: ***Streptococcus pneumoniae***

General Characters:

Streptococcus pneumoniae, also known as pneumococcus, is a Gram-positive, lancet-shaped bacterium that typically occurs in pairs (diplococci) or short chains. It's a facultative anaerobe, meaning it can survive with or without oxygen, and is non-motile and non-spore-forming. A key characteristic is its polysaccharide capsule, which helps it evade the host's immune system and is a major virulence factor. It's a common cause of pneumonia, especially in children and the elderly, and can also cause other infections like meningitis and sepsis.

Laboratory Diagnosis of *Streptococcus pneumoniae*:

Laboratory diagnosis of Streptococcus pneumoniae involves a combination of methods, including microscopy, culture, and biochemical tests. Microscopy reveals characteristic lancet-shaped, Gram-positive diplococci. Culture on blood agar shows α-hemolysis and characteristic colony morphology. Biochemical tests like optochin susceptibility and bile solubility further confirm the identification.

1- Microscopy: *S. pneumoniae* appears as lancet-shaped , Gram-positive diplococci or short chains under the microscope.

2- Culture: *S. pneumoniae* is typically grown on blood agar, where it exhibits α-hemolysis (a greenish discoloration around the colony) . Typical colonies are round , flat , smooth , and translucent . The quellung reaction, where the bacterial capsule becomes visually enhanced after reaction with specific antisera, can also be used for identification.

3- Biochemical Tests:

- Optochin susceptibility: *S. pneumoniae* is sensitive to optochin (ethylhydrocupreine hydro-chloride), while other viridans group streptococci are typically resistant.

- Bile solubility: *S. pneumoniae* is soluble in bile, which can be used to differentiate it from other streptococci.

- **Catalase test:** S. pneumoniae is catalase-negative, unlike some other respiratory bacteria.

4- Urinary antigen tests: these tests detect pneumococcal antigens in urine and are useful for diagnosing pneumococcal pneumonia, particularly in adults.

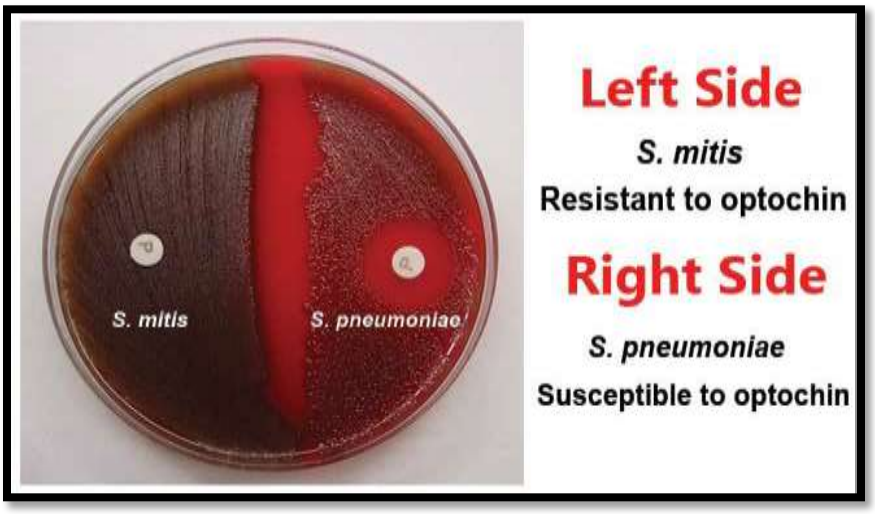
5- Molecular assays: PCR tests can quickly identify *S. pneumoniae* DNA from various samples, including CSF in cases of suspected meningitis.

6- Procalcitonin and CRP: these biomarkers can be helpful in distinguishing bacterial from viral pneumonia , and higher levels may be associated with more severe pneumococcal

pneumonia.

Optochin Sensitive Test:

This test is used to distinguish *Streptococcus pneumoniae* (optochin sensitive) from other α-hemolytic streptococci (optochin resistant) *Streptococcus mitis*.



الأسبوع الرابع

الهدف التعليمي: التعرف على البكتريا الوتدية وطرق تشخيصها.

مدة المحاضرة: 4 ساعات.

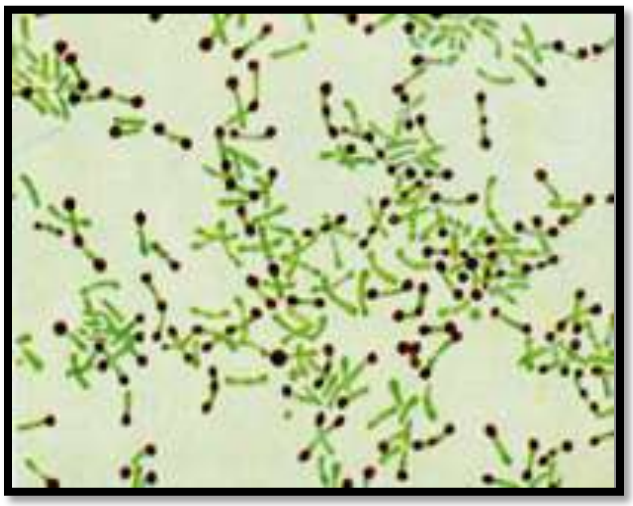
الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Genus: *Corynebacterium***

General Characters:

Corynebacterium is a genus of Gram-positive, non-spore-forming, rod-shaped bacteria commonly found in various environmental as well as on the skin and mucous membranes of humans and animals. While many Corynebacterium species are harmless and part of the normal microbiota, some can cause diseases in humans and animals.



The Laboratory Diagnosis :

1-Sample Collection:

Clinical specimens, such as throat swabs, nasal swabs, or lesions from suspected diphtheria cases, are collected.

2- Culture:

Clinical specimens are cultured on selective media that support the growth of *Corynebacterium diphtheriae* while inhibiting the growth of other bacteria. Löffler's serum medium and tellurite-containing media (e.g., Tinsdale agar) are commonly used. Plates are incubated at 35-37°C in an aerobic atmosphere for 24-48 hours.

3- Gram Stain:

In Gram staining, *Corynebacterium diphtheriae* appears as Gram-positive, non- spore-forming, irregularly shaped rods (bacilli). It often Laboratory diagnosis of *Corynebacterium diphtheriae*.

