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:

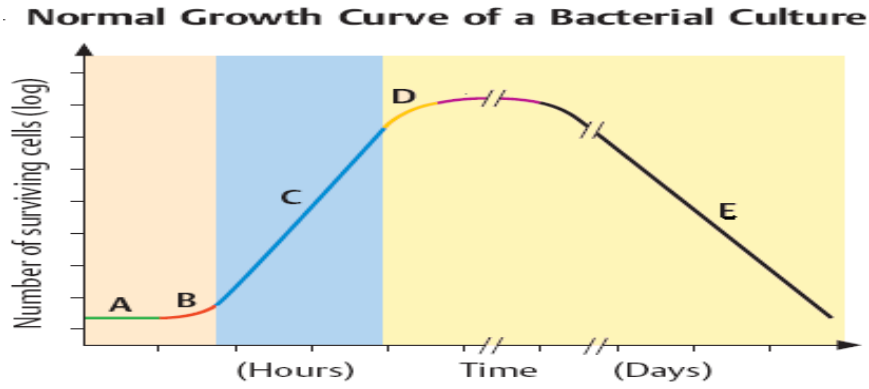
1-Lag phase: occurs when bacteria are inoculated (تزرع) into a fresh, nutritionally enriched medium (محيط غني بالغذاءالطازج).

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 غير لحم الخنزير 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°, 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:-

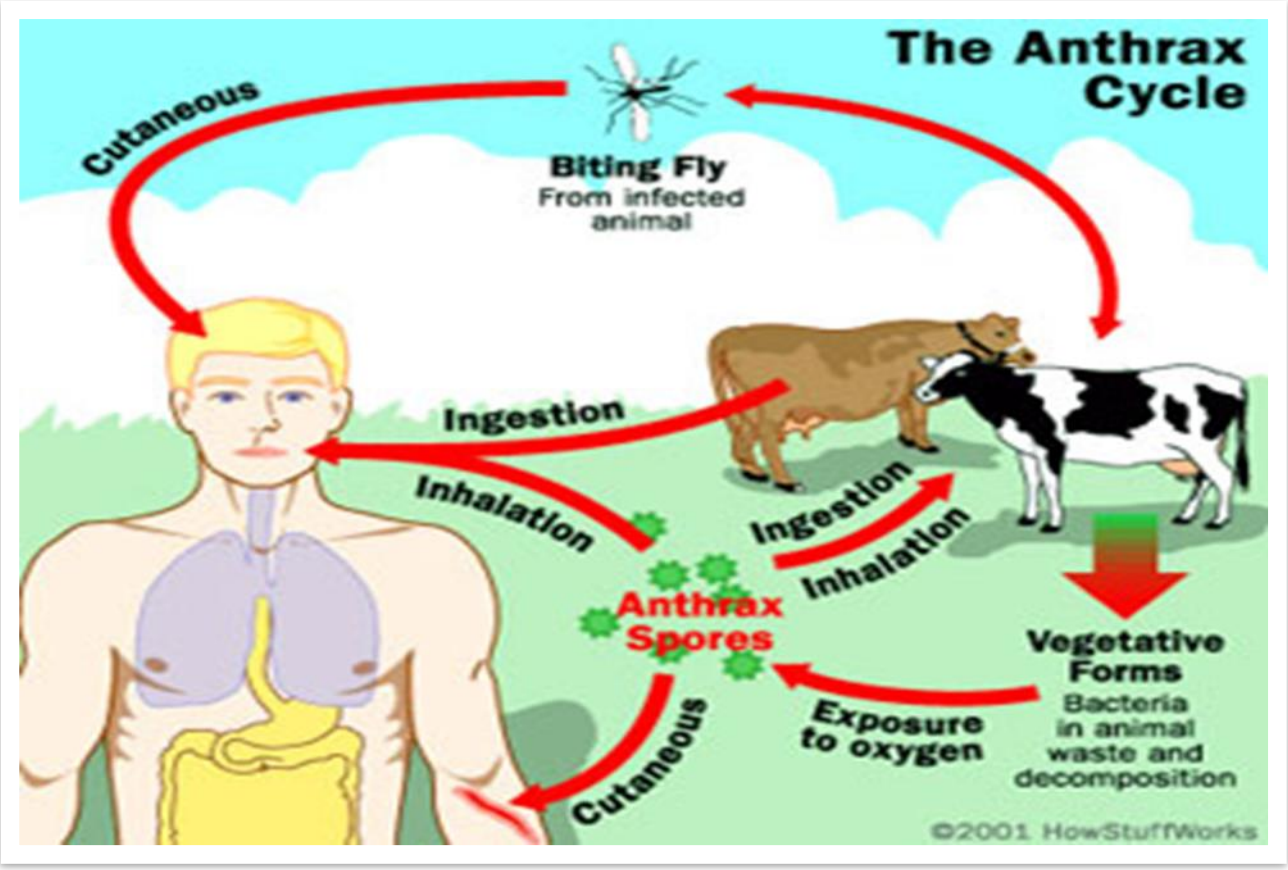
1- *B. anthracis* possesses an antiphagocytic capsule essential for virulence.

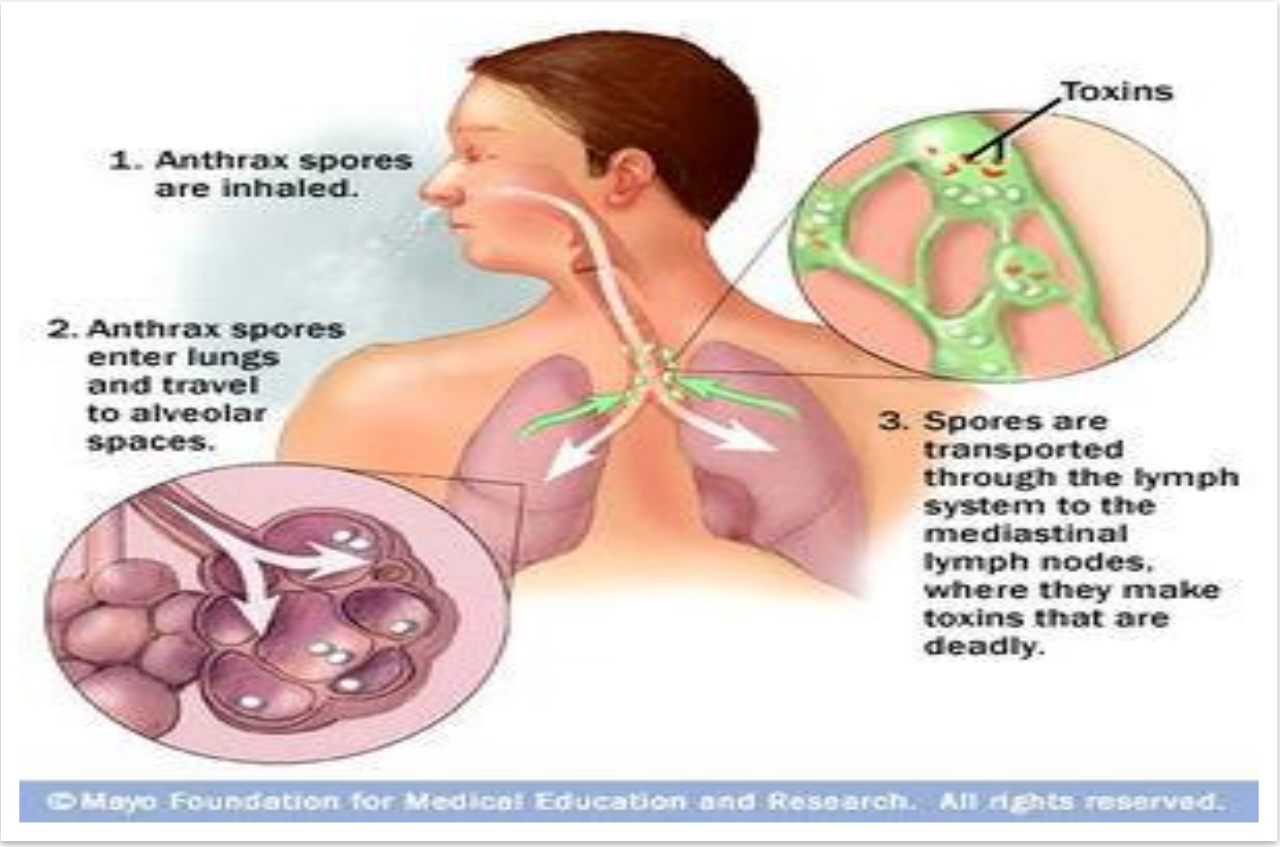
2- The organism also produces three plasmid-coded exotoxins:

- **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.





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- Cutaneous, the most common form (95%), causes a localized, inflammatory, black, necrotic lesion (eschar).
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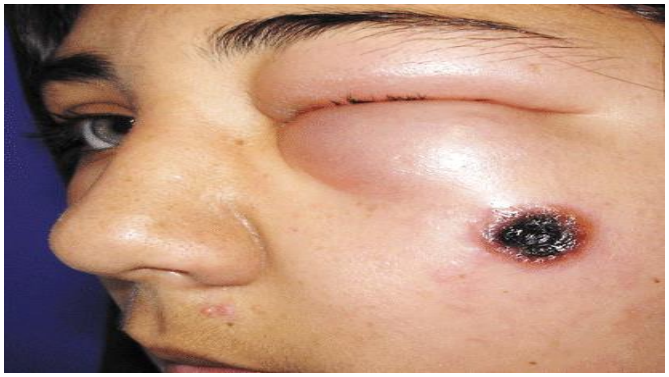
- Gastrointestinal, a rare but also fatal (causes death to 25%) type, results from ingestion of spores.



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

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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 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.

d- 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

e- 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.

f- 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**

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

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

8- Protein G:- prevent effective phagocytosis.

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

10- 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°, 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).
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Primary treatment is surgical, accompanied by antibiosis (penicil lins, cephalosporins).

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4- neurotoxins was an extremely potent toxin (botulin is heat sensitive but total in activation requires boiling for 20 minutes).
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4- tetanospasmin, a neurotoxic exotoxin that disrupts nerve impulses to muscles.(spastic paralysis) oxygen stable, heat labile.

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Drug of choice:-

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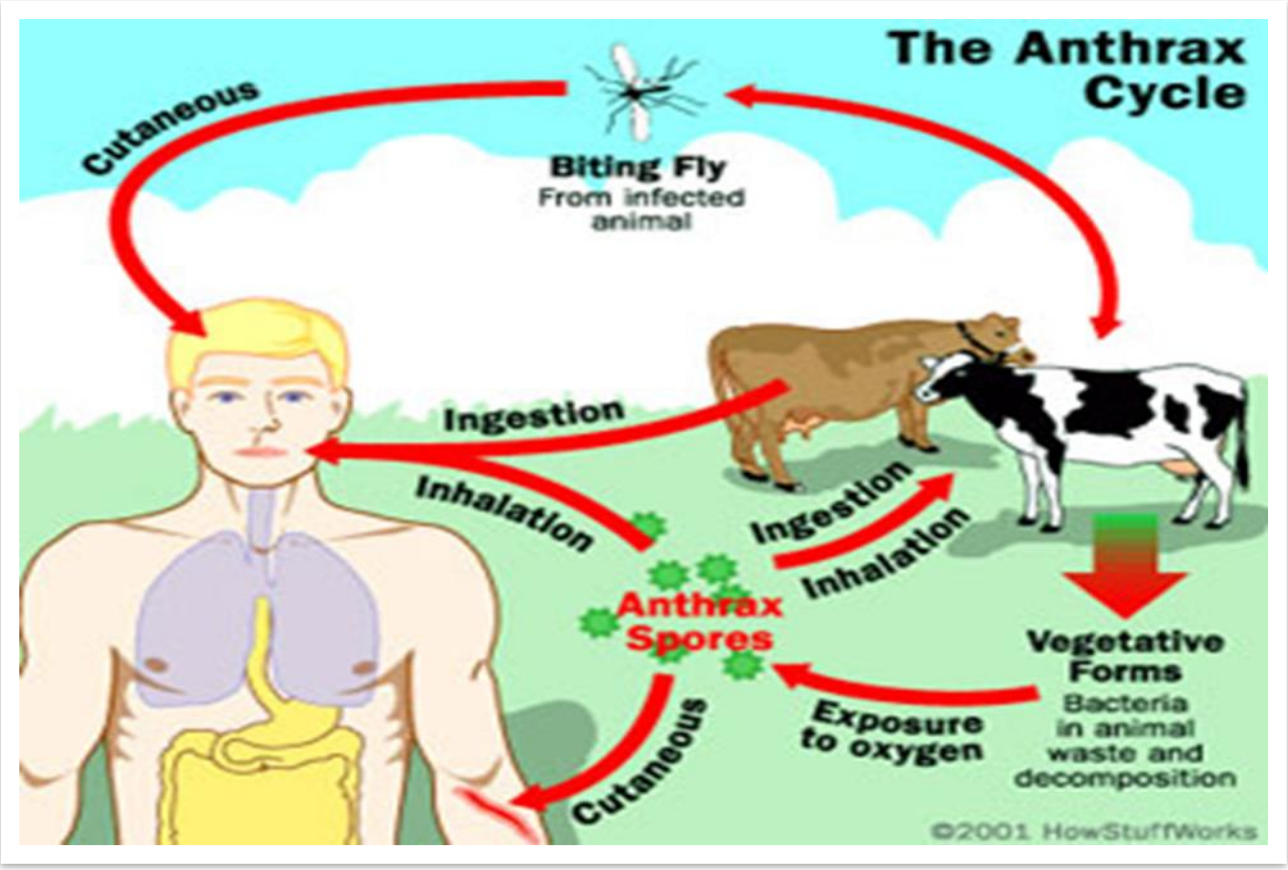

