

**Bacterial toxins ( By HOZA, A.S ):****TOXIGENESIS :**

Two types of bacterial toxins :

1. **Lipopolysaccharides:** are associated with the cell walls of Gram-ve bacteria.

The lipopolysaccharide (LPS) component of the Gram-ve bacterial outer membrane bears the name endotoxin because of **its association with the cell wall of bacteria.**

2. **Proteins:** may be released into the extracellular environment of pathogenic bacteria. **Most of the protein toxins are thought of as exotoxins, since they are "released" from the bacteria and act on host cells at a distance.**

**A: BACTERIAL PROTEIN TOXINS ( Exotoxins )**

- The protein toxins are soluble proteins secreted by living bacteria during **exponential** growth.
- The production of protein toxins is specific to a particular bacterial species - (e.g. only *Clostridium tetani* produces tetanus toxin. - only *Corynebacterium diphtheriae* produces the diphtheria toxin) .
- Usually, virulent strains of the bacterium produce the toxin (or range of toxins) while non-virulent strains do not.
- Toxin is the major determinant of virulence.
- Both Gram-positive and Gram-negative bacteria produce soluble protein toxins. Bacterial protein toxins are the most potent poisons known and **may show activity at very high dilutions.**

**Exotoxins :**

The protein toxins resemble enzymes.

- **Like enzymes, bacterial exotoxins are:**
  1. proteins .
  2. denatured by heat, acid, proteolytic enzymes .
  3. have a high biological activity (most act catalytically) .
  4. exhibit specificity of action.
- Bacterial protein toxins are highly specific in the substrate utilized and in their mode of action.
- Usually the site of damage caused by the toxin indicates the location of the substrate for that toxin.
- Terms such as "**enterotoxin**", "**neurotoxin**", "**leukocidin**" or "**hemolysin**" are used to indicate the target site of some well-defined protein toxins.

**Exotoxins :**

- Certain protein toxins have very **specific cytotoxic** activity (i.e., they attack specific cells, for example, tetanus or botulinum toxins).
- Some (as produced by staphylococci, streptococci, clostridia, etc.) have **fairly broad cytotoxic** activity and cause nonspecific death of tissues (necrosis).
- Toxins that are phospholipases may be relatively nonspecific in their cytotoxicity.
- A few protein toxins cause death of the host and are known as "lethal toxins", (e.g. anthrax toxin).

**Exotoxins :**

- Protein toxins are strongly antigenic.
- In vivo, specific antibody (antitoxin) neutralizes the toxicity of these bacterial proteins.
- In vitro, specific antitoxin may not fully inhibit their enzymatic activity.
- **Toxoids:** are detoxified toxins which retain their antigenicity and their immunizing capacity (first discovered by Ehrlich).

**Exotoxins :**A + B Subunit Arrangement of Protein Toxins :**Many protein toxins, consist of two components:**

1. **Subunit A:** responsible for the enzymatic activity of the toxin.
  2. **Subunit B:** concerned with binding to a specific receptor on the host cell membrane and transferring the enzyme across the membrane.
- The enzymatic component is not active until it is released from the native toxin.

**B. ENDOTOXINS:**

Endotoxins are:

- Invariably associated with Gram-ve bacteria as constituents of the outer membrane of the cell wall.

**ENDOTOXINS:**

1. The biological activity of endotoxin is associated with the lipopolysaccharide (LPS). **Toxicity** is associated with the lipid component (Lipid A) and **Immunogenicity** (antigenicity) is associated with the polysaccharide components.
2. The cell wall antigens (O antigens) of Gram-negative bacteria are components of LPS.
3. Compared to the classic exotoxins of bacterial endotoxins are **less** potent and **less** specific in their action, since they do not act enzymatically.
4. Endotoxins are heat stable (boiling for 30 min does not destabilize endotoxin).
5. But certain powerful oxidizing agents such as , superoxide, peroxide and hypochlorite degrade them.
6. Endotoxins, although strongly antigenic, cannot be converted to toxoids.

**Lipopolysaccharides:**

Are complex **amphiphilic** molecules with a mw of about 10kDa, that vary widely in chemical composition both between and among bacterial species.

LPS consists of three components:

1. **Lipid A .**
2. **Core polysaccharide .**
3. **O polysaccharide.**

**Lipid A :**

- Lipid A consists of a phosphorylated N-acetylglucosamine (NAG) dimer with 6 or 7 fatty acids (FA) attached.
- The structure of Lipid A is **highly conserved** among Gram-ve bacteria.
- Among **Enterobacteriaceae** Lipid A is **virtually constant** .

**Core (R) polysaccharide:**

- Is attached to the 6 position of one N-acetylglucosamine ( NAG) .
- Is common to all members of a bacterial genus with minor variations (e.g. *Salmonella*).
- *Salmonella*, *Shigella* and *Escherichia* have similar but not identical cores.

**O polysaccharide (also referred to as the O antigen or O side chain):**

- Is attached to the core polysaccharide.
- Consists of repeating oligosaccharide subunits made up of 3-5 sugars.
- A **major antigenic** determinant (antibody-combining site) of the Gram-negative cell wall **resides in the O polysaccharide**.

**LPS and virulence of Gram-negative bacteria :**

- Endotoxins are toxic to most mammals.
- They are strong antigens but they seldom elicit immune responses which give full protection to the animal against secondary challenge with the endotoxin.
- Cannot be toxoided.
- Endotoxins released from multiplying or disintegrating bacteria significantly contribute to the symptoms of Gram-ve bacteremia and septicemia important pathogenic factors in Gram-ve infections.

**Regardless of the bacterial source :**

- All endotoxins produce the same range of biological effects in the animal host.
- Injection of living or killed Gram-ve cells, or purified LPS, into experimental animals wide spectrum of nonspecific pathophysiological reactions .

**The role of Lipid A :**

- Physiological activities of endotoxins- mediated mainly by the Lipid A component of LPS.
- Lipid A is the toxic component of LPS.
- Lipid A is known to react at the surfaces of macrophages release **cytokines** that mediate the pathophysiological response to endotoxin.

**The role of the O polysaccharide :**

- Although nontoxic, the polysaccharide side chain (O antigen) of LPS may act as a determinant of virulence in Gram-ve bacteria.
- polysaccharide is responsible for the property of "smoothness" of bacterial cells, which may contribute to their resistance to phagocytic engulfment.
- O-polysaccharide is antigenic, and the usual basis for antigenic variation in Gram-ve bacteria rests in differences in their O polysaccharides.

**Pathogenicity Islands (PAI):**

- Are a distinct class of genomic islands which are acquired by horizontal gene transfer.
- Incorporated in the genome of pathogenic bacteria
- usually absent from non-pathogenic organisms of the same or closely related species.
- They occupy relatively large genomic regions ranging from 10-200 kb.
- encode genes which contribute to virulence of the pathogen.
- One species of bacteria may have **more** than one pathogenicity island.
  - For example, in *Salmonella*, five pathogenicity islands have been identified.
- Found **mainly** in Gram-ve bacteria, but have been shown in **a few** Gram-positives.
- May be located on the bacterial chromosome or may be a part of a plasmid.
- Play a vital role in the virulence of bacterial pathogens of humans , animals and plants.
- May also represent a mechanism that contributes to the appearance of new pathogens.
- Genomic islands play an important role in microbial evolution.

Knowledge about PAI, their **structure**, their **mobility**, and the **pathogenicity** factors they encode is helpful:

A- In gaining a better understanding of bacterial evolution and interactions of pathogens with **eucaryotic** host cells.

B - Also may have important practical implications such as providing delivery systems for vaccination and tools for the development of new strategies for therapy of bacterial infections.

**Resistance to disease:**

Immune system : Two intrinsic systems:

- **Innate** (nonspecific) defense system.
- **Adaptive** (specific) defense system.
- Functional system rather than organ system.
- Innate and adaptive defenses intertwined.
- Release and recognize many of same defensive molecules.
- Innate defenses do have specific pathways for certain substances.
- Innate responses release proteins that alert cells of adaptive system to foreign molecules.

**Innate defense system has two lines of defense :**

**First** - external body membranes (skin and mucosae).

**Second** - antimicrobial proteins, phagocytes, and other cells  
Inhibit spread of invaders.

- Inflammation most important mechanism .
- **Adaptive defense system :**

**Third line** of defense attacks *particular* foreign substances ,takes longer to react than innate system.



**Innate defenses :****Surface barriers:**

- Skin.
- Mucous membranes.

**Internal defenses :**

- Phagocytes.
- Natural killer cells.
- Inflammation.
- Antimicrobial proteins.
- Fever.

**Adaptive defenses :**Humoral immunity:

- B cells.

Cellular immunity:

- T cells.

**Innate Defenses :**

- Surface barriers ward off invading pathogens .

Skin, mucous membranes, and their secretions.

- Physical barrier to most microorganisms Keratin resistant to weak acids and bases, bacterial enzymes, and toxins.
- Mucosae provide similar mechanical barriers.

