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

الحقيبة التدريسية لمادة بكتريا مرضية نظري

الصف: الثاني

تدريسي المادة

م.د. رواء صادق مجيد

الفصل الدراسي الثاني

جدول مفردات مادة بكتريا مرضية نظري

Theoretical syllabus	
weeks	Topics
1	Systemic bacteriology, Genus Staphylococcus, General characters , toxin production , enzyme , immunity, Sensitivity test.
2	Genus Streptococcus General characters. Biochemical test, Antigenic characters , M protein Streptococcus group A, diseases, toxin, and immunity.
3	Streptococcus group B, C, D. Biochemical reaction immunity, diseases. Streptococcus pneumonia and Streptococcus variance disease, antigenic structure.
4	Gram positive bacilli – Corynebacterium diphtheria. Shape of bacteria, virulence, toxin, immunity, shick test. Antitoxin, skin test.
5	Genus Mycobacterium , general characters, Classification of bacteria , growth , antigenic structure , Disease, immunity.
6	Genus Bacillus, Bacillus anthraces. General characters, biochemical reaction, antigenic structure, toxin, immunity.
7	Anaerobic bacteria – Clostridium, general characters. Clostridium perifringeus , general characters . Antigen structure, biochemical reaction, virulence, toxin. Clostridium tetani , disease , immunity, antigenic structure
8	Genus Neisseria, general characters, biochemical reaction. Neisseria gonorrhea, antigenic structure, virulence. Neisseria meningitides, immunity, sensitivity test. Antigenic structure , virulence , immunity
9	Genus Haemophilus , general characters , growth factors , Virulence, immunity. Genus Bordetella, general characters, disease.
10-11	Family Enterobacteriaceae , General characters , classification , biochemical reaction , Antigenic characters, sugar fermentation, sensitivity test. Genus Escherichia coli, Klebsiella, diseases, virulence, Immunity.

12	Genus Vibirio, history of disease, general characters, Antigenic structure, virulence, immunity, treatment. Classical Vibirio EL-TOR biotype. Vibirio parahaemical. Campylobacter jejuni.
13	Genus Brucella , general characters , diseases , species , Zoonosis. Yersinia pestis , general characters , virulence , diseases
14	Francisella , general characters , transmition diseases , Virulence, syphilis, VDRL. Nocardia , general characters , stin-direct smear . Mycoplasma, shape, virulence, Lab.dignosis .
15	Chlamydia , general characters , shape , biochemical test , Virulence, immunity.

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 .

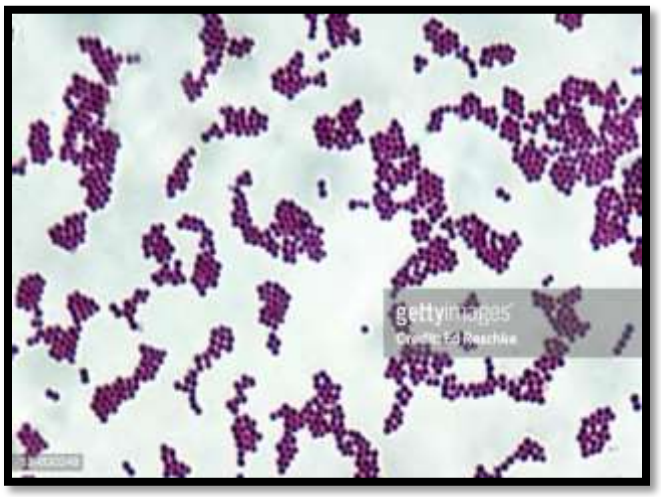
مدة المحاضرة: ساعاتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة **Staphylococci**

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.



1. Gram-Positive Bacteria: staphylococci are Gram-positive bacteria, which means they have a thick peptidoglycan cell wall that retains the purple stain in the Gram staining process.

2. Shape and Arrangement: staphylococci are typically round (cocci) and occur in clusters. The term "staphylo" comes from the Greek word for "bunch of grapes," describing their

clustered appearance.

3. Common Species: some of the most clinically relevant species of staphylococci include:

***Staphylococcus aureus*:** this species is known for causing a wide range of infections, including skin infections (e.g., boils, impetigo), respiratory infections, food poisoning, and more. It produces toxins and enzymes that contribute to its pathogenicity.

***Staphylococcus epidermidis*:** often found on the skin and mucous membranes, this species is usually harmless but can cause infections, especially in individuals with weakened immune systems or those with implanted medical devices (e.g., catheters, prosthetic joints).

4. Disease and Infections: staphylococci can cause infections when they enter the body through cuts, wounds, or other means. Infections can range from minor skin conditions like pimples and boils to more serious infections like cellulitis, abscesses, and bloodstream infections. *Staphylococcus aureus*, in particular, is associated with a broad spectrum of diseases.

5. Antibiotic Resistance: staphylococci, especially *Staphylococcus aureus*, have developed resistance to many antibiotics over time. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known antibiotic-resistant strain that poses a significant challenge in healthcare settings.

6. Treatment: infections caused by staphylococci are typically treated with antibiotics. However, the choice of antibiotics depends on the specific strain and its susceptibility to drugs. MRSA infections, for example, may require different antibiotics than methicillin- sensitive *Staphylococcus aureus* (MSSA) infections.

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.

الأسبوع الثاني والثالث

الهدف التعليمي: التعرف على بكتريا المكورات المسبحية.

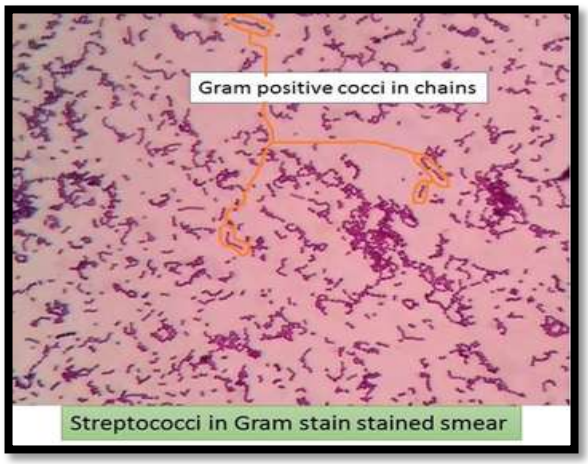
مدة المحاضرة: ساعتان.

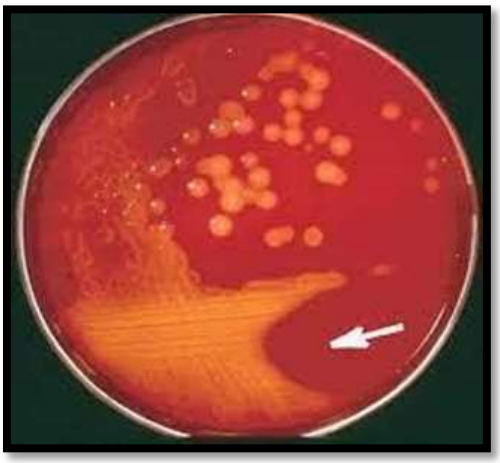
الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Streptococci**

General characters: they are Gram positive cocci arranged in chains, non-motile and nonsporing. They require media enriched with blood, serum or ascitic fluid for their growth. 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.





1. Gram-Positive Bacteria: streptococci are Gram-positive bacteria, meaning they have a thick peptidoglycan cell wall that retains the purple stain in the Gram staining process.