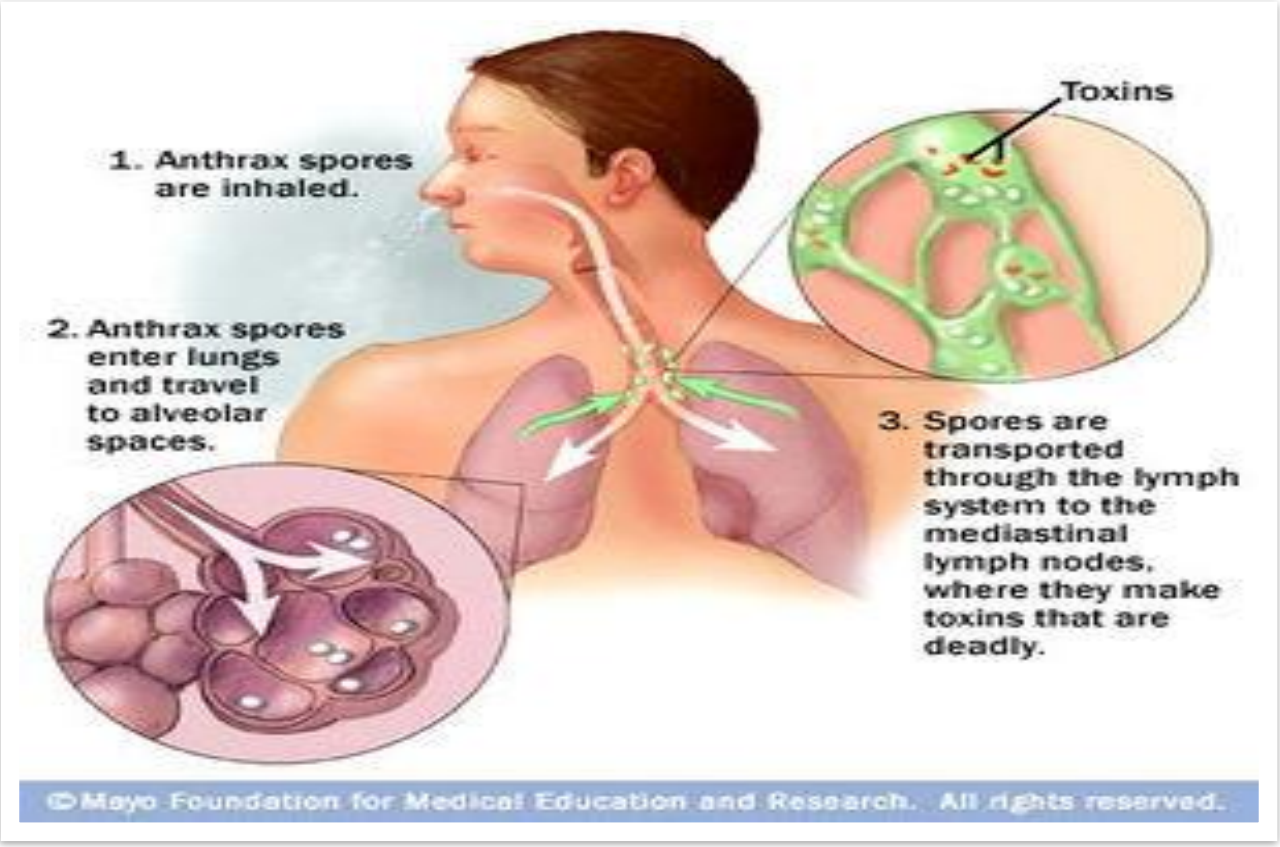

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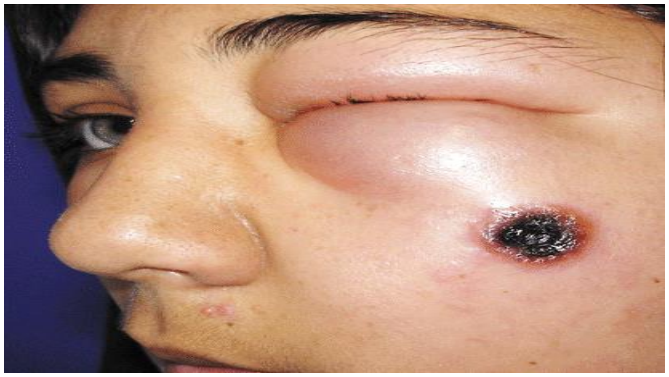

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Treatment:-

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

2- systemic infection treated with clindamycin.

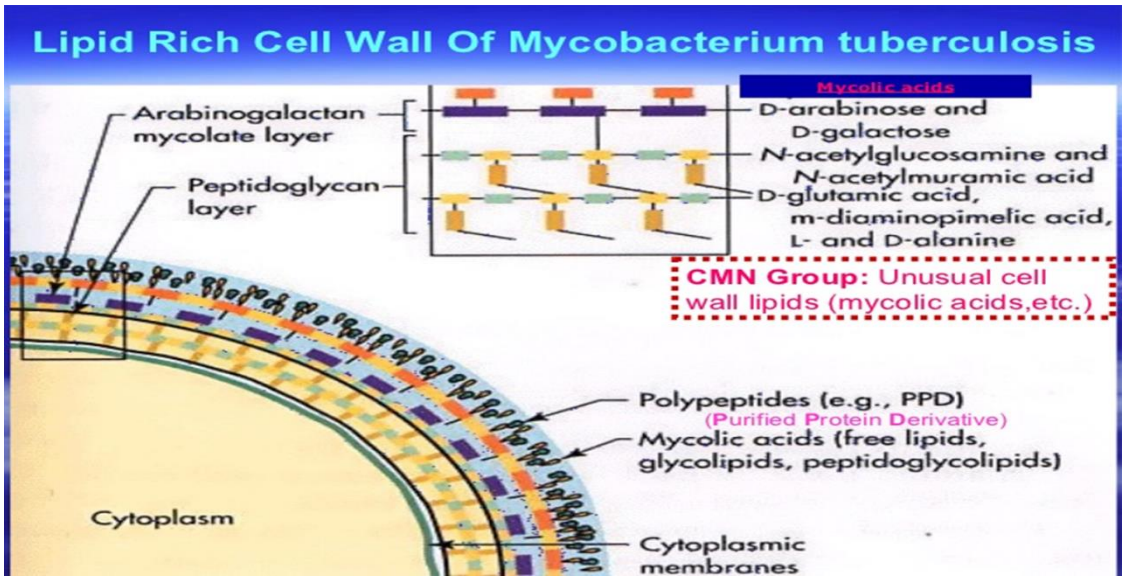
Diagnosis:-

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***,

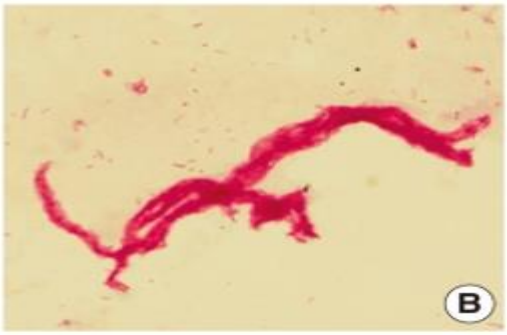
6- **Non Tuberculous mycobacteria (NTM)** are all the other mycobacteria, which can cause pulmonary disease resembling tuberculosis, lymphadenitis, skin disease, or disseminated disease.

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.

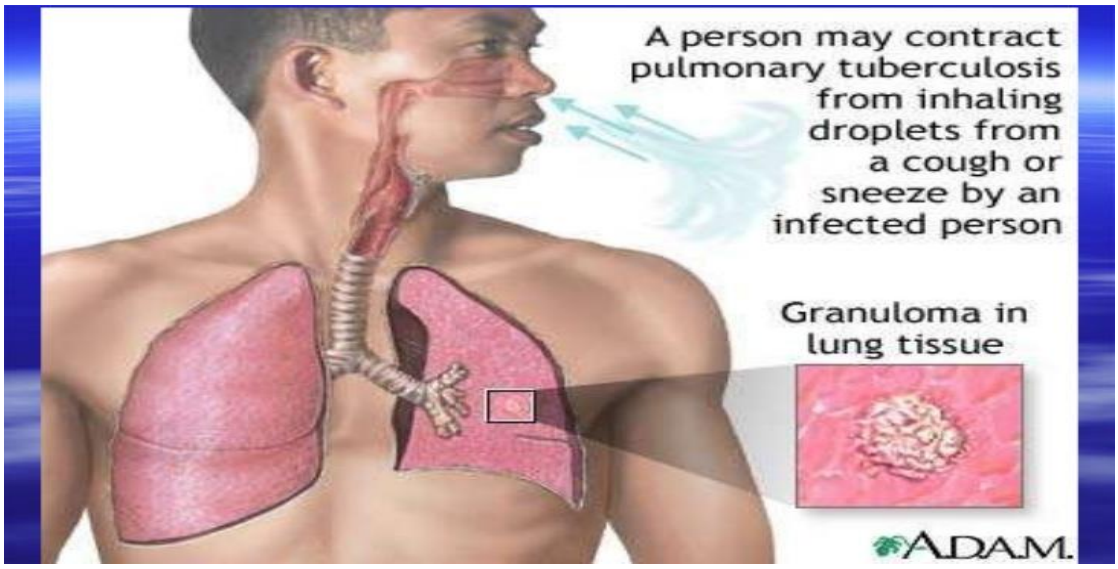
1- **Cording factor**: is a glycolipid derivative of mycolic acid that is present on the outer surface of *M. tuberculosis.* It's have immunogenic properties. Its cause's bacilli to grow in culture contain serpentine" cords" observation of this type of growth is usually indicative of pathogenicity.



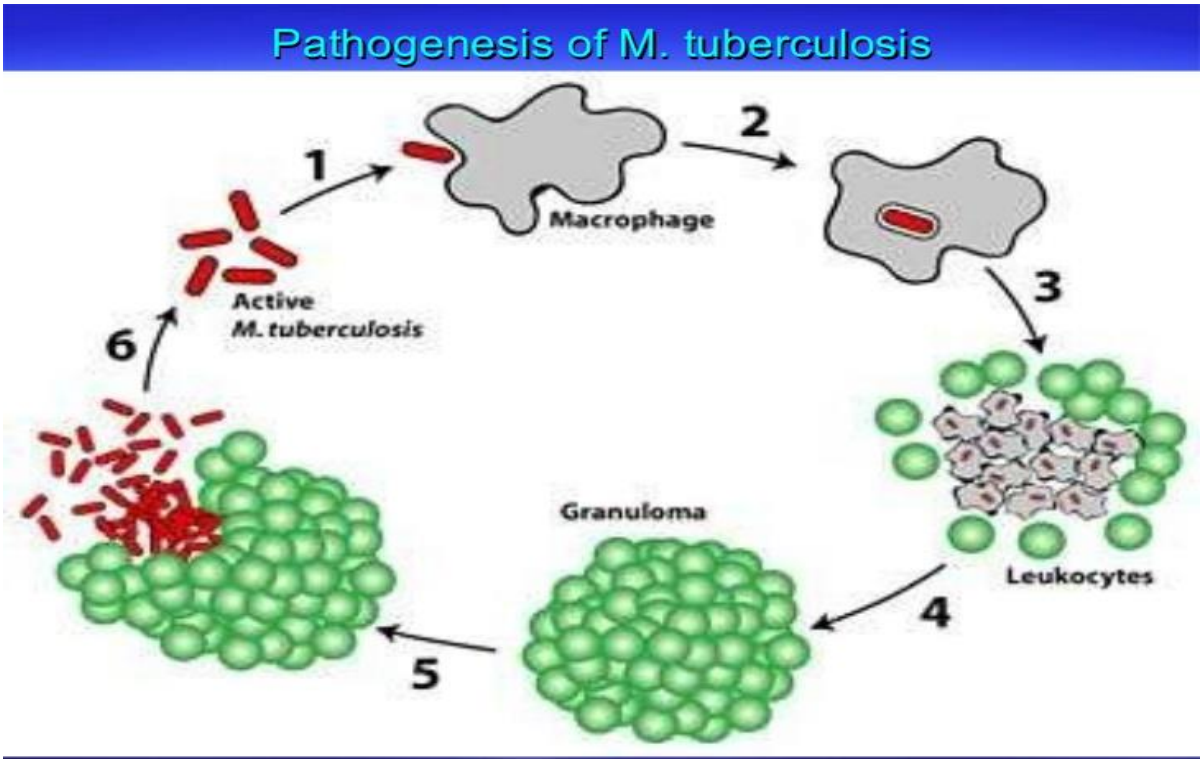
2- **Sulfatides**: glycolipids inhabit phagolysosome formation, permitting the bacterium to survive intra-cytoplasmic after being ingested by macrophages.