**Surface Barriers :**

- Protective chemicals inhibit or destroy microorganisms .
  - Acidity of skin and secretions – acid mantle – inhibits growth .
  - Enzymes - lysozyme of saliva, respiratory mucus, and lacrimal fluid – kill many microorganisms .
  - Defensins – antimicrobial peptides – inhibit growth.
  - Other chemicals - lipids in sebum, dermcidin in sweat – toxic .

**Respiratory system modifications :**

- Mucus-coated hairs in nose.
- Cilia of upper respiratory tract sweep dust- and bacteria-laden mucus toward mouth .

Surface barriers breached by nicks or cuts - second line of defense must protect deeper tissues .

**Internal Defenses: Cells and Chemicals:**

- Necessary if microorganisms invade deeper tissues.
  - Phagocytes.
  - Natural killer (NK) cells.
  - Antimicrobial proteins (interferons and complement proteins).
  - Fever.

**Inflammatory response :** (macrophages, mast cells, WBCs, and inflammatory chemicals).

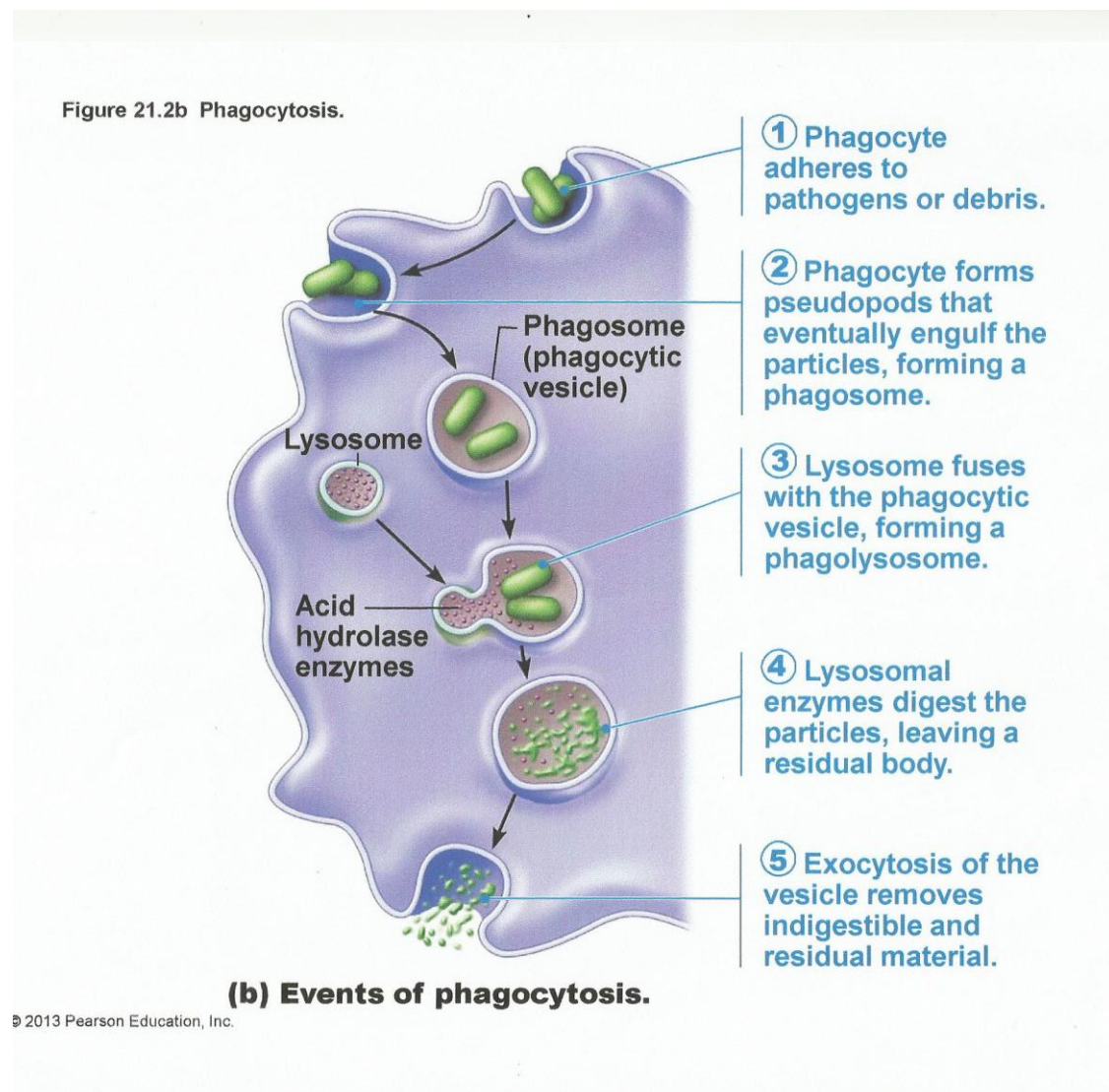
**Phagocytes:**

- **Neutrophils** most abundant but die fighting , become phagocytic on exposure to infectious material.
- **Macrophages** develop from monocytes – chief phagocytic cells – robust cells .
  - **Free macrophages** wander through tissue spaces, e.g., alveolar macrophages.
  - **Fixed macrophages** permanent residents of some organs; e.g., stellate macrophages (liver) and microglia (brain).

**Mechanism of Phagocytosis :**

- Phagocyte must adhere to particle.
  - Some microorganisms evade adherence with capsule .
  - **Opsonization** marks pathogens—coating by complement proteins or antibodies.
- Cytoplasmic extensions bind to and engulf particle in vesicle called **phagosome**.
- Phagosome fuses with lysosome → **phagolysosome**.
- Pathogens killed by acidifying and digesting with lysosomal enzymes.
- Helper T cells cause release of enzymes of **respiratory burst**, which kill pathogens resistant to lysosomal enzymes by :
  - Releasing cell-killing free radicals.
  - Producing oxidizing chemicals (e.g.,  $H_2O_2$ ) .
  - Increasing pH and osmolarity of phagolysosome.

## Phagocytosis :



## Phagocyte Mobilization:

- Neutrophils lead macrophages follow as attack continues, monocytes arrive , 12 hours after leaving bloodstream → macrophages, these "late-arrivers" replace dying neutrophils and remain for cleanup prior to repair .

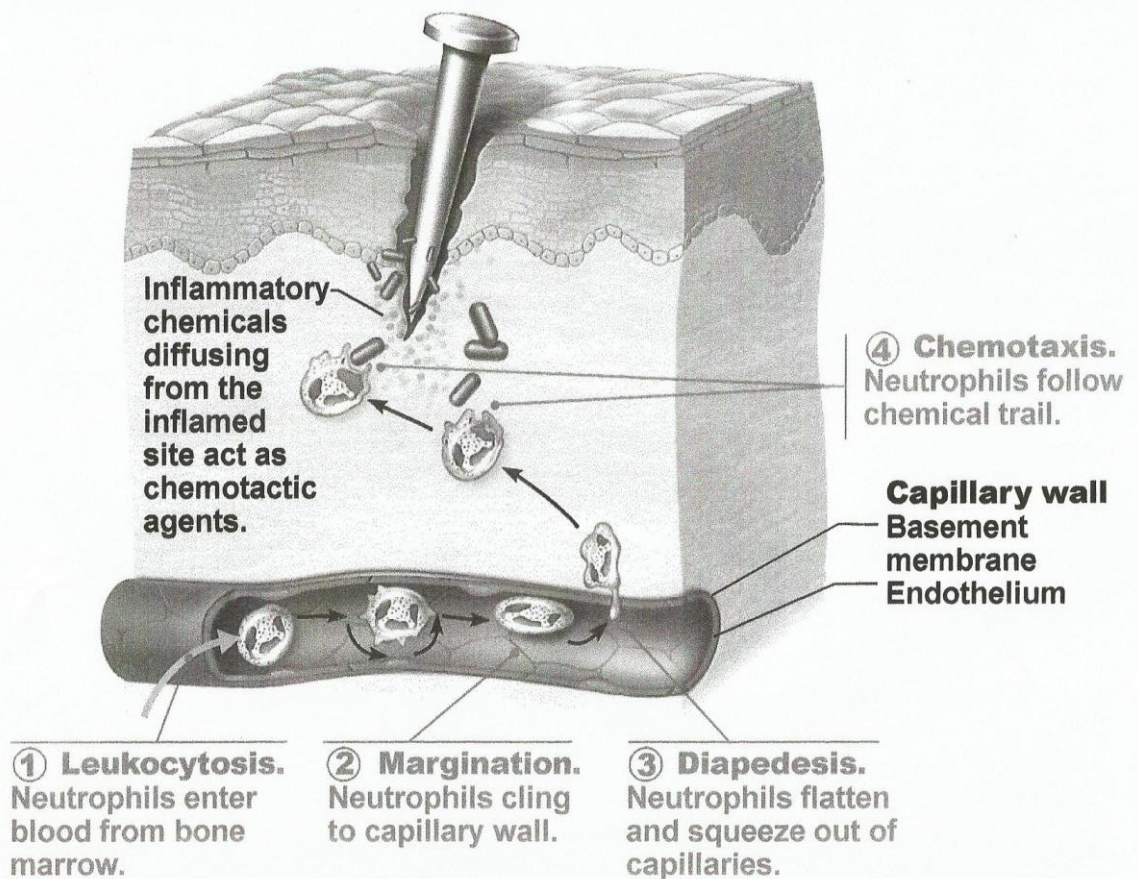
If inflammation due to pathogens, complement activated; adaptive immunity elements arrive.

