Microbiology

Definition and historical preview

Microbiology is a Branch of biology concerned with the study of microscopic forms of life, i.e. life forms too small to be seen with the unaided eye. Also it deals with structure, morphology, and the relationships between microorganisms and other organisms.

Branches of microbiology

Bacteriology: Is the science that deals with study of bacteria, this subdivision of microbiology involves the identification, classification, and characterization of bacterial species.

Mycology: (from the Greek μύκης, mukēs, meaning "fungus") is the branch of biology concerned with the study of fungi, including their genetic and biochemical properties, their taxonomy and their use to humans as a source for tinder, medicinals (e.g., penicillin), food (e.g., beer, wine, cheese, edible mushrooms) and entheogens, as well as their dangers, such as poisoning or infection.

Virology : is the study of viruses and virus-like agents: their structure, classification and evolution, their ways to infect and exploit host cells for virus reproduction, their interaction with host organism physiology and immunity, the diseases they cause, the techniques to isolate and culture them, and their use in research and therapy. Virology is considered to be a subfield.

Immunology: is the science that covers the study of all aspects of the immune system in all organisms.[1] It deals with the physiological functioning of the immune system in states of both health and diseases; malfunctions of the immune system in

immunological disorders (autoimmune diseases, hypersensitivities, immune deficiency, transplant rejection); the physical, chemical and physiological characteristics of the components of the immune system in vitro, in situ, and in vivo. Immunology has applications in several disciplines of science, and as such is further divided.

Parasitology: is the study of parasites that infect humans, the diseases caused by them and clinical picture. It is also concerned with the various methods of their diagnosis, treatment and finally their prevention & control. A parasite is an organism that live on or within another organism called the host.

There are two types of microorganisms:

Eukaryotic cell microorganisms (الأحياء المجهريه حقيقية النواة); which include protozoa and fungi.

Prokaryotic cell microorganisms (الأحياء المجهريه البدائية النواة); which include bacteria

History of Microbiology

ANTONY VAN LEEUWENHOEK (1632-1723)

- He was the first Person, who invented the microscope and discovered the microbial world.
- The microscopes of Leeuwenhoek could magnify objects about 200-300 times

LOUIS PASTEUR (1822-1895)

He was a Professor of Chemistry, he considered as "**Father of Microbiology**",.

- He was invent a method for sterilization called **"Pasteurization"**, now widely used in dairy units, to kill pathogenic microorganisms in milk. Also discovered steam sterilizer, autoclave and hot air oven.
- He was achieve the **"Germ theory of disease"** as he visualized that diseases are caused by microorganisms.
- He differentiated between aerobic and anaerobic bacteria and coined the term "**anaerobic**" to refer to the organisms that do not require oxygen for growth.
- He developed the process of **"attenuation"** during his work on "chicken cholera" in fowls. He found that cultures which had been stored in the laboratory for sometime would not kill the animals as fresh cultures did.
- He developed a live attenuated **anthrax vaccine**, by incubation at 40-42°C, which proved to be useful in protecting animals against anthrax.

ROBERT KOCH (1843-1912)/KOCH'S POSTULATES

He was a German country Doctor who later became the Professor of hygiene and Director of institute of infective diseases at Berlin.

He perfected many bacteriological techniques and known as "**Father of Practical Bacteriology**".

- He introduced staining techniques. He prepared dried bacterial films (Smears) on glass slides and stained them with aniline dyes for producing a better contrast under microscope.-
- He discovered tubercle bacillus (*Mycobacterium tuberculosis*) which is popularly called as **Koch's bacillus**. He injected tubercle

bacilli into laboratory animals and reproduced the disease, satisfying all Koch's postulates.

- He discovered *Vibrio cholerae*, the causative agent of cholera disease.

- He developed pure culture techniques by introducing solid media. The use of agar-agar obtained from dried sea weeds (*Gelidium Sp.).*

- He establish the causative role between a particular microorganism and a particular disease. They are popularly known as **Koch's postulates** (Henle-Koch's Posulates). They are :

1. A specific organism should be found constantly in association with the disease.

2. The organism should be isolated and grown in a pure culture in the laboratory.

3. The pure culture when inoculated into a healthy susceptible animal should produce symptoms/lesions of the same disease.

4. From the inoculated animal, the microorganism should be isolated in pure culture.

5. An additional criterion introduced is that specific anitbodies to the causative organism should be demonstrable in patient's serum.

ALEXANDER FLEMMING (1881-1955)

He was an English scientist worked at St. Mary's hospital in London. Flemming was associated with two major discoveries:-

- **lysozyme**. In 1922, he discovered lysozyme by demonstrating that the nasal secretion has the power of dissolving or lasing certain

kinds of bacteria. Subsequently, he showed that lysozyme was present in many tissues of the body. –

- **penicillin** In 1929, Flemming made an accidental discovery that the fungus *Penicillium notatum* produces an antibacterial substance which he called penicillin. Flemming was culturing Staphylococci in petridishes and some of his cultures were contaminated with a mold, subsquently indetified as *Penicillium notatum*. Around the mold colony, there were clear zones, where Staphylococci disappeared. Flemming attributed this to the production of an antibacterial susbstance by the mold. Flemming cultured the fungus *Penicilium notatum* in broth cultures, filtered the fungal mat and obtained the penicillin in soluble form in the culture filtrate.

Bacterial structure

Bacteria: it is a prokaryotic cell that cannot be seen by unaided eye there size between 0.3 and 5 µm. They have three basic forms: cocci, straight rods, and curved or spiral rods. There nucleoid consists of a very thin circular double strand DNA molecular that is not surrounded by a membrane. It may be contain additional genetic structures known as (plasmids).

Fine Structures of Bacteria

Cell envelope: most bacterial cell envelope consisting of a cell wall and an underling cytoplasmic membrane.

Cell Wall

The rigid cell wall which provides protection and imparts (يمنح) shape to most bacterial cells also it facilitate communication with surrounding environment. It was entirely absent in a few unusual bacteria ex: Mycoplasmas.

Peptidoglycan (murein) is the principle structure component of the cell wall; this compound is found in both Gram-positive and Gram-negative organisms, although it is more abundant in Gram-positive bacteria. Peptidoglycan polymers consist of repeating disaccharides formed by N-acetylglucosamine and N-acetylmuramic acid.

The cell wall of Gram-positive bacteria:-

Gram positive bacteria have a simpler but thicker cell wall consisting primarily of:-

1- Multiple layers of peptidoglycan that may consist of as many as 40 layers (15–80 nm thick) and account for as much as 30% of the dry mass of the cell wall.
2- teichoic acid polymers that dispersed throughout the peptidoglycan layers and covalently coupled to the murein , while The membrane lipoteichoic acids are anchored in the cytoplasmic membrane. the role of the teichoic acids is not known in detail; possibly they regulate the activity of the autolysins that steer (يحدد)growth and transverse fission (الانفلاق المستعرض) processes in the cell.
3- Cell wall-associated proteins such as protein A, the clumping factor, the fibronectin- binding protein of *Staphylococcus aureus* and the M protein of *Streptococcus pyogenes*. These proteins frequently function as pathogenicity determinants (محددات الامرا) (specific adherence; phagocyte protection).

The cell wall of Gram-negative bacteria:-

The cell wall of gram negative bacteria is thinner than that of Gram-positive bacteria, it composed of:-

1- Bilayer of peptidoglycan, the murein is only about 2 nm thick and contributes up to 10% of the dry cell wall mass.
2- The outer membrane an additional membrane lies above the peptidoglycan layer. it is much thicker than the single peptidoglycan It contains numerous proteins (50% by mass), it composed of lipid bilayer, proteins (porins), and lipopolysaccharide(LPS, endotoxin).

The Cytoplasmic Membrane

This elementary (أولي) membrane, also known as the plasma membrane, It is basically a double layer of phospholipids with

numerous proteins integrated (مغروسة) into its structure. It is the physical and metabolic barrier between the interior and exterior of the bacterial cell. The cytoplasmic membrane exhibits a well-defined selective permeability.

Cytoplasmic component include:-

Genetic material:-

The cellular nucleus in prokaryotes consists of a tangle (متشابك) of double-stranded DNA, not surrounded by a membrane and localized in the cytoplasm. In E. coli (and probably in all bacteria), it takes the form of a single circular molecule of DNA. The genomic sequence of many bacteria is known.

The plasmids are a small portion of the DNA persists as extrachromosomal elements. These circular, twisted DNA molecules are smaller than the nucleoid genome and reproduce autonomously (ذاتيا). The plasmids of human pathogenic bacteria often bear important genes that determined the phenotype (النمط الظاهري) of bacteria (resistance genes, virulence genes).

Ribosomes:-

Bacterial cell contain approximately 20 000 ribosomes per cell. The type of ribosomes were 70S comprising 30S and 50S subunits, there functions was protein synthesis.

Storage granules :-Temporarily hold excess metabolites. Their presence and amount depend vary with the species of bacteria and its metabolic activity.

External structures:

1- **Capsule**

An additional stricture present outside the cell wall of some kind of bacteria including pathogenic bacteria contain some sort of a polysaccharide layer(طبقة سكريات متعددة) .

The important of capsule:-

1- It can prevent bacterial phagocytosis.
2- Its play an important role in the adherence (التصاق) of bacteria to tissues, or artificial devices.
3- also the bacteria of a single species can be classified in to serotypes based on the fine chemical structure of this capsule

2-Flagella

Are present in many bacteria, its responsible for there motility , its composed exclusively of linear proteins called flagellin and are driven by rotary of swivel –like basal hook, depending on how the flagella are arranged its classified in to several types :-

1- Monotrichous:- some bacteria have a single flagellum
2- Lophotrichous:-
3- Peritrichous:-are destreputed over the surface of the bacterium.

3- **Attachment Pili (Fimbriae** (الخملة)**), Conjugation Pili**

Many Gram-negative bacteria possess short, hair-like structure made of proteins (0.1–1.5 nm thick, 4–8 nm long). They are anchored in the outer membrane of the cell wall and extend radially (بشكل شعاعي) from the surface. Fimbriae are shorter and stiffer (أقوى) than flagella, and slightly smaller in diameter

1- sex pili : that are specifically involve in bacterial conjugation .

2- Common pili (الخملة الاعتيادية) : are usually involved in adherence (التصاق) of bacterial cells to mucosal surfaces which is essential step in colonization and infection of a host .

Bacterial physiology

Nutritional Requirements of Cells: Every organism must find in its environment. All of the substances required for energy generation and cellular biosynthesis (والتمثيل الحيوي للخلية). In the laboratory, bacteria are grown in culture media (وسط زرعي) which are designed (مصمم) to provide all the essential nutrients (الغذاء الاساسي) in solution for bacterial growth.

Macronutrients (العناصر الاساسية): these are required in relatively large quantities and play important role in cell structure (بناء الخلية) and metabolism(العمليات الايضية)

Micronutrient (العناصر النادرة): these are required in small quantities for function of certain enzyme system.

Bacterial requirement for optimum growth:

1-water
2-source of carbon and nitrogen
3-inorganic salts
4-growth factor in some cases
5-source of energy

1-water: it is the most important requirement because it is the principal constituent of bacterial cell. It constitutes about 80% of the total weight, it is vehicle (وسيلة النقل) for the entry of all nutrients in to the cells and for the elimination (إزالة) of all waste products, it is participates in metabolic reaction and it forms an integral part (عنصر مكمل) of protoplasm.

2-Source of carbon and nitrogen: bacterial are classified in to four groups based on the carbon and nitrogen sources they utilize.

A- Autotrophs: use carbon dioxide as the sole source of carbon.

b- Heterotrophs: require more complex organic compounds, such as carbohydrates and amino acids.

C-phototrophs: drive energy from the sunlight. Ex: *rhodospirillum* .

D-chemotrophs:. Is the derivation of biological energy (مشتقات الطاقة الحياتية) from reactions taking place without light (من التفاعلات التي تتم بدون ضوء); in bacteria two types of Chemotrophy prevail:

3- Inorganic salts: these are required for osmotic regulation and to provide trace elements essential for certain enzyme system ex: phosphate, sodium, potassium.

4- Growth factors: many pathogenic species require certain key substances for their growth known as growth factors these include:

a. purines and pyrimidines: required for synthesis of nucleic acids (لتركيب الأحماض النوويه) (DNA and RNA).

b. amino acids (الأحماض الأمينيه): required for the synthesis of proteins (البروتينات).

c. vitamins: needed as coenzymes(عامل مساعد) and functional groups of certain enzymes.