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.

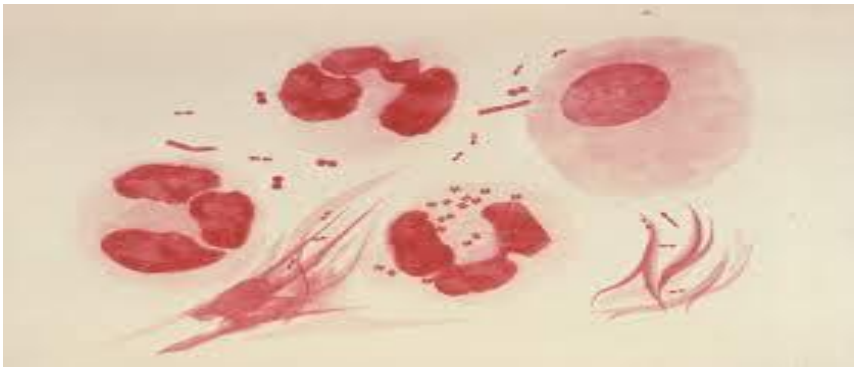
Therapy. Paucibacillary forms are treated with dapson plus rifampicin for six months. Multibacillary forms require treatment with dapson, rifampicin, and clofazimine over a period of at least two years.

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.

Escherichia coli:

E. coli is the head of the large bacterial family, ***Enterobacteriaceae***, the **enteric bacteria**, which are facultatively anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease. The *Enterobacteriaceae* are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella, Shigella, Yersinia*). Several others are normal colonists of the human gastrointestinal tract (e.g. *Escherichia*, *Enterobacter, Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of

humans.Cells are able to survive outside the body for a limited amount of time, wich makes them ideal indicator organisms to test environmental samples for fecal contamination. Depnding on the virulence factors they possess, virulent E.coli strains cause either non- inflammatory diarrhea (watery diarrhea) or inflammatory diarrhea (dysentery with stool usually containing blood, mucus, and leukocytes).

Pathogenesis of *E.coli*:

Over 700 antigenic types (**serotypes**) of *E. coli* are recognized based on **O, H, and K antigens**. At one time serotyping was important in distinguishing the small number of strains that actually cause disease. Thus, the serotype O157:H7 (O refers to somatic antigen; H refers to flagellar antigen) is uniquely responsible for causing HUS (hemolytic uremic syndrome). Nowadays, particularly for diarrheagenic strains (those that cause diarrhea) pathogenic *E. coli* are classified based on their unique virulence factors and can only be identified by these traits. Hence, analysis for pathogenic *E. coli* usually requires that the isolates first be identified as *E. coli* before testing for virulence markers.

Pathogenic strains of *E. coli* are responsible for three types of infections in humans: **urinary tract infections (UTI)**, **neonatal meningitis**, and **intestinal diseases (gastroenteritis)**. The diseases caused (or not caused) by a particular strain of *E. coli* depend on distribution and expression of an array of virulence determinants, including adhesins, invasins, toxins, and abilities to withstand host defenses.

Summary of the Virulence Determinants of Pathogenic *E. coli*

Adhesins

CFAI/CFAII
Type 1 fimbriae
P fimbriae
S fimbriae
Intimin (non-fimbrial adhesin)
EPEC adherence factor

Invasins

hemolysin
Shigella-like "invasins" for intracellular invasion and spread

Motility/chemotaxis
flagella

Toxins
LT toxin
ST toxin
Shiga toxin
cytotoxins
endotoxin (LPS)

Antiphagocytic surface properties
capsules
K antigens
LPS

Defense against serum bactericidal reactions
LPS
K antigens

Defense against immune responses
capsules
K antigens
LPS
antigenic variation

Genetic attributes
genetic exchange by transduction and conjugation
transmissible plasmids
R factors and drug resistance plasmids
toxin and other virulence plasmids

siderophores and siderophore uptake systems
pathogenicity islands

Urinary Tract infections:

Uropathogenic *E. coli* (UPEC) cause 90% of the urinary tract infections (UTI) in anatomically-normal, unobstructed urinary tracts. The bacteria colonize from the feces or perineal region and ascend the urinary tract to the bladder. Bladder infections are 14-times more common in females than males by virtue of the shortened urethra. The typical patient with uncomplicated cystitis is a sexually-active female who was first colonized in the intestine with a uropathogenic *E. coli* strain. The organisms are propelled into the bladder from the periurethral region during sexual intercourse. With the aid of specific adhesins they are able to colonize the bladder.

The adhesin that has been most closely associated with uropathogenic *E. coli* is the **P fimbria** (or **pyelonephritis-associated pili [PAP]**). The letter designation is derived from the ability of P fimbriae to bind specifically to the P blood group antigen which contains a D-galactose-D-galactose residue. The fimbriae bind not only to red cells but to a specific galactose dissaccharide that is found on the surfaces uroepithelial cells in approximately 99% of the population.

The frequency of the distribution of this host cell receptor plays a role in susceptibility and explains why certain individuals have repeated UTI caused by *E. coli*. Uncomplicated *E. coli* UTI virtually never occurs in individuals lacking the receptors.

Uropathogenic strains of *E. coli* possess other determinants of virulence in addition to P fimbriae. *E. coli* with P fimbriae also possess the gene for Type 1 fimbriae, and there is evidence that P fimbriae are derived from Type 1 fimbriae by insertion of a new fimbrial tip protein to replace the mannose-binding domain of Type 1 fimbriae. In any case, **Type 1 fimbriae** could provide a supplementary mechanism of adherence or play a role in aggregating the bacteria to a specific manosyl-glycoprotein that occurs in urine.

Uropathogenic strains of *E. coli* usually produce **siderophores** that probably play an essential role in iron acquisition for the bacteria during or after colonization. They also produce **hemolysins** which are cytotoxic due

to formation of transmembranous pores in host cell membranes. One strategy for obtaining iron and other nutrients for bacterial growth may involve the lysis of host cells to release these substances. The activity of hemolysins is not limited to red cells since the alpha-hemolysins of *E. coli* also lyse lymphocytes, and the beta-hemolysins inhibit phagocytosis and chemotaxis of neutrophils.

Another factor thought to be involved in the pathogenicity of the uropathogenic strains of *E. coli* is their resistance to the complement-dependent bactericidal effect of serum. The presence of K antigens is associated with upper urinary tract infections, and antibody to the **K antigen** has been shown to afford some degree of protection in experimental infections. The K antigens of *E. coli* are "capsular" antigens that may be composed of proteinaceous organelles associated with colonization (e.g., CFA antigens), or made of polysaccharides. Regardless of their chemistry, these capsules may be able to promote bacterial virulence by decreasing the ability of antibodies and/or complement to bind to the bacterial surface, and the ability of phagocytes to recognize and engulf the bacterial cells. The best studied K antigen, K-1, is composed of a polymer of N-acetyl neuraminic acid (sialic acid), which besides being antiphagocytic, has the additional property of being an antigenic disguise.

NeonatalMeningitis

Neonatal meningitis affects 1/2,000-4,000 infants. Eighty percent of *E. coli* strains involved synthesize K-1 capsular antigens (K-1 is only present 20-40% of the time in intestinal isolates).

E. coli strains invade the blood stream of infants from the nasopharynx or GI tract and are carried to the meninges.

The **K-1 antigen** is considered the major determinant of virulence among strains of *E. coli* that cause neonatal meningitis. K-1 is a homopolymer of sialic acid. It inhibits phagocytosis, complement, and responses from the host's immunological mechanisms. K-1 may not be the only determinant of virulence, however, as **siderophore** production and **endotoxin** are also likely to be involved.

Epidemiologic studies have shown that pregnancy is associated with increased rates of colonization by K-1 strains and that these strains become involved in the subsequent cases of meningitis in the newborn. Probably, the infant GI tract is the portal of entry into the bloodstream. Fortunately,

although colonization is fairly common, invasion and the catastrophic sequelae are rare.

Neonatal meningitis requires antibiotic therapy that usually includes ampicillin and a third-generation cephalosporin.

Intestinal Diseases Caused by *E. coli*

As a pathogen, *E. coli* is best known for its ability to cause intestinal diseases. Five classes (virotypes) of *E. coli* that cause diarrheal diseases are now recognized: **enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC)**. Each class falls within a serological subgroup and manifests distinct features in pathogenesis.

Enterotoxigenic *E. coli* (ETEC)

ETEC is an important cause of diarrhea in infants and travelers in underdeveloped countries or regions of poor sanitation. In the U.S., it has been implicated in sporadic waterborne outbreaks, as well as due to the consumption of soft cheeses, Mexican-style foods and raw vegetables. The diseases vary from minor discomfort to a severe cholera-like syndrome. ETEC are acquired by ingestion of contaminated food and water, and adults in endemic areas evidently develop immunity. The disease requires colonization and elaboration of one or more enterotoxins. Both traits are plasmid-encoded.

ETEC may produce a **heat-labile enterotoxin (LT)** that is similar in molecular size, sequence, antigenicity, and function to the cholera toxin (Ctx). It is an 86kDa protein composed of an enzymatically active (A) subunit surrounded by 5 identical binding (B) subunits. It binds to the same identical ganglioside receptors that are recognized by the cholera toxin (i.e., GM1), and its enzymatic activity is identical to that of the cholera toxin.

ETEC may also produce a **heat stable toxin (ST)** that is of low molecular size and resistant to boiling for 30 minutes. There are several variants of ST, of which ST1a or STp is found in *E. coli* isolated from both humans and animals, while ST1b or STh is predominant in human isolates only.

The ST enterotoxins are peptides of molecular weight about 4,000 daltons. Their small size explains why they are not inactivated by heat. ST causes an increase in cyclic GMP in host cell cytoplasm leading to the same effects as an increase in cAMP. ST1a is known to act by binding to a guanylate cyclase that is located on the apical membranes of host cells, thereby activating the enzyme. This leads to secretion of fluid and electrolytes resulting in diarrhea.

The infective dose of ETEC for adults has been estimated to be at least 10^8 cells; but the young, the elderly and the infirm may be susceptible to lower numbers.

ETEC adhesins are **fimbriae** which are species-specific. For example, the K-88 fimbrial Ag is found on strains from piglets; K-99 Ag is found on strains from calves and lambs; CFA I, and CFA II, are found on strains from humans. These fimbrial adhesins adhere to specific receptors on enterocytes of the proximal small intestine.

Symptoms ETEC infections include **diarrhea without fever**. The bacteria colonize the GI tract by means of a fimbrial adhesin, e.g. CFA I and CFA II, and are **noninvasive**, but produce either the LT or ST toxin.

Enteroinvasive *E. coli* (EIEC)

EIEC closely resemble *Shigella* in their pathogenic mechanisms and the kind of clinical illness they produce. EIEC penetrate and multiply within epithelial cells of the colon causing widespread cell destruction. The clinical syndrome is identical to *Shigella* dysentery and includes a **dysentery-like diarrhea with fever**. EIEC apparently lack fimbrial adhesins but do possess a specific adhesin that, as in *Shigella*, is thought to be an outer membrane protein. Also, like *Shigella*, EIEC are **invasive** organisms. They do not produce LT or ST toxin.

There are no known animal reservoirs of EIEC. Hence the primary source for EIEC appears to be infected humans. Although the infective dose of *Shigella* is low (in the range of 10 to few hundred cells), volunteer feeding studies showed that at least 10^6 EIEC organisms are required to cause illness in healthy adults. Unlike typical *E. coli*, EIEC are non-motile, do not decarboxylate lysine and do not ferment lactose. Pathogenicity of EIEC is primarily due to its ability to invade and destroy colonic tissue. The invasion phenotype, encoded by a high molecular weight plasmid, can be

detected by PCR and probes for specific for invasion genes.

Enteropathogenic *E. coli* (EPEC)

EPEC induce a profuse **watery, sometimes bloody, diarrhea**. They are a leading cause of infantile diarrhea in developing countries. Outbreaks have been linked to the consumption of contaminated drinking water as well as some meat products. Pathogenesis of EPEC involves a plasmid-encoded protein referred to as **EPEC adherence factor** (**EAF**) that enables localized adherence of bacteria to intestinal cells and a non fimbrial adhesin designated **intimin**, which is an outer membrane protein that mediates the final stages of adherence. They do not produce ST or LT toxins.

Adherence of EPEC strains to the intestinal mucosa is a very complicated process and produces dramatic effects in the ultrastructure of the cells resulting in rearrangements of actin in the vicinity of adherent bacteria. The phenomenon is sometimes called "attachment and effacing" of cells. EPEC strains are said to be "moderately-invasive", meaning they are not as invasive as *Shigella*, and unlike ETEC or EAEC, they cause an inflammatory response. The diarrhea and other symptoms of EPEC infections probably are caused by bacterial invasion of host cells and interference with normal cellular signal transduction, rather than by production of toxins.

Through volunteer feeding studies the infectious dose of EPEC in healthy adults has been estimated to be 10^6 organisms.

Some types of EPEC are referred to as **diffusely adherent *E. coli* (DAEC)**, based on specific patterns of adherence. They are an important cause of traveler's diarrhea in Mexico and in North Africa.