### Steps for phagocyte mobilization :

1. **Leukocytosis:** release of neutrophils from bone marrow in response to **leukocytosis-inducing factors** from injured cells .
2. **Margination:** neutrophils cling to walls of capillaries in inflamed area in response to called cell adhesion molecules (CAMs).
3. **Diapedesis** of neutrophils .
4. **Chemotaxis:** inflammatory chemicals (**chemotactic agent**) promote positive chemotaxis of neutrophils.

Figure 21.4 Phagocyte mobilization.

Innate defenses → Internal defenses



**Natural Killer (NK) Cells :****Nonphagocytic large granular lymphocytes**

1. attack cells that lack "self" cell-surface receptors.
2. Induce **apoptosis** in cancer cells and virus-infected cells .
3. Secrete potent chemicals that enhance inflammatory response.

**Inflammatory Response :**

- Triggered whenever body tissues injured.
- Prevents spread of damaging agents.
- Disposes of cell debris and pathogens.
- Alerts adaptive immune system.
- Sets the stage for repair .

**Inflammatory Response:**

- Cardinal ( major or main ) signs of acute inflammation:
  1. Redness.
  2. Heat.
  3. Swelling.
  4. Pain.

(Sometimes 5. Impairment of function of ..... , ..... , ..... ).

**Inflammatory Response:**

- Begins with chemicals released into Extracellular fluid ( ECF ) by injured tissues, immune cells, blood proteins.
- Macrophages and epithelial cells of boundary tissues bear Toll-like receptors (TLRs).
- Activated TLRs trigger release of cytokines that promote inflammation.

**Inflammatory Response:**

- Inflammatory mediators :

Kinins, prostaglandins (PGs), and complement .

- Dilate local arterioles (**hyperemia**).
  - Causes redness and heat of inflamed region .
- Make capillaries leaky.
- Many attract leukocytes to area.
- Some have inflammatory roles.

**Inflammatory Response: Edema ( الإستسقاء )**

Capillary permeability → **exudate** to tissues .

- Fluid containing clotting factors and antibodies.
- Causes local swelling (**edema**).
- Swelling pushes on nerve endings → pain.
  - Pain also from bacterial toxins, prostaglandins, and kinins.
- Moves foreign material into lymphatic vessels.
- Delivers clotting proteins and complement.

**Inflammatory Response:**

- Clotting factors form fibrin mesh.
  - Scaffold for repair.
  - Isolates injured area so invaders cannot spread.

**Antimicrobial Proteins:**

- Include interferons and complement proteins.
- Some attack microorganisms directly.
- Some hinder microorganisms' ability to reproduce.

## **The Normal Bacterial Flora of Humans**

In healthy human, there are many of bacteria are consistently associated with the body

1- Body surfaces.

2- Mucus membranes.

skin and mucous membranes, are always contact with environmental organisms and become colonized by various bacterial species.

3- Intestinal linings of humans.

The mixture of organisms or the bacteria and other microbes that are consistently associated with human and found at any site are called the **normal flora**, or the "**indigenous microbiota**". These bacteria have a symbiotic interactions with their hosts.

The normal flora of humans consists of a few fungi, but bacteria are the most numerous and obvious microbial components of the normal flora

**The internal tissues, e.g. blood, brain, muscle, etc., are normally free of microorganisms.**

**The normal flora** derive from their host a steady supply of nutrients, a stable environment, protection and transport .

**The host** obtains from the normal flora certain nutritional and digestive benefits, stimulation of the development and activity of immune system, and protection against colonization and infection by pathogenic bacteria.



**Endogenous diseases : ( En do geno us )**

Diseases that are produced by the normal flora in their host .

**Most endogenous** bacterial diseases are **opportunistic infections**, meaning that the organism must be given a special opportunity of weakness in the host defenses in order to infect.

**Harmful Effects of the Normal Flora:**

The overall harmful effects of microbes are summarized below:

**1- Bacterial synergism:**

Between a member of the normal flora and a potential pathogen helps in establishment of infection.

There are examples of a member of the normal flora supplying a **vitamin** or some other growth factor that a pathogen needs in order to grow.

**This is called cross-feeding between microbes**

**2- Competition:**

For nutrients Bacteria in the gastrointestinal tract may get to some of our utilizable nutrients before we are able to absorb them.

**3- Induction of a low grade toxemia:**

Minute amounts of bacterial toxins (e.g. endotoxin) may be found in the circulation. **Of course**, it is these small amounts of bacterial antigen that stimulate the formation of natural antibodies.

**4- The normal flora may be agents of disease:**

Members of the normal flora may cause **endogenous disease** if they reach a site or tissue where they cannot be restricted or tolerated by the host defenses.

Many of the normal flora are **potential pathogens**, and if they gain access to a compromised tissue from which they can invade, disease may result.

**5- Transfer to susceptible hosts :**

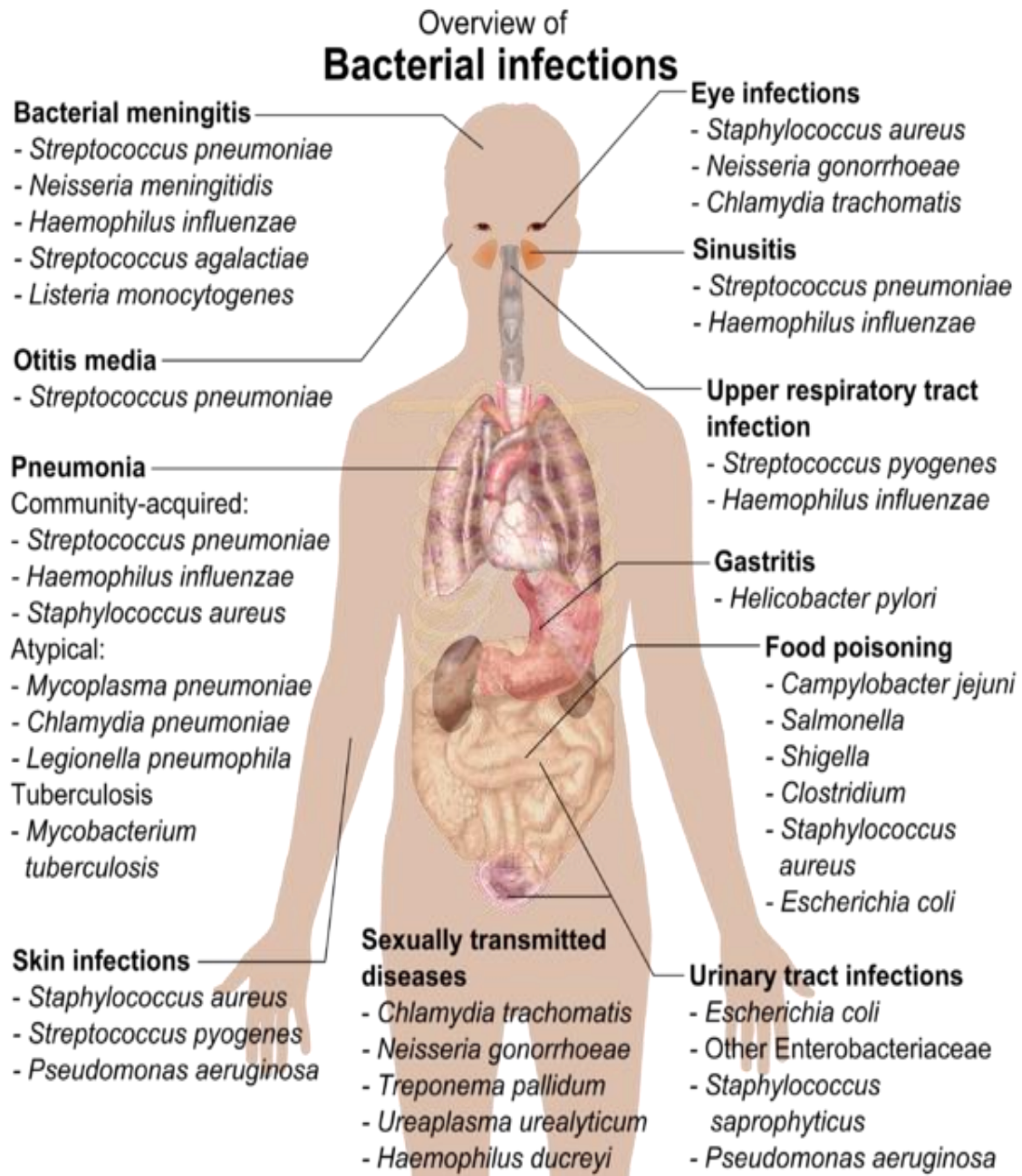
Some pathogens of humans that are members of the normal flora may also rely on their host for transfer to other individuals where they can produce disease.