4- Catalase Test: corynebacterium species are typically catalase-positive, meaning they produce the enzyme catalase, which can break down hydrogen peroxide into water and oxygen.

5- Schick test: in 1913 Schick described test based on the fact that when a minute amount of diphtheria toxin is introduced intradermal it exerts a local destructive or necrotic effect on the cells of the skin and the underling tissue, if the blood passing through the tissue contains sufficient antitoxin , 1/500 to 1/250 or more of a unit of antitoxin per ml , the injected toxin is neutralized and thus no reaction occurs . The reaction in susceptible person having less than certain amount of the antitoxin in the blood , show visible local reaction . this reaction has been widely applied with a view of gauging immunity or susceptibility to diphtheria.

Elek Test:

This test is used to determine the toxigenic and nontoxigenic strains of *C. diphtheriae*.

Method:

1. A filter paper strip previously immersed in diphtheria antitoxin is incorporated onto serum agar in the center.
2. *Corynebacterium diphtheriae* is streaked onto the agar at right angles to the filter paper strip.

3. Incubate the plate for 24 hours at 37° C.

4. Observe for lines of precipitation on serum agar.

الأسبوع الخامس

الهدف التعليمي: التعرف على البكتريا المتفطرة وطرق تشخيصها.

مدة المحاضرة: 4 ساعات.

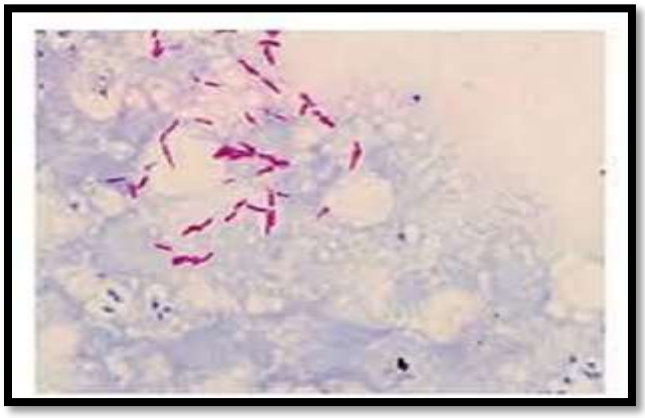
الأنشطة المستخدمة: أسئلة عصف ذهني.

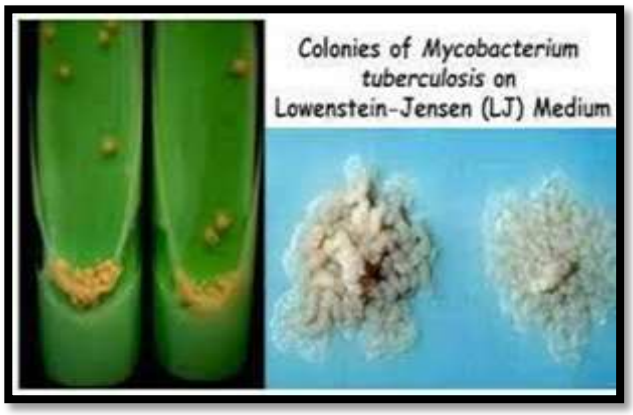
أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Genus: *Mycobacterium***

General Characters:

Mycobacteria are a group of bacteria that belong to the family Mycobacteriaceae. They are characterized by their unique cell wall structure, which includes a high lipid content, particularly mycolic acids, giving them distinctive staining properties and resistance to many common bacterial staining methods. Mycobacteria can be divided into two main groups: pathogenic and non-pathogenic.





Laboratory Diagnosis of Mycobacterium:

1- Staining: the most common staining method for mycobacteria is the acid-fast stain, also known as the Ziehl-Neelsen stain. This stain highlights the mycolic acid in the cell wall, causing the bacteria to appear red against a blue background.

2- Culture: mycobacteria are slow-growing organisms, and cultures may take several weeks to yield results. The culture is performed on selective media, such as Lowenstein- Jensen agar, and is incubated at a specific temperature (usually 37°C) for an extended period.

3- Molecular Tests: polymerase chain reaction (PCR) and nucleic acid amplification tests (NAATs) are used for the rapid detection of mycobacterial DNA, making them valuable for diagnosing tuberculosis and other mycobacterial infections.

4- Serological Tests: serological tests are not as commonly used for mycobacterial diagnosis compared to other bacteria. However, they may be employed in some cases, such as serological tests for leprosy.

5- Imaging: radiological imaging, such as chest X-rays, may be used to assess the extent of mycobacterial infections, especially in cases of tuberculosis.

6- Drug Susceptibility Testing: testing the susceptibility of mycobacterial isolates to various antibiotics, including first-line and second-line drugs for tuberculosis, is essential for guiding treatment.

Ziehl-Neelsen's Stain(Acid Fast Stain):

It is used for staining *Mycobacterium tuberculosis* which is hardly stained by gram stain because its walls contain in addition to the components of its basic cellular wall, a waxy lipid substance called mycolic acid make it not permeable for stains. The Mycobacteria is stained with primary stain, it cannot be decolorized with acid, so named as acid-fast bacteria. Acid fast bacilli (red or pink) and back ground (blue).

Stain Reagents:

1- Carbol-fuchsin (primary stain).

2- Acid-alcohol (decolorizer).

3- Methylene blue or malachite green (counter stain).

Procedure:

1- Prepare the smear from the culture or sputum and fix it by passing through the flame.

2- Cover the slide with carbol-fuchsin.

3- Heat gently until vapor rises (do not boil it) and wait for 3-5 minutes.

4- Wash the carbol-fuchsin with tap water.

5- Cover the slide with acid-alcohol for short time, repeat this step until the slide appear pink in color.

6- Wash the acid-alcohol with tap water.

7- Cover the slide with methylene blue for 1 minute.

8- Wash the methylene blue with tap water.

9- Let the slide air-dry.

10- Examine the slide under the oil immersion objective lens.

Petroff's Method:

The sputum is transfered to a sterile test tube and equal amount of sterile 4% NaOH is added on it. The tube is incubated at 37° C for 30 minutes with strong shaking every 5 minutes. The mixture is centrifuged at 3000 rpm for 30 minutes and supernatant is discard. The deposit is naturalized by 10 N HCl using a drop of phenol red as indicator. From the deposit, Lowenstein-Jensen medium is inoculated. The culture slant is incubated at 37° C. The growth is observed at the first week and then at weekly intervals for 8 weeks.