Environmental factor that effect growth of bacteria

1-moisture: the capacity to survive in dry environment varies from organism to organism. Some bacteria like Gonococci and *T. palladium* die quickly in dry conditions, while *staphylococcus aureus* and tubercle bacilli can survive drying for weeks and months.

2-gaseaus requirement: bacteria require oxygen for their growth based on oxygen requirements bacteria can be classified in to four types.

a- Obligate or strict aerobe-grow in presence of oxygen.

B- microaerophilic – requires low oxygen concentrations.

C- Obligate or stric anaerobic- grow only in absence of oxygen.

D- Facultative anaerobe –ordinary aerobes, grow in the presence of oxygen but can also grow in absence of oxygen.

3- temperature:

Pathogenic bacteria grow best at body temperature 37c◦

A- psychrophiles: grow optimally below 15c and are capable of growing at 0c generally do not grow above 20c most of them are soil and water saprophytes.

B- mesophiles: grow at moderate temperature they grow best at 20-40c majority of them are pathogenic organisms.

b- Thermophiles: grow optimally at temperature greater than 45c (range 45-80c) most of them are spore forming ex: bacillus and clostridia. They live in soil and water.

4-Carbon dioxide

5- PH: most pathogenic bacteria grow best (optimum pH) at a neutral or slightly alkaline pH (7.2-7.6), some bacteria grow at acidic pH ex: lactobacillus sp.

6- Light: bacteria grow well in dark.

Bacterial growth (نمو البكتريا):

Growth: The increase in size and division of any organisms or cell has been the main indicator (مؤشر) of microbial viability (حيوية). Bacteria are known to multiply (تتضاعف) by binary fission (الانشطار العرضي), the division of a single bacterium into two daughter bacteria, in suitable environment. After 18-24 hours (hr) of cultivation (زرعها) in the laboratory under ideal conditions (ظروف مثالية) of nutrition, oxygen availability, and buffering.

Growth curve:

When a bacterium is inoculated in to suitable medium and incubated at stable temperature and PH. Bacterial culture passes through different phases of growth when bacterial count of such culture is determined at different intervals and plotted in relation to time. We obtained the following phases:

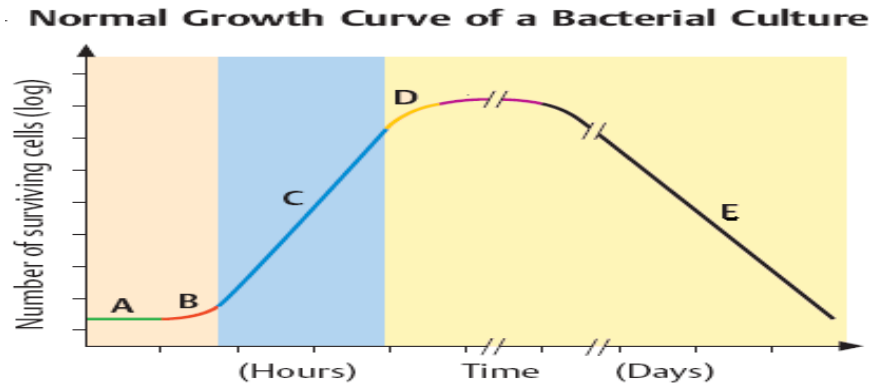
1-Lag phase: occurs when bacteria are inoculated (تزرع) into a fresh, nutritionally enriched medium (محيط غني بالغذاءالطازج).

2-Exponential growth phase: the next step in bacterial proliferation (تضاعف), represents the peak of growth activity (منحنى فعالية النمو) in a culture medium.

3-Stationary growth phase: depending on the bacterial species (أنواع البكتريا) and specific nutrient environment.

4-Decline phase: depending on the accumulation of toxic material (تجمع المواد اسمية) and deficiency of nutrient material (وقلة المواد الغذائية).

Generation time: it is the time required for a bacterium to form two daughter cells under optimum conditions, the generation time in most bacteria is 20 mints, however in tubercle bacilli it is 20 hours and in lepra it is 20 days.



A= lag phase, B = acceleration phase, C = log (exponential) phase

D = stationary phase, E = death phase

Infection:- is the invasion (اجتياح)of a host body tissues by microorganisms and the reaction between host tissues and microorganisms and there toxins. Infections are caused by microorganisms such as viruses, bacteria and fungi.

Disease transmission

1- **Direct transmission include**

- **Horizontal disease transmission :-**from one individual to another in the same generation. Horizontal transmission can occur by either direct contact, or indirect contact.

Direct contact: - The term usually refers to the transmission of microorganisms directly from one person to another by one or more of the following means:

- droplet contact – coughing or sneezing on another person
- direct physical contact – touching an infected person, including sexual contact
- indirect physical contact – usually by touching soil contamination or a contaminated surface
- airborne transmission – if the microorganism can remain in the air for long periods
- Fecal-oral transmission – usually from contaminated food or water sources.

Indirect contact:-transmission occurs, via another organism, either a vector الناقل (e.g. a mosquito) or an intermediate host (e.g. tapeworm in pigs can be transmitted to humans who ingest improperly cooked pork لحم الخنزير غير المطبوخ جيدا).

- **Vertical disease transmission** – passing a disease causing agent vertically from parent to offspring الذرية, such as prenatal.

Virulence (الضراوة):- it is the pathogen capacity to harm the host. Virulence is measured in term of number of M.O. or microgram of toxin necessary to kill a given host when administrated by certain route.

Virulence factor include:-

1-toxins: microbial toxins are usually grouped as exotoxins and endotoxins.

The essential features of each group are listed in the following table.

Endotoxins	exotoxins
1-integral part of microbial cell wall in Gve- bacteria is librated upon its disintegration.(تتفكك) 2-in LPS, lipid A may be responsible for toxicity. 3-relatively stable with stand heat above 60c for hours without loss of toxicity. 4-don't stimulate formation of antibody. 5-don't convert to toxoids. 6-weakly toxic, fatal to animal in hundred micrograms 100 Mg. 7-often produce fever.	1-execreated by living cell found in high conc. In fluid medium. 2-polypeptide with M.W.10,000-900,000 Dalton. 3-relatively unstable, loss of toxicity take place when exposed to temp. above 60c. 4-Highly antigenic, stimulate formation of antitoxin (high titer). 5-convert to antigenic nin-toxic toxoids by using formalin, heat acids. 6-highly toxic fatal to lab animals in microgram or less. 7-don't produce fever.

2-extracellular enzymes:

Collagenase: it hydrolyzed collagen, this promote spread of bacteria in tissue ex: *closterdium perifringens.*

Coagulase: this enzyme in conjugation of serum factors cause plasma coagulation leading to the formation of fibrin wall around staph lesion which help them to persist.

Hyaluronidase: hydrolyze hyalouronic acid (a constituent of ground substance of connective tissue) produced by clostridium and staphylococci.

Streptokinase: dissolve and coagulate plasma, also aid in the spread of streptococci.

Protease: hydrolyze immunoglobulin.

Invasiveness (الاجتياح):- it is the ability of M.O. to enter the host tissue, multiply and spread. Microorganisms that cause tetanus and diphtheria are toxin producers but they are non-invasive, anthrax &plague bacteria are highly invasive, staphylococcus &streptococcus Are moderately invasive. Certain microorganisms may be invasive and virulent because they survive within phagocytic cells and resist enzymatic attack.

Genus staphylococcus

They are invasive microorganism, resist high concentration of NaCl, drugs and dryness, capable to survive outside of the body for extend periods. They are normal flora on skin surface and mucous membranes of the upper respiratory tract, any break or injury in the skin or mucus membrane may lead to infect underlying tissues, they are moderately resist to heat (50-60c for 1-1.5 hr) and salt (10%NaCl).

Classification:- there are three medically important species in this group.

1-*staphylococcus aureus*:-is responsible for most staphylococcal infection in humans.

2-*S. epidermidis* :- often causes opportunistic infection in debilitated or immunocompromised patients.

3-*S.saprophyticus*:-anther opportunistic organism, may cause urinary tract infections in women.

General characteristic:-

G+ve cocci grape like appearance or cluster some time appear in short chain pairs or single, non motile, non spore forming some form capsule when freshly isolated from tissue.

Extracellular toxins and enzymes:-

1- Plasma coagulase is an enzyme that functions like thrombin to convert fibrinogen into fibrin. Tissue microcolonies surrounded by fibrin walls are difficult to phagocytose.

2- A toxin can have lethal CNS effects, damages membranes (resulting in, among other things, hemolysis), and is responsible for a form of dermonecrosis.

3- Leukocidin damages microphages and macrophages by degranulation.

4- Exfoliatins are responsible for a form of epidermolysis.

5- Food poisoning symptoms can be caused by eight serologically differentiated enterotoxins (A-E, H, G, and I). These proteins are not inactivated by heating to 100 8C for 15–30 minutes. Staphylococcus enterotoxins are superantigens.

6- Toxic shock syndrome toxin-1 (TSST-1) is produced by about 1% of Staphylococcus strains. TSST-1 is a superantigen that induces clonal expansion of many T lymphocyte types (about 10%), leading to massive production of cytokines, which then give rise to the clinical symptoms of toxic shock.

7- haemolysin:- acts on cell membrane of RBCs, platelets, macrophages causing lysis-fatal infection.

Pathogenesis and clinical pictures:-

1- Invasive infections. In this type of infection, the pathogens tend to remain in situ after penetrating through the derma or mucosa and to cause local infections characterized by purulence (. Examples include furuncles, carbuncles, wound infections, sinusitis, otitis media, and mastitis puerperalis. Other kinds of invasive infection include postoperative or posttraumatic ostitis/ osteomyelitis, endocarditis following heart surgery (especially valve replacement), postinfluenza pneumonia, and sepsis in immunocompromised patients. S. aureus and E. coli are responsible for approximately equal shares of nearly half of all cases of inpatient sepsis.

2- Toxicoses. Food poisoning results from ingestion of food contaminated with enterotoxins. The onset a few hours after ingestion takes the form of nausea, vomiting, and massive diarrhea.

3- Mixed forms. Dermatitis exfoliativa (staphylococcal scalded skin syndrome, Ritter disease), pemphigus neonatorum, and bullous impetigo are caused by exfoliatin-producing strains that infect the skin surface. Toxic shock syndrome (TSS) is caused by strains that produce TSST-1. These strains can cause invasive infections, but may also only colonize mucosa. The main symptoms are hypotension, fever, and a scarlatiniform rash.

Treatment :- the staph has the ability to produce B-lactamase enzyme which cause cleavage of B-lactam bond, this character is controlled by genetics and the plasmid transfer this character. So the drug of choice for staph are cephalosporin& methicillin. Vancomycin is the drug of choice for methicillin-resistant staphylococci.

Immunity:- no vaccine is available .

Genus streptococci

Include a large number of species, some of which are pathogenic and others are member of the normal flora of oropharynx and gastrointestinal tract.

Classification:- Streptococci can be classified according to:

1- Oxygen requirements
   - Anaerobic (*Peptostreptococcus*)
   - Aerobic or facultative anaerobic (*Streptococcus*)

2- Serology (Lanciefield Classification)

3- Hemolysis on Blood Agar (BA)

2- Lancefieled system:- determination of antigenicity of streptococcal cell wall carbohydrate called the C substance allows grouping of streptococcus in to groups A through R. species can be grouped on the basis of antigenic differences of the cell wall proteins (M,R,T protein).serious human pathogens fall into groups A,B,C,D and G.

3- Hemolytic pattern.

a- Alpha hemolytic streptococci: these species are called viridians. Most species in this group lack a polysaccharide capsule except *S. pneumonia*. Colonies on blood agar are surrounded by a green zone. This "greening" is caused by H2O2, which converts hemoglobin into methemoglobin

b- Beta hemolytic streptococci: these species responsible for the majority of streptococcal diseases, although not all of them are pathogenic. Colonies on blood agar are surrounded by a large, yellowish hemolytic zone in which no more intact erythrocytes are present and thehemoglobin is decomposed.

c- Gamma hemolytic streptococci: these species usually not pathogenic.

This term indicates the absence of macroscopically visible hemolytic zones

Group A streptococci (*S. pyogenes*)

Pathogenesis and Virulence Factors:-

- **Structural components**

1- **protein M,** antiphagocytic, anticomplement and strogly immunogenic which interferes with opsonization and lysis of the bacteria .

2- **Lipoteichoic acid & F protein,** is an adhesion factor that, together with protein M, enables group A streptococci to bind to pharyngeal epth. Cells.