**This includes the pathogens that colonize the upper respiratory tract such as :**

*Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus*,

**And potential pathogens such as:**

*E. coli*, *Salmonella* or *Clostridium* in the gastrointestinal tract.



## Overview of Bacterial infection:

### 1- Bacterial meningitis : التهاب السحايا الجرثومي

*Streptococcus pneumoniae* ( strep to co ccus - p neu mon iae ).

*Neisseria meningitidis* ( neis ser ia - men ing ti dis ) .

*Haemophilus influenzae* ( Haemo philus - in ful en zae ).

*Streptococcus agalactiae* ( strep to co ccus - aga lac ti ae ).

*Listeria monocytogenes* ( lis ter ia - mono cy to ge nes) .

### 2- Otitis media :O ti tis التهاب الإذن الوسطي

*Streptococcus pneumoniae* ( Strep to co ccus - p neu mon iae ).

### 3- Pneumonia : الالتهاب الرئوي

#### Community-acquired :

*Streptococcus pneumoniae* ( Strep to co ccus - p neu mon iae ).

*Haemophilus influenzae* ( Haemo philus - in ful en zae ).

*Staphylococcus aureus* ( Sta phy lo coc cus - aur eus ) .

#### Atypical:

*Mycoplasma pneumoniae* ( My co plasma - p neu mon iae ) .

*Chlamydia pneumoniae* ( Ch la my dia - p neu mon iae ).

*Legionella pneumophila* ( Legi o nella - p neu mo phila ).

#### Tuberculosis :

*Mycobacterium tuberculosis* ( My co bac ter ium - tu ber cu lo sis).

### 4- Skin infection :

*Staphylococcus aureus* ( Sta phy lo coc cus - aur eus ) .

*Streptococcus pyogenes* ( Strep to coc cus – pyo gen es ) .

*Pseudomonas aeruginosa* ( P sedo mo nas aer u gi no sa ) .

**5- Eye infection :**

*Staphylococcus aureus* ( Sta phy lo coc cus - aur eus ) .

*Neisseria gonorrhoeae* ( Neis seria - go nor rho eae ) .

*Chlamydia trachomatis* ( Ch la my dia - trac homa tis ) .

**6- Sinusitis : si nusi tis التهاب الجيوب الأنفية**

*Streptococcus pneumoniae* ( strep to co ccus - p neu mon iae ) .

*Haemophilus influenzae* ( Haemo philus - in ful en zae ) .

**7- Upper respiratory tract infection :**

*Streptococcus pneumoniae* ( strep to co ccus - p neu mon iae ) .

*Haemophilus influenzae* ( Haemo philus - in ful en zae ) .

**8- Gastritis : Gas tri tis التهاب المعدة .**

*Helicobacter pylori* ( He li co bac ter - py lo ri ) .

**9- Food poisoning :**

*Campylobacter jejuni* ( Cam py lo bacter - je ju ni ) .

*Salmonella* . ( Sal mon ella ) .

*Shigella* . ( Shi g ella ) .

*Clostridium* . ( Clos tri di um ) .

*Staphylococcus aureus* ( Sta phy lo coc cus - aur eus ) ..

*Escherichia coli* .

**10- Urinary tract infection :**

*Escherichia coli* . ( Es che ri chia - co li ).

Other Enterobacteriaceae .

*Staphylococcus saprophyticus*. (Sta phy lo coc cus - sap ro phyti cus ).

*Pseudomonas aeruginosa* ( P sedo mo nas aer u gi no sa ) .

**11- Sexually transmitted diseases:**

*Chlamydia trachomatis* . ( Ch la my dia - tra cho ma tis ).

*Neisseria gonorrhoeae* ( Neis seria - go nor rho eae ) .

*Treponema pallidum*. ( Tre po ne ma - pal lid um ).

*Ureaplasma urealyticum* . ( Urea plasma - urea ly ti cum ).

*Haemophilus ducreyi* . ( Haemo phi lus - duc reyi ).

=====

*Ureaplasma urealyticum*

Definitions of ureaplasma

*noun*

a small bacterium related to the mycoplasmas, characterized by the ability to metabolize urea.

"In 40 cases the other bacteria made it impossible to recognise the results of ureaplasmas in culture."

# External and Internal Barriers

## Physical and Chemical Barriers :

Physical and chemical barriers protect against a wide variety of invaders.

- Few pathogens can pass through the tough layer of dead skin cells that surrounds the body.
- Tears and saliva contain enzymes that destroy or disable many pathogens.
- Mucus secreted by mucous membranes carries trapped pathogens to other areas of the body for disposal.
- Cilia sweep mucus and pathogens to the throat, where they can be swallowed or coughed out.
- Gastric juice in the stomach destroys many pathogens that enter the body through the nose and mouth.

## Defense Strategies of the Immune System:

The immune system has **two** major defense strategies:

1. The **inflammatory response** is general, or nonspecific; it works against all types of pathogens.
2. **Specific defenses** work against particular pathogens.

## Host Defenses :

### **Nonspecific Resistance :**

#### First line of defense include :

1. Intact skin.
2. Mucous membranes and their secretions.
3. Normal microbiota.

#### Second line of defense include :

1. Phagocytic white blood cells.
2. Inflammation.
3. Fever.
4. Antimicrobial substances.

### **Specific Resistance :**

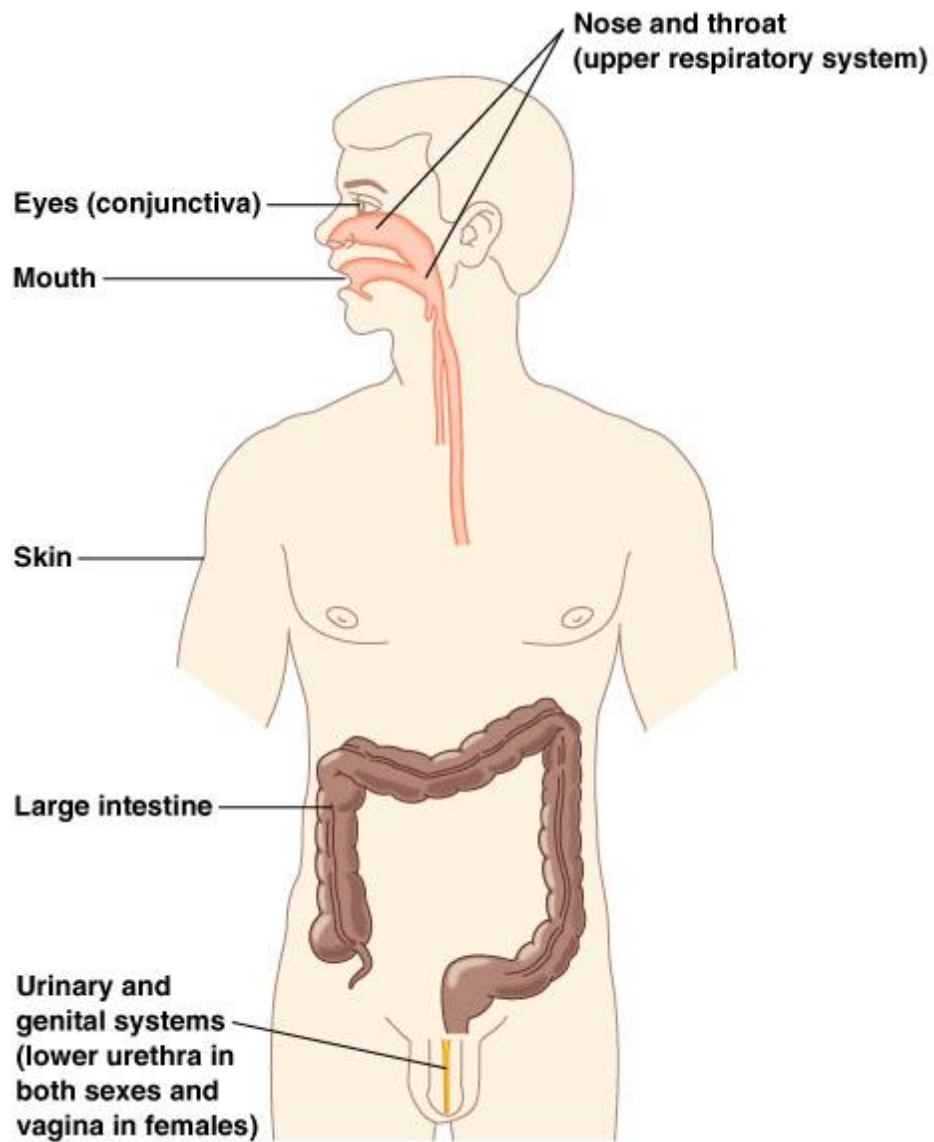
#### **Responses of the Immune system:**

#### Third line of defense include :

1. Specialized lymphocytes.
2. B cell and T cell.
3. Antibodies.



## First line of defense – physical & chemical barriers :



Intact, unbroken **skin** (Broken skin = port of entry):

Almost all bacteria are incapable to penetrate skin , predominantly inhabited by *Staphylococcus epidermidis* .