Enteroaggregative *E. coli* (EAEC)

The distinguishing feature of **EAEC** strains is their ability to attach to tissue culture cells in an aggregative manner. These strains are associated with persistent diarrhea in young children. They resemble ETEC strains in that the bacteria adhere to the intestinal mucosa and cause non-bloody diarrhea without invading or causing inflammation. This suggests that the organisms produce an enterotoxin of some sort. Recently, a distinctive heat-labile plasmid-encoded toxin has been isolated from these strains, called the **EAST** (**EnteroAggregative ST**) **toxin**. They also produce a

hemolysin related to the hemolysin produced by *E. coli* strains involved in urinary tract infections. The role of the toxin and the hemolysin in virulence has not been proven. The significance of EAEC strains in human disease is controversial.

Enterohemorrhagic *E. coli* (EHEC)

EHEC are recognized as the primary cause of **hemorrhagic colitis (HC)** or bloody diarrhea, which can progress to the potentially fatal **hemolytic uremic syndrome (HUS)**. EHEC are characterized by the production of verotoxin or **Shiga toxins** (Stx). Although Stx1 and Stx2 are most often implicated in human illness, several variants of Stx2 exist.

There are many serotypes of Stx-producing *E. coli* , but only those that have been clinically associated with HC are designated as EHEC. Of these, **O157:H7** is the prototypic EHEC and most often implicated in illness worldwide. The infectious dose for O157:H7 is estimated to be 10 - 100 cells; but no information is available for other EHEC serotypes. EHEC infections are mostly food or water borne and have implicated undercooked ground beef, raw milk, cold sandwiches, water, unpasteurized apple juice and vegetables

EHEC are considered to be "**moderately invasive**". Nothing is known about the colonization antigens of EHEC but **fimbriae** are presumed to be involved. The bacteria do not invade mucosal cells as readily as *Shigella*, but EHEC strains produce a toxin that is virtually identical to the Shiga toxin. The toxin plays a role in the intense inflammatory response produced by EHEC strains and may explain the ability of EHEC strains to cause HUS. The toxin is phage encoded and its production is enhanced by iron deficiency.

Diarrheagenic *E. coli*: virulence determinants and characteristics of disease

1-ETEC
fimbrial adhesins e.g. CFA I, CFAII, K88. K99 ,non invasive ,produce LT and/or ST toxin ,watery diarrhea in infants and travelers; no inflammation, no fever

2-EIEC
nonfimbrial adhesins, possibly outer membrane protein invasive (penetrate and multiply within epithelial cells) does not produce shiga toxin dysentery-like diarrhea (mucous, blood), severe inflammation, fever

3-EPEC

non fimbrial adhesin (intimin)EPEC adherence factor (EAF) enables localized adherence of bacteria to intestinal cells moderately invasive (not as invasive as *Shigella* or EIEC) does not produce LT or ST; some reports of shiga-like toxin usually infantile diarrhea; watery diarrhea with blood, some inflammation, no fever; symptoms probably result mainly from invasion rather than toxigenesis.

4-EAEC

adhesins not characterized non invasive produce ST-like toxin (EAST) and a hemolysin persistent diarrhea in young children without inflammation or fever.

5-EHEC

adhesins not characterized, probably fimbriae moderately invasive does not produce LT or ST but does produce shiga toxin pediatric diarrhea, copious bloody discharge (hemorrhagic colitis), intense inflammatory response, may be complicated by hemolytic uremia.

Enterobacter :

Enterobacter is a genus of common Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacteria of the family *Enterobacteriaceae*. Several strains of these bacteria are pathogenic and cause opportunistic infections in immunocompromised (usually hospitalized) hosts and in those who are on mechanical ventilation. The urinary and respiratory tracts are the most common sites of infection. The genus *Enterobacter* is a member of the coliform group of bacteria. It does not belong to the fecal coliforms (or thermotolerant coliforms) group of bacteria, unlike *Escherichia coli*, because it is incapable of growth at 44.5 °C in the presence of bile salts. Two clinically important species from this genus are *E. aerogenes* and *E. cloacae*.

Structure and Metabolism

Enterobacter bacteria are motile, rod-shaped cells, some of which are encapsulated. They also possess peritrichous flagella. As facultative anaerobes, some *Enterobacter* bacteria ferment both glucose and lactose as a carbon source. Gas is produced from the metabolic processes, but they do not produce hydrogen sulfide. *Enterobacter cloacae* A-11 and other closely related bacteria are prototrophic, glycolytic strain of *Enterobacter* that are found on a number of different seeds and plants.

Pathology

Some symptoms of *Enterobacter* infections include bacteremia, lower respiratory tract infections, skin infections, soft tissue infections, urinary tract infections, UTI, endocarditis, intraabdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections. They are an opportunistic pathogens that rarely cause disease in otherwise healthy individuals. This bacterium's virulence seems to be due largely to an endotoxin that it produces. Nosocomial infections are the most frequent type of *Enterobacter* infections, but community-acquired infections are sometimes observed. The bacteria usually infects people who stay in the hospital, especially on the ICU, for long periods of time as well as people how have used many antimicrobial agents, have serious underlying conditions (eg: diabetes, malignancies, burns, mechanical ventilation, etc.), use foreign devices such as intravenous catheters, and immunosuppression.These infections can be contracted endogenously via colonization of the skin, gastrointestinal tract, or urinary tract or exogenously from the "ubiquitous nature of these bacteria". In many cases, the hands of personnel, intravenous solutions, endoscopes, blood products, devices for monitoring intra-arterial pressure, and stethoscopes have been deemed the source for the infection. (Sinave) One specific species of this bacteria, *Enterobacter sakazakii*, is well-known for causing infections in infants who were fed milk-based powered infant formulas. The bacteria, which was called a "yellow pigmented *Enterobacter cloacae*" until 1980, survives when the contaminated powered formula is heated and prepared. Since it has caused several outbreaks of infection in the past, baby formulas are now effectively screened for *E. sakazakii* before they are sold. The infections caused in infants are neonatal meningitis, sepsis, and necrotizing enterocolitis; the reported case-fatality rate varies from 40-80% among infants who contract this bacterial infection .

Virulence factors:

the pathogenic mechanisms expressed by strains of *Enterobacter* are unknown. Like other strains such as *Klebsiella*, they express type 1 and

type 3 fimbraie. Most strains also express an aerobactin-mediated iron uptake systems, commonly associated with extra-intestinal human bacterial pathogens. Some strains may produce a haemolysin resembling the α-haemolysin produced by strains of *E. coli*. Additionally, an outer membrane protein, OmpX, may be a pathogenic factor for strains *E. cloacae*. This particular protein appears to reduce the production of porins on the gram-negative bacteria, leading to decreased sensitivity to β-lactam antibiotics and therefore might play a role in cell invasion of the host .

Enterobacter species produce type 1 or type 3 mannose sensitive hemagglutinins (MSHA) and rarely produce mannnose-resistant hemagglutinins. The only exception being *E. gergoviae*. Additionally, production of a variety of siderophores by enterobacters is also commonly seen. *E. cloacae* generate the hydroxyamate siderophore aerobactin, which is commonly used with microbial species that cause invasion disease. Additionally, several toxins have been found to be produced by *Enterobacter* species. Usually these toxins are described to having single strains or are limited in the number of isolates

E.cloacea:

Enterobacter cloacae infections have the highest mortality rate compared to other *Enterobacter* infections. Many of the clinical samples of the *Enterobacter* infections are hard to distinguish from other bacterial infections, so having its genome sequenced would be very useful for treating these infections.

Enterobacter cloacae are nosocomial pathogens that can cause a range of infections such as bacteremia, lower respiratory tract infection, skin and soft tissue infections, urinary tract infections, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections . This organism affects mostly the vulnerable age groups such as the elderly and the young and can cause prolonged hospitalization in the intensive care unit (ICU) . ICU pathogens can cause morbidity and mortality and the management of this bacteria infection is complicated by the organism's multiple antibiotic resistance. These bacteria contain beta-lactamase, which is undetectable in vitro and is highly resistant to antibiotics such as third generation cephalosporins.

This organism is mainly isolated as nosocomial infections in the ICU for those who stay in the hospital for prolonged periods. The infection may be contracted through the skin, gastrointestinal tract, urinary tract, or cross-contamination. Outbreaks can also be traced back to hands of personnel,

endoscopes, blood products, total parenternal nutrition solutions, albumin, and hospital equipment such as stethoscopes and dialysis.

E.aerogenes:

The genus *Enterobacter* is more specifically a nosocomial opportunistic pathogen and is sought out to be one of the many key causes for extraintestinal infections next to *E. coli*. Infections commonly attributed to *E. aerogenes* are respiratory, gastrointesntinal, and urinary tract infections, specifically cystits, in addition to wound, bloodstream, and central nervous system infections .Furthermore, *E. cloacea* and *E. aerogenes* are the species most commonly associated with aduilt cases of meningitis. Colonies of *Enterobacter* strains may be slightly mucoid.

In the clinical setting, *Enterobacter aerogenes* and *Enterobacter cloacae* are the most frequently isolated in samples of infected hospitalized patients. The majority of the infections are etiologically due to inadvertent transfer of bacteria during surgery or prolonged treatment in hospitals in patients who use venous or urethral catheters. Enterobacteriaceae may account for 80% of clinically significant isolates of gram-negative bacilli and for 50% of clinically significant bacteria in clinical microbiology laboratories. Additionally, they account for nearly 50% of septicemia cases and more than 70% of urinary and intestinal tract infections. The severity of these infections thus create an importance to target, isolate, identify and test for susceptibility for the causes of these nosocomial infections. *Enterobacter aerogenes* causes disease in humans through inadvertent bacteria transfer in hospital settings. A selection of enteric bacteria like *E. aerogenes* are opportunistic and only infect those who already have suppressed host immunity defenses. Infants, the elderly, and those who are in the terminal stages of other disease or are immunosuppressed are prime candidates for such infections .

E. aerogenes as well as other enteric bacteria, is known to have drug-resistant characteristics. There has been some success in dealing with infections through antibiotics, however, the fast development of multidrug resistence has become an increasingly growing problem .These multiresistant strains have caused outbreaks in intensive care units (ICUs) in Belgium, France, Austria, and the United States and has further become more emergent than its sister species *E. cloacaw* . *E. aerogenes* is resistant to ampicillin and it has been more recently discovered that it is resistant to imipenem

Escherichia coli:

E. coli is the head of the large bacterial family, ***Enterobacteriaceae***, the **enteric bacteria**, which are facultatively anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease. The *Enterobacteriaceae* are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella*, *Shigella*, *Yersinia*). Several others are normal colonists of the human gastrointestinal tract (e.g. *Escherichia*, *Enterobacter*, *Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of humans.Cells are able to survive outside the body for a limited amount of time, wich makes them ideal indicator organisms to test environmental samples for fecal contamination. Depnding on the virulence factors they possess, virulent E.coli strains cause either non- inflammatory diarrhea (watery diarrhea) or inflammatory diarrhea (dysentery with stool usually containing blood, mucus, and leukocytes).

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EPEC adherence factor

Invasins
hemolysin
Shigella-like "invasins" for intracellular invasion and spread

Motility/chemotaxis
flagella

Toxins
LT toxin
ST toxin
Shiga toxin
cytotoxins
endotoxin (LPS)

Antiphagocytic surface properties
capsules
K antigens
LPS

Defense against serum bactericidal reactions
LPS
K antigens

Defense against immune responses
capsules

K antigens
LPS
antigenic variation

Genetic attributes
genetic exchange by transduction and conjugation
transmissible plasmids
R factors and drug resistance plasmids
toxin and other virulence plasmids
siderophores and siderophore uptake systems
pathogenicity islands

Urinary Tract infections:

Uropathogenic *E. coli* (UPEC) cause 90% of the urinary tract infections (UTI) in anatomically-normal, unobstructed urinary tracts. The bacteria colonize from the feces or perineal region and ascend the urinary tract to the bladder. Bladder infections are 14-times more common in females than males by virtue of the shortened urethra. The typical patient with uncomplicated cystitis is a sexually-active female who was first colonized in the intestine with a uropathogenic *E. coli* strain. The organisms are propelled into the bladder from the periurethral region during sexual intercourse. With the aid of specific adhesins they are able to colonize the bladder.

The adhesin that has been most closely associated with uropathogenic *E. coli* is the **P fimbria** (or **pyelonephritis-associated pili [PAP]**). The letter designation is derived from the ability of P fimbriae to bind specifically to the P blood group antigen which contains a D-galactose-D-galactose residue. The fimbriae bind not only to red cells but to a specific galactose dissaccharide that is found on the surfaces uroepithelial cells in approximately 99% of the population.

The frequency of the distribution of this host cell receptor plays a role in susceptibility and explains why certain individuals have repeated UTI caused by *E. coli*. Uncomplicated *E. coli* UTI virtually never occurs in individuals lacking the receptors.

Uropathogenic strains of *E. coli* possess other determinants of virulence in addition to P fimbriae. *E. coli* with P fimbriae also possess the gene for

Type 1 fimbriae, and there is evidence that P fimbriae are derived from Type 1 fimbriae by insertion of a new fimbrial tip protein to replace the mannose-binding domain of Type 1 fimbriae. In any case, **Type 1 fimbriae** could provide a supplementary mechanism of adherence or play a role in aggregating the bacteria to a specific manosyl-glycoprotein that occurs in urine.