3- **Protein G:-** prevent effective phagocytosis.

4- **Hyaluronic acid capsule,** this capsule is not immunogenic but has antiphagocytic properties.

5- **C substance and cytoplasmic membrane antigens.** These molecules are structurally similar to human tissue antigens, particularly those of the heart, kidney, and joints.

- **Enzymes**
  - Streptokinases
  - Deoxynucleases
  - C5a peptidase
- **Pyrogenic toxins (**erythrogenic toxins, exotoxin A, exotoxin B, cardiohepatic toxin **)**that stimulate macrophages and helper T cells to release cytokines.
- **Streptolysins**

1- **Streptolysin O:-** oxygen –label, lyse red blood cells, white blood cells, and platelets, acts as an antigen. Past infections can be detected by measuring the antibodies to this toxin (antistreptolysin titer).

2- **Streptolysin S:- oxygen** – stable and nonimmunogenic , its hemolytic and cytotoxic

- **Spreading factors :-**(hyaluronidase, proteinases, streptokinase, and nucleas).

Diseases caused by group A streptococci

- **Suppurative**
  1- **Non-Invasive**
    - Pharyngitis ("strep throat")-inflammation of the pharynx
    - Skin infection, Impetigo
  2- **Invasive**
    - Scarlet fever-rash that begins on the chest and spreads across the body
    - Pyoderma-confined, pus-producing lesion that usually occurs on the face, arms, or legs
    - Necrotizing fasciitis-toxin production destroys tissues and eventually muscle and fat tissue
- **Non Suppurative**
  1- Rheumatic fever: Life threatening inflammatory disease that leads to damage of heart valves muscle
  2- Glomerulonephritits
    - Immune complex disease of kidney
    - inflammation of the glomeruli and nephrons which obstruct blood flow through the kidneys

Treatment:-

Penicillin (patients who are allergic to penicillin the drug of choice is erythromycin).

Group B streptococci (*S. agalactiae)*

Are often isolated from the nasopharynx, oral cavity, intestinal tract, and vaginal of healthy individual.

Diseases caused by group B streptococci

They are a significant cause of neonatal infections, acquired during passage through the birth canal. (bacteremia, pneumonia and meningitis).

Treatment :- Ampicillin is the drug of choice.

Streptococcus pneumoniae (pneumococcus).

There morphology is distinctive in that the cocci are ovoid or lancet-shaped and are often seen in pairs on Gram-stained samples. *S. pneumonia* lacks group- specific cell wall antigens, therefore it cannot be classified using the lancefield system.

- **Virulence factors :-**
  1- polysaccharide capsule (which has antiphagocytic properties).
  2- IgA protease (inactivates secretory LgA antibodies.
- **Clinical disease :-** (bacterial pneumonia in adults and children, otitis media, meningitis, sinusitis, and bronchitis).

Treatment :-

Penicillin, third generation cephalosporins and vancomycin is the drug of choice for highly resistant strains of *S. pneumonia.*

Species of Corynebacteria

General characters:-

Most the members of genus corynebacteriumarm are normal flora of the skin, nasophyarynx, oropharynx, urogenital tract, and gastrointestinal tract. These species are collectively known as diphtheroids. They are G +ve pleomorphic bacilli (club-like appearance). Non motile, non-spore forming, non-capsulated bacilli, catalase +ve. Aerobic or facultative anaerobic optimum temperature 37c^{o}, PH 7.2

Corynebacterium diphtheriae (Diphtheria)

Determinants of pathogenicity:-

C. diphtheria is not an invasive organism, systemic symptoms are attributable to production of an exotoxin**,** diphtheria toxin. After binding to the host cells, the active subunit will interrupt the protein synthesis of the target host cell and results in cell death.

Toxin production occurs only when the bacillus is itself infected by a specific virus (bacteriophage, a lysogenic β-phage) carrying the genetic information for the toxin (toxin gene).

Only toxigenic strains can cause severe disease. So, all isolates of C. diphtheriae should be tested by the laboratory for toxigenicity (ELISA or the Elek tests).

Pathogenesis and Clinical Picture.

Diphtheria is an acute, toxin-mediated disease caused by toxigenic *Corynebacterium diphtheriae* . It's a very contagious and potentially life-threatening bacterial disease. The incubation period of diphtheria is 2-4

days (range, 1-7 days). This disease can involve almost any mucous membrane.

It's cause a localized infectious, which usually attacks the throat and nose mucous membrane. In serious cases, it can attack the heart and nerves which cause a the major complications of myocarditis and neuritis, and can also cause low platelet counts (thrombocytopenia) and protein in the urine (proteinuria).

Common symptoms: malaise, sore throat, anorexia, and low-grade fever With lymph nodes enlargement in the submandibular areas of neck .

Typical sign: specific membrane formation, The pseudomembrane consists of coagulated fibrin, inflammatory cells, destructed mucous tissues and bacteria. The formation of pseudomembrane in larynx, trachea or bronchia may have the potential for airway obstruction.

Transmission:-

Transmission is most often person-to-person spread from the respiratory tract (by small droplet when coughing or sneezing). Rarely, transmission may occur from skin lesions or articles soiled with discharges from lesions of infected persons. (children are most often affected).

Treatment:-

Suppression of bacterial growth (antibiotic) , neutralization of the toxin (antitoxin) , and supportive measures. The patient should be admitted to a hospital and isolated. penicillin is the drug of choice.

Control and prevention:-

Vaccination with diphtheria toxoid will effectively prevent diphtheria. Diphtheria toxoid is included in the diphtheria-pertussis-tetanus(DPT)vaccine.

Genus Clostridium

General characteristic :-

- Gve+ large bacilli
- Colony morphology is variable.
- anaerobic
- vegetative forms are slightly motile.
- spore forming (Spores are resistance to heat ,other physical agents & resist oxygen except *C. tetani*).
- saprophytic in the external environment (soil), although some are part of the intestinal flora of humans& animals.
- they produce exotoxin& enzymes (lack catalase, superoxide dismutase).
- vegetative forms are slightly motile.
- some species swarms like *proteus*,
- few of them are capsulated.
- hemolysis on blood agar is frequent.

All species of this genus are human pathogens and cause severe infection.

Classification :-

Family : bacillaceae

Genus : *clostridium*

Spp: *C. tetani*…….tetanus

C. perifringens…….welchii – gas gangarine

C. botulinum ……. Food poisoning

C. difficili …….severe diarrhea

C. septicum …….associated with wound infection.

C. perferingens

Determinant of pathogenicity:-

1- Exotoxins (alph-toxin) is most important and mediates destruction of host cell membranes.
2- Enterotoxin inserts and disrupts membranes of mucosal cell.
3- The capsular materiel is polysaccharide. ***C. perferingens*** can be classified in to five serotypes (A TO B) according to the properties of capsule and specific toxin production.

Clinical diseas :-

1- suppurative infection and abscesses.
2- Localized cellulitis.
4- Enteritis necroticans.
5- Gas gangrene (myonecrosis).
6- Food poisoning.

Therapy:-

Primary treatment is surgical, accompanied by antibiosis (penicil lins, cephalosporins).

C. botulinum

Determinant of pathogenicity:-

1- neurotoxins was an extremely potent toxin (botulin is heat sensitive but total in activation requires boiling for 20 minutes).
2-

Clinical diseas:-

1- Food poisoning
2- Infant botulism
3-Wound botulism

C.tetani

Determinant of pathogenicity:-

1- tetanospasmin, a neurotoxic exotoxin that disrupts nerve impulses to muscles.(spastic paralysis) oxygen stable, heat labile.

2- Flagellar antigen(I-X) type I and III cause of human infection

3- Haemolysin : its heat label and oxygen labile .

Clinical disease:-

1- local tetanus

2-cephalic tetanus

3- generalized tetanus

Treatment:-

Antitoxin – after any injury Antibiotic penicillin , tetracycline , Erythromycin.

C. difficile

Determinant of pathogenicity:-

1- toxin A, which is an enterotoxin that is thought to be primarily responsible for the gastrointestinal disease caused by this organism.

2- toxin B a cytotoxin has a less clear role in the infection.

Clinical diseas:-

1- antibiotic – associated diarrhea.

2- pseudomembranous colitis.

Drug of choice:-

Vancomycin, pencillin G.

Genus bacillus

This genus include 48 recognizable species the defining characteristic of bacillus are Gve^{+} rod, form oval central located spores, non-motile, mostly obligate aerobes, some facultative anaerobes. Two Bacillus species are considered medically significant: *B. anthracis*, which causes anthrax, and *B. cereus*, which causes food poisoning similar to that caused by Staphylococcus.

B. anthracis :- was the first bacterium conclusively demonstrated to cause disease by Robert Koch in 1877, the species name anthracis is form of the disease , cutaneuos anthrax in which large black skin lesions are formed

The bacterium can be cultivated in ordinary nutrient medium under aerobic or anaerobic conditions .