- How?
  - Dryness.
  - Temperature.
  - Low pH (acidic) of skin.
  - bacteriocidal secretion by the sebaceous glands .
  - Desquamation – sloughing of epithelium .
  - Perspiration (sweat contain lysozymes – attack bacterial cell wall) .
- Exception: *Staphylococcus aureus* in moist area
- **Eyes :**
  - Blinking of eyelids .
  - Tears containing lysozymes.
- **Outer ear canal:**
  - Wax contains antibacterial components .
- **Mucus membranes :**
  - layers of mucosal cells that line body cavities that open to the outside (digestive, genitourinary and respiratory tracts).
  - Mucus is produced by the mucosal cells:
    - Contains antimicrobial substance such as lysozymes, lactoferrin .
    - Mucosal cells are rapidly dividing and work to flush out of body along with attached bacteria.

**Digestive tract :**

- Mouth and lower digestive tract – lots of bacteria (mostly anaerobes e.g. *Bacteroides*, anaerobic *Streptococci* [*Streptococcus mutans* in mouth] and *Clostridium* in colon .
- How?
  - Mucus .
  - Saliva (contains lysozyme).
  - Bile (alkaline) in small intestine.
  - Stomach acids.
  - Defecation (feces contains up to 50% bacteria !).
  - Mucus contain antibacterial agents, antibodies and immune cells called phagocytes.

**• Genitourinary tract :**

- Urinary tract is sterile in a health person except the distal urethra .
- How?
  - Urination.
  - Secretion (vaginal and seminal fluid).
  - Low pH of vagina (presence of several *Lactobacillus* sp., *Candida albicans*).

- **Respiratory tract :**
  - Nose - nasal hair, mucus secretions (phagocytes and antibacterial enzymes), irregular chambers .
  - ciliated epithelium (nasal cavity, sinuses, bronchi and trachea).
  - Cough reflexes.
  - Alveolar macrophages.
- **Microbial antagonism :**
  - Normal flora vs. invaders. ( Normal flora against invaders ).
    - Compete for colonization sites .
    - Compete for nutrients.
    - Produce bacteriocins .
  - Administration of broad spectrum antibiotics may kill only certain members of the normal flora, leaving the others to overgrow → superinfection

e.g. yeast in vagina – yeast vaginitis .

*Clostridium difficile* in colon – diarrhea and colitis .

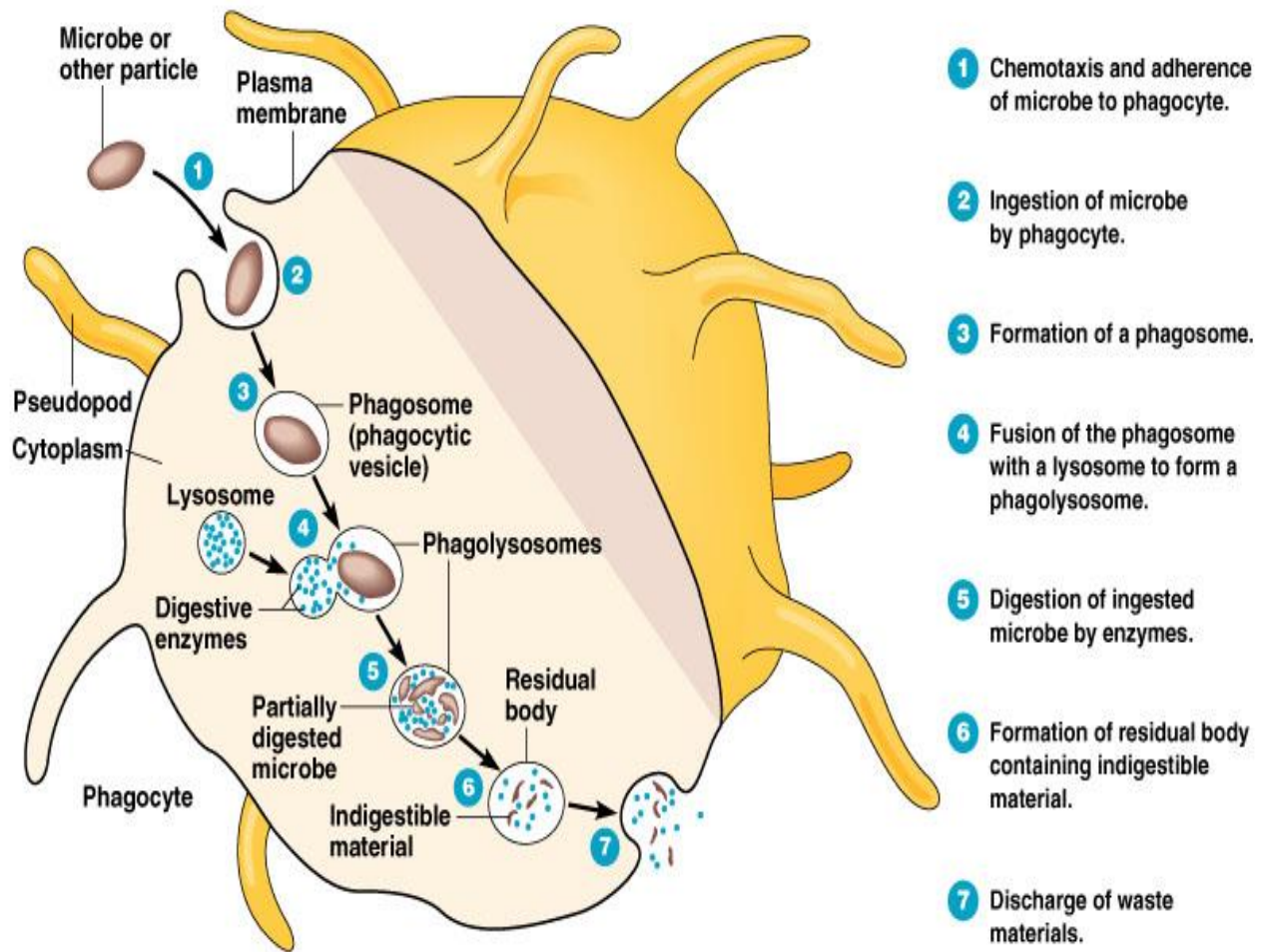
**Second line of defense :**

Once beyond the protective outer barrier of the body, the invading microbes will encounter a series of nonspecific cellular and chemical defense mechanisms.

- Mechanisms:
  - Inflammation – a series of events that removes or contain the offending agent and repair the damage.
  - Chemotaxis – movement of cells toward a chemical influence (chemokines or chemotatic agents).
  - Phagocytosis – process in which cell ingest foreign particulate matter e.g. microbes.
- Many are carried out by the white blood cells in blood.

**Phagocytosis** is the ingestion of microorganisms or other matter by a cell. Many white blood cells **engulf** invasive microorganisms by the process of phagocytosis. The steps in phagocytosis are:

- 1. **Chemotaxis** is the process by which phagocytes are attracted to microorganisms.
- 2. **Attachment:** The phagocyte then adheres to the microbial cell. This adherence may be facilitated by **opsonization** – coating the microbe with plasma proteins.
- 3. **Ingestion:** Pseudopods of phagocytes engulf the microorganism and enclose it in a phagosome to complete ingestion.
- 4. **Digestion:** Lysosomes fuse with the phagosome to form a digestive vacuole. The microbe is killed and digested.



(a) Phases of phagocytosis

**Blood Components :**

- Fluid portion :
  - **Serum**: liquid portion of clotted blood.
  - **Plasma**: liquid portion with clotting factors.
  - “Plasma can clot; Serum cannot”
  - Contains antibodies & other proteins
- Clotting factors (proteins):
  - Fibrinogen.
  - Prothrombin .
- Formed elements:
  - **Erythrocytes** – red blood cells (RBC) – carry oxygen and carbon dioxide; no nucleus.
  - **Leukocytes** – white blood cells (WBC) – defense.
  - **Platelets** – thrombocyte particles – clotting; no nucleus.

# Streptococci :

The streptococci are :

- Gram positive spherical bacteria characteristically arranged in pairs or chains.
- Lengths of chains are conditioned by environmental factors.