2. Arrangement: streptococci are often arranged in chains or pairs, which is a characteristic feature used for their classification.

3. Classification: streptococci are classified into different groups based on their hemolytic properties (the effect they have on red blood cells when grown on blood agar). The two primary groups are:

Alpha-hemolytic streptococci: these bacteria partially break down red blood cells, leading to a greenish discoloration on blood agar plates. Examples include *Streptococcus pneumoniae* and some oral streptococci.

Beta-hemolytic streptococci: these bacteria completely break down red blood cells, causing a clear zone (beta-hemolysis) around their colonies on blood agar plates. The beta-hemolytic group is further divided into subgroups, including Group A, Group B, Group C, and Group G streptococci.

4. Pathogenic Species:

***Streptococcus pyogenes (Group A Streptococcus or GAS)*:** this bacterium is a significant human pathogen that can cause a wide range of infections, including strep throat, scarlet fever, impetigo, cellulitis, and invasive diseases such as necrotizing fasciitis and toxic shock syndrome.

***Streptococcus agalactiae (Group B Streptococcus or GBS)*:** GBS is often found in the female genital and gastrointestinal tracts and can cause infections in newborns, pregnant women, and adults with weakened immune systems.

***Streptococcus pneumoniae*:** is resides asymptomatically in the nasopharynx of healthy carriers. However, in susceptible individuals, such as elderly and immunocompromised people and children, the pathogen can spread to other locations and cause disease. It is the main cause of community acquired pneumonia and meningitis in children and the elderly, and of septicemia in HIV-infected persons. Despite the name, the organism causes many types of pneumococcal infections other than pneumonia. These invasive pneumococcal diseases include acute sinusitis, otitis media, conjunctivitis, meningitis, bacteremia, sepsis, osteomyelitis, septic

arthritis, endocarditis, peritonitis, pericarditis, cellulitis, and brain abscess. It is one of the most common causes of bacterial meningitis in adults and young adults.

Disease and Infections: streptococci can cause infections in various parts of the body, including the throat, skin, respiratory tract, and bloodstream. The severity of the infection and the symptoms depend on the species and the specific strain of streptococcus involved.

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.

Prevention: preventing streptococcal infections may involve good hygiene practices, such as handwashing and covering the mouth when coughing or sneezing. For specific populations, such as pregnant women, screening for Group B Streptococcus during pregnancy is recommended to prevent neonatal infections.

Understanding the different species and groups of streptococci is essential for diagnosing and treating streptococcal infections effectively, as the choice of antibiotics and treatment strategies can vary based on the specific strain and the type of infection.

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.

الأسبوع الرابع

الهدف التعليمي: التعرف على البكتريا الوتدية.

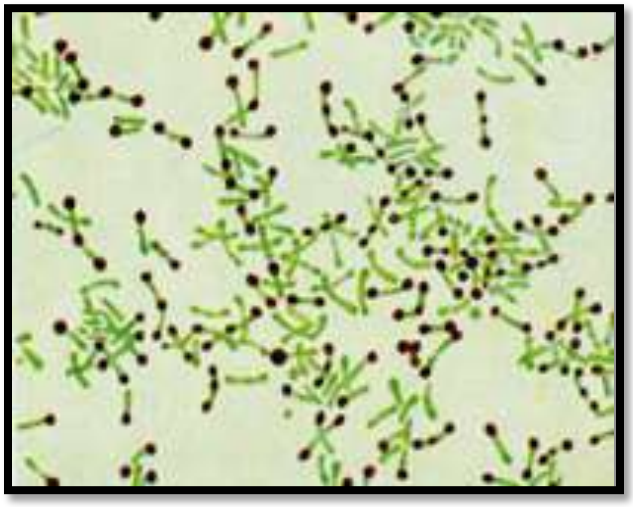
مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Corynebacterium**

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.



Cell Shape and Arrangement: corynebacterium bacteria are typically rod-shaped (bacilli) and can occur singly, in pairs, or in irregular formations. In some species, they may exhibit club-like or pleomorphic shapes.

Gram Stain: corynebacterium species are Gram-positive, meaning they retain the crystal violet stain in the Gram staining process due to their thick peptidoglycan cell wall.

Aerobic or Facultatively Anaerobic: corynebacterium species are generally aerobic, meaning they require oxygen for growth. However, some species are facultative anaerobes and can grow

in the absence of oxygen.

Non-Motile: most corynebacterium species are non-motile, meaning they do not possess flagella for movement.

Clinical Significance: while the majority of corynebacterium species are harmless and part of the normal microbiota of the skin and mucous membranes, some species are opportunistic pathogens. For example:

Corynebacterium diphtheriae is the causative agent of diphtheria, a potentially severe respiratory infection.

Corynebacterium urealyticum can cause urinary tract infections and other infections in immunocompromised individuals.

Growth on Solid Media:

Corynebacterium diphtheriae grows on various types of solid media commonly used in clinical microbiology labs. Blood agar, Löffler's serum medium, Tinsdale agar, and trypticase soy agar supplemented with blood are among the media that support the growth of *C. diphtheriae*.

Colonial Morphology:

Colonies of *Corynebacterium diphtheriae* typically appear as small, gray, or gray- white colonies. They may exhibit a characteristic "black dot" or "bull's-eye" appearance at the center of the colony due to the presence of metachromatic granules (polymetaphosphate granules) that can stain dark.

Hemolysis:

On blood agar, *C. diphtheriae* often displays β-hemolysis, which is complete hemolysis of red blood cells surrounding the colony. This hemolytic zone is clear and transparent.

Incubation Conditions:

Cultures of *C. diphtheriae* are typically incubated at 35-37°C in an aerobic atmosphere, as the

bacterium is strictly aerobic and requires oxygen for growth.

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.

Diseases of Diphtheria:

Respiratory Diphtheria: this is the most common form of diphtheria. It involves the respiratory tract, including the throat, larynx (voice box), and nasal passages. The characteristic symptom is the formation of a thick, grayish pseudomembrane composed of dead tissue and bacteria on the mucous membranes of the throat and airways. This pseudomembrane can obstruct the airway, causing difficulty in breathing and, in severe cases, suffocation.

Cutaneous Diphtheria: in this form, diphtheria affects the skin, leading to the development of painful, ulcerating skin lesions. Cutaneous diphtheria is typically less severe than respiratory diphtheria but can serve as a reservoir for transmission.

Pharyngeal Diphtheria: this form primarily involves the pharynx and tonsils, leading to symptoms similar to respiratory diphtheria, including the formation of a pseudomembrane.

Nasopharyngeal Diphtheria: diphtheria can also affect the nasopharynx, leading to symptoms such as nasal congestion, difficulty swallowing, and the presence of a pseudomembrane in the nasal passages.

Diphtheria toxin

Diphtheria toxin is a single polypeptide chain consisting of two subunits known as an A-B toxin. Binding to the host cell surface of the B subunit by specific receptor (heparin-binding epidermal growth factor), that is present on many human cells allows the A subunit to penetrate the host cell by endocytosis. After that, the A fragment separates from the B fragment and becomes an active enzyme. The B fragment is released from the host cell. This enzyme catalyzes a chemical reaction that inactivates substance required for movement of the ribosome on mRNA (inactivate elongation factor 2). It halts protein synthesis by blocking the transfer of amino acids from tRNA to the growing polypeptide chain on the ribosome and the cell dies. Cells that lack the appropriate receptor do not take up the toxin and are unaffected by it. This receptor specify explains why some tissues of the body are not affected in diphtheria, while others such as heart, spleen, muscles, liver, kidney and nerves are severely damaged.