Uropathogenic strains of *E. coli* usually produce **siderophores** that probably play an essential role in iron acquisition for the bacteria during or after colonization. They also produce **hemolysins** which are cytotoxic due to formation of transmembranous pores in host cell membranes. One strategy for obtaining iron and other nutrients for bacterial growth may involve the lysis of host cells to release these substances. The activity of hemolysins is not limited to red cells since the alpha-hemolysins of *E. coli* also lyse lymphocytes, and the beta-hemolysins inhibit phagocytosis and chemotaxis of neutrophils.

Another factor thought to be involved in the pathogenicity of the uropathogenic strains of *E. coli* is their resistance to the complement-dependent bactericidal effect of serum. The presence of K antigens is associated with upper urinary tract infections, and antibody to the **K antigen** has been shown to afford some degree of protection in experimental infections. The K antigens of *E. coli* are "capsular" antigens that may be composed of proteinaceous organelles associated with colonization (e.g., CFA antigens), or made of polysaccharides. Regardless of their chemistry, these capsules may be able to promote bacterial virulence by decreasing the ability of antibodies and/or complement to bind to the bacterial surface, and the ability of phagocytes to recognize and engulf the bacterial cells. The best studied K antigen, K-1, is composed of a polymer of N-acetyl neuraminic acid (sialic acid), which besides being antiphagocytic, has the additional property of being an antigenic disguise.

NeonatalMeningitis

Neonatal meningitis affects 1/2,000-4,000 infants. Eighty percent of *E. coli* strains involved synthesize K-1 capsular antigens (K-1 is only present 20-40% of the time in intestinal isolates).

E. coli strains invade the blood stream of infants from the nasopharynx or GI tract and are carried to the meninges.

The **K-1 antigen** is considered the major determinant of virulence among strains of *E. coli* that cause neonatal meningitis. K-1 is a homopolymer of

sialic acid. It inhibits phagocytosis, complement, and responses from the host's immunological mechanisms. K-1 may not be the only determinant of virulence, however, as **siderophore** production and **endotoxin** are also likely to be involved.

Epidemiologic studies have shown that pregnancy is associated with increased rates of colonization by K-1 strains and that these strains become involved in the subsequent cases of meningitis in the newborn. Probably, the infant GI tract is the portal of entry into the bloodstream. Fortunately, although colonization is fairly common, invasion and the catastrophic sequelae are rare.

Neonatal meningitis requires antibiotic therapy that usually includes ampicillin and a third-generation cephalosporin.

Intestinal Diseases Caused by *E. coli*

As a pathogen, *E. coli* is best known for its ability to cause intestinal diseases. Five classes (virotypes) of *E. coli* that cause diarrheal diseases are now recognized: **enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC)**. Each class falls within a serological subgroup and manifests distinct features in pathogenesis.

Enterotoxigenic *E. coli* (ETEC)

ETEC is an important cause of diarrhea in infants and travelers in underdeveloped countries or regions of poor sanitation. In the U.S., it has been implicated in sporadic waterborne outbreaks, as well as due to the consumption of soft cheeses, Mexican-style foods and raw vegetables. The diseases vary from minor discomfort to a severe cholera-like syndrome. ETEC are acquired by ingestion of contaminated food and water, and adults in endemic areas evidently develop immunity. The disease requires colonization and elaboration of one or more enterotoxins. Both traits are plasmid-encoded.

ETEC may produce a **heat-labile enterotoxin (LT)** that is similar in molecular size, sequence, antigenicity, and function to the cholera toxin (Ctx). It is an 86kDa protein composed of an enzymatically active (A) subunit surrounded by 5 identical binding (B) subunits. It binds to the same

identical ganglioside receptors that are recognized by the cholera toxin (i.e., GM1), and its enzymatic activity is identical to that of the cholera toxin.

ETEC may also produce a **heat stable toxin (ST)** that is of low molecular size and resistant to boiling for 30 minutes. There are several variants of ST, of which ST1a or STp is found in *E. coli* isolated from both humans and animals, while ST1b or STh is predominant in human isolates only. The ST enterotoxins are peptides of molecular weight about 4,000 daltons. Their small size explains why they are not inactivated by heat. ST causes an increase in cyclic GMP in host cell cytoplasm leading to the same effects as an increase in cAMP. ST1a is known to act by binding to a guanylate cyclase that is located on the apical membranes of host cells, thereby activating the enzyme. This leads to secretion of fluid and electrolytes resulting in diarrhea.

The infective dose of ETEC for adults has been estimated to be at least 10^8 cells; but the young, the elderly and the infirm may be susceptible to lower numbers.

ETEC adhesins are **fimbriae** which are species-specific. For example, the K-88 fimbrial Ag is found on strains from piglets; K-99 Ag is found on strains from calves and lambs; CFA I, and CFA II, are found on strains from humans. These fimbrial adhesins adhere to specific receptors on enterocytes of the proximal small intestine.

Symptoms ETEC infections include **diarrhea without fever**. The bacteria colonize the GI tract by means of a fimbrial adhesin, e.g. CFA I and CFA II, and are **noninvasive**, but produce either the LT or ST toxin.

Enteroinvasive *E. coli* (EIEC)

EIEC closely resemble *Shigella* in their pathogenic mechanisms and the kind of clinical illness they produce. EIEC penetrate and multiply within epithelial cells of the colon causing widespread cell destruction. The clinical syndrome is identical to *Shigella* dysentery and includes a **dysentery-like diarrhea with fever**. EIEC apparently lack fimbrial adhesins but do possess a specific adhesin that, as in *Shigella*, is thought to be an outer membrane protein. Also, like *Shigella*, EIEC are **invasive** organisms. They do not produce LT or ST toxin.

There are no known animal reservoirs of EIEC. Hence the primary source

for EIEC appears to be infected humans. Although the infective dose of *Shigella* is low (in the range of 10 to few hundred cells), volunteer feeding studies showed that at least 10^6 EIEC organisms are required to cause illness in healthy adults. Unlike typical *E. coli*, EIEC are non-motile, do not decarboxylate lysine and do not ferment lactose. Pathogenicity of EIEC is primarily due to its ability to invade and destroy colonic tissue. The invasion phenotype, encoded by a high molecular weight plasmid, can be detected by PCR and probes for specific for invasion genes.

Enteropathogenic *E. coli* (EPEC)

EPEC induce a profuse **watery, sometimes bloody, diarrhea**. They are a leading cause of infantile diarrhea in developing countries. Outbreaks have been linked to the consumption of contaminated drinking water as well as some meat products. Pathogenesis of EPEC involves a plasmid-encoded protein referred to as **EPEC adherence factor** (**EAF**) that enables localized adherence of bacteria to intestinal cells and a non fimbrial adhesin designated **intimin**, which is an outer membrane protein that mediates the final stages of adherence. They do not produce ST or LT toxins.

Adherence of EPEC strains to the intestinal mucosa is a very complicated process and produces dramatic effects in the ultrastructure of the cells resulting in rearrangements of actin in the vicinity of adherent bacteria. The phenomenon is sometimes called "attachment and effacing" of cells. EPEC strains are said to be "moderately-invasive", meaning they are not as invasive as *Shigella*, and unlike ETEC or EAEC, they cause an inflammatory response. The diarrhea and other symptoms of EPEC infections probably are caused by bacterial invasion of host cells and interference with normal cellular signal transduction, rather than by production of toxins.

Through volunteer feeding studies the infectious dose of EPEC in healthy adults has been estimated to be 10^6 organisms.

Some types of EPEC are referred to as **diffusely adherent *E. coli* (DAEC)**, based on specific patterns of adherence. They are an important cause of traveler's diarrhea in Mexico and in North Africa.

Enteroaggregative *E. coli* (EAEC)

The distinguishing feature of **EAEC** strains is their ability to attach to tissue culture cells in an aggregative manner. These strains are associated with persistent diarrhea in young children. They resemble ETEC strains in that the bacteria adhere to the intestinal mucosa and cause non-bloody diarrhea without invading or causing inflammation. This suggests that the organisms produce an enterotoxin of some sort. Recently, a distinctive heat-labile plasmid-encoded toxin has been isolated from these strains, called the **EAST** (**EnteroAggregative ST**) **toxin**. They also produce a **hemolysin** related to the hemolysin produced by *E. coli* strains involved in urinary tract infections. The role of the toxin and the hemolysin in virulence has not been proven. The significance of EAEC strains in human disease is controversial.

Enterohemorrhagic *E. coli* (EHEC)

EHEC are recognized as the primary cause of **hemorrhagic colitis (HC)** or bloody diarrhea, which can progress to the potentially fatal **hemolytic uremic syndrome (HUS)**. EHEC are characterized by the production of verotoxin or **Shiga toxins** (Stx). Although Stx1 and Stx2 are most often implicated in human illness, several variants of Stx2 exist.

There are many serotypes of Stx-producing *E. coli* , but only those that have been clinically associated with HC are designated as EHEC. Of these, **O157:H7** is the prototypic EHEC and most often implicated in illness worldwide. The infectious dose for O157:H7 is estimated to be 10 - 100 cells; but no information is available for other EHEC serotypes. EHEC infections are mostly food or water borne and have implicated undercooked ground beef, raw milk, cold sandwiches, water, unpasteurized apple juice and vegetables

EHEC are considered to be "**moderately invasive**". Nothing is known about the colonization antigens of EHEC but **fimbriae** are presumed to be involved. The bacteria do not invade mucosal cells as readily as *Shigella*, but EHEC strains produce a toxin that is virtually identical to the Shiga toxin. The toxin plays a role in the intense inflammatory response produced by EHEC strains and may explain the ability of EHEC strains to cause HUS. The toxin is phage encoded and its production is enhanced by iron deficiency.

Diarrheagenic *E. coli*: virulence determinants and characteristics of disease

1-ETEC
fimbrial adhesins e.g. CFA I, CFAII, K88. K99 ,non invasive ,produce LT and/or ST toxin ,watery diarrhea in infants and travelers; no inflammation, no fever

2-EIEC
nonfimbrial adhesins, possibly outer membrane protein invasive (penetrate and multiply within epithelial cells) does not produce shiga toxin dysentery-like diarrhea (mucous, blood), severe inflammation, fever

3-EPEC
non fimbrial adhesin (intimin)EPEC adherence factor (EAF) enables localized adherence of bacteria to intestinal cells moderately invasive (not as invasive as *Shigella* or EIEC) does not produce LT or ST; some reports of shiga-like toxin usually infantile diarrhea; watery diarrhea with blood, some inflammation, no fever; symptoms probably result mainly from invasion rather than toxigenesis.

4-EAEC
adhesins not characterized non invasive produce ST-like toxin (EAST) and a hemolysin persistent diarrhea in young children without inflammation or fever.

5-EHEC
adhesins not characterized, probably fimbriae moderately invasive does not produce LT or ST but does produce shiga toxin pediatric diarrhea, copious bloody discharge (hemorrhagic colitis), intense inflammatory response, may be complicated by hemolytic uremia.

Enterobacter :

Enterobacter is a genus of common Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacteria of the family *Enterobacteriaceae*. Several strains of these bacteria are pathogenic and cause opportunistic infections in immunocompromised (usually

hospitalized) hosts and in those who are on mechanical ventilation. The urinary and respiratory tracts are the most common sites of infection. The genus *Enterobacter* is a member of the coliform group of bacteria. It does not belong to the fecal coliforms (or thermotolerant coliforms) group of bacteria, unlike *Escherichia coli*, because it is incapable of growth at 44.5 °C in the presence of bile salts. Two clinically important species from this genus are *E. aerogenes* and *E. cloacae*.

Structure and Metabolism

Enterobacter bacteria are motile, rod-shaped cells, some of which are encapsulated. They also possess peritrichous flagella. As facultative anaerobes, some *Enterobacter* bacteria ferment both glucose and lactose as a carbon source. Gas is produced from the metabolic processes, but they do not produce hydrogen sulfide. *Enterobacter cloacae* A-11 and other closely related bacteria are prototrophic, glycolytic strain of *Enterobacter* that are found on a number of different seeds and plants.

Pathology

Some symptoms of *Enterobacter* infections include bacteremia, lower respiratory tract infections, skin infections, soft tissue infections, urinary tract infections, UTI, endocarditis, intraabdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections. They are an opportunistic pathogens that rarely cause disease in otherwise healthy individuals. This bacterium's virulence seems to be due largely to an endotoxin that it produces. Nosocomial infections are the most frequent type of *Enterobacter* infections, but community-acquired infections are sometimes observed. The bacteria usually infects people who stay in the hospital, especially on the ICU, for long periods of time as well as people how have used many antimicrobial agents, have serious underlying conditions (eg: diabetes, malignancies, burns, mechanical ventilation, etc.), use foreign devices such as intravenous catheters, and immunosuppression.These infections can be contracted endogenously via colonization of the skin, gastrointestinal tract, or urinary tract or exogenously from the "ubiquitous nature of these bacteria". In many cases, the hands of personnel, intravenous solutions, endoscopes, blood products, devices for monitoring intra-arterial pressure, and stethoscopes have been deemed the source for the infection. (Sinave) One specific species of this bacteria, *Enterobacter sakazakii*, is well-known for causing infections in infants who were fed milk-based powered infant formulas. The bacteria, which was called a "yellow pigmented *Enterobacter cloacae*" until 1980, survives when the contaminated powered formula is heated and prepared.