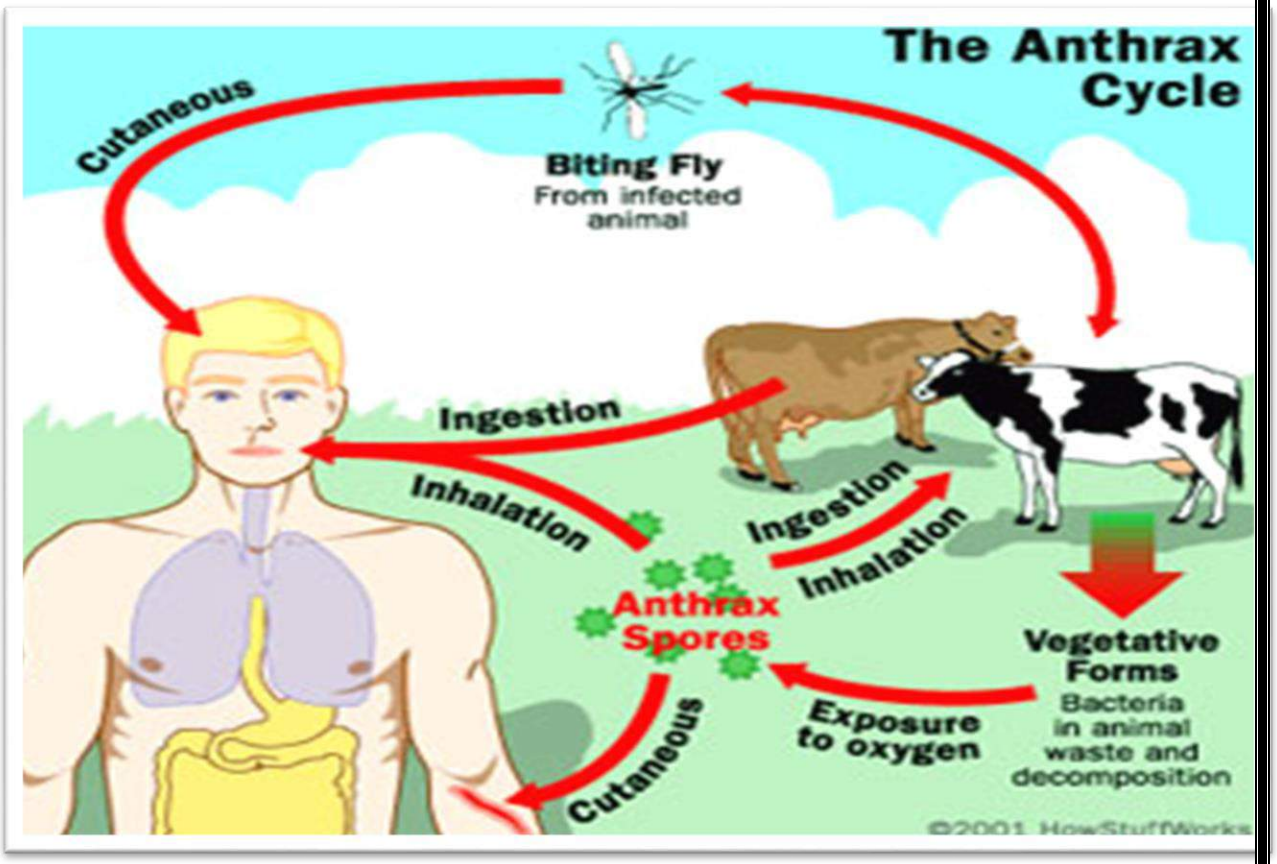
Virulence factor:-

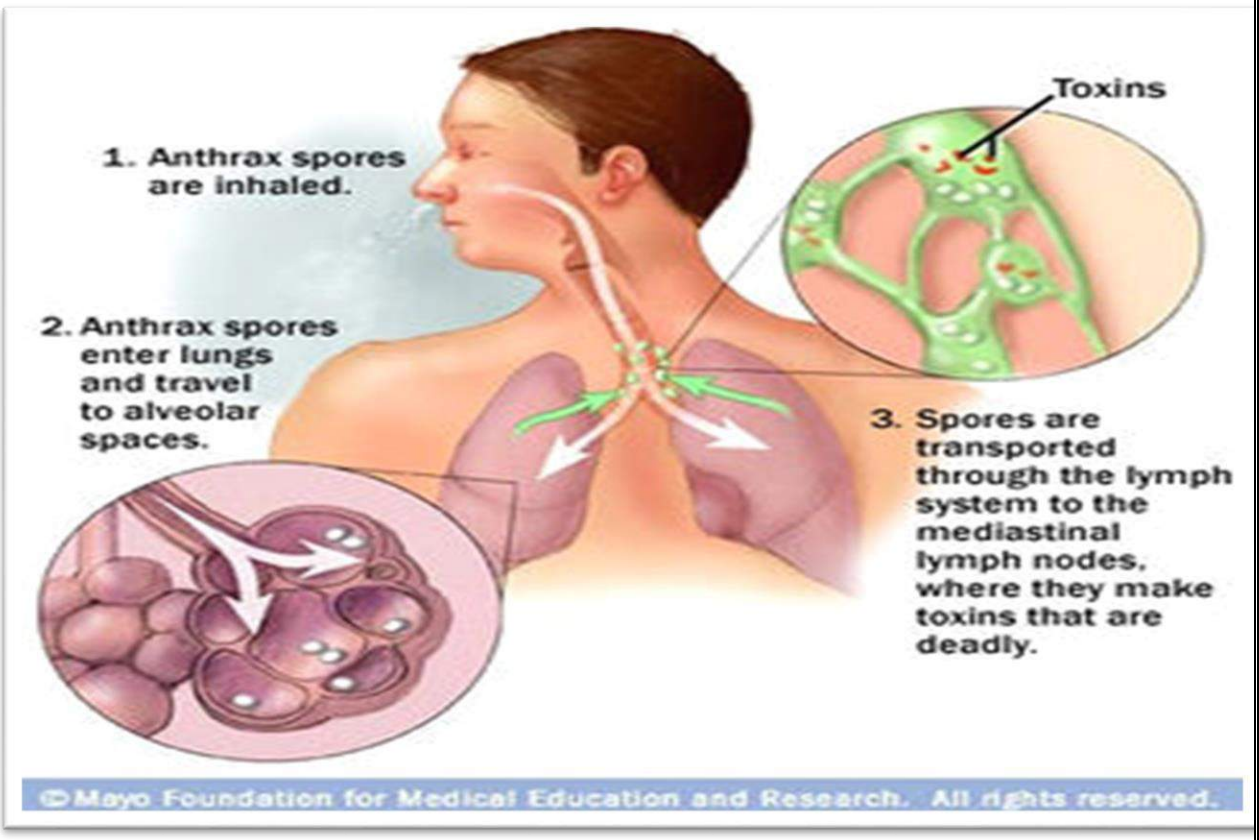
1- *B. anthracis* possesses an antiphagocytic capsule essential for virulence.
2- The organism also produces three plasmid-coded exotoxins:

- **edema factor**:- a calmodulin-dependent adenylate cyclase, causes elevation of intracellular cAMP, and is responsible for the severe edema usually seen in *B. anthracis* infections
- **lethal toxin:-** is responsible for tissue necrosis;
- **Protective antigen:-** (so named because of its use in producing protective anthrax vaccines) mediates cell entry of edema factor and lethal toxin.

Mode of transmission:-

Humans acquire it as a result of contact with infected animals or animal products. In humans the disease takes one of three forms, depending on the route of infection. Cutaneous anthrax, which accounts for more than 95 percent of cases worldwide, results from infection through skin lesions; intestinal anthrax results from ingestion of spores, usually in infected meat; and pulmonary anthrax results from inhalation of spores.





Clinical disease :-

Three forms of human anthrax disease are recognized based on their portal of entry.

- Cutaneous, the most common form (95%), causes a localized, inflammatory, black, necrotic lesion (eschar).
- Pulmonary, the highly fatal form, is characterized by sudden, massive chest edema followed by cardiovascular shock.
- Gastrointestinal, a rare but also fatal (causes death to 25%) type, results from ingestion of spores.

Treatment:-

Penicillin is the drug of choice.

Bacillus cereus* and *bacillus subtilis

Are widely distributed in the environment and may cause human disease, particularly in immunocompromised individuals.

Virulence factors:-

Produce enterotoxins and pyogenic toxin.

Clinical disease:-

1-food poisoning (short incubation, nausea, and vomiting as the predominant symptoms). *Bacillus cereus* can cause two distinct types of food poisoning. The *diarrheal type* is characterized by diarrhea and abdominal pain occurring 8 to 16 hours after consumption of the contaminated food. It is associated with a variety of foods, including meat and vegetable dishes, sauces, pastas, desserts, and dairy معمل البان products. In *emetic disease*, on the other hand, nausea and vomiting begin 1 to 5 hours after the contaminated food is eaten. Boiled rice that is held for prolonged periods at ambient temperature and then quick-fried before serving is the usual offender, although dairy products or other foods are occasionally responsible. The symptoms of food poisoning caused by other *Bacillus* species (*B subtilis, B licheniformis*, and others) are less well defined. Diarrhea and/or nausea occurs 1 to 14 hours after consumption of the contaminated food. A wide variety of food types have proved responsible in recorded instances.

A *Bacillus* food poisoning episode usually occurs because spores survive cooking or pasteurization and then germinate and multiply when the food is inadequately refrigerated. The symptoms of *B cereus* food poisoning are caused by a toxin or toxins produced in the food during this multiplication. Toxins have not yet been identified for other *Bacillus* species that cause food poisoning.

2-systemic infection can also cause systemic infection in immunocompromised patients.

Treatment:-

1- food poisoning is usually a self-limiting situation that requires supportive treatment only.

2- systemic infection treated with clindamycin.

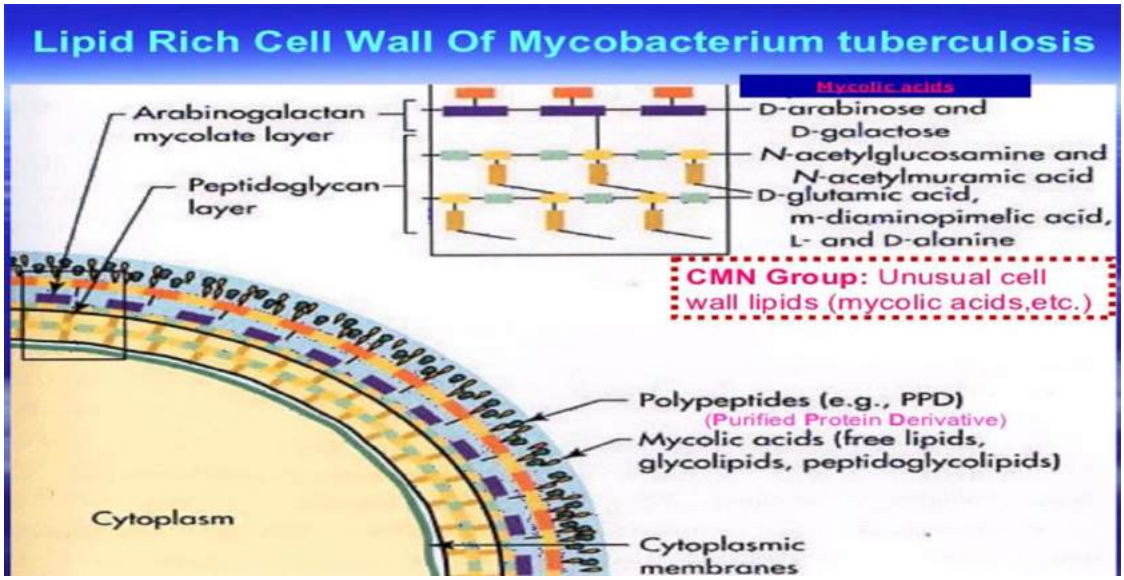
Diagnosis:-

Grow well on 5% sheep blood agar (large, feathery, spreading, beta-hemolytic) chocolate agar, routine blood culture media and commonly used nutrient broths.

Genus Mycobacterium

General characteristic:-

They are slow-growing, aerobic bacteria, with an unusual cell wall composition, they are slender, strait or slightly curved rode, some species are saprophytic .the most significant human pathogens are *M. tuberculosis*, *M. avium*-intracellulare, and *M. leprae*. Mycobacteria characteristically survive after ingestion by macrophages and behave as facultative intracellular organisms.



Taxonomy:-

- Phylum:- Actinobacteria
- family:- Mycobacteriaceae
- Genus *Mycobacteria*

Medical classification

Mycobacteria can be classified into several major groups for purpose of diagnosis and treatment:-

1- ***M. tuberculosis*** **(MTB)** , which can cause tuberculosis

2- ***M. leprae***, which causes Hansen's disease or leprosy**;**

3- ***M. bovis*****,**

4- ***M. africanum*****,**

5- ***M. microti***,

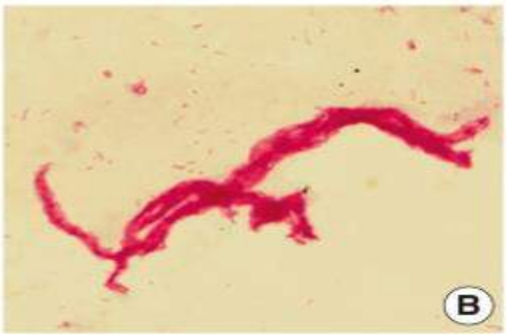
6- **Non Tuberculous mycobacteria (NTM)** are all the other mycobacteria, which can cause pulmonary disease resembling tuberculosis, lymphadenitis, skin disease, or disseminated disease.

Mycobacterium tuberculosis

In the entire history of humankind, it is believed that tuberculosis has killed more people than any other disease. In 1882, the microbiologist Robert Koch discovered the tubercle bacillus, at a time when one of every seven deaths in Europe was caused by TB. It's the causative agents of Tuberculosis which is highly contagious and spreads through the air from coughing.

Determinants of pathogenicity:- the virulence factors for M. tuberculosis have not been as well defined as those for many other bacteria.

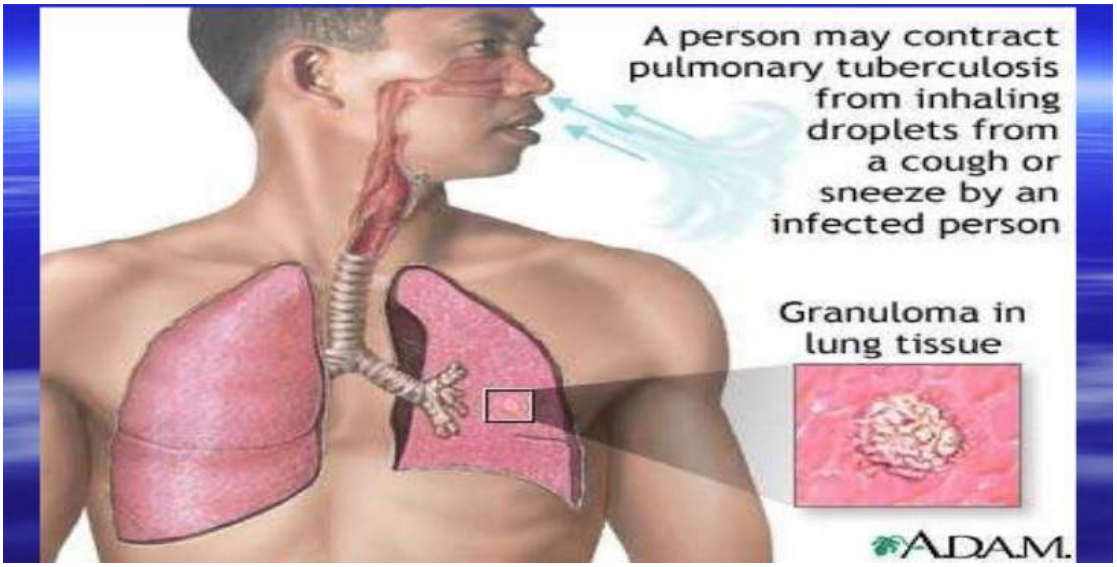
1- **Cording factor**: is a glycolipid derivative of mycolic acid that is present on the outer surface of *M. tuberculosis.* It's have immunogenic properties. Its cause's bacilli to grow in culture contain serpentine" cords" observation of this type of growth is usually indicative of pathogenicity.