الأسبوع السادس

الهدف التعليمي: التعرف على البكتريا العصوية وطرق تشخيصها .

مدة المحاضرة: 4 ساعات.

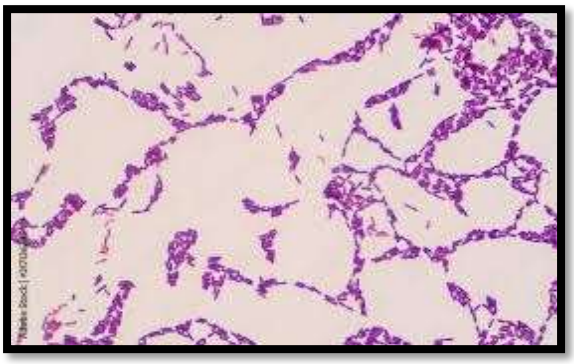
الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Genus: *Bacillus***

General Characters:

Bacillus is a genus of gram-positive, rod-shaped bacteria that are commonly found in various environments, including soil, water, and the gastrointestinal tracts of animals. While many Bacillus species are harmless and play important roles in nature, some can be pathogenic to humans and animals.



Spore Formation: one of the notable features of many Bacillus species is their ability to form endospores, also known as bacterial spores. These spores are highly resistant to harsh environmental conditions, such as heat, radiation, and desiccation, allowing Bacillus to survive in adverse conditions for extended periods.

Aerobic Nature: most Bacillus species require oxygen for growth and are classified as aerobic bacteria.

Laboratory Diagnosis of Bacillus:

The laboratory diagnosis of Bacillus infections or the identification of Bacillus species in various contexts typically involves a combination of microbiological and molecular techniques. Here are the key steps involved in the laboratory diagnosis of Bacillus:

1- Sample Collection: clinical or environmental samples suspected of containing Bacillus bacteria are collected and properly labeled. The source of the sample will vary depending on the context, such as clinical specimens (e.g., blood, tissue, wound swabs) or environmental samples (e.g., soil, water, food).

2- Culture: the sample is streaked onto appropriate culture media. For Bacillus, common culture media include nutrient agar and blood agar. These bacteria typically grow as colonies that are cream-colored and can be identified based on their morphology (rod- shaped) and other characteristics.

3- Gram Staining: a Gram stain of the sample can provide initial information about the bacterial morphology and whether the bacteria are gram-positive (as Bacillus species typically are) or gram-negative.

4- Biochemical Tests: biochemical tests are used to confirm the identity of the isolated Bacillus species. These tests may include catalase testing (Bacillus species are usually catalase-positive) and other metabolic test.

5- Spore Staining: to confirm the presence of endospores, spore staining techniques such as the Schaeffer-Fulton or Malachite green staining methods can be used. This helps differentiate Bacillus species from other bacteria.

6- Molecular Identification: for more accurate species-level identification and differentiation, molecular methods such as Polymerase Chain Reaction (PCR) and DNA sequencing can be employed. Specific genes, such as the 16S rRNA gene, can be targeted for sequencing to determine the exact species.

7- Antibiotic Susceptibility Testing: if the Bacillus strain is associated with an infection, antibiotic susceptibility testing can be performed to determine the most effective antibiotics for treatment.

8- Toxin Detection: in cases where the Bacillus species is known to produce toxins, such as Bacillus cereus, toxin assays can be conducted to confirm toxin presence.

9- Serological Tests (If applicable): for certain Bacillus-related diseases, such as anthrax caused by Bacillus anthracis, serological tests for specific antibodies may be conducted.

الأسبوع السابع

الهدف التعليمي: التعرف على البكتريا المطثية وطرق تشخيصها .

مدة المحاضرة: 4 ساعات.

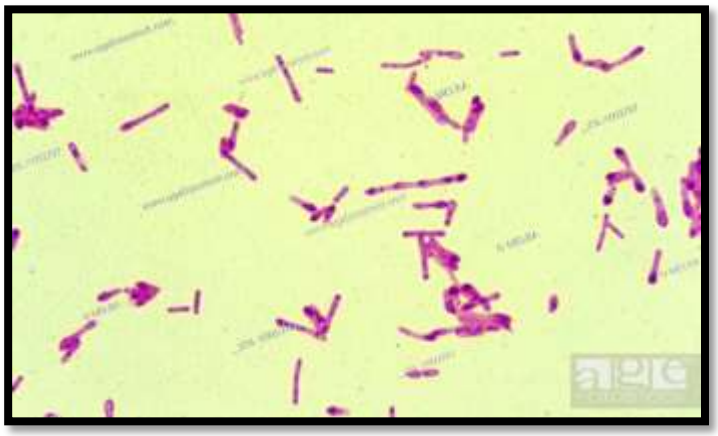
الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Genus: *Clostridium***

General Characters:

Clostridium is a genus of gram-positive, anaerobic bacteria known for their ability to form endospores (resistant, dormant structures) under unfavorable conditions. This genus encompasses a wide range of species, some of which are harmless and play essential roles in natural environments, while others can be pathogenic to humans and animals. Here are some key points about Clostridium:



Anaerobic Nature: clostridium species are obligate anaerobes, meaning they thrive in environments devoid of oxygen. They can cause infections in deep tissues and body cavities where oxygen levels are low.

Endospore Formation: one of the hallmark features of Clostridium is their ability to form endospores. These spores are highly resistant to heat, desiccation, and disinfectants, allowing Clostridium species to survive harsh conditions.