Diphtheroids:

"Diphtheroids" is a term used in clinical microbiology to describe a group of bacteria that are similar in appearance or characteristics to Corynebacterium diphtheriae but are non-pathogenic or opportunistic pathogens. These bacteria belong to the same genus as Corynebacterium but do not possess the diphtheria toxin and are typically part of the normal microbiota of the skin and mucous membranes.

الأسبوع الخامس

الهدف التعليمي: التعرف على البكتريا المتفطرة.

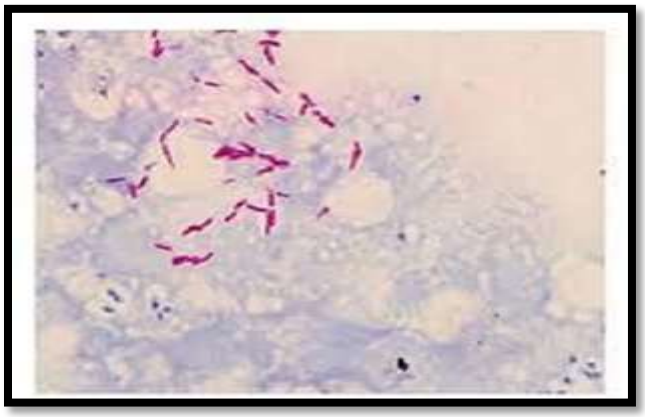
مدة المحاضرة: ساعتان.

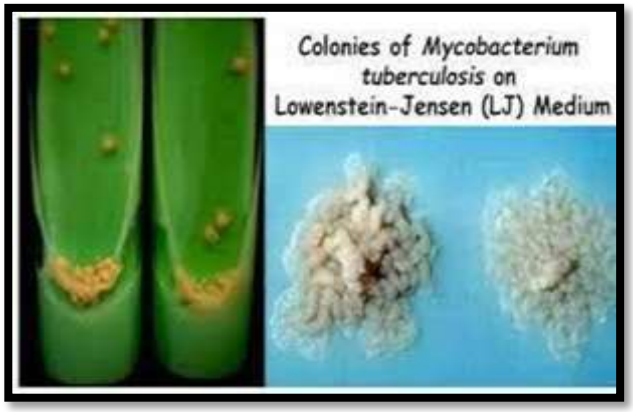
الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Mycobacteria**

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.





1- Pathogenic Mycobacteria:

***Mycobacterium tuberculosis*:** this species is responsible for tuberculosis (TB), a highly contagious and potentially fatal disease that primarily affects the lungs but can also affect other organs. TB remains a significant global health concern.

***Mycobacterium leprae*:** this bacterium causes leprosy, also known as Hansen's disease. Leprosy primarily affects the skin and peripheral nerves and can lead to deformities if left untreated. It is less common today but still exists in some regions.

Other Pathogenic Mycobacteria: besides *M. tuberculosis* and *M. leprae*, there are other pathogenic mycobacteria that can cause various diseases, including *Mycobacterium avium* complex (MAC), Mycobacterium bovis (which can cause bovine tuberculosis and can infect humans), and *Mycobacterium ulcerans* (causing Buruli ulcer).

2- Non-pathogenic Mycobacteria:

Some mycobacteria are part of the normal microbiota in humans and animals and do not cause disease. They are often found in environmental sources, including soil and water. Examples include *Mycobacterium smegmatis* and *Mycobacterium fortuitum*.

Laboratory diagnosis of mycobacterial infections, especially tuberculosis, involves specialized techniques due to the unique characteristics of these bacteria:

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.

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.

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.

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.

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

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.

Mycobacterial infections can be challenging to diagnose and treat due to their slow growth and the development of drug resistance in some strains. Early and accurate diagnosis is crucial for effective management and control of mycobacterial diseases.

الأسبوع السادس

الهدف التعليمي: التعرف على البكتريا العصوية.

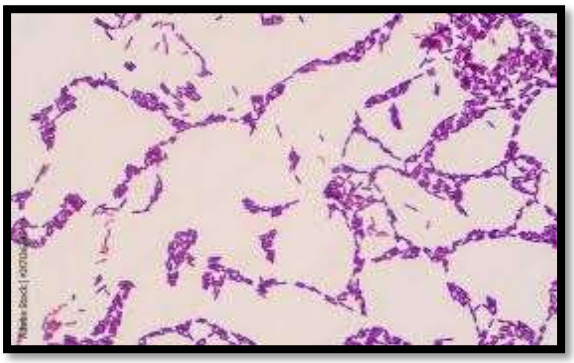
مدة المحاضرة: ساعات (ساعتان نظري+ 4 ساعات عملي).

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Bacilli**

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.



Morphology: bacillus bacteria are characterized by their rod-shaped (bacillary) morphology. They are typically gram-positive, meaning they retain the violet crystal stain in the Gram stain procedure.

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.

Habitat: bacillus species are ubiquitous and can be found in a wide range of environments, including soil, water, air, and the digestive tracts of humans and animals. They are known for their ability to form spores and persist in soil and other natural settings.

Pathogenic Species: while most Bacillus species are harmless, some can be opportunistic pathogens in humans and animals. The most notable pathogenic species is Bacillus anthracis, which causes anthrax, a potentially fatal disease that can affect humans and livestock. Other species, such as Bacillus cereus, can cause food poisoning when certain conditions are met.

Industrial and Biotechnological Uses: some Bacillus species have industrial and biotechnological significance. Bacillus subtilis, for example, is used in the production of various enzymes, antibiotics, and other bioproducts. It is also used as a model organism in scientific research.

Bioremediation: certain Bacillus species have the ability to degrade organic compounds and contaminants in the environment. They are sometimes used in bioremediation processes to clean up contaminated sites.

Disease Diagnosis: bacillus species can be identified and characterized using various microbiological and molecular techniques, including culture, biochemical tests, DNA sequencing, and polymerase chain reaction (PCR).

Research: Bacillus species, particularly Bacillus subtilis, have been extensively studied in microbiological research and are used as model organisms to investigate various aspects of bacterial physiology, genetics, and molecular biology.

Probiotics: some bacillus species are used as probiotics, which are beneficial microorganisms that can support gut health in humans and animals.

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:

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).

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.

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.

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.

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.

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.

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.

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.

Environmental Monitoring: in industrial or environmental contexts, Bacillus species may be monitored for their ability to degrade contaminants or produce valuable enzymes. In such cases, growth and activity assays specific to the application may be employed.

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

Probiotic Characterization (If applicable): in the case of Bacillus species used as probiotics, their identification and characterization ensure their safety and effectiveness as probiotic supplements.

الأسبوع السابع

الهدف التعليمي: التعرف على البكتريا المطثية.

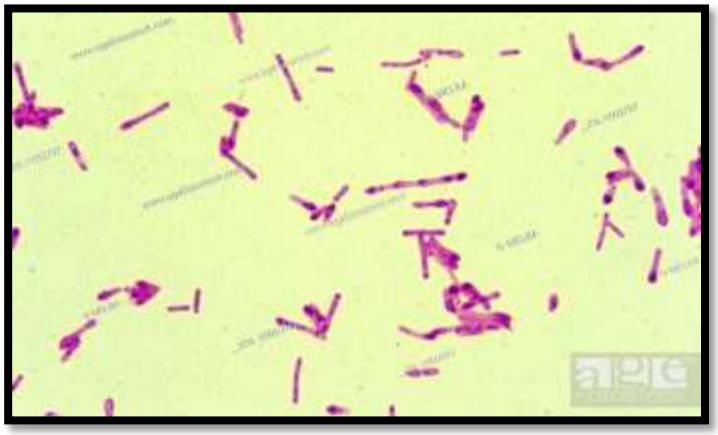
مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Clostridium**

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.