Since it has caused several outbreaks of infection in the past, baby formulas are now effectively screened for *E. sakazakii* before they are sold. The infections caused in infants are neonatal meningitis, sepsis, and necrotizing enterocolitis; the reported case-fatality rate varies from 40-80% among infants who contract this bacterial infection .

Virulence factors:

the pathogenic mechanisms expressed by strains of *Enterobacter* are unknown. Like other strains such as *Klebsiella*, they express type 1 and type 3 fimbraie. Most strains also express an aerobactin-mediated iron uptake systems, commonly associated with extra-intestinal human bacterial pathogens. Some strains may produce a haemolysin resembling the α-haemolysin produced by strains of *E. coli*. Additionally, an outer membrane protein, OmpX, may be a pathogenic factor for strains *E. cloacae*. This particular protein appears to reduce the production of porins on the gram-negative bacteria, leading to decreased sensitivity to β-lactam antibiotics and therefore might play a role in cell invasion of the host .

Enterobacter species produce type 1 or type 3 mannose sensitive hemagglutinins (MSHA) and rarely produce mannnose-resistant hemagglutinins. The only exception being *E. gergoviae*. Additionally, production of a variety of siderophores by enterobacters is also commonly seen. *E. cloacae* generate the hydroxyamate siderophore aerobactin, which is commonly used with microbial species that cause invasion disease. Additionally, several toxins have been found to be produced by *Enterobacter* species. Usually these toxins are described to having single strains or are limited in the number of isolates

E.cloacea:

Enterobacter cloacae infections have the highest mortality rate compared to other *Enterobacter* infections. Many of the clinical samples of the *Enterobacter* infections are hard to distinguish from other bacterial infections, so having its genome sequenced would be very useful for treating these infections.

Enterobacter cloacae are nosocomial pathogens that can cause a range of infections such as bacteremia, lower respiratory tract infection, skin and soft tissue infections, urinary tract infections, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections . This organism affects mostly the vulnerable age groups such as the elderly and the young and can cause prolonged hospitalization in the

intensive care unit (ICU) . ICU pathogens can cause morbidity and mortality and the management of this bacteria infection is complicated by the organism's multiple antibiotic resistance. These bacteria contain beta-lactamase, which is undetectable in vitro and is highly resistant to antibiotics such as third generation cephalosporins.

This organism is mainly isolated as nosocomial infections in the ICU for those who stay in the hospital for prolonged periods. The infection may be contracted through the skin, gastrointestinal tract, urinary tract, or cross-contamination. Outbreaks can also be traced back to hands of personnel, endoscopes, blood products, total parenternal nutrition solutions, albumin, and hospital equipment such as stethoscopes and dialysis.

E.aerogenes:

The genus *Enterobacter* is more specifically a nosocomial opportunistic pathogen and is sought out to be one of the many key causes for extraintestinal infections next to *E. coli*. Infections commonly attributed to *E. aerogenes* are respiratory, gastrointesntinal, and urinary tract infections, specifically cystits, in addition to wound, bloodstream, and central nervous system infections .Furthermore, *E. cloacea* and *E. aerogenes* are the species most commonly associated with aduilt cases of meningitis. Colonies of *Enterobacter* strains may be slightly mucoid.

In the clinical setting, *Enterobacter aerogenes* and *Enterobacter cloacae* are the most frequently isolated in samples of infected hospitalized patients. The majority of the infections are etiologically due to inadvertent transfer of bacteria during surgery or prolonged treatment in hospitals in patients who use venous or urethral catheters. Enterobacteriaceae may account for 80% of clinically significant isolates of gram-negative bacilli and for 50% of clinically significant bacteria in clinical microbiology laboratories. Additionally, they account for nearly 50% of septicemia cases and more than 70% of urinary and intestinal tract infections. The severity of these infections thus create an importance to target, isolate, identify and test for susceptibility for the causes of these nosocomial infections. *Enterobacter aerogenes* causes disease in humans through inadvertent bacteria transfer in hospital settings. A selection of enteric bacteria like *E. aerogenes* are opportunistic and only infect those who already have suppressed host immunity defenses. Infants, the elderly, and those who are in the terminal stages of other disease or are immunosuppressed are prime candidates for such infections .

E. aerogenes as well as other enteric bacteria, is known to have drug-resistant characteristics. There has been some success in dealing with infections through antibiotics, however, the fast development of multidrug resistence has become an increasingly growing problem .These multiresistant strains have caused outbreaks in intensive care units (ICUs) in Belgium, France, Austria, and the United States and has further become more emergent than its sister species *E. cloacaw* . *E. aerogenes* is resistant to ampicillin and it has been more recently discovered that it is resistant to imipenem

Klebsiella

The genus Klebsiella belongs to the tribe Klebsiellae, a member of the family Enterobacteriaceae. The organisms are named after Edwin Klebs, a 19th century German microbiologist. Klebsiellae are nonmotile, rodshaped, gram-negative bacteria with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defence mechanisms.

Members of the Klebsiella genus typically express 2 types of antigens on their cell surface. The first is a lipopolysaccharide (O antigen); the other is a capsular polysaccharide (K antigen). Both of these antigens contribute to pathogenicity. About 77 K antigens and 9 O antigens exist. The structural variability of these antigens forms the basis for classification into various serotypes. The virulence of all serotypes appears to be similar.

The genus was originally divided into 3 main species based on biochemical reactions. Today, 7 species with demonstrated similarities in DNA homology are known. These are (1) Klebsiella pneumoniae, (2) Klebsiella ozaenae, (3) Klebsiella rhinoscleromatis, (4) Klebsiella oxytoca, (5) Klebsiella planticola, (6) Klebsiella terrigena, and (7) Klebsiella ornithinolytica. K. pneumoniae is the most medically important species of the group. K. oxytoca and K. rhinoscleromatis have
also been demonstrated in human clinical specimens. In recent years, Klebsiellae have become important pathogens in nosocomial infections.

Clinical Manifestations:

Klebsiellae are ubiquitous in nature. In humans, they may colonize the skin, pharynx, or gastrointestinal tract. They may also colonize sterile wounds and urine. Klebsiellae may be regarded as normal flora in many parts of the colon, intestinal tract and in the biliary tract. Oropharyngeal carriage has been associated with endotracheal intubation, impaired host defences, and antimicrobial use.

K. pneumoniae and *K. oxytoca* are the 2 members of this genus responsible for most human infections. They are opportunistic pathogens found in the environment and in mammalian mucosal surfaces. The principal pathogenic reservoirs of infection are the gastrointestinal tract of patients and the hands of hospital personnel. Organisms can spread rapidly, often leading to nosocomial outbreaks.

Infection with Klebsiella organisms occurs in the lungs, where they cause destructive changes. Necrosis, inflammation, and haemorrhage occur within lung tissue, sometimes producing a thick, bloody, mucoid sputum described as currant jelly sputum. The illness typically affects middle-aged and older men with debilitating diseases such as alcoholism, diabetes, or chronic bronchopulmonary disease. This patient population is believed to have impaired respiratory host defences. The organisms gain access after the host aspirates colonizing oropharyngeal microbes into the lower respiratory tract. Klebsiellae have also been incriminated in nosocomial infections. Common sites include the urinary tract, lower respiratory tract, biliary tract, and surgical wound sites. The spectrum of clinical syndromes includes pneumonia, bacteraemia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrhoea, upper respiratory tract infection, wound infection, osteomyelitis, and meningitis. The presence of invasive devices, contamination of respiratory support equipment, use of urinary catheters, and use of antibiotics are factors that increase the likelihood of nosocomial infection with Klebsiella species. Sepsis and septic shock may follow entry of organisms into the blood from a focal source.

PATHOGENICITY FACTORS OF *KLEBSIELLA*:

The pathogenic mechanisms of *Klebsiella* infections has identified a number of bacterial factors that contribute to the pathogenesis of these bacteria. Both in vitro and in vivo models have been established to investigate the interaction of bacterial cells and the host. The use of animal models has been a critical element in the study of *Klebsiella* pathogenicity by providing vital information that cannot be obtained from in vitro studies. In particular, animal models have been established to study *Klebsiella* virulence factors in UTIs; mice and rats seem to be appropriate animal types. Lower UTIs have been investigated in the diuresis mouse or rat model of cystitis by intravesicular injection of organisms .To study *Klebsiella*-mediated upper UTI, a rat model of experimental retrograde pyelitis has been established .Frequently, both models include scanning electron microscopy of the surface of the bladder or renal pelvis.

The pathogenicity of *Klebsiella* focuses on the group of five factors :

Capsular Antigens:

Klebsiellae usually develop prominent capsules composed of complex acidic polysaccharides. The capsular repeating subunits, consisting of four to six sugars and, very often, uronic acids (as negatively charged components), can be classified into 77 serological types .Capsules are essential to the virulence of *Klebsiella* .The capsular material forms thick bundles of fibrillous structures covering the bacterial surface in massive layers. This protects the bacterium from phagocytosis by polymorphonuclear granulocytes, on the one hand ,and prevents killing of the bacteria by bactericidal serum factors, on the other ..Apart from their antiphagocytic function, *Klebsiella* capsule polysaccharides have been reported to inhibit the differentiation and functional capacity of macrophages in vitro .Moreover, injection of large doses of *Klebsiella* capsular polysaccharide (CPS) may even produce immunological paralysis, as has been demonstrated in mice that showed a dose-dependent decrease in the production of antibodies to the specific capsular antigenWhile *Klebsiella* CPS were generally considered to mediate virulence properties, this consideration has recently been abandoned because of the great differences in virulence observed among different capsular types: strains expressing the capsule antigens K1 and K2 were found to be especially virulent in a mouse peritonitis model, whereas isolates of other serotypes showed little or no virulence . *Klebsiella* strains of serotypes K1, K2, K4, and K5 were more virulent than were those expressing other capsule types .At present, strains expressing capsule types K1 and K2 are considered especially likely to be virulent.

The degree of virulence conferred by a particular K antigen might be connected to the mannose content of the CPS. Capsular types with low virulence, such as the K7 or K21a antigen contain repetitive sequences of mannose-α-2/3-mannose or l-rhamnose-α-2/3-l-rhamnose. These sequences are recognized by a surface lectin of macrophages, which mediates opsonin-independent (i.e., complement- and antibody-independent) phagocytosis, known as lectinophagocytosis .Lectinophagocytosis has been defined as nonopsonic phagocytosis that is based on recognition between surface lectins on one cell and surface carbohydrates on the opposing cell .Lectinophagocytosis may be mediated either by bacterial surface lectins such as fimbriae or by phagocyte lectins that act as receptors. Macrophages with the mannose-α-2/3-mannose-specific lectin or mannose receptor recognize, ingest, and subsequently kill *Klebsiella* serotypes containing the CPS repeating sequences Manα2/3Man or l-Rhaα2/3l-Rha. In contrast, strains that lack these repeating sequences are not recognized by macrophages and hence phagocytosis does not take

place. This model is consistent with the marked virulence of K2, which completely lacks mannose-α-2/3-mannose structures .Thus, *Klebsiella* strains bearing capsule types devoid of these mannose or rhamnose sequences should be more closely associated with infectious diseases.

the K2 serotype is among the most common capsule types isolated from patients with UTI, pneumonia, or bacteremia. It can be assumed, therefore, that K2 is the predominant serotype of human clinical isolates worldwide whereas K2 strains are very rarely encountered in the environment .Thus, the observed predominance of the K2 serotype in *Klebsiella* infections is quite consistent with the concept of lectinophagocytosis.

Pili (Fimbriae):

As a critical first step in the infectious process, microorganisms must come as close as possible to host mucosal surfaces and maintain this proximity by attaching to the host cell (adherence). The adhesive properties in the *Enterobacteriaceae* are generally mediated by different types of pili. Pili (otherwise known as fimbriae) are nonflagellar, filamentous projections on the bacterial surface. These structures are up to 10 μm long and have a diameter of 1 to 11 nm ,they consist of polymeric globular protein subunits (pilin) with a molecular mass of 15 to 26 kDa .

Pili are demonstrated mainly on the basis of their ability to agglutinate erythrocytes of different animal species. Depending on whether the reaction is inhibited by d-mannose, these adhesins are designated as mannose-sensitive or mannose-resistant hemagglutinins (MSHA and MRHA), respectively .Of the different types of pili described in enterobacteria, there are two predominant types in *Klebsiella* spp.

Type 1 (common) pili.