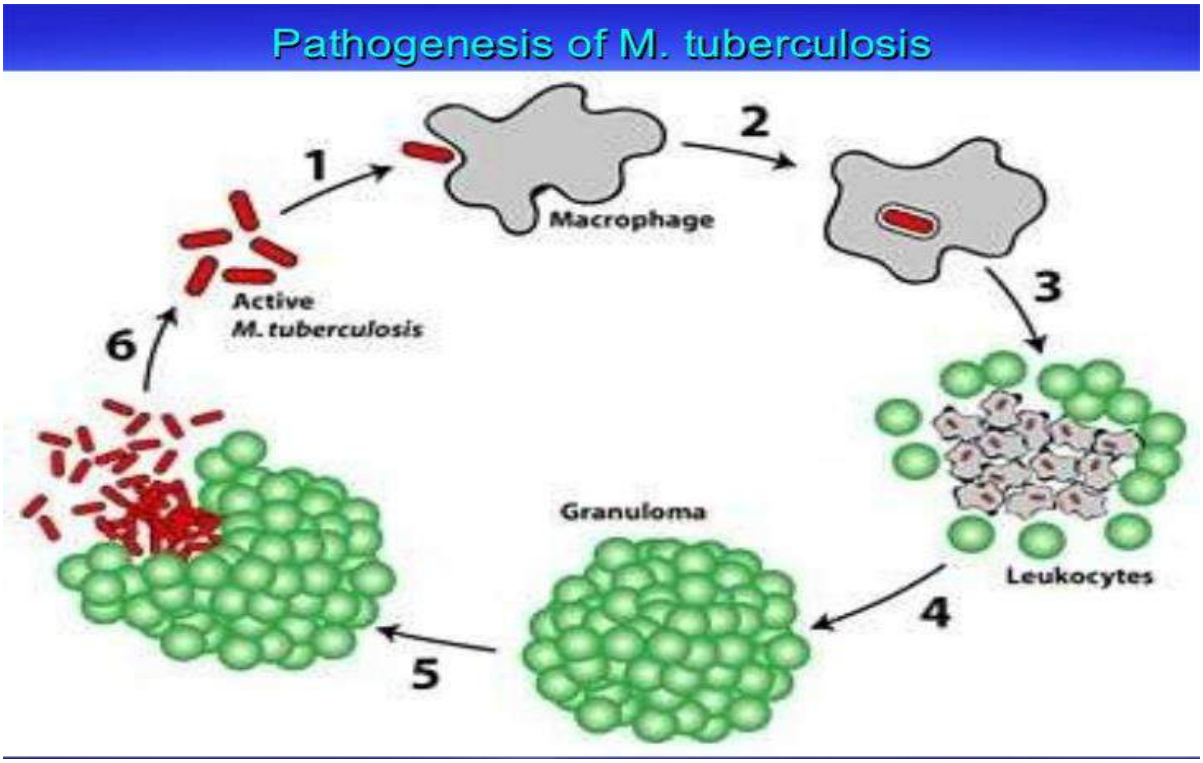
2- **Sulfatides**: glycolipids inhabit phagolysosome formation, permitting the bacterium to survive intra-cytoplasmic after being ingested by macrophages.

Clinical disease:-

1- **Primary tuberculosis**. In the majority of cases, the pathogens enter the lung in droplets, granulomas form at the primary infection site and in the affected lymph nodes, and macrophages are activated by the cytokine MAF (macrophage activating factor).



2- They are phagocytosed by alveolar macrophages. TB bacteria are able to reproduce in these macrophages due to their ability to inhibit formation of the phago-lysosome. Within 10–14 days a reactive inflammatory focus develops, this called primary focus from which the TB bacteria move into the regional lymph nodes, where they reproduce and stimulate a cellular immune response.



3- A tuberculin allergy also developed . The further course of the disease depends on the outcome of the battle between the TB and the specific cellular immune defenses.

4- Mycobacteria may also be transported to other organs via the lymph vessels or bloodstream and produce dissemination foci there. The host eventually prevails in over 90% of cases: the granulomas and foci fibrose, scar, and calcify, and the infection remains clinically silent.

2- Secondary tuberculosis.:- In about 10% of infected persons the primary tuberculosis reactivates to become an organ tuberculosis, either within months (5 %) or after a number of years (5 %).

Treatment:-

• Isoniazid (called INH)

• Rifampin

• Pyrazinamide (PZA)

• Ethambutol

Mycobacterium leprae :-

It's the causative agent of leprosy. There are two major form of leprosy .

1-tuberculoid leprosy: is characterized by macules or extensive plaques on the trunk, face, and limbs.

2-lepromatous leprosy: the bacteria probably disseminate hematogenously, although the disease does not appear to be manifest in the deeper organs.

Diagnosis :- is based primarily on clinical signs and symptoms.

Pathogenesis. The pathomechanisms of LB are identical to those of TB. The

host organism attempts to localize and isolate infection foci by forming granulomas. Leprous granulomas are histopathologically identical to tuberculous granulomas. High counts of leprosy bacteria are often found in the macrophages of the granulomas.

Clinical picture. Leprosy is manifested mainly on the skin, mucosa, and peripheral nerves. A clinical differentiation is made between tuberculoid leprosy(TL) and lepromatous leprosy(LL). There are many intermediate forms. TL is the benign, non-progressive form characterized by spotty dermal lesions. The LL form, on the other hand, is characterized by a

malignant, progressive course with nodular skin lesions and cordlike nerve thickenings that finally lead to neuroparalysis. The inflammatory foci contain large numbers of leprosy bacteria.

Therapy. Paucibacillary forms are treated with dapson plus rifampicin for six

months. Multibacillary forms require treatment with dapson, rifampicin, and

clofazimine over a period of at least two years.

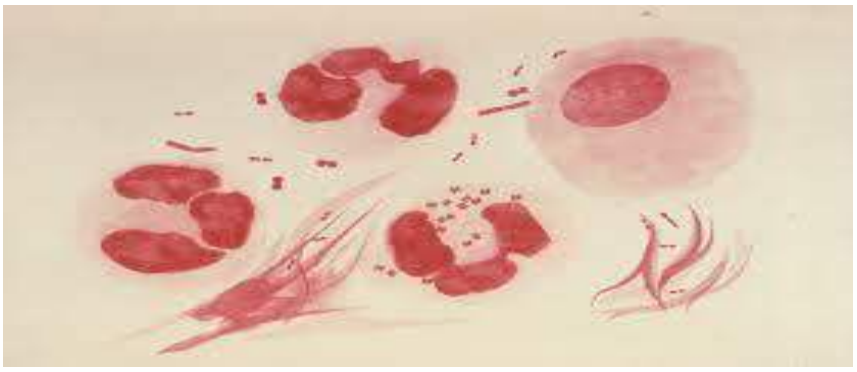
Immunity. The immune defenses mobilized against a leprosy infection are

strictly of the cellular type. The lepromin skin test can detect a postinfection allergy. This test is not, however, very specific (i.e., positive reactions in cases in

Genus neisseria

General characteristic:-

The typical Neisseria is a gram-negative, non-motile diplococcus. Individual cocci are coffee bean-shaped or kidney-shaped; when the organisms occur in pairs, the flat or concave sides are adjacent, it need enriched media. Some it capsulated and piliated. The genus Neisseria includes two pathogenic species N. meningitides and N. gonorrhoeae as well as several nonpathogenic species.



N . gonorrhoeae

also known as ***gonococci*** (plural), or ***gonococcus*** (singular), is a species of Gram-negative coffee bean-shaped diplococci bacteria responsible for the sexually transmitted infection gonorrhea.

Clinical deases

1- local infection (urethritis, cervicits, neonatal ophthalmia, pharyngeal gonorrhea, perihepatitis)

2- disseminated infection (DGI)

Determinant of pathogenicity

1- pili

2-capsule it does not prevent phagocytosis, but it does seem to allow intracellular survival of ingested organisms.

3- endotoxin disseminated infection, is much less toxic than the endotoxin produced N. meningitides.

4- enzymes (IgAase, beta-lactamase).

5-outer memprene protein (I,II)

Protein I (invasion, disseminated infection).

Protein II (primary virulence factore, mediate attachment to mucosal cells and have antiphagocytic properties.

6- peptidoglycan has pro- inflammatory properties.

Pathogenesis:-

The bacteria adhere to the surface of epithelial cells, particularly those of the urethra, genital tract, rectum, and throat. Then invade the epithelial cells and penetrate the sub mucosal space, causing a suppurative infection. Disseminated via direct propagation or hematogenous spread.

Treatment

The agent of choice used to be penicillin G. In recent years, however, the percentage of penicillinase-producing strains has increased considerably all over the world. For this reason, third-generation cephalosporins are now used to treat uncomplicated cases of gonorrhea. They are applied in a single dose (e.g., ceftriaxone, 250–500mg i.m.). Good results have also been reported with single-dose oral application of fluorinated 4-quinolones (e.g., 0.5 g ciprofloxacin or 0.4 g ofloxacin).

N. meningitides

One of the most virulent human pathogens, is the etiologic agent of meningitis التهاب السحايا.

Classification :-

1- serogroups: divided in to nine serogrouups according to antigenic differences in capsular polysaccharide(A,B,C,X,Y,Z,29e,W-135).

2-serotypes: the serogroupe B,C are subdivided in to serotypes according to outer membrane proteins.

Clinical disease:-

1- a febrile illness.

2- meningitis.

3- acute meningoccemia

4-hypotension, multiple organ failure and septic shock الصدمة النتنة

Determinants of pathogenicity:-

1- adhesion factors pili serve as adhesion factors for the oropharyngeal and probably mediate attachment to the meningeal tissues as well.

2- polysaccharide capsule has antiphagocytic properties.

3-LPS, endotoxin is ten times more potent than most other endotoxins.

4-IgA proteases cleave IgA protect the bacteria against the effect of secretary IgA.

Pathogenesis :-

Attaches to the cells of the nasopharynx following the inhalation of contaminated droplets. Disseminates hematogenously and reaches the meninges they proliferate causing inflammation. Onset of the meningitis is usually sudden, after an incubation period of two to three days, with severe headache, fever, neck stiffness, and severe malaise. Severe hemorrhagic sepsis sometimes develops

Immunity :-

Complement- fixing, IgM and IgG antibodies can promote bacterial elimination from circulation and tissue.

Treatment :-

The antibiotic of choice is penicillin G. Very good results have also been obtained with third-generation cephalosporins, e.g., cefotaxime or ceftriaxone. It is important to start treatment as quickly as possible to prevent delayed damage. The advantage of cephalosporins is that they are also effective against other meningitis pathogens due to their broad spectrum of action (with the exception of Listeria monocytogenes).

Immunoprophylaxis:-

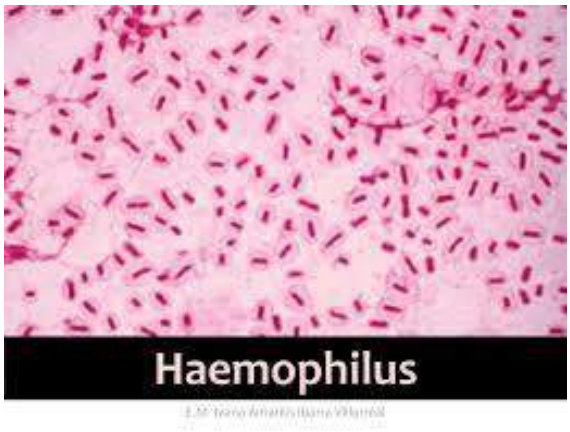
1-A vaccine constituted by the polysaccharide of serotypes A and C is available.

2- A quadrivalennt vaccine :- constituted by the polysaccharide of A,C,Y,W-135

3-conjugate vaccine:- constituted by the polysaccharide of A and C + non toxic mutant of diphtheria toxin.

Genus haemophilus

This genus includes several human pathogenic species of which *H. influenza* is the most important.



Species :-

1- ***H. influenza***

2- *H. parainfluenzae*(cause pneumonia and endocarditis**)**

3- *H. haemolyticus*

4- *H. suis*

5- *H. ducreyi*

H. influenza

Is found in the mucous membrane of the upper respiratory tract in human. It is an important cause of several diseases in infant and young children .