Habitat: clostridium bacteria are commonly found in soil, water, and the gastrointestinal tracts of humans and animals. They are also present in decaying organic matter.

Pathogenic Species: some clostridium species are known pathogens. Notable pathogenic species include:

- *Clostridium botulinum*: the causative agent of botulism, a potentially lethal illness caused by a neurotoxin produced by the bacteria.

- *Clostridium tetani*: responsible for tetanus, a disease characterized by muscle stiffness and spasms due to the production of tetanospasmin toxin.

- *Clostridium difficile*: a leading cause of healthcare-associated diarrhea and colitis, often associated with antibiotic use.

- *Clostridium perfringens*: can cause gas gangrene, food poisoning, and various soft tissue infections.

Industrial and Biotechnological Uses: some non-pathogenic clostridium species have industrial applications. For example, *Clostridium acetobutylicum* is used in the production of acetone and butanol through fermentation.

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.

Vaccine Development: vaccines have been developed for certain Clostridium-associated diseases, such as tetanus and botulism.

Clostridial Toxins: clostridium species often produce potent toxins that contribute to the pathology of the diseases they cause. Understanding these toxins is critical for diagnosis and treatment.

Treatment: treatment of clostridium infections typically involves antibiotics and supportive care. In cases of botulism, antitoxin may be administered. Infections may also require wound debridement and surgery in severe cases.

Clostridium bacteria have diverse roles in nature and can be both beneficial and harmful to humans. While some species are responsible for severe diseases, others have valuable industrial applications, highlighting the importance of understanding and managing these bacteria.

الأسبوع الثامن

الهدف التعليمي: التعرف على بكتريا النيسيريا.

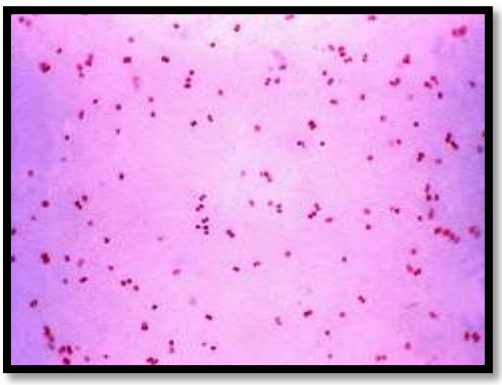
مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Neisseria**

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*.



***Neisseria meningitidis*:** this bacterium is responsible for causing meningococcal disease, which can manifest as meningitis (inflammation of the membranes covering the brain and spinal cord) and septicemia (bloodstream infection). Meningococcal infections can be severe and even fatal if not treated promptly with antibiotics. Vaccines are available to protect against some of the most common strains of *Neisseria meningitidis*.

Neisseria gonorrhoeae: this bacterium is the causative agent of gonorrhea, a sexually transmitted infection (STI) that primarily affects the genital and reproductive tract.

Bacteriological Study of Neisseria:

A bacteriological study of Neisseria involves the examination and analysis of these bacteria for various purposes:

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.

8- Vaccine Development: understanding the biology and genetics of Neisseria is crucial for developing vaccines against diseases caused by these bacteria, such as meningococcal vaccines.

الأسبوع التاسع

الهدف التعليمي: التعرف على البكتريا المستدمية وبكتريا البورديتيلا.

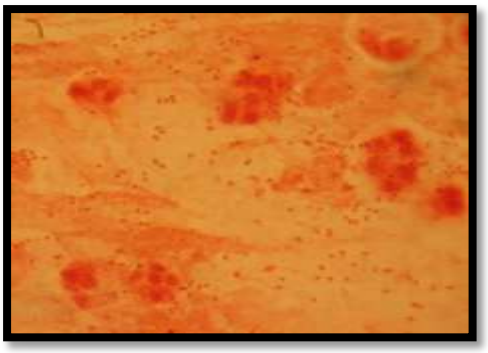
مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Haemophilus**

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.



Clinical Significance: Haemophilus species can cause a range of diseases in humans, depending on the specific species and strain. Some of the notable diseases caused by Haemophilus include:

***Haemophilus influenzae*:** commonly associated with respiratory infections such as bronchitis, sinusitis, and pneumonia. It can also cause invasive diseases like bacteremia, meningitis, and epiglottitis, especially in young children.

***Haemophilus ducreyi*:** the causative agent of chancroid, a sexually transmitted infection characterized by genital ulcers.

***Haemophilus parainfluenzae*:** often found in the upper respiratory tract and can be associated with respiratory infections, but it is generally less pathogenic than H. influenzae.

***Haemophilus haemolyticus*:** although it is considered a commensal bacterium, it can occasionally cause infections, including bacteremia.

Bacteriological Study of Hemophilus:

A bacteriological study of Haemophilus involves the isolation, identification, and characterization of Haemophilus bacteria from clinical samples, typically obtained from patients with suspected infections. Here are the key steps typically involved in such a study:

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.

Genus: *Bordetella*

Morpholog:y

The Bordetella are small, Gram-negative, encapsulated, strict aerobic coccobacilli. It is arranged singly or in small groups and is not easily distinguished from *Haemophilus* species. The most important species related to human are:

1. *Bordetella pertussis.*

2. *Bordetella parapertussis.*

Pathogenicity and clinical findings:

Bordetella pertussis causes whooping cough (pertussis), an acute respiratory infection marked by severe, spasmodic coughing episodes characterized by a “whooping” sound when the person breathes in. Pertussis is a common and dangerous childhood disease in unvaccinated infants and children. The bacteria colonize only ciliated cells of the respiratory mucosa, and they multiply rapidly, leukocytosis with lymphocytosis is also common during this phase of the illness.

Bordetella parapertussis can cause a milder form of pertussis. *Bordetella pertussis* produces a number of virulence factors, including pertussis toxin, adenylate cyclase toxin, filamentous hemagglutinin, and hemolysin. Agglutinogens and other outer membrane proteins are important antigens.

Laboratory diagnosis:

1. Culture: is the gold standard because it is the only 100% specific method for identification of *Bordetella pertussis*. This bacterium can be cultured on modified Bordet-Gengou medium, charcoal-horse blood agar (Regan-Lowe)**.** Nasopharyngeal (NP) specimens collected during the first 2 weeks of cough. This is when viable bacteria are still present in the nasopharynx.

2. *Bordetella* DNA can also be detected by PCR.

3. Serological diagnosis.

4. Vitek

Treatment:

Pertussis can sometimes be very serious, requiring treatment in the hospital. Babies are at greatest risk for serious complications from pertussis. Treatment for pertussis if started early, it can help reduce severity, duration and the risk of complications, particularly in infants. So, once a diagnosis is made, the treatment should start on antibiotics immediately. The most popular antibiotics used are azithromycin, clarithromycin and erythromycin.

الأسبوع العاشر والحادي عشر

الهدف التعليمي: التعرف على بكتريا العائلة المعوية.

مدة المحاضرة: ساعتان.

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.