Type 1 pili are the best investigated of the bacterial adhesins. They are MSHA which agglutinate guinea pig erythrocytes. The adhesion protein in this pilus type is located on the fimbrial shaft and is capable of binding to mannose-containing trisaccharides of the host glycoproteins .The sugar structures presumably consist of short oligomannose chains bound via N-glycosidic linkages to the glycoproteins .The relevance of these pili to bacterial virulence is thought to arise mainly from binding of the bacteria to mucus or to epithelial cells of the urogenital, respiratory, and intestinal tracts .Their role in the pathogenesis primarily with the pathogenesis of lower UTI ,type 1 pili may also be involved in the pathogenesis of pyelonephritis .In this setting, these structures have been shown to bind

effectively to proximal tubulus cells .Type 1 fimbriae are also capable of binding to soluble, mannosyl-containing glycoproteins in urine, such as the Tamm-Horsfall protein ,or in saliva .These findings provide an explanation for the fact that type 1 pili mediate bacterial colonization of the urinogenital and respiratory tracts .Adherence of bacteria to cells of the respiratory tract leads to impairment of colonization resistance in the upper airways, with a subsequent proliferation of facultative pathogenic bacteria. This impairment may result in the development of pneumonia, especially in patients undergoing long-term mechanical ventilation

This type of adhesin mediates bacterial colonization of the host mucosal surfaces via a rather nonspecific binding. In pathogenic microorganisms, colonization of the mucous membrane is followed by invasion of the underlying tissue, with all of the subsequent events of infectious pathogenesis. Adhesin-binding triggers stimulation of the leukocyte ,which ultimately leads to phagocytosis and intracellular killing of the bacterium .The bacterium counters this form of host defense by switching off the expression of type 1 pili in tissue .Thus, while type 1 pili are important for host colonization, their contribution to subsequent steps of pathogenesis is less clear.

Type 3 pili.

Unlike other fimbriae, type 3 pili agglutinate only erythrocytes that have been treated with tannin. Although its name, mannose-resistant, *Klebsiella*-like hemagglutination (MR/K-HA), implies that this fimbrial type is synthesized only by *Klebsiella*, later studies demonstrated that type 3 pili occur in many enteric genera,, these pili were later found to be capable of binding to various human cells. Strains of *K. pneumoniae* expressing type 3 pili adhere to endothelial cells, epithelia of the respiratory tract, and uroepithelial cells .In the kidneys, these pili mediate bacterial adhesion to tubular basement membranes, Bowman's capsules, and renal vessels .Binding to tannic acid-treated erythrocytes is inhibited by spermidine, a polyamine that is also secreted in urine .Since spermidine is exposed on the cell surface of damaged erythrocytes, it has been suggested that MR/K hemagglutination is mediated by spermidine .This might explain why type 3 pili bind to tannic acid- or heat-treated erythrocytes but not to untreated erythrocytes.

The role of this fimbrial type in the pathogenetic process is largely unknown. So far, the only evidence of a correlation between the type 3 MrkD hemagglutinin and disease has been the observation of expression of type 3 pili in *Providencia stuartii* in catheter-associated bacteriuria .This

species is not a common cause of UTI in short-term-catheterized or noncatheterized persons but has a much higher prevalence in the urine of patients with long-term indwelling catheters. In the above-mentioned study, it was demonstrated that the higher prevalence of *P. stuartii* in catheter-associated bacteriuria was due to its ability to adhere and persist to the catheter in the catheterized urinary tract by expression of the MR/K hemagglutinin.

Three new types of *Klebsiella* adhesins have been recently reported. The R-plasmid-encoded CF29K adhesin of *K. pneumoniae* has been demonstrated to mediate adherence to the human intestinal cell lines Intestine-407 and CaCo-2 .This adhesin type seems to be identical to the A adhesive protein of human diarrheal *E. coli* strains.While the two adhesins mentioned above are nonfimbrial, a third putative colonization factor of the human gut is a new fimbria that has been termed KPF-28 .Interestingly, this fimbrial type has been found in the majority of *K. pneumoniae* strains producing CAZ-5/SHV-4 type ESBL.

Lipopolysaccharides (LPS):

For *Klebsiella*, two hypotheses have been propounded .First, capsule polysaccharides may cover and mask the underlying LPS and exhibit a surface structure that does not activate complement. On the other hand, the O side chains of the LPS may reach through the capsule layer and be exposed to the exterior milieu in certain *Klebsiella* capsule types .Since LPS is generally able to activate complement, C3b is subsequently deposited onto the LPS molecules. However, since it is fixed preferentially to the longest O-polysaccharide side chains, C3b is far away from the bacterial cell membrane. Thus, the formation of the lytic membrane attack complex (C5b–C9) is prevented, and subsequent membrane damage and cell death do not take place.

Siderophores:

The growth of bacteria in host tissue is limited not only by the host defense mechanisms but also by its supply of available iron. Iron is an essential factor in bacterial growth, functioning mainly as a redox catalyst in proteins participating in oxygen and electron transport processes. The supply of free iron available to bacteria in the host milieu is extremely low, since this element is bound intracellularly to proteins such as hemoglobin, ferritin, hemosiderin, and myoglobin and extracellularly to high-affinity iron-binding proteins such as lactoferrin and transferrin. The level of free, bioavailable iron (10^{-18} M) is several thousandfold too low for normal bacterial growth.

Many bacteria attempt to secure their supply of iron in the host by secreting high-affinity, low-molecular-weight iron chelators, called siderophores, that are capable of competitively taking up iron bound to host proteins .Under iron-deficient conditions, e.g., in the host milieu, enterobacteria synthesize a variety of siderophores, which belong to two different chemical groups, one consisting of the phenolate-type siderophores and one consisting of the hydroxamate-type siderophores.

In the genus *Klebsiella*, the production of both enterobactin and aerobactin has been demonstrated. However, while enterobactin is synthesized by almost all strains ,aerobactin-positive *Klebsiella* isolates, irrespective of the species or source of isolation, have been observed rarely.

Aerobactin-producing *Klebsiella* ~ndicates that this siderophore does not play a central role in the pathogenicity of the genus *Klebsiella*. It should be pointed out, however, that clinical *K. pneumoniae* isolates, which do not synthesize aerobactin themselves, are entirely capable of using exogenously introduced aerobactin as their sole source of iron .By synthesizing only the intrinsically expressed aerobactin receptor, such strains could derive an advantage over other aerobactin-synthesizing bacteria in mixed infections. The aerobactin-mediated iron uptake system would thus be an indirect contributor to the pathogenicity of the genus *Klebsiella*.

As in many enterobacteria, and as has been especially well studied in *E. coli*, other factors have also been demonstrated in *Klebsiella* spp. Although the production of cytotoxins enterotoxins and hemolysin has been sporadically described, these features probably play a rather minor role in *Klebsiella*.

Proteus:

Proteus species are part of the *Enterobacteriaceae* family of gram-negative bacilli. *Proteus* organisms are implicated as serious causes of infections in humans, along with *Escherichia, Klebsiella*, *Enterobacter*, and *Serratia*

species. *Proteus* species are most commonly found in the human intestinal tract as part of normal human intestinal flora, along with *Escherichia coli* and *Klebsiella* species, of which *E coli* is the predominant resident. *Proteus* is also found in multiple environmental habitats, including long-term care facilities and hospitals. In hospital settings, it is not unusual for gram-negative bacilli to colonize both the skin and oral mucosa of both patients and hospital personnel. Infection primarily occurs from these reservoirs. However, *Proteus* species are not the most common cause of nosocomial infections.

Proteus mirabilis causes 90% of *Proteus* infections and can be considered a community-acquired infection. *Proteus vulgaris* and *Proteus penneri* may be isolated from individuals in long-term care facilities and hospitals and from patients with underlying diseases or compromised immune systems.

Patients with recurrent infections, those with structural abnormalities of the urinary tract, those who have had urethral instrumentation, and those whose infections were acquired in the hospital have an increased frequency of infection caused by *Proteus* and other organisms (eg, *Klebsiella*, *Enterobacter*, *Pseudomonas*,enterococci, staphylococci).

Pathophysiology

Proteus species possess an extracytoplasmic outer membrane, a feature shared with other gram-negative bacteria. In addition, the outer membrane contains a lipid bilayer, lipoproteins, polysaccharides, and lipopolysaccharides. Infection depends on the interaction between the infecting organism and the host defense mechanisms. Various components of the membrane interplay with the host to determine virulence. Inoculum size is important and has a positive correlation with the risk of infection.

Certain virulence factors have been identified in bacteria. The first step in the infectious process is adherence of the microbe to host tissue. Fimbriae facilitate adherence and thus enhance the capacity of the organism to produce disease. *E coli*, *P mirabilis*, and other gram-negative bacteria contain fimbriae (ie, pili), which are tiny projections on the surface of the bacterium. Specific chemicals located on the tips of pili enable organisms to attach to selected host tissue sites (eg, urinary tract endothelium). The presence of these fimbriae has been demonstrated to be important for the attachment of *P mirabilis* to host tissue.

The attachment of *Proteus* species to uroepithelial cells initiates several events in the mucosal endothelial cells, including secretion of interleukin 6 and interleukin 8. *Proteus* organisms also induce apoptosis and epithelial cell desquamation. Bacterial production of urease has also been shown to increase the risk of pyelonephritis in experimental animals. Urease production, together with the presence of bacterial motility and fimbriae, may favor the production of upper urinary tract infections (UTIs) by organisms such as *Proteus.*

Enterobacteriaceae (of which *Proteus* is a member) and *Pseudomonas* species are the microorganisms most commonly responsible for gram-negative bacteremia. When these organisms invade the bloodstream, endotoxin, a component of gram-negative bacterial cell walls, apparently triggers a cascade of host inflammatory responses and leads to major detrimental effects. Because *Proteus* and *Pseudomonas* organisms are gram-negative bacilli, they can cause gram-negative endotoxin-induced sepsis, resulting in **systemic inflammatory response syndrome (SIRS),** which carries a mortality rate of 20%-50%.

Although other organisms can trigger a similar response, it is useful to consider gram-negative bacteremia as a distinct entity because of its characteristic epidemiology, pathogenesis, pathophysiology, and treatment. The presence of the sepsis syndrome associated with a UTI should raise the possibility of urinary tract obstruction. This is especially true of patients who reside in long-term care facilities, who have long-term indwelling urethral catheters, or who have a known history of urethral anatomic abnormalities.

The ability of *Proteus* organisms to produce urease and to alkalinize the urine by hydrolyzing urea to ammonia makes it effective in producing an environment in which it can survive. This leads to precipitation of organic and inorganic compounds, which leads to struvite stone formation. Struvite stones are composed of a combination of magnesium ammonium phosphate (struvite) and calcium carbonate-apatite. Struvite stone formation can be sustained only when ammonia production is increased and the urine pH is elevated to decrease the solubility of phosphate. Both of these requirements can occur only when urine is infected with a urease-producing organism such as *Proteus.* Urease metabolizes urea into ammonia and carbon dioxide: Urea $\rightarrow$ $2NH_3$ + CO_2. The ammonia/ammonium buffer pair has a pK of 9.0, resulting in the combination of highly alkaline urine rich in ammonia.

Proteus exhibits interesting behavior that may harm its host.

- The bacterium contains an endotoxin in its cell wall. The toxin causes an inflammatory response in the host when it's released.
- Fimbriae on the cell wall of the bacterium help it to adhere to the urinary tract lining and other surfaces.
- The bacterium can form stationary films on top of surfaces, including urinary catheters. Bacteria in films (biofilms) are harder to eradicate than free-living bacteria because they secrete a slime that protects their bodies.
- Proteus triggers the formation of mineral stones and crusts in the urinary tract of its host.

Proteus mirabilis:

Proteus mirabilis is part of the normal flora of the human gastrointestinal tract. It can also be found free living in water and soil. When this organism, however, enters the urinary tract, wounds, or the lungs it can become pathogenic. *Proteus mirabilis* commonly causes urinary tract infections and the formation of stones.

Virulence Factors:

The flagellum of *P. mirabilis* is crucial to its motility, a characteristic that helps the organism colonize. The flagellum has also been linked to the ability of *P. mirabilis* to form biofilms, aiding in the bacteria's resistance to defenses of the host and select antibiotics. *P. mirabilis* also relies on its pili for adhesion to avoid being flushed out of the urinary tract system. Important to *Proteus mirabilis* is urease, responsible for raising the pH and consequently making it easier to thrive. Increased pH allows stone formation to take place. On occasion the stones fill the entire renal pelvis. Also present are endotoxins, responsible for induction of the inflammatory response system and pore-forming hemolysins.

Diseases caused by *P.mirabilis*:

The most common infection involving *Proteus mirabilis* occurs when the bacteria moves to the urethra and urinary bladder. Although *Proteus mirabilis* mostly known to cause urinary tract infections, the majority of urinary tract infections are due to *E. coli.* One-hundred thousand cfus per milliliter in the urine are usually indicative of a urinary tract infection. Urinary tract infections caused by *P. mirabilis* occur usually in patients under long-term catherization. The bacteria have been found to move and create encrustations on the urinary catheters. The encrustations cause the catheter to block.

Symptoms for urethritis are mild including frequency of urination and pyuria (presence of white blob cells in the urine). Cystitis (bladder infection) symptoms are easier to distinguish and include back pain, concentrated appearance, urgency, hematuria (presence of red blood cells in the urine), and suprapubic pain as well as increased frequency of urination and pyuria.
Pyelonephritis (kidney infection) can occur when the bacteria migrates from the lower urinary tract. Although it is seen as a furtherance of infections, not all patients have the symptoms associated with urethritis and cystitis. Pyelonephritis is marked by nausea and vomiting.
Proteus mirabilis can enter the bloodstream through wounds. This happens with contact between the wound and an infected surface. The bacteria induce inflammatory response that can cause sepsis and systemic inflammatory response syndrome (SIRS). SIRS has a mortality rate between 20 and 50 percent.
P. mirabilis can also, though less common, colonize the lungs. This is the result of infected hospital breathing equipment and causes pneumonia. Symptoms for pneumonia include fever, chills, chest pain, rales, and cough.
Prostatitis can occur as a result of *P. mirabilis* infection, causing fever, chills, and tender prostate in men.