## Growth characteristics :

Streptococci grow in solid media, supplemented with blood, as discoid colonies, usually one to two mm in diameter.

## Some streptococci :

- ( **$\beta$  hemolytic**) can lyse blood cells and cause a complete clearing of blood all around the colonies.
- Other strains cause no change in blood agar (  **$\gamma$  or nonhemolytic** ) .
- Other reduce hemoglobin and cause a greenish discolouration of agar ( **$\alpha$  hemolytic**).

## Hemolytic reactions on culture media:

- In **beta hemolytic** reaction red blood cells are completely lysed.
- In **alpha hemolytic** reaction red blood cells are not completely lysed but colonies are surrounded by greenish discolouration of agar due to streptococcal action on haemoglobin.
- Nonhemolytic or **gamma hemolytic** streptococci have no effect on blood agar.



**Lancefield classification:**

- An important method of distinguishing between pyogenic streptococci is the serological classification pioneered by the American bacteriologist Rebecca Lancefield 1895-1981. .
- She detected different versions of the major cell wall polysaccharide among the pyogenic streptococci.
- The polysaccharide is referred to as the group polysaccharide identifies a number of different groups labeled by capital letters ( Lancefield groups A,B,C . etc. ).
- Lancefield subdivided streptococci based on cell wall antigen (antigen C). This carbohydrate forms the basis of serologic grouping .
- For group A streptococci, antigen C is rhamnose-N-acetylglucosamine.
- For group B, streptococci, antigen C is rhamnose-glucosamine polysaccharide etc.
- **Group A contains most of the streptococci that cause infection in humans.**
- Groups B to O are less pathogenic and are often present without causing disease.
- Most are hemolytic; of those, the beta subgroup is the most likely to be the cause of infection.

<b>Complexity</b>	<b>Simple Carbohydrates</b>	<b>Complex Carbohydrates</b>
	monosaccharides	disaccharides, oligosaccharides & polysaccharides

***Streptococcus pyogenes* (Group A streptococcus):**

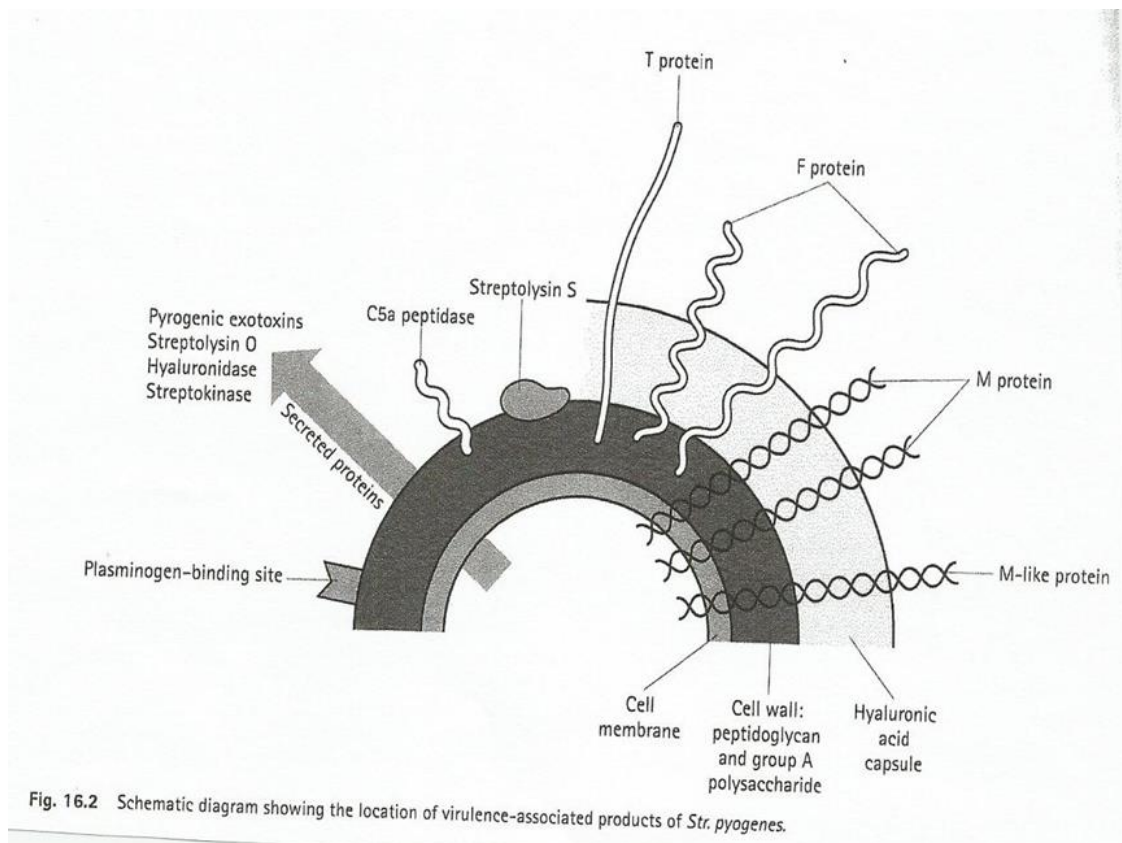
- Is a Gram-positive, nonmotile, nonsporeforming coccus that occurs in chains or in pairs of cells.
- Individual cells are round-to-ovoid cocci, 0.6-1.0 micrometer in diameter.
- Streptococci divide in one plane and thus occur in pairs or (especially in liquid media or clinical material) in chains of varying lengths.
- **The metabolism of *Str. pyogenes* is fermentative; the organism is a catalase-negative aerotolerant anaerobe (facultative anaerobe), and requires enriched medium containing blood in order to grow.**
- Group A streptococci typically have a capsule composed of hyaluronic acid and exhibit beta (clear) hemolysis on blood agar.

**Group A Streptococcus (*Str. pyogenes*) Virulence Factors:**

- **Capsule** - composed of hyaluronic acid identical to that found in connective tissue (non-immunogenic); antiphagocytic.
- **M protein** - the most important virulence factor, located on the end of the hairlike fimbriae, a major antiphagocytic component.
- **M-like proteins** bind the Fc portion of IgG and IgM.
- **F protein** - fibronectin-binding protein (the major adhesin for bacterial attachment to the epithelial cells of the pharynx and the skin).
- **Pyrogenic exotoxins** - erythrogenic toxins - produced by lysogenic strains of streptococci (mediate pyrogenicity [fever], superantigens, responsible for red rash observed in scarlet fever).
- **Streptolysin S** - non-immunogenic cell-bound hemolysin (lyses erythrocytes, leukocytes, platelets; kills phagocytes by autolysis).
- **Streptolysin O** - immunogenic, kills leukocytes by autolysis, used for the ASO test (a recent infection).
- **Streptokinase** (fibrinolysin) - lysing blood clots (fibrin).
- **DNase** - depolymerizes DNA present in pus, reduces the viscosity of pus, facilitates spread of the organisms.
- **Hyaluronidase** ("spreading factor") - degrades connective tissue.
- Two major outer proteins M and T.
- *Streptococcus pyogenes* belong to Lancefield group A.
- M protein chief virulent factor.

*Streptococcus pyogenes* produces a wide array of **virulence factors** and a very large number of diseases. Virulence factors of Group A streptococci include: (1) **M protein**, fibronectin-binding protein (**Protein F**) and **lipoteichoic acid** for adherence; (2) **hyaluronic acid capsule** as an immunological disguise and to inhibit phagocytosis; **M-protein** to inhibit phagocytosis (3) **invasins** such as **streptokinase**, **streptodornase** (DNase B), **hyaluronidase**, and **streptolysins**; (4) exotoxins, such as **pyrogenic (erythrogenic) toxin** which causes the rash of **scarlet fever** and systemic **toxic shock syndrome**.

The **cell surface** of *Streptococcus pyogenes* accounts for many of the bacterium's determinants of virulence, especially those concerned with colonization and evasion of phagocytosis and the host immune responses. The surface of *Streptococcus pyogenes* is incredibly complex and chemically-diverse. Antigenic components include **capsular polysaccharide (C-substance)**, cell wall **peptidoglycan** and **lipoteichoic acid (LTA)**, and a variety of surface proteins, including **M protein**, **fimbrial proteins**, **fibronectin-binding proteins**, (e.g. **Protein F**) and cell-bound **streptokinase**.