Lab Diagnosis:

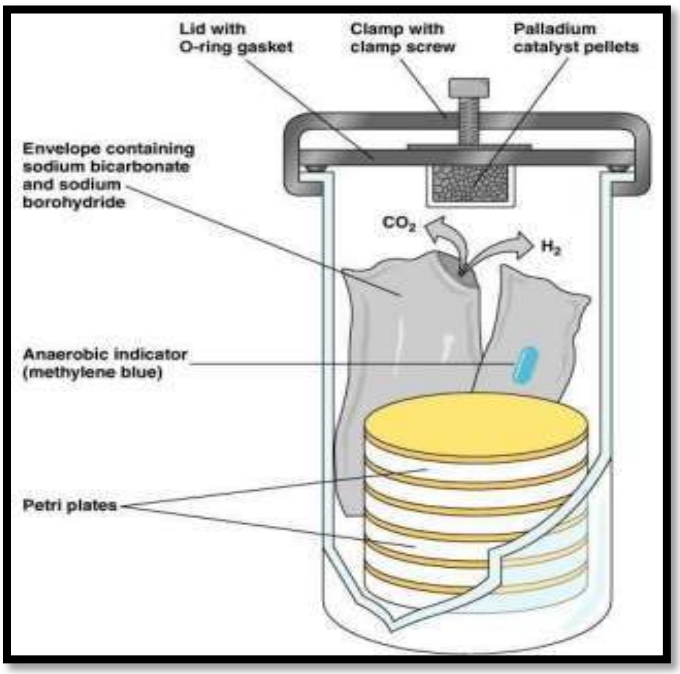
The laboratory diagnosis of clostridium infections typically involves the isolation of the bacteria from clinical samples, followed by confirmation and identification. This may include:

1- Culture: clostridium species are cultured under anaerobic conditions using specialized media like anaerobic blood agar or Reinforced Clostridial Agar (RCA).

2- Biochemical Testing: biochemical tests, such as the indole test and hydrogen sulfide production test, can help identify clostridium species.

3- Molecular Identification: PCR and DNA sequencing of specific genes are used for precise identification, especially in cases of suspected pathogenic species.

McIntosh and Filde's Jar:



الأسبوع الثامن

الهدف التعليمي: التعرف على بكتريا النيسيريا وطرق تشخيصها .

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

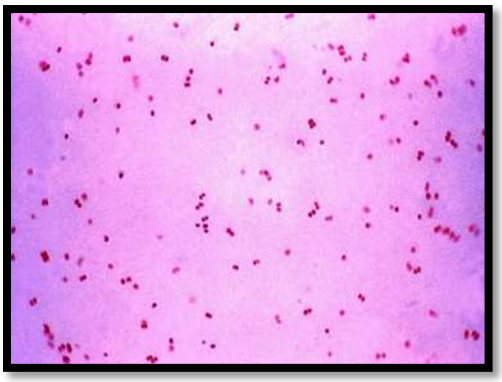
أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة:

Genus: *Neisseria*

General Characters:

Neisseria is a genus of bacteria that includes several species, some of which can be pathogenic to humans. These bacteria are gram-negative cocci, which means they have a spherical shape and a double membrane structure. The two most well-known species within the Neisseria genus are *Neisseria meningitidis* and *Neisseria gonorrhoeae*.



Laboratory Diagnosis:

1- Isolation and Cultivation: the first step is to isolate Neisseria bacteria from clinical samples or laboratory cultures. Neisseria species typically grow best in a selective medium, such as Thayer- Martin agar, which contains antibiotics to inhibit the growth of other bacteria. The bacteria are incubated at an appropriate temperature, usually around 35-37°C in an environment with increased carbon dioxide (CO_2) levels.

Morphological Characteristics: observing the shape and arrangement of Neisseria cells under a microscope can provide important information. Neisseria are typically cocci (spherical) and

often appear in pairs (diplococci).

2- Gram Staining: Neisseria bacteria are gram-negative, so they will appear pink or red under a Gram stain. This staining method helps in their initial identification.

3- Biochemical Tests: Various biochemical tests are used to further characterize Neisseria species. These tests may include catalase, oxidase, and carbohydrate fermentation tests. For example, *Neisseria gonorrhoeae* is oxidase-positive, while *Neisseria meningitidis* is oxidase-positive and ferments glucose.

4- Serological Testing: serological methods can be used to identify specific strains or serogroups of Neisseria, especially in the case of *Neisseria meningitidis*. This is important for tracking outbreaks and selecting appropriate vaccines.

5- Antibiotic Susceptibility Testing: determining the antibiotic susceptibility profile of Neisseria strains is crucial for guiding treatment. *Neisseria gonorrhoeae*, in particular, has developed resistance to multiple antibiotics, so susceptibility testing is important.

6- Molecular Techniques: polymerase chain reaction (PCR) and DNA sequencing can be employed to confirm the identity of Neisseria isolates and to study specific genes or genetic elements associated with virulence or antibiotic resistance.

7- Epidemiological Studies: bacteriological studies of Neisseria often play a vital role in epidemiological investigations during outbreaks of diseases like meningococcal disease or gonorrhea. Molecular typing techniques, such as pulsed-field gel electrophoresis (PFGE) or multilocus sequence typing (MLST), are used to trace the source and spread of infections.

Oxidase Test:

The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme.

Reagents: Kovacs oxidase reagent**.**

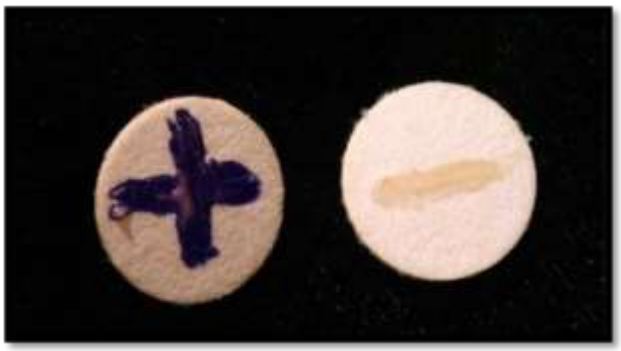
Procedure:

1- Soak a small piece of filter paper in 1% Kovacs oxidase reagent and let dry.

2- Use a loop and select a well-isolated colony from a fresh (18 - 24 hours) culture plate and rub onto treated filter paper.

3- Observe for color change.

4- Microorganisms are oxidase positive when the color changes to dark purple within 5 to10 seconds . Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds . Microorganisms are oxidase negative if the color does not changes or it takes longer than 2 minutes.



Growth Requirements:

1- Temperature: optimal growth occurs between 35-37°C (95-98.6°F).

2- Atmosphere: most Neisseria species are carboxyphilic (capnophilic), requiring a 5-10% CO_2 enriched atmosphere. This can be achieved using a CO_2 incubator or a CO_2 generating kit.

3- pH: the ideal pH for Neisseria growth is around 7.0-7.5.

4- Media: Neisseria require enriched media for growth , such as chocolate agar , blood agar , or Thayer-Martin agar (which also contains antimicrobials to inhibit other bacteria).