Classification:

1- Based on action on lactose:

It is an old method. It has practical value in diagnostic bacteriology.

a- Lactose fermenter.

e.g. *Escherichia coli, Klebsiella.*

b- Late lactose fermenter.

e.g. *Shigella sonnei , Paracolons*

c- Non-lactose fermentation.

e.g. *Salmonella, Shigella.*

2- Modern taxonomical concept:

Enterobacteriaceae may be classified into tribes, genera and species by their cultural and biochemical characters. The species are further classified as: biotypes, serotypes, bacteriophage types and colicin types. At present there are five tribes as under:

Enterobacteriaceae:

Tribe I Escherichieae.

Genus: *Escherichia, Edwardsiella , Citrobacter, Salmonella , Shigella.*

Tribe II Klebsielleae.

Genus: *Klebsiella, Enterobacter , Hafnia , Serratia.*

Tribe III Proteeae.

Genus: *Proteus.*

Tribe IV Erwinieae.

Genus: *Erwinia.*

Tribe V Yersinae.

Yersinia pestis , Yersinia enterolitico, Yersinia pseudotuberculosis.

Escherichia coli :

It lives only in human or animal intestine. Detection of *E. coli* in drinking water is taken as evidence of recent pollution with human or animal excreta. *Escherichia coli* in contaminated water may be detected using PCR (rapid and sensitive), DNA probes, plating and biochemical tests.

Morphology:

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.

Biochemical reactions:

It ferments lactose, glucose, sucrose, maltose and mannitol forming acid and gas. Indole and methyl red (MR) is positive. VP and citrate is negative. Urea is not hydrolyzed. H2S is not produced.

Antigenic structure:

There are 4 types of antigens:

1- Somatic antigen (O antigen): they are heat stable.

2- Surface antigen (K antigen). they are heat labile.

3- Flagellar antigen (H antigen): they are thermolabile .

4- Fimbrial antigen (F antigen): they are thermolabile .

Toxin production:

It produces endotoxin. Besides that, it also produces two types of exotoxins:

a- Enterotoxin which is heat labile, filtrable

b- Hemolysin which may be: heat labile, filtrable and lethal for animals.

Endotoxin may cause fever, leukopenia, hypotension,

Pathogenesis:

1- Gastroenteritis: certain serotypes produce fatal type of gastroenteritis in infants

2- Urinary tract infection.

3- Pyogenic infection, e.g. wound infection, abscess, peritonitis, and meningitis.

4- Septicemia: it is one of the most common cause of septicemia.

***Klebsiella*:**

It is found in the mucosa of upper respiratory tract, intestine and genitourinary tract. It is non-motile, capsulated, growing in ordinary media forming large mucoid colonies of varying degree of stickiness. It has been classified into many species on the basis of biochemical reactions.but the important species is *K. pneumoniae:* It ferments sugar (glucose, lactose, mannitol) with production of acid and gas. Indole and MR are negative, VP and citrate are

positive. It hydrolysis urea. It may cause pneumonia, urinary tract infection and pyogenic infections.

Proteus:

Morphology: 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.

Biochemical reactions: it forms acid and gas from glucose . It characteristically deaminates phenylalanine to phenylpyruvic acid (PPA) .Hydrolysis of urea is another characteristic property of proteus.

Antigenic structure: A number of O and H antigens are produced in proteus.

Pathogenicity: it is an opportunist pathogen.It may cause urinary tract infection. It may produce pyogenic lesions like abscess, infection of wound, ear or respiratory tract. *Proteus morganii* is reported to cause infantile diarrhea.

Shigella:

It is Gram-negative, nonmotile, facultatively anaerobic, non-spore-forming rods it is genus of rod-shaped bacteria in the family Enterobacteriaceae, species of which are normal inhabitants of the human intestinal tract and can cause dysentery, or shigellosis. Shigella are microbiologically characterized as gram-negative, non- spore-forming, nonmotile bacteria. Their cells are 0.4 to 0.6 micrometer across by 1 to 3 micrometer long.

Morphology: it is non-motile, non-capsulated, about 0.5 × 1 to 3 μ in size.

Biochemical Reactions :

It is MR positive and reduces nitrates to nitrites. It does not form H2S, 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 .

Antigenic structure: it has one or more major antigens and large number of minor somatic antigens. There is antigenic sharing between some members of genus and between *Shigella*

and *E. coli*. The somatic O antigen of *Shigella* is lipopolysaccharide. Their serologic specificity depends on the polysaccharides. Common fimbrial antigen may occur. For identification of Shigella both antigenic and biochemical properties should be considered.

Toxin:

Following toxins are produced:

Endotoxin: on autolysis endotoxin is released which is lipopolysaccharide.

Enterotoxin of Shigella dysenteriae inhibits sugar and amino acid absorption in small intestine of man.

Pathogenesis:

Shigellae cause bacillary dysentery.. Bacillary dysentery has short incubation period (1 to 7 days). There is frequent passage of loose motion containing blood and mucus with griping pain and tenesmus. *Shigella dysenteriae* type I may cause complications like arthritis, toxic neuritis.

Salmonella:

Genus *Salmonella* is found in the intestine of man, animals and birds. Sometimes food (egg and meat) may be contaminated with this organism. It may cause enteric fever, gastroenteritis and septicemia.

Morphology: it 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.

Biochemical reactions: it ferments glucose, mannitol and maltose forming acid and gas except *Salmonella typhi*.

Antigenic structure:

Somatic antigen (O): it is phospholipid protein polysaccharide complex.

Flagellar antigen (H): it is heating labile protein.

Vi antigen: it is surface and heat labile antigen.

Pathogenesis:

Enteric fever: it is caused by *S. typhi* (70 to 85% in India), *S. paratyphi A* (15 to 21%) and *S. paratyphi B S. paratyphi A* and *S. paratyphi B* may cause paratyphoid fever resembling enteric fever.

Food poisoning*:* it is by ingestion of contaminated food, e.g. meat and egg. Food poisoning is caused by *S. typhimurium, S. enteritides, S. newport,* etc. Incubation period is 12 to 48 hours. There is fever, vomiting, diarrhea (mucus and blood in stool).

الأسبوع الثاني عشر

الهدف التعليمي: التعرف على بكتريا الضمات.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

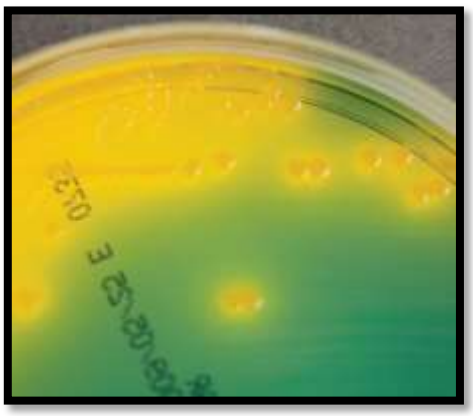
أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Vibirio**

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

***Vibrio cholerae*:**

The bacterium *V. cholerae* is the cause of cholera. The epidemiology of cholera closely parallels the recognition of *V. cholerae* transmission in water and the development of sanitary water systems . Cholera is associated with poor sanitation, as well as direct contact with or consumption of contaminated water and/or food (e.g., water used for drinking, cooking, bathing, and crop irrigation).



General Characters:

V. cholerae regularly ferments sucrose and mannose but not arabinose. A positive oxidase test result is a key step in the preliminary identification of *V. cholerae* and other vibrios. While most *Vibrio* species are halophilic, requiring the presence of NaCl (range from < 0.5-4.5%) to grow, *V. cholerae* can grow on most agar media without additional salt.