Determinant of pathogenicity:-

1- Encapsulated *H.influenzae* contains capsule polysaccharides, used for its typing. Of the six serotypes (a-f) type b causes most of the sever invasive diseases. Also it has antiphagocytic properties and is the prime virulence factor for H. influenza type b

2- membrane lipoligosaccharide: play role in bacterial attachment, invasiveness and paralysis of the ciliated respiratory epithelium.

3- IgA protease.

Pathogenicity

It enter the body through the upper respiratory tract (by inhalation) resulting in either asymptomatic colonization or infections such as otitis media or sinusitis et al.

The organism produces IgA protease that degrades secretory IgA, thus facilitates attachment to the respiratory mucosa. After establishment in the upper respiratory tract the organism may enter the blood stream and spread to meninges or joints.

Clinical disease:-

Meningitis, otitis media, sinusitis, acute bacterial epiglottises, cellulitis, bacterimia and chronic bronchitis pneumonia.

Transmission:-

Is by inhalation of infected droplets. Close contact favors transmission.

Treatment :-

The mortality rate of untreated *H.influenzae* meningitis may be up to 90%. The drug of choice for meningitis or other serious systemic infection is ceftriaxone and related cephalosporin followed by chloramphenicol, ampicillin and trimethoprim sulfamethoxazole.

H. parainfluenzae

It is resembles to *H.influenzae* closely, but requires V factor. It is a normal flora of respiratory tract. It may be cause sub-acute endocarditis, conjunctivitis and urethritis.

H. haemolyticus

It is resembles to *H.influenzae* closely, requires X& V factor but it is hemolytic organism, it form beta hemolytic on blood agar. It is a normal flora in the nasopharynx, it may cause urinary tract infections in childhood or rarely upper respiratory tract infections.

H. ducreyi

It is resembles to *H.influenzae* closely, but requires X factor only for growth. It is the etological agent of a sexually transmitted disease called chancroid, (soft chancre). Chancroid is ulcer on the genital with marked tenderness and swelling- the regional (bubo), lymph nodes are enlarged and painful.

Treatment:-

The drug of choice is erythromycin followed by ciprofloxacin, then ceftriaxone.

Genus brucella:-

Morphology and culture:-

Brucellae are slight, coccoid, Gram-negative rods with no flagella. They only reproduce aerobically. In the initial isolation the atmosphere must contain 5–10% CO2. Enriched mediums such as blood agar are required to grow them in cultures. The genus Brucella includes three medically relevant species—B. abortus, B. melitensis, and B. suis—besides a number of others. These three species are the causative organisms of classic zoonoses.

Pathogenesis and clinical picture:-

Human brucellosis infections result from direct contact with diseased animals or indirectly by way of contaminated foods, in particular unpasteurized milk and dairy products. The bacteria invade the body either through the mucosa of the upper intestinal and respiratory tracts or through lesions in the skin, then enter the subserosa تحت المصلية or subcutis تحت الجلد. From there they are transported by microphages or macrophages, in which they can survive, to the lymph nodes, where a lymphadenitis develops. The pathogens then disseminate from the affected lymph nodes, at first lymphogenously and then hematogenously, finally reaching the liver, spleen, bone marrow, and other RES tissues, in the cells of which they can survive and even multiply. The granulomas typical of intracellular bacteria develop. From these inflammatory foci, the brucellae can enter the bloodstream intermittently بشكل متقطع, each time causing one of the typical febrile episodes, which usually occur in the evening and are accompanied by chills. The incubation period is one to four weeks. B. melitensis infections are characterized by more severe clinical symptoms than the other brucelloses.

Diagnosis.:- This is best achieved by isolating the pathogen from blood or biopsies in cultures, which must be incubated for up to four weeks. The laboratory must therefore be informed of the tentative diagnosis. Brucellae are identified based on various metabolic properties and the presence of surface antigens, which are detected using a polyvalent Brucella-antiserum in a slide agglutination reaction. Special laboratories are also equipped مجهزة to differentiate the three Brucella species. Antibody detection is done using the agglutination reaction according to Gruber-Widal in a standardized method. In doubtful cases, the complement binding reaction and direct Coombs test can be applied to obtain a serological diagnosis.

Therapy.:- Doxycycline is administered in the acute phase, often in combination with gentamicin. A therapeutic alternative is cotrimoxazole. The antibiotic regimen must be continued for three to four weeks.

Mycoplasma:-

General Characteristics

The mycoplasmas are essentially bacteria lacking a rigid cell wall during their entire life cycle, although they are also much smaller than bacteria. With Giemsa stain, they

appear as tiny pleomorphic cocci, short rods, short spirals, and sometimes as hollow ring forms. Their diameter ranges from 0.15 u to 0.30 u.

Structure

The cell is enclosed by a limiting membrane which is more similar to that of animal cells than that of bacterial cells because of sterols present in the membrane. The cytoplasm contains ribosomes, but lacks mesosomes. There is no nuclear membrane. In some strains, amorphous material المواد غير المتبلورة on the outer surface of the membrane suggests the existence of a capsule.

Pathogenesis

M. pneumoniae is an extracellular pathogen that adheres to the respiratory epithelium by a specialized terminal protein attachment factor. This adherence protein interacts specifically with neuraminic acid residues on the epithelial cell surface. Cilia stasis ركود الاهداب occurs following attachment and then destruction of the superficial layer of epithelial cells. Destruction is due to release of hydrogen peroxide and superoxide anion**.**

Laboratory Diagnosis

The laboratory diagnosis of mycoplasma infection can be accomplished by:

1. Culturing the organism from sputum, mucous membrane swabbings or other specimens by direct inoculation into
   liquid or solid media containing serum, yeast extract and penicillin to inhibit contaminating bacteria. Colonies will
   become detectable in one to three weeks. They stain intensely with neutral red or tetrazolium or methylene blue.
   The organism can be presumptivelyفرضا identified by its hemabsorption or B-hemolysis of guinea pig red blood cells. It
   is conclusively identified by staining its colonies with homologous fluorescein-labelled antibody.

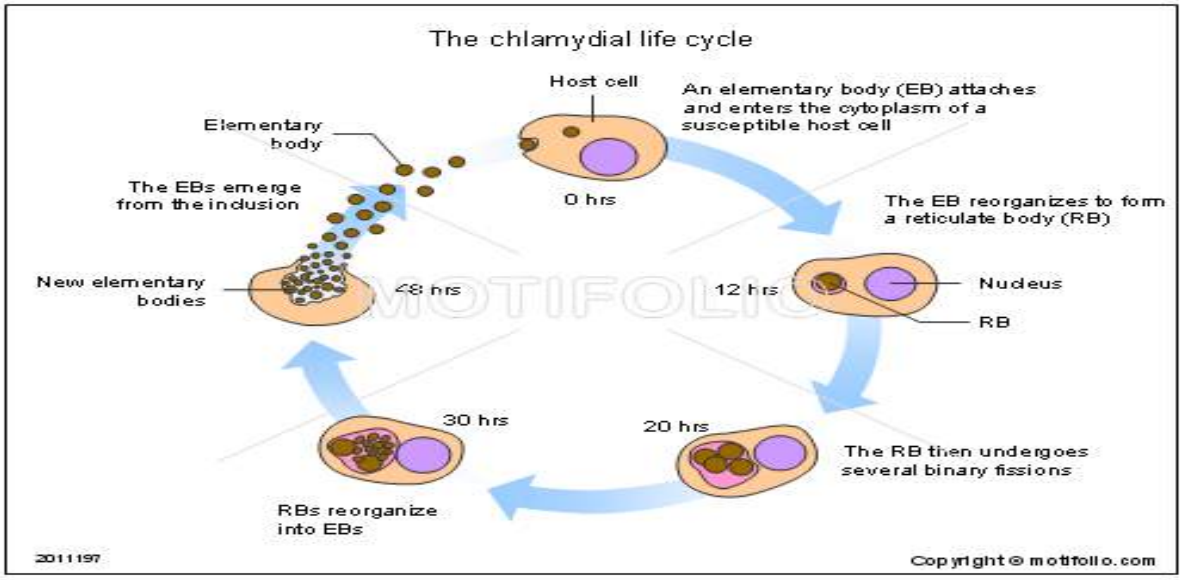
2. Quantitation of the patient antibody response to mycoplasma by complement fixation tests on acute and
   convalescent serum. Cold agglutinins to human O erythrocytes may also be measured.

Chlamydia:-

General characteristic:-

The bacteria in the taxonomic family Chlamydiaceae are small (0.3–1 lm) obligate cell parasites with a Gram-negative cell wall. The reproductive cycle of the chlamydiae comprises يتالف two developmental stages: The elementary bodies (300nm) are optimally adapted to survival outside of host cells and it is the infectious form. The initial bodies (1000nm), also known as reticulate bodies, are the form in which the chlamydiae reproduce inside the host cells by means of transverse fission. Cell wall contains LPS but does not have a peptidoglycan Three human pathogen species of chlamydiae are known:

C. psittaci (psittacosis or parrot fever).(tetracycline), C. trachomatis (trachoma), C. pneumoniae. Influenza like infections, sinusitis, pharyngitis, bronchitis, pneumonias (atypical



Culture:-

Chlamydiae exploit energy metabolism processes in their host cells that they themselves are lacking (ATP synthesis). For this reason, they can only be grown in special cell cultures, in the yolk sacs of embryonated hen eggs, or in experimental animals.

Chlamydia psittaci (Ornithosis داء الطيور, Psittacosis)

Pathogenesis and clinical picture.:- The natural hosts of C. psittaci are birds. This species causes infections of the respiratory organs, the intestinal tract, the genital tract, and the conjunctiva of parrots and other birds. Humans are infected by inhalation of dust (from bird excrements) containing the pathogens,
more rarely by inhalation of infectious aerosols. After an incubation period of one to three weeks, ornithosis presents with fever, headache, and a pneumonia that often takes an atypical clinical course.
The infection may, however, also show no more than the symptoms of acommon cold, or even remain clinically silent. Infected persons are not usually sources of infection.

Diagnosis. The pathogen can be grown from sputum in special cell cultures. Direct detection in the culture is difficult and only possible in specially
equipped laboratories. The complement binding reaction can be used to identify antibodies to a generic antigen common to all chlamydiae, so that this test would also have a positive result in the presence of other chlamydial infections. The antibody test of choice is indirect microimmunofluorescence.

Therapy. Tetracyclines (doxycycline) and macrolides.

Epidemiology and prevention. Ornithosis affects birds worldwide. It is also observed in poultry. Diagnosis of an ornithosis in a human patient necessitates يتطلب a search for and elimination of the source, especially if the birds in question are household pets.

Family Enterobacteriaceae

Enterobacteriaceae are Gram negative, motile or non motile, some are capsulate, non spore former. All members of family ferment glucose with or with out gas production, they are natural habitat in the intestinal tract of man and animals, they are aerobic or Facultative anaerobic, catalase positive and oxidase negative.

Important genus:-

The most important genus of this family are :-

1-Escherichia
2-klebsiella
3- proteus
4- salmonella
5- shigellae
6- vibrio

Classification Based on action lactose:-

1- Lactose fermenter e.g Escherichia coli -klebsiella
2- Late lactose fermenter e.g shigella sonnei
3- non lactose fermenter e.g salmonella – shigella

Esherichia coli

Gram negative ,non capsulated, coccobacilli or bacilli, motile , spores are not formed, facultative anaerobic.

Antigenic structure :-

1- Somatic antigen (o Ag) they are heat stable . they are divided into 160 groups designted as 1,2,3 and so on
2- surface antigen (K)

They are heat labile . they interfere with o agglutination unless destroyed by heating at 100-121 c they are of 3 types .