5- Oxygen: while generally aerobic, *N. gonorrhoeae* can grow anaerobically when a suitable electron acceptor is present.

الأسبوع التاسع

الهدف التعليمي: التعرف على البكتريا المستدمية وطرق تشخيصها.

مدة المحاضرة: 4 ساعات.

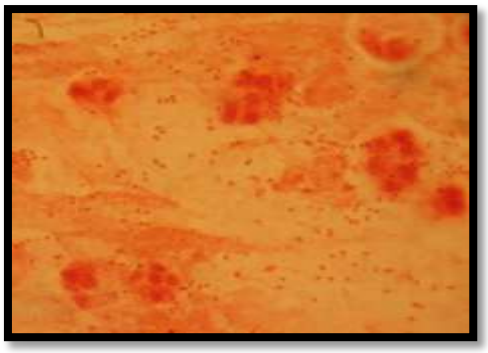
الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Genus: *Haemophilus***

General Characters:

Haemophilus is a genus of bacteria composed of small, gram-negative, facultatively anaerobic or microaerophilic organisms. Haemophilus species are known for their ability to cause various infections in humans, including respiratory tract infections, ear infections, and invasive diseases.



Laboratory Diagnosis:

1- Sample Collection: collection clinical samples that may contain Haemophilus bacteria. These samples can include throat swabs, sputum, blood, cerebrospinal fluid (CSF), or other relevant specimens, depending on the suspected infection site.

2- Sample Processing: process the collected samples to prepare them for laboratory analysis. This may involve dilution, homogenization, or other techniques to release the bacteria from the sample.

3- Culture: inoculate the processed samples onto selective culture media suitable for the growth of Haemophilus species. Chocolate agar is a commonly used medium that supports the growth of Haemophilus. Some variants of chocolate agar, known as "factor X" and "factor V" agar, provide the necessary growth factors (hemin and nicotinamide adenine dinucleotide, NAD) required for Haemophilus growth.

4- Incubation: incubate the culture plates at an appropriate temperature (typically 35-37°C) in a controlled atmosphere with increased levels of carbon dioxide (CO_2) or in a candle jar. Haemophilus species require an enriched atmosphere with higher CO_2 levels for optimal growth.

Colonial Morphology: examine the culture plates for the presence of colonies with typical Haemophilus morphology. Haemophilus colonies are often small, grayish or translucent, and may have a convex or domed appearance.

5- Gram Staining: perform a Gram stain on the isolated colonies to confirm that they are gram-negative bacteria. Haemophilus species are gram-negative.

6- Biochemical Testing: conduct various biochemical tests to further characterize the isolated bacteria and confirm their identity. Common tests include oxidase testing, catalase testing, and sugar fermentation tests.

X and V Factor Testing: confirm the requirement for X (hemin) and V (NAD) factors by conducting tests to determine if the isolated Haemophilus strains grow on media without these factors but not on media without both factors.

7- Serotyping: in some cases, serotyping may be performed to distinguish different strains of Haemophilus, especially for epidemiological or vaccine-related studies.

8- Antibiotic Susceptibility Testing: perform antibiotic susceptibility testing to determine the susceptibility of the isolated Haemophilus strains to various antibiotics. This information is crucial for guiding treatment decisions.

Satellitism Test:

It is used to identify *Haemophilus* species on blood agar.

Procedure:

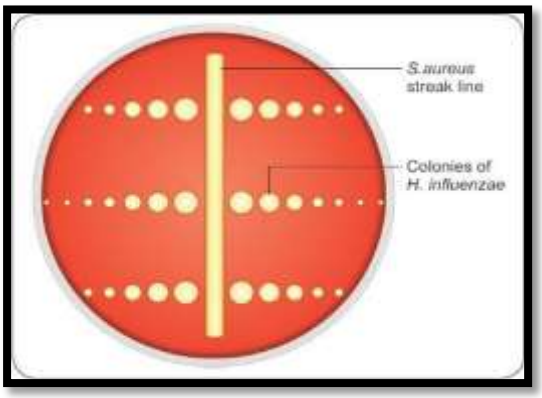
1- Mix a loop full of *Haemophilus* growth in 2 ml of sterile normal saline.

2- Inoculate the bacteria suspension on a plate of blood agar using a sterile swab.

3- Streak a pure culture of *Staphylococcus aureus* across the inoculated plate which provides V-factor for *Heamophilus*.

4- Incubate the plate overnight in a CO_2-enriched environment at 35-37° C.

5- Look for growth and satellite colonies in next morning.



الأسبوع العاشر

الهدف التعليمي: التعرف على بكتريا العائلة المعوية وطرق تشخيصها .

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Enterobacteriaceae**

General Characters:

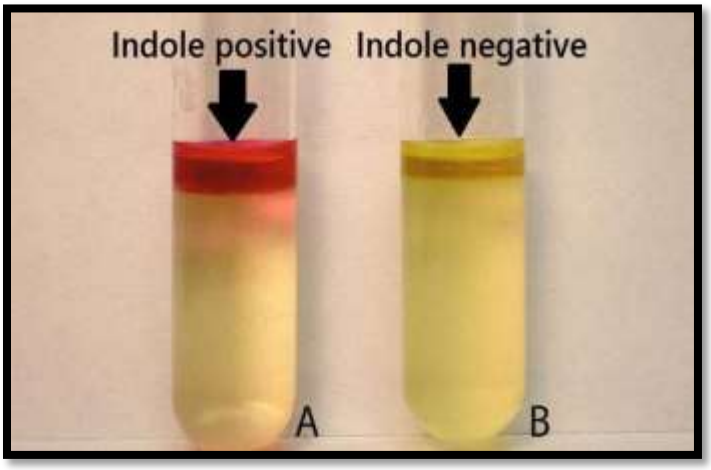
Numerous interrelated bacterial flora of intestine are Gram-negative rods, motile only with peritrichous flagella or non-motile, non-sporing, non-acid fast, ferment glucose with or without formation of gas, reduce nitrates into nitrites, form catalase, oxidase negative and aerobic or anaerobic.

IMViC Tests:

Each of the letters in "IMViC" represent one of four tests; "**I"** is for indole; "**M**" is for methyl red; **"V"** is for Voges-Proskauer and **"C"** is for citrate utilization. Lowercase **"i"** is added for the ease of pronunciation. IMViC tests are employed in the identification / differentiation of members of family enterobacteriaceae.