Antigenic Structure:

Many vibrios share a single heat-labile flagellar H antigen. Antibodies to the H antigen are probably not involved in the protection of susceptible hosts. *V. cholerae* has O lipopoly-saccharides that confer serologic specificity. Based on the O antigen, there are over 200 serogroups; however, only *V. cholerae* strains of serogroup O1 and O139 cause epidemic and pandemic cholera . Occasionally, non-O1/non-O139 *V. cholerae* strains have been described as causes of cholera-like diarrheal disease. Antibodies to the O antigens tend to protect laboratory animals against infections with *V. cholerae*. The *V. cholerae* serogroup O1 antigen has determinants that make possible further subtyping; these serotypes are Ogawa, Inaba, and Hikojima . Furthermore, two biotypes of epidemic *V. cholerae* have been defined, classic and El Tor. The El Tor biotype produces a hemolysin , gives positive results on the Voges-Proskauer test , and is resistant to polymyxin B. Molecular techniques can also be used to type *V. cholerae* . Typing is used for epidemiologic studies, and tests generally are done only

in reference laboratories. *V. cholerae* O139 is very similar to *V. cholerae* O1 El Tor biotype. *V. cholerae* O139 does not produce the O1 lipopolysaccharide and does not have all the genes necessary to make this antigen . *V. cholerae* O139 and other non-O1 *V. cholerae* strains, as well as *V. vulnificus* produce acidic polysaccharide capsules; however, *V. cholerae* O1does not make a capsule.

Virulence:

V. cholerae produce a heat-labile enterotoxin with a molecular weight (MW) of about 84000 , consisting of subunits A (MW , 28000) and B. Ganglioside GM1 serves as the mucosal receptor for subunit B, which promotes entry of subunit A into the cell.

Activation of subunit A1 yields increased levels of intracellular cyclic adenosine mono-phosphate (cAMP) and results in prolonged hypersecretion of water and electrolytes. There is increased sodium-dependent chloride secretion , and absorption of sodium and chloride by the microvilli is inhibited. Electrolyte-rich diarrhea occurs with as much as 20‑30 L/day, resulting in dehydration, shock, acidosis, and death. The genes for *V. cholerae* enterotoxin are located on the bacterial chromosome. Cholera enterotoxin is antigenically related to LT of *Escherichia coli* and can stimulate the production of neutralizing antibodies. However, the precise role of antitoxic and antibacterial antibodies in protection against cholera is not clear.

Immunity:

Gastric acid provides some protection against vibrios, including *V. cholerae*. An attack of cholera is followed by immunity to reinfection, but the duration and degree of immunity are not known . In experimental animals, specific IgA antibodies occur in the lumen of the intestine. Similar antibodies in serum develop after infection but last only a few months. Vibriocidal antibodies in serum (titer ≥1:20) have been associated with protection against colonization and disease. The presence of antitoxin antibodies has not been associated with protection.

Treatment:

The most important part of treating cholera patients consists of water and electrolyte replacement to correct the severe dehydration and salt depletion. Many antimicrobial agents are effective against *V. cholerae*, but these play a secondary role in patient management.

Appropriate antimicrobial therapy can also reduce the duration and amount of shedding of *Vibrio* organisms in the stool. The antibiotic choice should be based on the local antimicrobial resistance profiles . Tetracycline has shown to be very effective treatment for cholera, and generally has better efficacy than furazolidone and chloramphenicol.

Erythromycin and/or azithromycin are an appropriate choice of antimicrobial therapy in children and in pregnant women ; other antimicrobial agents that are effective include trimethoprim‑sulfamethoxazole, fluoroquinolones, and doxycycline.

***Vibrio parahaemolyticus*:**

V. parahaemolyticus is a halophilic bacterium that causes acute gastroenteritis after ingestion of contaminated seafood such as raw fish or shellfish . After an incubation period of 12‑24 hours , nausea and vomiting , abdominal cramps , fever , and an explosive watery diarrhea occur. Except in severe cases , grossly evident blood and/or mucus is not found in stool specimens . Clinically , the enteritis ranges from mild watery diarrhea to a forthright , dysentery-like syndrome, but then tends to subside spontaneously within 1‑4 days with no treatment other than restoration of water and electrolyte balance. No enterotoxin has yet been isolated from this organism. *V. parahaemolyticus* is a facultatively anaerobe, Gram-negative rod , and does not grow well on some of the routine differential media used to grow salmonellae and shigellae, but it does grow well on blood agar. The organism grows well on TCBS agar , where it yields green colonies (does not ferment sucrose) . The final organism identification is achieved by use of various standard biochemical tests. Usually no specific treatment other than rehydration is required since the gastroenteritis is self-limited. However,

antimicrobial therapy could be considered for patients in whom the diarrheal illness does not resolve within 5 days ; doxycycline and/or fluoroquinolones are appropriate choice for antibiotic therapy and would shorten the duration of the illness.

***Campylobacter jejuni*:**

C. jejuni has emerged as a common human pathogen , causing mainly gastroenteritis and occasionally systemic infections. This organism is the most common cause of bacterial gastroenteritis in the United States ; according to the CDC surveillance data, an estimated 2 million cases occur in the United States each year. *C. jejuni* and other campylobacters are curved , comma- , or S-shaped, Gram-negative, non-spore-forming rods; they have also been described as having (sea gull wing) shapes . Campylobacters are motile, with a single polar flagellum at one or both ends, but some organisms may lack flagella all together.

C. jejuni as well as *C. coli* are positive for both oxidase and catalase . Campylobacters do not oxidize or ferment carbohydrates. Gram stained smears show typical morphology. Nitrate reduction , hydrogen sulfide production , hippurate hydrolysis tests, and antimicrobial susceptibilities can be used for further identification of species . A positive hippurate hydrolysis test distinguishes *C. jejuni* from the other *Campylobacter* species . The campylobacters have lipopolysaccharides with endotoxic activity. Cytopathic extracellular toxins and enterotoxins have been found, but the significance of the toxins in human disease is not well defined.

الأسبوع الثالث عشر

الهدف التعليمي: التعرف على بكتريا البروسيلا.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: ***Brucella*:**

The brucellae are obligate parasites of animals and humans and are characteristically located intracellularly . They are relatively inactive metabolically. *Brucella melitensis* typically infects goats; *Brucella suis*, swine; *Brucella abortus*, cattle; and *Brucella canis*, dogs. Other species are found only in animals . Although named as species , DNA relatedness studies have shown there is only one species in the genus , *B. melitensis*, with multiple biovars. The disease in humans brucellosis (undulant fever, Malta fever), is characterized by an acute bacteremic phase followed by a chronic stage that may extend over many years and may involve many tissues.