Treatment and Prevention:

Proteus mirabilis infections can be treated with broad-spectrum penicillins or cephalosporins except in severe cases. It is not susceptible to nitrofurantoin or tetracycline and has experienced increasing drug resistance of ampicillin, trimethoprim, and ciprofloxin. In cases with severe stone formation, surgery is necessary to remove the blockage.
Proteus mirabilis is part of the normal flora of the gastrointestinal tract, and as a result the bacteria enters the urinary tract or infects medical equipment by the fecal route. Consequently, prevention includes good sanitation and hygiene, including proper sterilization of medical equipment. It is also suggested that patients not requiring catherization should not receive catherization, despite its convenience for the caretaker.

Genus *VIBRIO*

Vibrio cholerae, the major pathogen in this genus, is the cause of cholera.

Vibrio parahaemolyticus causes diarrhea associated with eating raw or improperly cooked seafood.

Vibrio vulnificus causes cellulitis and sepsis.

Important Properties

Vibrios are curved, **comma-shaped,** gram-negative rods. *V. cholerae* is divided into two groups according to the nature of its O cell wall antigen. Members of the O1 group cause epidemic disease, whereas non-O1organisms either cause sporadic disease or are nonpathogens. The O1 organisms have two biotypes, called classic

and El Tor, and three serotypes, called Ogawa, Inaba, and Hikojima. (Biotypes are based on differences in biochemical reactions, whereas serotypes are based on antigenic differences.

Vibrio parahaemolyticus and *V. vulnificus* are **marine organisms;** they live primarily in the ocean, especially in warm salt water. They are **halophilic** (i.e., they require a high NaCl concentration to grow).

1. Vibrio cholera

Pathogenesis

Vibrio cholerae is transmitted by **fecal contamination** of water and food, primarily from human sources. Human carriers are frequently asymptomatic and include individuals who are either in the incubation period or convalescing. The main animal reservoirs are marine shellfish. Ingestion of these without adequate cooking can transmit the disease.

The pathogenesis of cholera is dependent on colonization of the small intestine by the organism and secretion of enterotoxin. For colonization to occur, large numbers of bacteria must be ingested because the organism is particularly sensitive to stomach acid. Persons with little or no stomach acid, such as those taking antacids or those who have had gastrectomy, are much more susceptible. Adherence to the cells of the brush border of the gut, which is a requirement for colonization, is related

to secretion of the bacterial enzyme mucinase, which dissolves the protective glycoprotein coating over the intestinal cells.

After adhering, the organism multiplies and secretes an **enterotoxin** called choleragen (cholera toxin). This exotoxin can reproduce the symptoms of cholera even in the absence of the *Vibrio* organisms.

Choleragen consists of an A (active) subunit and a B (binding) subunit. resulting

in the loss of water and ions from the cell. The watery efflux enters the lumen of the gut, resulting in a massive watery diarrhea that contains neither neutrophils nor red

blood cells. Morbidity and death are due to **dehydration**an d **electrolyte imbalance.**

Laboratory Diagnosis

a culture of the diarrhea stool containing *V. cholerae* will show colorless colonies on MacConkey's agar because lactose is fermented slowly. The organism is oxidase-positive, which distinguishes it from members of the Enterobacteriaceae. On TSI agar, an acid slant and an acid butt without gas or H2S are seen because the organism ferments sucrose. A presumptive diagnosis of *V. cholerae* can be confirmed by agglutination of the organism by polyvalent O1 or non-O1antiserum.

Treatment

Treatment consists of prompt, adequate replacement of water and electrolytes, either orally or intravenously. Glucose is added to the solution to enhance the uptake ofwater and electrolytes. Antibiotics such as tetracycline are necessary, but they do shorten the duration of symptoms and reduce the time of excretion of the organisms.

Gram-Negative Rods Related to Animal Sources (Zoonotic Organisms)

BRUCELLA

Disease

Brucella species cause brucellosis (undulant fever).

Important Properties

Brucellae are small gram-negative rods without a capsule. The three major human pathogens and their animal reservoirs are *Brucella melitensis* (goats and sheep), *Brucella abortus* (cattle), and *Brucella suis* (pigs).

Pathogenesis

The organisms enter the body either by ingestion of **contaminated milk products** or **through the skin** by direct contact, They localize in the **reticuloendothelial system,** namely, the lymph nodes, liver, spleen, and bone marrow. Many organisms are killed by macrophages, but some survive within these cells, where they are protected from antibody. The host response is granulomatous, with lymphocytes and epithelioid giant cells, which can progress to form focal abscesses. The mechanism of pathogenesis of these organisms is not well defined, except that endotoxin is involved. No exotoxins are produced.

Clinical Findings

After an incubation period of 1 to 3 weeks, nonspecific symptoms such as fever, chills, fatigue, malaise, anorexia, and weight loss occur. The onset can be acute or gradual. The undulating (rising-and-falling) fever pattern that gives the disease its name occurs in a minority of patients. Enlarged lymph nodes, liver, and spleen are frequently found.

Laboratory Diagnosis

Recovery of the organism requires the use of enriched culture media and incubation in 10% CO2. The organisms can be presumptively identified by using a slide agglutination test with *Brucella* antiserum, and the species can be identified by biochemical tests. If organisms are not isolated, analysis of a serum sample from the patient for a rise in antibody titer to *Brucella* can be used to make a diagnosis. In the absence of an acute-phase serum specimen, a titer of at least 1:160 in the convalescent-phase serum sample is diagnostic.

Treatment

The treatment of choice is tetracycline plus rifampin. There is no significant resistance to these drugs.

Prevention

Prevention of brucellosis involves pasteurization of milk, immunization of animals, and slaughtering of infected animals. There is no human vaccine.

- ## Campylobacter

Classification

There are several species, but only Campylobacter jejuni and C. coli commonly infect humans (cause acute enterocolitis). C. fetus, occasionally causes systemic infection in persons with immune deficiency.

Epidemiology

Campylobacter spp. they are the most common cause of acute bacterial enterocolitis. The infection is essentially a zoonosis with a wide range of reservoirs, including wild birds, poultry, cattle, sheep, pigs and pets. Modes of transmission are correspondingly varied.

General characteristics

Spirally curved, Gram-negative bacilli; motile with single polar flagella; non sporing; non-capsulated; microaerophilic. They are oxidase and catalase positive, but do not metabolise sugars.

Pathogenicity

The Campylobacter cell wall contains endotoxins. Cytopathic extracellular toxins and enterotoxins have also been demonstrated, but their exact role is unclear. They cause acute inflammation of the intestinal mucosa, like that caused by Salmonella and Shigella spp. Infection is self limiting and seldom lasts for more than a week. Mean incubation period is 3 days.

Diseases

Campylobacter enteritis: this can mimic acute appendicitis; occasionally genuine appendicitis is present. It can also mimic an acute attack of ulcerative colitis.

Reactive arthritis and Guillain Barr e syndrome: (polyneuropathy with paralysis) are complications that may arise during the recovery phase of illness.

Laboratory diagnosis

Culture of faeces on selective media(charcoal) at 42 _C under microaerophilic conditions;. Serology for serum antibodies to Campylobacter spp. can be of value in culture-negative patients with suspected late sequelae and in the investigation of outbreaks.

Treatment

Rehydration. Erythromycin or ciprofloxacin indicated only for severe illness.

- ## Helicobacter

Classification

There are many Helicobacter species, each adapted to a particular animal or niche. H. pylori is the species associated with man.

H. pylori

Epidemiology

H. pylori infects human gastric epithelial cells and humans are the main reservoir.

The microorganism is acquired most commonly in early childhood and usually becomes chronic, often life-long. Transmission is by the faucal-oral or oral–oral routes and is associated with close contact and poor sanitation.

General characteristics

Curved Gram-negative bacilli; motile; non-sporing; non-capsulated; H. pylori grows slowly at 37 _C on enriched media in a microaerophilic (characteristic of gastric mucus), urease positive.

Pathogenicity and associated infections

Urease is an important colonization factor, enabling the microorganism to neutralize gastric acid through the production of ammonia from urea. H. pylori is the most common cause of duodenal ulceration and gastric cancer. H. pylori damages antral D cells that release somatostatin, thereby interrupting the negative feedback inhibition of gastric acid from the gastric corpus. Gastric acid thus increases, thereby inducing gastric metaplasia in the duodenum. Witch are susceptible to H. pylori infection, Extension of H. pylori into the gastric corpus causes chronic active inflammation and eventually atrophy of cells, a precursor to metaplasia and cancer.

Diagnosis

1- Gastric biopsy to identify typical histopathological appearance
2- culture of H. pylori on selective medium in a microaerobic atmosphere
3- urease test, H. pylori is unique in producing a urease with a high affinity and rate of activity.
4- The urea breath test is highly sensitive and specific.
5- . The stool antigen test, also highly sensitive and specific, detects the presence of H. pylori in stools using monoclonal antibodies.

Treatment

Combination treatment is required due to poor antibiotic penetration into the gastric mucosa, gastric acidity and increasing antimicrobial resistance.

Spirochaetes

These bacteria, in the order Spirochaetales, are thin (0.1–0.5_5.0–20.0 mm), helical and weakly Gram-negative. Treponema, Borrelia and Leptospira are three genera which cause human diseases . Laboratory diagnoses are based primarily on serological tests, as few spirochaetes can be cultured readily in vitro.

- ## Treponema

T. pallidum

General characteristic

Gram-negative spiral bacteria; motile; non-capsulated; non-sporing; non-culturable in vitro, and grows only slowly in vivo. Motile forms can be visualized in clinical specimens by dark field microscopy or with specialized staining.

Associated infection and epidemiology

Syphilis is mainly an STI.

Laboratory diagnosis

Diagnosis is principally based on serology, but microscopy can be useful in early infection.

1- Microscopy Primary, secondary and congenital syphilis can be diagnosed by dark field examination for spirochaetes in fresh material from skin lesions or lymph node aspirates.
2- Non-specific antibody tests are commonly used, including the Venereal Disease Research Laboratory (VDRL) and rapid plasma reagin (RPR) tests.
3- Specific antibody tests are based on T. pallidumderived antigens. Commonly used tests include syphilis enzyme immunoassays, the fluorescent treponemal antibody (FTA-abs) test a\\\\\nd the T. pallidum particle or haemagglutination assays (TPPA, TPHA).
4- Polymerase chain reaction is being developed for diagnosis.

• Borrelia

General charecteristics

Gram-negative, spiral bacteria; motile; non-capsulated; non-sporing; require specialised media for culture; anaerobic or microaerophilic. Borrelia species are associated with two important human infections: relapsing fever (primarilyB. recurrentis and B. duttoni), and Lyme borreliosis (B. burgdorferi group).

Borrelia species Associated infections and epidemiology

1- Relapsing fever occurs worldwide; characterised by episodes of fever and spirochaetaemia, which can cause severe multi-system effects, separated by periods when the patient is a pyrexial. The relapsing picture is related to the ability of Borrelia species to vary their outer surface protein antigenic structure as many as 30 times, thus evading specific host antibodies for some time.
2- Epidemic relapsing fever is caused exclusively by B. recurrentis and is spread via human body lice, Pediculus humanus. There is no known nonhuman reservoir host. An untreated patient may have a relapse 7–10 days after an initial febrile episode of 3–6 days.
3- Endemic relapsing fever is caused by many Borrelia species and is spread from rodent reservoir hosts via infected Ornithodoros soft ticks during blood meals. Multiple relapses, tending to become shorter and less severe with succeeding episodes, can occur in untreated patients.

Laboratory diagnosis

Borreliae can be identified in stained blood or buffy coat smears taken when the patient is pyrexial. Serological tests are not readily available.

Treatment

Tetracycline is the treatment of choice.

Mycoplasmataceae

Definition

Small (0.15–0.25 mm), Gram-negative microorganisms; pleomorphic with no cell wall; grow slowly on enriched media; aerobic or facultatively anaerobic. The family Mycoplasmataceae consists of two genera: Mycoplasma (69 species) and Ureaplasma (2 species). Only a few species have been identified as human pathogens, including: Mycoplasma pneumoniae, M. hominis, M. genitalium, M. fermentans and Ureaplasma urealyticum.

Associated infections

. Respiratory: pharyngitis; community-acquired pneumonia (M. pneumoniae)

. Central nervous system: meningitis, encephalitis, transverse myelitis (M. pneumoniae)

. Genitourinary (debated): urethritis, pelvic inflammatory disease (U. urealyticum,M. hominis, M. genitalium: urethritis)

Laboratory diagnosis

1-Culture: mycoplasmas and ureaplasmas can be grown on enriched media; penicillin is often added to inhibit other microorganisms. Although M. pneumoniae can be isolated fromsputum after incubation for up to 3 weeks, diagnosis is normally by serology. M. hominis grows after about 4 days, producing colonies
with a ‘fried egg’ appearance. U. urealyticum requires urea for growth and forms small colonies.

2-Serology:M. pneumoniae infections can be diagnosed by serological tests (complement fixation test or enzyme immunoassays) for IgG (4-fold rise in titres is indicative of current infection) or IgM.

Treatment

Macrolides (e.g. erythromycin) or tetracycline