Indole Test:

It is performed in tryptophan broth (peptone water). It is used to determine the ability of bacteria to produce the enzyme tryptophanase. Tryptophanase breakdown tryptophan to release indole. Tryptophan is an amino acid that can suffer deamination and hydrolysis by bacteria that express tryptophanase enzyme.

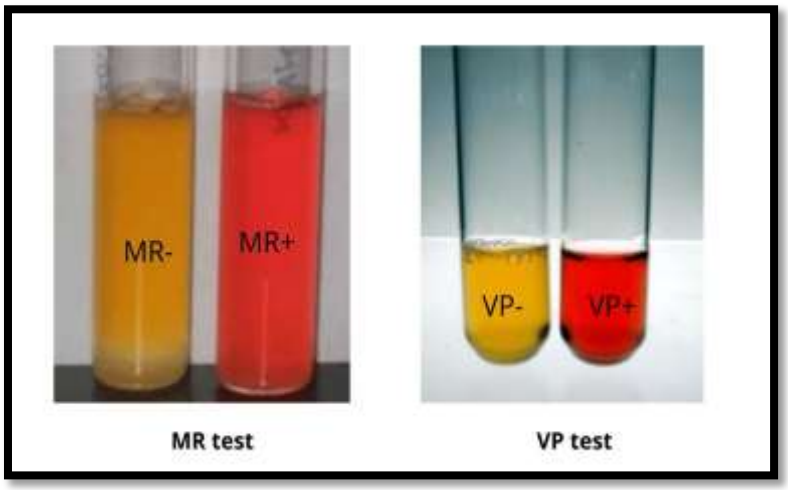


Methyl Red Test:

Some bacteria have the ability to utilize glucose and convert it to a stable acid like lactic, acetic or formic acid as the end product. These bacteria initially metabolize the glucose to pyruvic acid , which is further metabolized through the mixed acid pathway to produce the stable acid **(**glucose is fermented and produces several organic acids "lactic, acetic and formic acids"). The type of acid produced differs from species to species and depends on the specific enzymatic pathways present in the bacteria. The acid decreases the pH to 4.5 or below, which is indicated by a change in the color of methyl red from yellow to red.

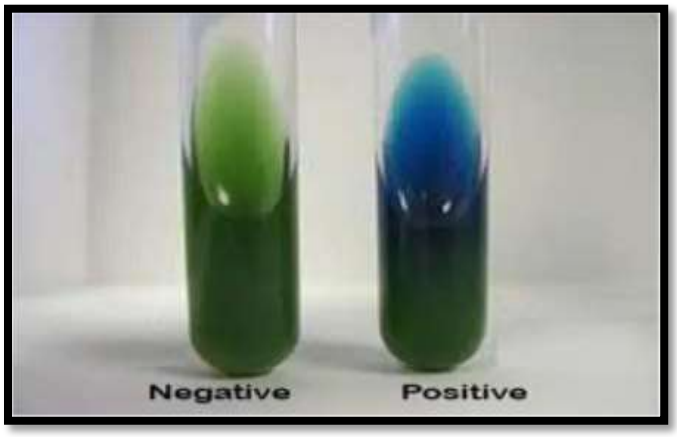
Voges-Proskauer Test:

This test done to determine the ability of the bacteria to produce a neutral end product acetylmethylcarbinol (acetone) from glucose fermentation (red color) . If the culture is negative for acetone, it will remain yellow.



Citrate Utilization Test:

It is used to determine the ability of bacteria to utilize sodium citrate as its only carbon source and inorganic.



Bacteria	Indole test	Methyl red test	Vogas-Proskauer test	Citrate test	Urease test	Motility test
E. coli	+ ve	+ ve	- ve	- ve	- ve	Motile
Klebsiella	- ve	- ve	+ ve	+ ve	+ ve	Non
Proteus mirabilis	- ve	+ ve	- ve	+ ve	+ ve	Motile
Proteus vulgaris	+ ve	+ ve	- ve	- ve	+ ve	Motile
Shigella	+ ve	+ ve	- ve	- ve	- ve	Non
Salmonella	- ve	+ ve	- ve	+ ve	- ve	Motile

Types of Culture Media:

1- Basal Media: e.g. Nutrient broth , nutrient agar , peptone water, is support the growth of a wide range of non-fastidious bacteria, including some enterobacteriaceae.

2- Enriched Media: e.g. Blood agar, chocolate agar , is support the growth of nutritionally demanding (fastidious) bacteria, but can also be used for enterobacteriaceae.

3- Selective Media: e.g. MacConkey agar , eosin methylene blue (EMB) agar , Hektoen enteric agar , violet red bile agar (VRBA) , GranuCult™ EE (enterobacteriaceae enrichment) MOSSEL broth , is inhibit the growth of certain bacteria while promoting the growth of others. Specifically, they are used to isolate enterobacteriaceae from mixed cultures.

الأسبوع الحادي عشر

الهدف التعليمي: التعرف على بكتريا الايشيركية القولونية وطرق تشخيصها.

مدة المحاضرة: 4 ساعات.

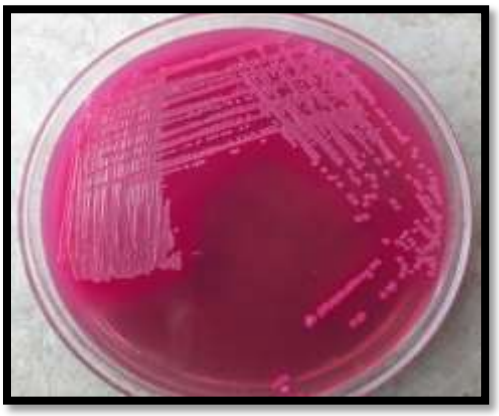
الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: ***Escherichia coli***

General Characters:

It is Gram-negative, non-capsulated, short, plump bacilli 2 to 4 μ × 0.4 to 0.7 μ in diameter and are motile. Spores are not formed. It is ferments lactose, glucose, sucrose, maltose and mannitol forming acid and gas. Urease is not hydrolyzed. It lives only in human or animal intestine.



Laboratory Diagnosis:

1- Sample Collection: depending on the suspected infection , samples of stool , urine, blood, or other infected material are collected.