3- Flagellar antigen (Hantigon) are thermolabile and nonophassic About 50 types have been described
4- Fimbrial antigen (F antigen) they are thermolabile and have no significance in antigenic classification of *E. coli.*

Clinical samples:-

1-feces
2-infected tissue
3-blood
4-urine

Laboratory diagnosis:-

1- isolation: grow on MacConkey agar, which contains lactose and a PH indicator. If lactose is fermented, acid will be generated and the colony will turn pink.
2- biochemical analysis: it ferment lactose, glucose , sucrose, maltose and monnital with acid and gas . Sorbitol fermentation, production of indole and H_2 S -ve, oxidase reaction, VP and citrateiss –ve, urease is not hydrolysed
3- serotyping
1- gram stain
2-macConkey
3-Eosin methylene blue(EMB).
4-TSI
5-IMVC test(indol, methyl red, vogas proskawer, citrate utlilization)
6-motility test
7-uras test
8- sensitivity test.

klebsiella

These are Gram negative Lactose Fermenting bacilli, non motile Capsulate both in the tissues and on ivitro cultivation , all strains produce large amounts of slime and so the colonies are mucoid most strains of klebsiella are saprophytic and are found in many parts of the inviroment . There are two important species:-
17-8-*Klebsiclla pneumoniae* cause pneumonia , U.T.I , meningitis its important pathogens in nosocomial infection.
Klebsiella oxytoca cause Diarrhaea and infants infection
Kleb.rhinoscleromatis , kleb ozacnae
Culture grow well on simple media form larg colonies high convex And mucoid on macconkey agar colonies due to lactose fermenter Aerobic or Facultive anerobic microrganismis opt temp 37 c , 18 -24 hr
17-9 Antigenic structure
There are two important bacteria antigens

1- somatic antigen O Ag . Lipopoly saccharide (No . OAg q)
2- Capsullar antigen KAg polysaccharide (No.KAg 77)

17-10 Lab Diagnosis
* Specimens sputum , urin , pus , pus swabs.stool,culture , macconkey agar , D.C.A

17-11 Biochemical reaction : Indole –ve . M.R –ve V.P + C+ve
T.S.I Acid slant +bulo, urease Leat +ve after 4 hr .
Sera – typing : Anti sera (OAg , KAg)
* stain :Gram stain & capsule stain

Salmonella

general characters:-

Salmonella rod – shaped, Gram – negative, non spore forming , non lactose fermenters , most species motile, onterobacteria with diameters around 0.7 to 1.5 Mm . Lengths from 2 to 5 Mm . peritrichous Flagella, facultative anaerobes most species produce hydrogen sulfide which can readily be detected by growing them on media containing furrow sulfate such as TSI.

Important species :-

Salmonella are found in warm and cold – blooded animals , in humans they cause illnesses in humans and many animals such as typhoid fever , paratyphoid fever , and food infection , food poisoning salmonellosis

Antigenic structure :-

There are 2000 serotype which classified this species and serotype to group and sub group by surface Antigens by Kauffmann & ushite
1- somatic (O) Antigen
*major (o) is group specific
*minor (o) is sub group specific
2- Flagellar H Antigen :- it's a protein Ag classified to 2 phas

Hag a-phase -1 (specific phase)
b-phase -2 (non specific phase)
This bacteria will poses 2 sets of genes . if the body produce Ab against H-Ag (specific) bacteria will change there Ag to phase -2
3- Virulence (Vi) Antigen

This Ag existed on the outer surface and this Ag will occur in some species of *salmonella* especially in *sal . typhi, sal.paratyphi c* ,this Ag have areolation with virulence and consider as poorly antiphagocytosis so the titer is low comparable with the titter of Ab against O, H antigen . so we must remove vi Ag by heating at 60c for 1 hour to make serological test.
*(O) Ag is heat resistance and alcohol resistance .

*(H) Ag is heat labil and consider as agood Antigenicity because it stimulate the immune system

Slamonella typhi

1- its high invasive it a good invader to intestine and will reach to lymph nodule then to the blood .
2- Intracellular : its have the ability to enter inside the cell body like W.B.C so it will be protect from host defense from phagocyte cell
3- Vi Ag .not all species have poses this Ag except *sal . typhi* and *sal . paratyphie*

Pathogenicity:-

Salmonella infections are zoonotic they can be transmitted by humans to animals vice versa.

1- Enteric fever :- consist of typhoid and paratyphoid . the incubator period from 3-25day according to the uptake Dose of bacteria and the avemge reach to 10 day

Cause by *sal.typhi , sal . paratyphi A,B.*

*fever :graduated fever : the fever will increase gradually for 2-3 days then will reach 40c and still at it

Which have reticulo endothelial system RES Liver , spleen , kidney , bone marrow , lungs

2-Salmonellosis : it a gastro enteritis caused by salmonella species exceptea *sal.typhi*

The source of infection are contaminated animal products like (meat,egg,milk) contaminated a food

Food infection	food poisoning
1-fever (pyrexia)	no fever
2-long symptoms dauration	short symptoms
3-long incubation period	short incubation
More than 48hr	period 8 hr

Salmonellosis: it share between food poisoning and food infection

Lab diagnosis:

Sample blood, feaces , urine

Sample	1st week	2nd week	3rd week
Blood	90%	75%	30%
Fecal	40%	50%	80%
Urine	____	10%	20%
Serology(serum)	20%	50%	80%

Widal test → agglutination test

The disadvantage of widal test is undistinguishable between carrier and infected person.

Shigella

general charactors

Gram negative bacilli , non spore forming facultatively anerobic non motile bacteria non lactose termenter except *shigella sonnii*
Give laet lactose formentar

Group

A *shigella dysenteriae*
B *shigella flexneri*
C *sh . bodyii*
D *sh. Sonnii*

Shigella dysenteriae

spread by contaminated water and food , causes the most severe dysentery because produce (shig a toxin) or severe diarrhea gastroenteritis in humans . this disease Known as (bacillary dysentery) shigellosis starts 12 hours to six days, usually one to two days, after exposure to the bacterium. Dehydration is also common symptom .

Lab.Diagnosis:-

Speciment stool
*Gram stain G- rods
*Culture on errochment media selenite – F broth.
Colonies on DCA agar are colorless non lactose fermenter T.S.I slant Alk Butt acid H2S –ve gas –ve IMVPC indole + ve , MR +ve , VP-ve , C-ve

Proteus:

some are free living in water and sewage and even vegetable soil, some are normal intestinal flora, pathogenic strains cause mainly UTI, otitis media, wound infection, it is one of the most causative agent of nosocomial infection.

Taxonomy:-

Family : enterobacteriaceae

Genus: *proteus*

Species belong to Genus proteus:-

1- ***Proteus vulgaris*** :- UTI, wound infection
2- ***Proteus mirabilis*** **:-** UTI, wound infection, nosocomial infection.
3- ***Proteus myxofaciens*** **:-** non pathogenic.
4- ***proteus inconstans :-*** non pathogenic.
5- ***Proteus rettgei :-*** gastroenteritis.
6- ***proteus morganii(morganella) :-*** summer diarrhea.

Classification :-

Serological classification is not dependable because of the cross reactivity with Rikettsia

General characteristic :-

Gve- bacilli, coccobacilli, , pleomorphic, actively motile, with peritrichous flagellae, non capsulated, non spore forming.

Antigenic composition:-

Lick most gram negative enterobacteriaceae, proteus species have O,H,and K antigens.

Culture:-

Aerobic but can grow anaerobically , Grow well on simple media, growth at (25-37c), grow on solid media and showing swarming phenomena. Ferment glucose , sucrose and maltose are but not lactose.

Some factors inhibites the swarming phenomena:-

1-percentage or age concentration (4%).
2- presence of bile salts(macconkey)
3- anaerobic condition.

Enzymes produce by proteus are:

1- gelatinase:- liquefaction of gelatin
2- phenylalanine deaminase:-
3- urease:- is an important factor determining its pathogencity in UTI
4- hemolysin:-

Pathogenesis (clinical disease):-

1- urinary tract infection . when urease producing strains become involved in urinary tract infection , the production of urease results in the liberation of ammonia and raises the PH of the urine. The alkalization of the urine reduces the solubility of calcium, creating conditions favorable for the deposition of calcium and magnesium salts and the production of kidney stones.
2-wound infection
3-septicemia

Lab diagnostic test:-

1-gram stain = Gve- bacilli or coccobacilli, pleomorphic.
2- inoculation macconkey agar.
3-blood agar (swarming & hemolysis).
4-TSI
5- ureas test.

6-IMVIC.
7-gelatine liquification.

8-phenyl alanine $\xrightarrow{\text{phenyl alanine deaminase}}$ phenyl pyravic + NH_3 $\xrightarrow{FeCl3}$ green complex
Phyneyl ala
9-phenyl alanine $\xrightarrow{\text{phynyl alanine deamilase}}$ phenyl pyruvic+ NH_3 the indicator converted frome green to blue.

test	Protus vulgaris	Protus mirabilis
Indol	+	-
Maltose	+	-
glucose	+	+
MR	+	+
VP	-	-
Citrate	+	+
Ureas	+	+
Motality	+	+
TSI	K\A++	K\A++
MACONKY	Pale colony	=
Blood agar	Swarming &hemolysis	=
Gelatine liq	+	+
Phenyl alanin	+\-	+

Pseudomonas
They found in soil , any moist area like water , they present in small NO. of normal intestinal flora and skin.

Taxonomy :-
Family : pseudomonadaceae
Genus : pseudomonas
Species :
1- pseudomonas aeruginosa
2- P. fluorescence.
3- P. putida.

General characteristics :-
G-ve bacill, motile with polar flagella (monotrichous or polytrichous) or some of them are non motile, non spor formers, non capsulated , aerobic but can grow anaerobically. They are characterized by extracellular pigments ,the color of these pigment deffer according to Spp.
1-pyocyanin :- has the ability to conversion of oxygen to superoxide and hydrogen peroxide, which inhabit the growth of other bacteria and cases cytotoxicity.(blue green pigment produces by P. aeruginosa).

2-pyoverdin:- is responsible for yellow to green discoloration of agar plates (P. fluorescence).
3-pyorubin red pigment & pycmelanin black pigment .

Determinant of pathogenicity:-

1- Alginat capsule some strains of Pseudomonas produce it, which give them mucoid appearance, its functions both as an adhesion factor and as an antifugocytic factor.
2- pilin:- are essential for initial colonization of epithelial tissue.
3-hemolysins.
4-proteases:-
5- toxins:- (LPS, endotoxin, enterotoxin, and two exotoxins S&A.

Pathogenicity:-

P.aeruginosa is the most mportant species, it is invasive and toxogenic produce infection in patient with abnormal host defence and is an important nosocomal pathogen they cause UTI, otitis media and septic shoke, the mian infection of it is burn infection and wound infection. They found in antiseptic solution, eye drops, grows well in dettol, heating 55c killit. So it coud be survive in detergents therefore its necessary to include this bacteria with nosocomal infection bacteria and this importance arise in cases of burns becauis it's the most isolated bacteria that cause burne infection and because its shows different or multiple antibiotic patterns and multiple colony types.

Classification :-
1- biochemical
2- serological (H, Ag O, Ag 110 serotype).
3- pyocin typing
4- phage typing
5- sensitivity pattern antibiotic.

Treatment :-
Pepiracillin, cefotaxim, or combination of pepiracillin with amimoglycoside like gentamycin drug of choice pyopen.

Lab diagnostic test:-
1- gram stain
2- milk agar for pigmentiation .
3- blood agar for heamolysis
4-king A, king B (selective and defferential).
5- MacConky agar.
6- TSI
7- IMVIC
8- motility
9- OF (oxidation – frementiation) contain glucose 1% bromothymol blue, K_2 HPO_4 buffering, add paraffin on the slant to produce anaerobic condition, inoculation by stabing, the color change to yellow.

10- nitrate broth.
11- oxidase and catalase.

Test	p. aeruginosa	P. fluorescence
Indol	-	-
MR	-	-
VP	-	-
SC	+	+
TSI	K\K--	K\K--
Nitrate	+	+
Motility	+	+
Growth at 42c	+	-
Growth at 4c	-	+
King A	+ Pyocyanin	(-,+), - PIGMENT
King B	+ fluorescen	+ FLUORESCEN
macConky	L.N.F transparence, irregular	L.N.F transparence, irregular
Oxidase	+	+
Catalase	+	+
OF medium	Oxidation (+), ferm(-)	Oxidation (+), ferm(-)

Genus vibrio

Family : Vibrionaceae
Genus : Vibrio
Ssp. :
1- *vibrio cholera* .
2- *vibrio parahaemolyticus.* (food-associated diarrhea disease).
3-v. vulnificus (wound infection, septicemia)
4- v. alginolyticus (otitis externa, wound infection).

vibrio cholera

Historical Background:-

In the nineteenth century, pandemic waves of cholera spread to many parts of the world. In 1961, a massive epidemic began in Southeast Asia; this is now recognized as the beginning of the seventh cholera pandemic. This pandemic was caused by the El Tor biotype of toxigenic *V. cholerae* O1. It spread rapidly through south Asia, the Middle East, and southeastern Europe, reaching Africa by 1970. In January 1991, epidemic cholera appeared in South America in several coastal cities of Peru and spread rapidly to adjoining countries. By the end of 1996, cholera had

spread to 21 countries in Latin America, causing over 1 million cases and nearly 12,000 deaths.