**Clinical features:**

1. *Streptococcus pyogenes* is one of the most frequent pathogens of humans.
2. It is estimated that between 5-15% of normal individuals harbor the bacterium, usually in the respiratory tract, without signs of disease.
3. As normal flora, *Str. pyogenes* can infect when defenses are compromised or when the organisms are able to penetrate the constitutive defenses.
4. When the bacteria are introduced or transmitted to vulnerable tissues, a variety of types of **suppurative infections** can occur.
5. In the last century, infections by *Str. pyogenes* claimed many lives especially since the organism was the most important cause of **puerperal fever** (sepsis after childbirth).
6. **Scarlet fever** was formerly a severe complication of streptococcal infection, but now, because of antibiotic therapy, it is little more than streptococcal **pharyngitis** accompanied by rash.
7. Similarly, **erysipelas** (a form of cellulitis accompanied by fever and systemic toxicity) is less common today.
8. However, there has been a recent increase in variety, severity and **sequelae** of *Streptococcus pyogenes* infections, and a resurgence of **severe invasive infections**, prompting descriptions of "flesh eating bacteria" in the news media.
9. A complete explanation for the decline and resurgence is not known. Today, the pathogen is of major concern because of the occasional cases of rapidly progressive disease and because of the small risk of serious sequelae in untreated infections.

10. These diseases remain a major worldwide health concern, and effort is being directed toward clarifying the risk and mechanisms of these sequelae and identifying rheumatogenic and nephritogenic strains of streptococci.
11. Acute *Streptococcus pyogenes* infections may present as **pharyngitis (strep throat)**, **scarlet fever** (rash), **impetigo** (infection of the superficial layers of the skin) or **cellulitis** (infection of the deep layers of the skin). Invasive, toxigenic infections can result in **necrotizing fasciitis**, **myositis** and **streptococcal toxic shock syndrome**.
12. Patients may also develop immune-mediated **post-streptococcal sequelae**, such as acute **rheumatic fever** and acute **glomerulonephritis**, following acute infections caused by *Streptococcus pyogenes*.

### **Treatment and prevention:**

Penicillin is still uniformly effective in treatment of Group A streptococcal disease. It is important to identify and treat Group A streptococcal infections in order to prevent sequelae. No effective vaccine has been produced, but specific M-protein vaccines are being tested.

(sequelae : a condition that is the consequence of a previous disease or injury . " the long-term sequelae of infection " ) .

**Staphylococci:**

- Staphylococci are Gram-positive spherical cells, usually arranged in irregular clusters (grape-like).
- They grow on many types of media, are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow .
- Some are members of normal flora of skin and mucous membranes of humans, others cause suppuration, abscess formation, toxin mediated diseases, and fatal septicemia

**Classification:**

Staphylococcus genus has about 30 species.

**The main species of clinical importance are:**

- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Staphylococcus saprophyticus*

*Staphylococcus aureus* is coagulase-positive, **which differentiates** it from the **other species** .

- *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* are coagulase –negative.

*Staphylococcus saprophyticus* is the cause of urinary tract infection in young women.

**Staphylococci culture:**

Staphylococci grow on most bacteriologic media under aerobic or microaerophilic condition at 37° C.

The colonies on solid media are round, smooth, raised, glistening.

*Staphylococcus aureus* form gray to deep golden yellow colonies.

*Staphylococcus epidermidis* form gray to white colonies.

**Staphylococci :**

- Produce **catalase** (which differentiates them from the streptococci).
- Ferment many carbohydrates.
- Are resistant to drying, heat and 7.5% sodium chloride.
- Are sensitive to many antimicrobial drugs.

***Staphylococcus aureus:***

1. *Staphylococcus aureus* is a major pathogen for humans.
2. Many people are asymptomatic carriers; they have staphylococci on the skin and in the throat (opportunistic pathogen).
3. As a nosocomial pathogen, *Staph. aureus* has been a cause of morbidity and mortality.
4. In hospitals, the areas at risk for severe staphylococcal infections are the newborns nursery, intensive care units, operating rooms and cancer chemotherapy wards.



## **Diseases caused by *Staph. aureus*:**

### **A-Skin and soft tissues:**

- 1-Abscesses, furuncles.
- 2-Wound infections.
- 3-Cellulitis.
- 4-Impetigo.

### **B-Blood and cardiovascular system:**

- 1-Bacteremia.
- 2-Endocarditis.

### **C-Musculoskeletal:**

- 1-Osteomyelitis.
- 2-Arthritis.

### **D-Toxin mediated diseases:**

- 1-Toxic shock syndrome.
- 2-Food poisoning.
- 3-Scalded skin syndrome.

### **E-Metastatic abscesses (brain).**

### **F-Pulmonary.**

**Virulence factors of *Staphylococci*:**

Staphylococci can produce diseases through their ability to multiply in tissues and through production of many extracellular and cellular substances.

**Toxins****Hemolysins (cytolytic) Hemolysins (cytolytic):**

- $\alpha$  it is potent hemolysin, degrades red blood cells of rabbits.
- $\beta$  it degrades sphingomyelin and is toxic for red blood cells of sheep.
- $\gamma$  it lyses red blood cells of humans.
- $\delta$  it disrupts biologic membranes and may have a detergent role.

**Leukocidin (Panton-Valentine toxin):**

This toxin has **two** components. It can kill white blood cell of humans and rabbits; two components act **together** to form pores and they increase cation permeability.

- **Panton-Valentine leukocidin (PVL)** is a cytotoxin—one of the  $\beta$ -pore-forming toxins.
- The presence of PVL is associated with increased virulence of certain strains (isolates) of *Staphylococcus aureus*.
- It is present in the majority of community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) isolates studied and is the cause of necrotic lesions involving the skin or mucosa, including necrotic hemorrhagic pneumonia.
- PVL creates pores in the membranes of infected cells.
- PVL is produced from the genetic material of a bacteriophage that infects *Staphylococcus aureus*, making it more virulent.
- It was initially discovered by Van deVelde in 1894 due to its ability to lyse leukocytes.
- **It was named after Sir Philip Noel Panton and Francis Valentine when they associated it with soft tissue infections in 1932.**

**Exfoliative toxins:**

Are two distinct proteins with same molecular weight, they cause generalized desquamation on staphylococcal scalded skin syndrome.

SSSS is typical in newborns and in infants younger than 1 year (superantigen).

=====

**Toxic Shock Syndrome toxin TSST-1** (superantigen). It binds to MHC class II molecules yielding T cell stimulation which promotes the manifestations of toxic shock syndrome.

This syndrome is associated with fever, shock and multisystem involvement including desquamative skin rash.

=====

**Enterotoxins** there are multiple enterotoxins; like TSST-1 they are superantigens.

**Enterotoxins** are heat-stable and resistant to action of enzymes. **Enterotoxins** cause food poisoning, they are produced when *S. aureus* grows in fatty foods; ingestion results in vomiting and diarrhea. Emetic effect probably is the result of central nervous system stimulation (vomiting centre) when the toxin acts on neuron receptors in intestinal tract.

**Enzymes :**

**Coagulase** this enzyme clots oxalated or citrated plasma.

**Coagulase** binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization.

**Coagulase** may deposit fibrin on surface of staphylococci altering their ingestion by phagocytic cells.

=====

**Catalase** : converts hydrogen peroxide into water and oxygen.

Catalase test differentiates staphylococci (positive) from streptococci (negative).

=====

**Hyaluronidase** it is spreading factor.

**Staphylokinase** fibrinolytic enzyme, but acting much more slowly than Streptokinase.

**Proteinases and lipases** they act on proteinic and lipidic components.

Table 4.1 Overview of the *Staphylococcus* Species That Affect Humans Most Frequently

Species	Parameter
<i>S. aureus</i>	Coagulase-positive; colonies golden yellow. Local purulent infections: furuncles, carbuncles, bullous impetigo, wound infections, sinusitis, otitis media, mastitis puerperalis, ostitis, postinfluenza pneumonia, sepsis. Toxin-caused illnesses: food poisoning, dermatitis exfoliativa, toxic shock syndrome
<i>S. epidermidis</i>	Coagulase-negative; sensitive to novobiocin; most frequent CNS* pathogen; opportunist; infection requires host predisposition; foreign body infections with discrete clinical symptoms
<i>S. saprophyticus</i>	Coagulase-negative; resistant to novobiocin. Urinary tract infections in young women (10–20%); occasional nonspecific urethritis in men

**Diagnostic laboratory tests:**

**Samples:** A throat swab, a samples of pus, blood, tracheal aspirate, or spinal fluid may be used (in according with the different localization of process).

**Direct examination :** with a direct microscopic examination is not possible to distinguish saprophyticus from pathogenic organisms; culture and appropriate identification techniques must confirm this report.

**Isolation:** Specimens planted on blood agar plates develop the typical colonies in 18 hours at 37° C, but hemolysis and pigment production are optimal at room temperature.

**Identification:** Isolated colonies on blood agar medium are planted on Mannitol salt agar. This medium contains: mannitol, 7,5% of Na Cl and a pH indicator.

***Staph. aureus* ferments mannitol :** The salt (7,5% NaCl) inhibits the most other normal flora but not staphylococci.

**Mannitol salt agar is medium used to screen *S. aureus* from *S. epidermidis***

**Biochemical identification:**

**Coagulate test** : two different coagulase tests can be performed:

A **tube** test for free coagulase, and a **slide** test for bound coagulase or clumping factor. **The slide** coagulase test is performed by making uniform suspension of growth, adding 1 drop of plasma and observing for clumping within 10s (this test may be used as rapid screening technique to identify *S. aureus*).