2- Bacterial Culture: the collected samples are cultured in the lab to grow *E. coli* bacteria. This helps confirm the presence of the bacteria and isolate it for further testing

3- Gram Staining: it is Gram-negative, non-capsulated, short, plump bacilli.

4- Biochemical Tests: it is ferments lactose, glucose, sucrose, maltose and mannitol forming acid and gas. Urease is not hydrolyzed. Indole and methyl red (MR) is positive. VP and citrate is negative.

5- Antimicrobial susceptibility testing: if the bacteria are identified , they may be tested to determine which antibiotics are effective against them.

6- Shiga toxin testing: if *E. coli* O157:H7 is suspected, stool samples are tested for Shiga toxins, which are produced by these bacteria

7- Molecular assays: these tests can detect specific genes associated with virulence factors like Shiga toxins, providing a rapid diagnosis.

Indole and methyl red (MR) is positive. VP and citrate is negative.

الأسبوع الثاني عشر

الهدف التعليمي: التعرف على بكتريا الكليبسيلا وطرق تشخيصها.

مدة المحاضرة: 4 ساعات.

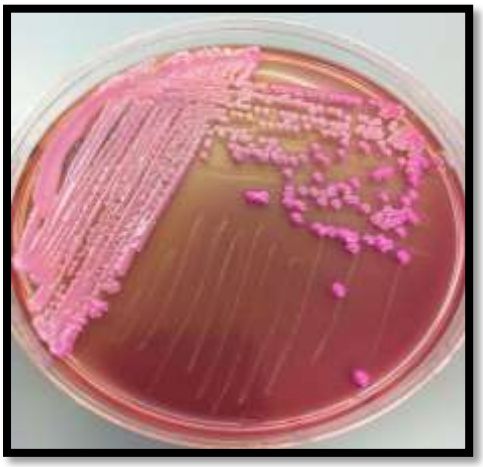
الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: ***Klebsiella***

General Characters:

It is found in the mucosa of upper respiratory tract, intestine and genitourinary tract. It is Gram-negative non-motile, capsulated, growing in ordinary media forming large mucoid colonies of varying degree of stickiness. It ferments sugar (glucose, lactose, mannitol) with production of acid and gas. It hydrolysis urea.



Laboratory Diagnosis:

1- Sample Collection: various clinical samples can be used for diagnosis , depending on the suspected infection site. These include sputum, blood, urine, wound swabs, and other relevant samples.

2-Culture Techniques: samples are inoculated onto blood agar and MacConkey agar plates and incubated aerobically. Klebsiella typically forms mucoid, non-hemolytic colonies on blood agar. On MacConkey agar , Klebsiella colonies are typically pink due to lactose fermentation.

3- Gram Staining: Klebsiella species appear as short , plump , Gram-negative bacilli. They are often encapsulated, which can be observed as a clear zone around the bacteria.

4- Biochemical Tests: Klebsiella is generally catalase-positive , oxidase-negative , and citrate-positive. It is indole-negative , except for some strains of *K. oxytoca*. Klebsiella is urease-positive.

5- Susceptibility Testing: antibiotic susceptibility testing (AST) is crucial to determine the appropriate treatment for Klebsiella infections.

6- Molecular Methods: PCR and qPCR assays targeting specific genes, such as the 16S rRNA gene or other virulence factors, can be used for rapid detection and identification of *Klebsiella pneumoniae*.

IMViC Tests:

Indole and MR are negative, VP and citrate are positive.

الأسبوع الثالث عشر

الهدف التعليمي: التعرف على البكتريا المتقلبة وطرق تشخيصها.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: ***Proteus***

General Characters:

it is Gram-negative rods showing great variation in size, 0.5 × 1 to 3 µ. It may be in long filaments or in granular form. It is actively motile and show swarming motility, best seen at 20°C. It is nonsporing and non-capsulated.

Laboratory Diagnosis:

1- Sample Collection : the type of sample collected depends on the suspected site of infection (e.g. , urine for urinary tract infections , pus or wound swabs for wound infections , blood for bloodstream infections).

2- Culture and Isolation : samples are cultured on standard media like blood agar and MacConkey agar , where Proteus species are typically non-lactose fermenters and exhibit a characteristic swarming motility.

3- Gram staining : is performed to visualize bacterial morphology . Proteus are Gram-negative bacilli (rod-shaped).

4- Biochemical Testing: it forms acid and gas from glucose . It characteristically deaminates phenylalanine to phenylpyruvic acid (PPA) .Hydrolysis of urea.

5- Susceptibility Testing: antibiotic susceptibility testing (AST) is crucial to determine the appropriate treatment for Proteus infections.

Classification:

1- *Proteus vulgaris.*

2- *Proteus mirabilis.*

3- *Proteus morganii.*

الأسبوع الرابع عشر

الهدف التعليمي: التعرف على لبكتريا السالمونيلا والشيكلا وطرق تشخيصها .

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

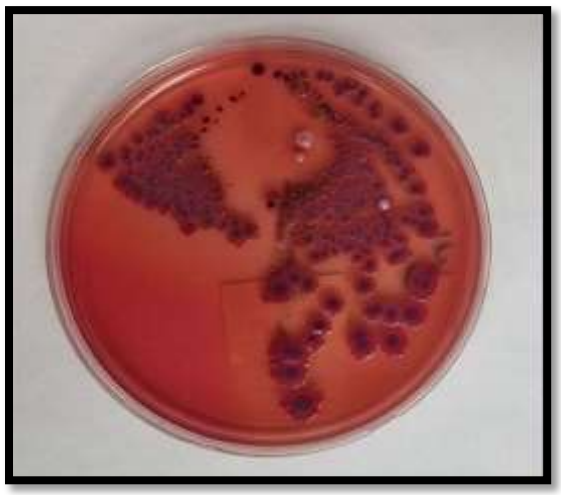
أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: ***Salmonella and Shigella***

General Characters:

Salmonella is Gram-negative rods, 2 to 4 μ × 0.6 μ in size, motile They are non-capsulated and non- sporing but may have fimbriae. is found in the intestine of man, animals and birds. Sometimes food (egg and meat) may be contaminated with this organism.

Shigella is Gram-negative , nonmotile , facultatively anaerobic , non-spore-forming rods and non-capsulated . It is genus of rod-shaped bacteria in the family enterobacteriaceae , species of which are normal inhabitants of the human intestinal tract.