General Characters:

Brucellae are adapted to an intracellular habitat , and their nutritional requirements are complex. Some strains have been cultivated on defined media containing amino acids, vitamins , salts , and glucose. Fresh specimens from animal or human sources are usually inoculated on trypticase-soy agar or blood culture media. Whereas *B. abortus* requires 5-10% CO_2 for growth, the other three species grow in air. Brucellae use carbohydrates but produce neither acid nor gas in amounts sufficient for classification . Catalase and

oxidase are produced by the four species that infect humans. Hydrogen sulfide is produced by many strains, and nitrates are reduced to nitrites. Brucellae are moderately sensitive to heat and acidity. They are killed in milk by pasteurization

Diseases:

Although each species of *Brucella* has a preferred host, all can infect a wide range of animals , including humans . The common routes of infection in humans are the intestinal tract (ingestion of infected milk), mucous membranes (droplets), and skin (contact with infected tissues of animals). Cheese made from unpasteurized goats' milk is a particularly common vehicle. The organisms progress from the portal of entry via lymphatic channels and regional lymph nodes to the thoracic duct and the bloodstream, which distributes them to the parenchymatous organs. Granulomatous nodules that may develop into abscesses form in lymphatic tissue, liver, spleen, bone marrow, and other parts of the reticuloendothelial system. In such lesions, the brucellae are principally intracellular. Osteomyelitis, meningitis, or cholecystitis also occasionally occurs. The main histologic reaction in brucellosis consists of proliferation of mononuclear cells, exudation of fibrin, coagulation necrosis, and fibrosis. The granulomas consist of epithelioid and giant cells, with central necrosis and peripheral fibrosis.

Species:

1- *Brucella abortus* usually causes mild disease without suppurative complications ; noncaseating granulomas of the reticuloendothelial system are found.

2- *Brucella canis* also causes mild disease.

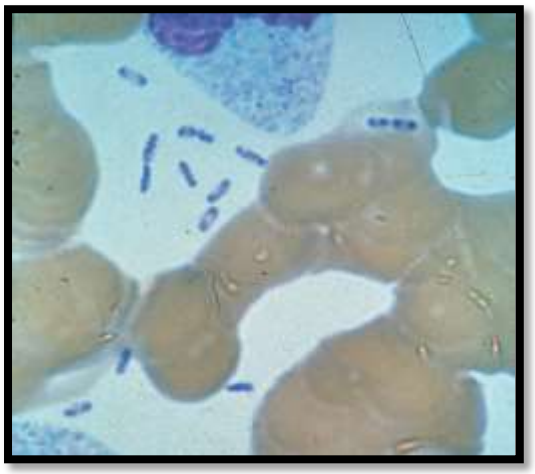
3- *Brucella suis* infection tends to be chronic with suppurative lesions ; caseating granulomas may be present.

4- *Brucella melitensis* infection is more acute and severe.

Yersinia pestis:

General Characters:

Y. pestis is a Gram-negative rod that exhibits striking bipolar staining with special stains such as Wright , Giemsa , Wayson , or methylene blue . It is nonmotile . It grows as a facultative anaerobe on many bacteriologic media and can be readily isolated when sterile specimens such as blood or lymph node aspirates are plated onto sheep blood agar. Growth is more rapid when agar plates are incubated at 28°C. In cultures on sheep blood agar incubated at 37°C, colonies may be smaller when compared to colonies from agar plates incubated at 28°C . To enhance the recovery of *Y. pestis* from a nonsterile site specimen (e.g. , sputum) , it is recommended to inoculate the specimen onto cefsulodin-irgasan-novobiocin (CIN) agar and incubate agar plates at 25‑28°C . Colonies of *Y. pestis* are typically gray to white , sometimes opaque, and are 1‑1.5 mm in diameter with irregular edges; the organism does not produce hemolysis.



Virulence:

All yersiniae possess lipopolysaccharides that have endotoxic activity when released. *Y. pestis* and *Y. enterocolitica* also produce antigens and toxins that act as virulence factors. They have type III secretion systems that consist of a membrane-spanning complex that allows the bacteria to inject proteins directly into cytoplasm of the host cells. The virulent yersiniae produce V and W antigens , which are encoded by genes on a plasmid of approximately 70 kb . This is essential for virulence ; the V and W antigens yield the requirement for calcium for growth at 37°C. Compared with the other pathogenic yersiniae,

Diseases:

When a flea feeds on a rodent infected with *Y. pestis* , the ingested organisms multiply in the gut of the flea and , helped by the coagulase , block its proventriculus so that no food can pass through . Subsequently , the " blocked " and hungry flea bites ferociously , and the aspirated blood , contaminated with *Y. pestis* from the flea , is regurgitated into the bite wound. The inoculated organisms may be phagocytosed by polymorphonuclear cells and macrophages. The *Y. pestis* organisms are killed by the polymorphonuclear cells but multiply in the macrophages ; because the bacteria are multiplying at 37°C , they produce the anti-phagocytic protein and subsequently are able to resist phagocytosis. The pathogens rapidly reach the lymphatics, and an intense hemorrhagic inflammation develops in the enlarged lymph nodes, which may undergo necrosis and become fluctuant. Although the invasion may stop there ,*Y. pestis* organisms often reach the bloodstream and become widely disseminated. Hemorrhagic and necrotic lesions may develop in all organs ; meningitis , pneumonia , and serosanguineous pleuropericarditis are prominent features.

الأسبوع الرابع عشر

الهدف التعليمي: التعرف على بكتريا فرانشيسلا.

مدة المحاضرة: ساعتان.

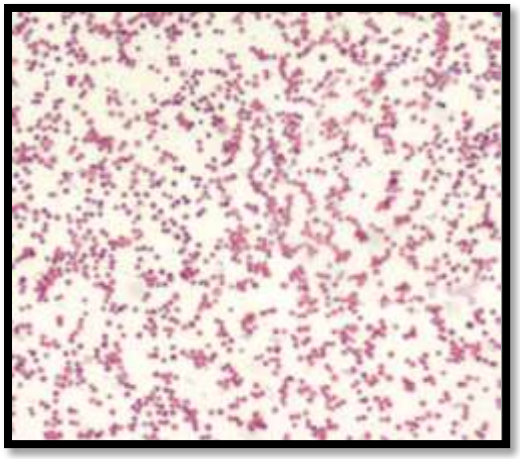
الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: ***Francisella***

General Characters:

F. tularensis is a small, Gram-negative coccobacillus. It is rarely seen in smears of tissue Growth requires enriched media containing cysteine. In the past, glucose-cysteine-blood agar was preferred , but *F. tularensis* grows on commercially available hemin-containing media , such as chocolate agar , modified Thayer-Martin agar, and buffered charcoal yeast extract (BCYE) agar used to grow *Legionella* species . Media should be incubated in CO_2 at 35-37° C for 2-5 days . Caution: to avoid laboratory-acquired infections, biosafety level three (BSL III) practices are required when working with live cultures suspected of containing *F. tularensis*. Clinical specimens require BSL II facilities and work practices.



Diseases:

F. tularensis is highly infectious: penetration of the skin or mucous membranes or inhalation of 50 organisms can result in infection . Most commonly , organisms enter through skin abrasions. In 2‑6 days , an inflammatory , ulcerating papule develops. Regional lymph nodes enlarge and may become necrotic, sometimes draining for weeks (ulceroglandular tularemia). Inhalation of an infective aerosol results in peribronchial inflammation and localized pneumonitis (pneumonic tularemia) . Oculoglandular tularemia can develop when an infected finger or droplet touches the conjunctiva. Yellowish granulomatous lesions on the eyelids may be accompanied by preauricular adenopathy . The other forms of the disease are glandular tularemia (lymphadenopathy but no ulcers) , oropharyngeal tularemia , and typhoidal tularemia (septicemia). All affected individuals have fever, malaise, headache, and pain in the involved region and regional lymph nodes.