Classification :

Isolates of *Vibrio cholerae* are classified into

1- Serogroups. O1, O139 Non-O1

2- Biotypes for serogroup O1 Classical and El Tor(on the basis of several phenotypic characteristics). Currently, the El Tor biotype is responsible for virtually all of the cholera cases throughout the world, and classical isolates are not encountered outside of Bangladesh.

3- Serotypes for serogroup O1 Inaba, Ogawa and HiKojima(based on agglutination in antiserum).

General characteristic :-

They found in nature mostly in water, fishes, food.They are curved G-ve (comma shape) aerobic rods, motile with single polar flagellum, found in single or cluster forming S shape, non spore formings, on prolonged culttvation vibrio may become coccoid.

Determinant of pathogencity:-

1-enterotoxin: secretes the potent enterotoxin choleragen.

2-adhesion factors.

Clinical Manifestations:-

Cholera is a secretory diarrheal disease. The enterotoxin produced by *V. cholerae* O1 and O139 causes a massive outpouring of fluid and electrolytes into the bowel. This rapidly leads to profuse watery diarrhea, loss of circulation and blood volume, metabolic acidosis, potassium depletion, and ultimately vascular collapse and death.

Treatment:-

Successful treatment of cholera patients depends on rapid replacement of fluid and electrolyte losses. With proper treatment, mortality is less than 1% of reported cases. Antimicrobial agents recommended by WHO for treating cholera patients include tetracycline, doxycycline, furazolidone, trimethoprim-sulfamethoxazole, erythromycin, or chloramphenicol.

Culture:

Vibrio produce convex, smoth round colonies opaque and granular in transmited light, most vibrio grow well at 37c on media contain mineral salts and amino acids (aspargin, arginin, lysine) as asource of carbon and nitrogene. These organisms grow at alkaline PH(8.5-9.5), they are rapidly killed by acid and by heating at 55c for 15 min,cultur containing carbonhydrates become sterile after few days.

-Vibrio cholera grows well on TCBS(thiosulfate citrate bile sucrose) media.

Lab diagnostic test:-

Stool or vomitus cultured on pepton water (pH=9), blood agar or TCBS, typical yellowish colonies can be picked after 18 hr.
The identification based on:-
1- sugar fermentation.
2-slide agglutination.
3-cholera red reaction.
4- string test: add5% na- deoxycholate solution to 1 drop of cultur, the culture convert to thread like when drows by loop disapper after 45-60sec.
5- IMVIC
6- gelatinase test.
7-kligler iron agar (TSI- without sucrose-pH7.4)
8- nitrate reduction test No3…….no2
9- blood agar.
10-pepton water Ph=9, 75NaCl.

vibrio parahaemolyticus

cause food poisoning characterized by diarrhea. Ti is a halophilic marine organism, it requires at least 2% sodium chloride to grow. pathogenic strain carry a thermostable cytotoxin that causes hemolysis .

Test	V.cholerae	V. parahaemolyticus
Catalase	+	+
Oxidase	+	+
No3 reduction	+	+
Indol	+	+
MR	+weak	-
VP	-	-
Citrate	+\-	+/-
D.W+ 7% NaCl	-	+
D.W+5% NaCl	+	-
TSI	a\a	k\a
Motility	+	+
Cholera red	+	-
Mannitol	+ weak	=
String test	+	=
O.F medium	Oxide-ferm	=
TCBS	Yellow colony	Green colony

Genus Campylobacter

Taxonomy :-

Family: campylobacteriaceae

Archobacter

Campylobacter

Species :

1- C. coli

2- C. fetus

3- C. hyointestinalis

4- C. lari

General characteristic :-

Gve-, curved, rod were formerly called vibrous, motile with singal polar flagellm, in pure cultur colonies have been recognized in two types.

1- observed large, flat, spread with uneven margin watery grayish, non hemolytic.

2- are also non hemolytic but are smaller with unbroken edges and they are convex and glistening.

The opt. growth temp. 42c, the organism required selective media for isolation (cary-blair or campyloblood agar) wich contain five antimicrobial agent such as cephalosporin, polymyxin B, rifampcin and enriched supplemented with 5-10% sheep or horse blood. This media selective for intestinal isolation of C. fetus sup sp. jejuni and inhibitis the sub sp. Intestinalis which is rarely responsible for enteric infection. They are microaerophilic, high concentration of O2 are toxic to these organisms are masked by the growth of other enteric bacteria.

Pathogenicity :-

C. fetus aub sp. Jejuni cause enteric infection such as sever diarrhea or bloody diarrhea, they may cause symptoms similar to that salmonellosis or shigellosis or food poisoning by staph, in contrast with salmonella or staph. The camp. Does not multiple in food.

The transmission of Camp. Is by contaminated and untreated water.
Isolation from blood of feces.

Isolation from feces:-

Samples should be transported to lab. Directly or transport media should be used cary blair or campy blood agar. Samples can be refrigerated for 24c because Camp. Is resistance to cold, the stool should be placed on the selective agar and incubated at 42c in a microaerobic atmosphere for 7 days.

Filtaration of fecesis an excellent method for isolation of Camp. Either 0.65 or 0.8 micrometer pore size, cellulose acetate filter is amodified way, afilter is placed on agar surface and adrop of stool is placed on the filter, the plate is incubated up right after 30 to 60 min at room temp. the filter is removed and the plate reincubated. The plate should be examined after 48 hrs. to 5 days for characteristic colony wich are gray to pinkish or yellowish gray, slightly mucoid looking, some show a tailing effect along the streak line.

Lab diagnosis :-

1- a wet preparation (direct examination) under dark or phase contrast microscop the identification dependent on basis of colonial morphology and microscope appearance of the organism obtained from typical colonies.

2- oxidase and catalase test may prephormed both. Should be positive in case of C.feuts jejun

3- most are a saccharolytic, unable to grow in 3.5 NaCl.

4- TSI: production of H2S

6- nitrate reduction (reduce NO3 ⟶ NO2

7- hydrolysis of indoxyl acetate (+ ve)

8-serological (agglutination) and nucleic acid probes.

Genus helicobacter

Taxanomy :-

Family : campylobacteriaceae

Genus : helicobacter

(causative agent of gastric ulcer)

General charectirestic :-

G-ve spirally shaped bacterium, fastidious, requires 3-7 day of incubation in microaerophilic condition (campy gas pak 55o2+ 10% co2) at 35-37c motile with polar flagellum.

Selective media for isolation should contain serum or blood, good growth of H. pylori may be obtained in presence of starch, charcoal. for example of selective media.

1- Colombia blood agar

2- Muller hinton agar

3- brucella agar: this media contain antibiotic (vancomycin, colomycin, colistin, trimetheprim and amphotericin).

4- beta horizonate agar

5- skirrows modified Thayer martin.

The culture incubated for 7 days in 37 c after that the colonies appear small translucent, yellowish, raised and have entire edges about 1mm in diameter.

Pathogenicity :-

H. pylori found in the stomach and is strongly associated with gastritis, gastric ulcer and duodenal ulceration, the mechanism of pathogenicity has

been identified by production of cytotoxin and the organism also produce large amount of urease, probably a useful strategy for survival in the acid environment of the gastric mucosa.

Treatment :-

Is with a combination of antiacids (omeprazole) and one or more broad-spectrum antibiotic (amoxicillin).

Diagnosis:-

For the detection of the presence of H.pylori, the tissue biopsy material should be transported to the lab. And may be reffergerater for 5hers. By using ureas test the result could be obtained after 5hr. (change in color from yellow to pink).

Procedure for obtaining culture from biopsy, by the following mehod:

1- tissue is rinsed in dextrose phosphate buffer.

Sterile gauze or filter to remove excess broth.

3- pressed into glass slide slide and prepare smear.

4- tissue is grinded in dextrose phosphate buffer and then inoculated to medium.

Lab diagnosis :-

1- gram stain (squash preparation of biopsy), for ex. Silver stain, giemsa stain or 1% basic fuchsin.

2- catalase , oxidase +ve

3- urease +ve (rapid urease 5% urea, the reaction takes for 4 hrs)

Diagnostic sample inoculation directly after crushing the biopsy material.

Genus Bordetella

genus Bordetella contains 3 species :-

1- *Bordetella pertussis*
2- *Bordetella para pertussis*
3- *Bordetella bronchisoptica*

Bordetella pertussis

the causative agent of whooping cough,

General characteristic:-

Gram negative , non motile coccobacilli (short rode) , they do not require x and y factors for growth, non spore forming, hemolytic, capsulated, strict aerobic and fastidious .

determinant of pathogenicity:-

1- adhesion : capsular, fimbriae or agglutinogens. The most important adhesion, filamentous hemagglutinin.
2- pretussis toxin : is the major toxin produced by B.pertussis.
3-adenylate cyclase-like toxin
4- tracheal cytotoxin
5- endotoxin : does not seem to play a major pathogenic role.

Antigenic structure

Several antigenic factors have been recognized the are antigenic haoenogenous and so are agglutinated by common anti serum follouning are antigenic factors

1) Aheat labile lipopolysaccharide endotoxin
Antibodies against it are not protective
2) Aheat labile protein toxin
3) A heat labile agglutinating antigen
4) Haemogglutinin
5) Heat labile antigen (histamine sensitizing factor)
6) lymphocytosis promoting factor

Pathogenceity

B. pertussis is local pathogen of the upper respiratory tract, most symptoms of whooping cough are directly related to mucosal destruction by this organism. Systemic effects are caused by either diffusion of *pertussis* toxin through the blood stream or cross- reactive immune reactions.

Clinical disease:

1- catharral phase:- after an incubation period of 7-14 days, the patient enters the catharral phase, characterized by a low-grade fever, rhinorrhea, and a progressively .

2-paroxysmal phase:- sever paroxysmal coughing episodes that involve an initial spasmodic phase followed by a rapid inhalation of air patient may also exhibit a leukemoid reaction, characterized by marked leukocytosis. This phase may last 6 weeks or longer, after 6 weeks, the patient is considered noninfectious, even if the cough persists.

3- convalescence:- lasts 1-3 weeks.

4- sequelae may be pulmonary:-

Transmission :-

Is mostly person to person by inhalation of bacteria-containing droplets expelled by a coughing patient.

Treatment :-

1- supportive measures are most important.

2- erythromycin is considered the drug of choice.

Immunity & prophylaxis:-

After a clinical infection, patients acquire long-lasting immunity, second infection are rare.

The classic vaccine is prepared with killed encapsulated organisms and is usually incorporated in the DPT vaccine.

Clinical sample :-

Specimen should be

1- a nasopharyngeal aspirate (best).

2- posterior nasopharyngeal swab.

3- Throat and anterior nasal swabs have unacceptably low rates of recovery.

* Swabs should be made of Dacron (not cotton becous it inhibits the growth of Bordetella). Calcium alginate swabs may also be used for culture, clinical specimens can be placed in Regan Lowetransport medium (one half strength charcoal agar supplemented with horse blood and cephalexin).

Laboratory diagnosis

1- Haemotological investigations

a- Total leucocyte count

b- Differential leucocyte count

2- Bacteriology

a- Microscopic examination by fluorescent antibody technique

b- culture:- specimen for culture should be collected during the catarrhal phase on calcium alginate nasal swabs moistened with aqueous pencillin to facilitate access to the posterior nares and also to limit the growth of commensals(cotton inhibits the growth of Bordetella).
Biochemically it is almost inactive it produces oxidase and catalase

1- culture and isolation :-

B. pertussis is an aerobic incubation at 35oC to 36oC, with sufficient humidity to avoid desiccation. Cultures require incubation for 3 to 4 days, andshould be incubated and checked for at least 7 days; incubation up to 12 days can increase yield.

for recovery of *B. pertussis* in clinical specimens recommend charcoal agar supplemented with 10% defibrinated horse blood and cephalexin (Regan-Lowe medium) while the Bordet-Gengou medium has the advantage of detecting the hemolysin characteristic for *B. pertussis*.

3- serological investigation.

a- direct immunofluorescence:- permits rapid diagnosis, smears of nasopharyngeal exudates are examined using fluorescein –labeled antibodies to *B. pertussis*.

b- enzyme immunoassay(ElA). That detect pertussis-specific IgA has been suggested to be valuable in the diagnosis of acute infection ,it is more useful during the paroxysmal phase.

4- PCR:- should be used in addition to, and not as a replacement for culture.

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Taxanomy :-

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Genus : helicobacter

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