Laboratory Diagnosis:

1- Salmonella:

a- Specimen Collection:

- Stool samples are typically collected early in the illness, before antibiotic treatment.
- Blood cultures are important for invasive infections.

b- Culture:

- Stool Culture: the most common method for diagnosing Salmonella gastroenteritis.
- Blood Culture : used when Salmonella is suspected to have entered the bloodstream , especially in cases of enteric fever or invasive disease.

c- Gram staining : is performed to visualize bacterial morphology. Salmonella are Gram-negative rod.

d- Biochemical Testing: it is ferments glucose, mannitol and maltose forming acid and gas.

e- Antimicrobial Susceptibility Testing: essential to determine which antibiotics will be effective for treatment.

f- Molecular Testing (PCR): becoming more common in clinical settings, particularly for detecting Salmonella in stool samples.

g- Serological Tests: while available , the CDC recommends against using them due to their unreliability according to the CDC.

2- Shigella:

a- Sample Collection:

- Fresh stool samples , ideally containing blood-tinged mucus, are collected from patients with suspected shigellosis.
- Rectal swabs can also be used, especially during the acute phase of the illness.

b- Stool Culture: stool samples are cultured on selective and differential media to isolate Shigella.

c- Gram staining : is performed to visualize bacterial morphology . Proteus are Gram-negative rod.

d- Biochemical Testing: It is MR positive and reduces nitrates to nitrites. It does not form H_2S, cannot utilize citrate and is inhibited in KCN. Catalase is positive except *Shigella dysenteriae* type I.Glucose is fermented with production of acid and no gas .

e- Antimicrobial Susceptibility Testing: essential to determine which antibiotics will be effective for treatment.

f- Serological Tests: is using specific antisera are performed to identify the Shigella species and serotype.

g- Molecular Methods: polymerase Chain Reaction (PCR) can be used to amplify Shigella DNA, allowing for faster detection.

الأسبوع الخامس عشر

الهدف التعليمي: التعرف على بكتريا الزائفة وبكتريا الضمات وطرق تشخيصها .

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: ***Pseudomonas***

General Characters:

Pseudomonas is slender, Gram-negative bacilli of 0.5 μ × 3.5 μ size, actively motile by polar flagellum. It is non- capsulated. It is aerobic growing on simple nutrient media with optimum temperature of 37°C. They are mostly saprophytes being found in water, soil and wherever decomposing matter is found.

Types of Pigments:

a. Pyocyanin (bluish green).

b. Fluorescein (yellowish green).

c. Proverdin (green).

d. Pyorubin (red).

e. Pyomelanin (black).

Laboratory Diagnosis:

1. Samples: pus , exudate , sputum , and swabs from conjunctiva are examined . Purulent discharge is usually greenish.

2. Culture: on nutrient agar media characteristic greenish blue colonies appear.

3- Gram staining: is performed to visualize bacterial morphology. Pseudomonas are Gram-negative bacilli.

4- Biochemical Testing: glucose is used oxidatively forming acid only. Nitrate are reduced to nitrites and nitrogen. Catalase and oxidase are positive.

5- Susceptibility Testing: antibiotic susceptibility testing (AST) is crucial to determine the appropriate treatment for Pseudomonas infections.

Oxidase test: is used to determine if an organism possesses the cytochrome oxidase enzyme.

Reagents: Kovacs oxidase reagent**.**

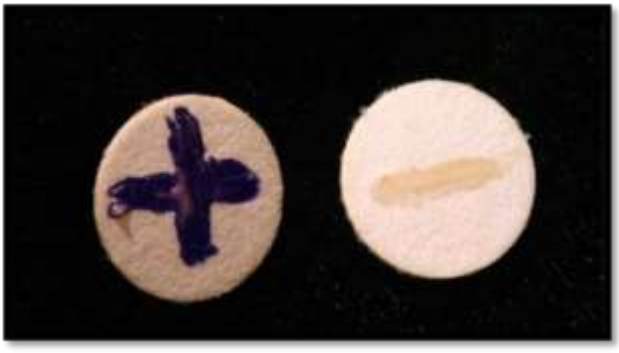
Procedure:

1- Soak a small piece of filter paper in 1% Kovacs oxidase reagent and let dry.

2- Use a loop and select a well-isolated colony from a fresh (18 - 24 hours) culture plate and rub onto treated filter paper.

3- Observe for color change.

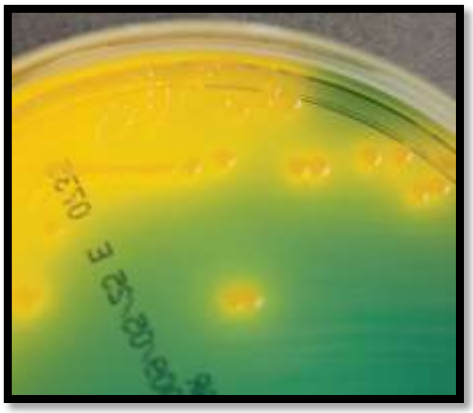
4- Microorganisms are oxidase positive when the color changes to dark purple within 5 to10 seconds . Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds . Microorganisms are oxidase negative if the color does not changes or it takes longer than 2 minutes.



Vibrio

General Characters:

Vibrios are among the most common bacteria in marine and estuarine waters, worldwide. They are comma-shaped , curved , and sometimes straight facultatively anaerobic , fermentative rods ; they are catalase and oxidase positive , and most species are motile by means of monotrichous or multitrichous polar flagella.



Laboratory Diagnosis:

1- Sample Collection:

Stool: samples should be collected during the acute stage of illness.

Other: vomitus or rectal swabs can be used if stool is unavailable.

2- Culture:

- TCBS Agar: this is the preferred selective media for isolating Vibrio species, especially *V. cholerae*, due to its ability to inhibit the growth of many other bacteria.

- Other Media: Vibrios can also grow on MacConkey agar and blood agar.

3- Gram staining: is performed to visualize bacterial morphology. Vibrio is Gram-negative comma-shaped.

4- Biochemical Testing: catalase and oxidase positive

5- Susceptibility Testing: antibiotic susceptibility testing (AST) is crucial to determine the appropriate treatment for vibrio infections.

7- Serotyping: for *V. cholerae* , serotyping (01 and 0139) is essential for confirming the diagnosis.

8- Molecular Methods: PCR assays are used to detect *V. cholerae* DNA directly in samples, offering higher sensitivity and speed.