Virulence:

While the prototypical LPS from enterobacteria contains a Lipid A part with six acyl chains (12-14 carbon atoms per chain), *Francisella* Lipid A is tetra-acylated, possesses long acyl chains (16-18 carbons), and is hypophosphorylated. These characteristics are key to evading the innate immune response.

Indeed, *Francisella* Lipid A is recognized neither by Toll-Like Receptor 4 nor by the murine intracytosolic LPS sensor, Caspase-11 and only poorly by its human counterpart Caspase-4. A capsule-like structure was observed around *F. tularensis* and characterized as a capsule of polysaccharide identical to the O-antigen subunit of LPS. The purified capsule lacked other LPS components, suggesting that it is a different entity. Unfortunately, to date, there is no convincing genetic way to invalidate the capsule without affecting LPS O-antigen, rendering the characterization of the capsule and its roles in virulence highly challenging.

The anti-inflammatory properties of highly virulent *F. tularensis* have been associated with membrane lipids enriched in this specific strain but absent in LVS. An atypical phosphatidylethanolamine with a very long-chain fatty acid (C24) and a 10 carbon chain was identified as a candidate lipid . As phosphatidylethanolamine is the main component of the inner membrane , this anti-inflammatory lipid may quantitatively contribute to the active inhibition of innate immune responses.

***Nocardia*:**

General Characters:

Nocardia species are aerobic and grow on a variety of media. Microscopically in clinical specimens, nocardiae appear as filamentous organisms with hyphae-like branching. On standard laboratory media, after incubation at 35–37°C for several days, they develop heaped, irregular, waxy colonies. Strains vary in their pigmentation from white to orange to red. These bacteria are Gram-positive and catalase positive, and they produce urease. Nocardiae form extensive branching substrates and aerial filaments that fragment, breaking into coccobacillary cells. The cell walls contain mycolic acids that are shorter chained than those of Mycobacteria. They are considered to be weakly acid fast, that is, they stain with the routine acid-fast reagent (carbolfuchsin) and retain this dye when decolorized with 1–4% sulfuric acid instead of the stronger acid-alcohol decolorant. The species of *Nocardia* are identified primarily by molecular methods such as 16S rRNA gene sequencing and restriction fragment length polymorphism (RFLP) analysis of amplified gene fragments such as *hsp* or *secA*.

Direct Smear:

A direct smear of Nocardia is a laboratory procedure used to examine a sample (like pus or sputum) for the presence of Nocardia bacteria. The procedure involves preparing a thin smear on a slide, heat-fixing it, and then staining it with a modified Ziehl-Neelsen or Kinyoun stain, which are types of acid-fast stains. Microscopic examination reveals branching, filamentous, and weakly acid-fast bacteria characteristic of Nocardia.

***Mycoplasma*:**

Shape:

After 2-6 days on biphasic (broth over agar) and agar medium incubated in a Petri dish that has been sealed to prevent evaporation , isolated colonies of the more rapidly growing mycoplasmas measuring 20-500 μ m can be detected with a hand lens. These colonies are round , with a granular surface and a dark center typically buried in the agar . They can be subcultured by cutting out a small square of agar containing one or more colonies and streaking this material on a fresh plate or dropping it into liquid medium.

Virulence:

At least 16 antigenically distinct species can be identified from humans , including *M. hominis* , *M. pneumoniae* , *M. genitalium* , and *U. urealyticum* . Most *Mycoplasma* species have high evolved systems for variation of outer membrane antigens presumably for evading the host immune response during infection. The species are classified by biochemical and serologic features . The complement fixation (CF) antigens of mycoplasmas are glycolipids. Antigens for enzyme-linked immunoassay (ELISA) tests are proteins. Some species have more than one serotype.

Diagnostic Laboratory:

a. Specimens: consist of throat swabs ; sputum ; inflammatory exudates ; and respiratory , urethral, or genital secretions.

b. Microscopic Examination: direct examination of a specimen for mycoplasmas is use-less. Cultures are examined as described earlier.

c. Cultures: the material is inoculated into broth and onto special solid media depending on the organism sought . Agar media are best incubated at 37°C with 5‑10% CO_2 (under micro-aerophilic conditions or even anaerobic conditions) . Broths require incubation at 37°C under atmospheric (aerobic) conditions. The duration of incubation varies from 2 to 4 days for organisms such as *M. hominis* and *U. urealyticum* to up to 4 weeks for *M. pneumoniae*.

d. Serology:

Antibodies develop in humans infected with mycoplasmas and can be demonstrated by several methods . CF tests can be performed with glycolipid antigens extracted with chloroform‑methanol from cultured mycoplasmas . *M. pneumoniae* and *M. genitalium* are serologically cross-reactive using CF tests. HI tests can be applied to tanned red blood cells with adsorbed *Mycoplasma* antigens. Indirect immunofluorescence may be used. The test that measures growth inhibition by antibody is quite specific. Enzyme immunoassays (EIAs) are available in most laboratories, but sensitivity and specificity are quite variable depending on the assay.

الأسبوع الخامس عشر

الهدف التعليمي: التعرف على بكتريا كلاميديا.

مدة المحاضرة: ساعتان.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: ***Chlamydia***

General Characters:

In chlamydiae , the outer cell wall resembles the cell wall of Gram-negative bacteria. It has a relatively high lipid content including lipopolysaccharide of low endotoxic activity . It is rigid but does not contain a typical bacterial peptidoglycan. As mentioned above, another important structural component is the MOMP encoded by *ompA*. MOMP antigenic variants of *C. trachomatis* are associated with different clinical syndromes . Penicillin-binding proteins occur in chlamydiae, and chlamydial cell wall formation is inhibited by penicillins and other drugs that inhibit transpeptidation of bacterial peptidoglycan. Lysozyme has no effect on chlamydial cell walls. *N*-acetylmuramic acid appears to be absent from chlamydial cell walls. Both DNA and RNA are present in EBsand RBs.

Shape:

Chlamydia bacteria have a distinctive shape and life cycle. They exist in two main forms: an elementary body (EB) and a reticulate body (RB). EBs are small and spherical, while RBs are larger and more oval, according to the stage of development. Both forms have cell walls In essence, chlamydia's similar to Gram-negative bacteria, but they differ in size and function shape and size change depending on its life cycle stage. EBs are small and spherical for transmission, while RBs are larger and more oval for intracellular replication.

Biochemical Tests:

1- Enzymatic assays:

Investigate the function of specific Chlamydial proteins and enzymes , like MurC , which is involved in peptidoglycan synthesis

2- Substrate specificity:

Studies the ability of Chlamydial enzymes to utilize different substrates , which can help in developing new drugs.

3- Metabolic pathways:

Understanding how Chlamydia synthesizes essential molecules like menaquinone (vitamin K2) can lead to the development of new antimicrobial targets

Virulence:

Chlamydiae possess shared group (genus) - specific antigens. These are heat-stable lipopoly-saccharides with 2-keto-3-deoxy-octanoic acid as an immunodominant component. Antibody to these genus-specific antigens can be detected by complement fixation (CF) and immuno-fluorescence . Species-specific or serovar-specific antigens are mainly outer membrane proteins . Specific antigens can best be detected by immunofluorescence , particularly using monoclonal antibodies. Specific antigens are shared by only a limited number of chlamydiae, but a given organism may contain several specific antigens.

Immunity:

Infected individuals often develop both group antibodies and serovar-specific antibodies in serum and in eye secretions . Immunofluorescence is the most sensitive method for their detection. Neither ocular nor serum antibodies confer significant resistance to reinfection.