**Catalase** staphylococci produce catalase, which forms water and oxygen from hydrogen peroxide. **The test differentiates** staphylococci, which are **positive**, from streptococci, which are **negative**.

**Antimicrobial treatment:**

Staphylococcal isolates should be tested for antimicrobial susceptibility because *S. aureus* has developed resistance to all antibiotic classes available for clinical use.

**Resistance to Penicillin:**

The most common mechanism of *Saph. aureus* resistance to  $\beta$ -lactam involves **penicillinase** an enzyme that hydrolyzes penicillin into inactive penicilloid acid.

**Penicillinase-producing** strains emerged rapidly after penicillin introduction in the mid 1940s and became prevalent in hospitals and in communities.

**(Penicillin G resistant *Saph. aureus* strains, producing penicillinase, now constitute about 90% of isolates in communities).**

In the late 1950 a new penicillinase-resistant penicillin called **Methicillin** was created.

**Hospital acquired methicillin- resistant *Staphylococcus aureus* :****Resistance to Methicillin:**

The first penicillinase-stable  $\beta$ -lactam such as methicillin and the isoxazolyl penicillins became available in the late 1950s. The first MRSA was described at about the same time.

The prevalence of MRSA in hospital has increased rapidly in the last periods (more than 60% in hospital centers with great geographic variations).

**Mechanism of Methicillin resistance:**

The mechanism is mediated by a new acquired penicillin binding protein 2A ( PBP2A ) , encoded by *mecA* gene.

( The *mecA* gene is a gene found in bacterial cells. The *mecA* gene allows a bacterium to be resistant to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics.)

**PBP2A**

A rapid immunochromatographic qualitative assay for the detection of penicillin binding protein 2a (PBP2a) direct from *S. aureus* culture isolates as an aid in detecting methicillin-resistant *Staphylococcus aureus* (MRSA).

Because of its low  $\beta$ -lactam affinity, PBP2A can complete cell wall synthesis.

**MRSA – Methicillin resistant *Staphylococcus aureus* :**

Methicillin resistant *Staph. aureus* are bacteria which have developed resistance to beta-lactam antibiotics – those which target cell wall synthesis of bacteria. Such antibiotics includes the penicillin family of drugs. Their resistance is conferred by the gene *mecA*, which encodes for the protein penicillin-binding protein 2a (PBP2).



**Alternative treatments for MRSA:**

Treatment of infections with hospital multiresistant MRSA appears problematic.

**Vancomycin** is the first choice in these situation.

Members of **aminoglycosides** associated with **vancomycin** increase their bactericidal activity.

**Quinupristin-Dalfopristin** is a combination of a streptogramin B and A , this association is synergic and shows a large activity against MRSA strains.

**Linezolid** belongs to a new oxazolidinone family of molecules, it is active against all multiresistant Gram positive pathogens.

**Daptomycin** is a cyclic lipopeptide antibiotic active against MRSA.

**Oxidase negative Gram negative Rods :****Enterobacteriaceae :**

coliforms or enterobacilli :

**Virulence and Antigenic Factors of Enterics :**

- Ability to colonize, adhere, produce various toxins and invade tissues.
- Some possess plasmids that may mediate resistance to antibiotics.
- Many enterics possess antigens that can be used to identify groups:
  - O antigen – somatic, heat-stable antigen located in the cell wall.
  - H antigen – flagellar, heat labile antigen.
  - K antigen – capsular, heat-labile antigen .

**It comprises the following bacterial groups :**

**Oxidase negative Enterobacteriaceae:****A . Lactose-fermenters:**

- *Escherichia spp.*
- *Klebsiella spp.*
- *Enterobacter spp.*
- *Citrobacter spp.*

**B . Non-lactose fermenters:**

- *Salmonella spp.*
- *Shigella spp.*
- *Proteus spp.*

**Pathogenic Enterobacteriaceae are often classified into three groups**

**1- Coliforms**, rapidly ferment lactose, part of the normal microbiota, may be opportunistic pathogens.

**Examples:** *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia* .

Presence of coliforms in water is indicate of impure water and of poor sewage treatment (i.e. one of the indicators of fecal pollution of water: *E. coli*)

**2- Non coliform opportunists**, do not ferment lactose .

**3- True pathogens.**

***Escherichia coli* :**

- Motile.
- Lactose-fermenting mucoid colonies on macconkey.
- Some strains are hemolytic on blood agar .
- Produce indole .
- aerobic or anaerobic.
- can grow in the presence or absence of O<sub>2</sub>.
- typically oxidase-negative.
- most strains ferment lactose & glucose with the production of acid and gas.

The most common and important of the coliforms **found in 100% of human intestines** , live in the intestinal tracts of animals in health and disease .

**Gastroenteritis** is the most common disease associated with *E. coli* (enteropathogenic, enterotoxigenic and enteroinvasive strains).

Often mediated by exotoxins that produce the symptoms associated with gastroenteritis.

**Most common disease associated with *E. coli* :**

- **Non-nosocomial urinary tract infections** (cystitis & pyelonephritis).
- Wound infections.
- Neonatal septicemia.
- **Meningitis.**
- Dysentery.
- Diarrhea of infants.
- Diarrhea of travelers.
- Pneumonia.
- Endocarditis.

Five classes (strains) of *E. coli* that cause diarrheal diseases:

1. ***Enteropathogenic E.coli* (EPEC).**
2. ***Enteroinvasive E.coli* (EIEC) .**
3. ***Enterotoxigenic E.coli* (ETEC).**
4. ***Enterohaemorrhagic E.coli* ( EHEC).**
5. ***Enteraggressive E.coli* ( EAEC).**

**Antigenic structure:**

- Over 700 antigenic types (**serotypes**) are recognized based on **O, H, and K antigens.**
- **Lipopolysaccharide LPS** (heat-stable somatic antigens O antigens ).
- Flagellar antigens ( H antigens ).
- Capsular antigens ( K antigens).

**Virulence Determinants of Pathogenic *E. coli* :**

- **Adhesins** fimbriae Intimin (non-fimbrial adhesin) EPEC adherence factor **Invasins** hemolysin Shigella-like "invasins" for intracellular invasion and spread .
- **Motility.**
- **Antiphagocytic surface properties .**
- **Defense against serum bactericidal reactions .**
- **Defense against immune responses .**

**Toxins and *E.coli* :*****E.coli* produce Exotoxins:**

- Hemolysins,

**Enterotoxins causes Diarrheas.**

Important toxins produces:

- Heat labile HL.
- Heat stable HS.
- Vero toxins VT ( Like Shigella toxins ).

Toxins Enterotoxins – produced by enterotoxigenic strains of *E. coli* (ETEC). Causes a movement of water and ions from the tissues to the bowel resulting in watery diarrhea.

**Treatment and Prophylaxis in Travelers' diarrhea:**

- Doxycycline, Trimethoprim, Norfloxacin , Fluroquinolones
- Avoid contaminated food,
- Safe protected water ,prefer bottled water.
- Hot foods.
- Hot drinks.

***Klebsiella*:**

- Usually found in GI tract.
- Four major species.
- *K. pneumoniae* is mostly commonly isolated species.
  - Possesses a polysaccharide capsule, which protects against phagocytosis and antibiotics AND makes the colonies moist and mucoid.
  - Has a distinctive “yeasty” odor.
  - Frequent cause of nosocomial pneumonia.

**Significant biochemical reactions :**

- Lactose positive.
- Most are urease positive.
- Non-motile.
- **Capsulated.**
- large gram-negative rods.
- Found in the digestive and respiratory systems of humans and animals.
- Can cause **opportunistic infections- hospital acquired (nosocomial).**
- No water borne disease ever associated with *Klebsiella* in drinking water .

**Main species of medical importance:**

- *K. pneumonia* ( **Pneumoniae** ).
- *K. rhinoscleromatis* ( rhinoscleroma ).
- *K. ozenae* ( **ozena** ).

***K. pneumoniae*:**

- most commonly isolated pathogenic species- meningitis-bacteremia.
- It is found as a commensal in the intestinal tract, and also found in
- moist environment in hospitals.
- It is an important **nosocomial pathogen**.
- It causes: **Pneumonia**- Urinary tract infection- Septicemia and meningitis (especially in neonates) Wound infection and peritonitis.

**Treatment:**

Based on sensitivity testing.

***Serratia* :**

- Produce a **red pigment when grown at room temperature**
- Can grow on catheters, in saline solutions, and other hospital supplies .
- Doesn't ferment lactose on MacConkey agar media .
- Can cause life-threatening **opportunistic infections** in the urinary and respiratory tracts of immunocompromised patients .
- Difficult to treat due to **resistance to various antimicrobial drugs.**
- Seven species, but *S. marcescens* is the only one clinically important .
- Frequently found in nosocomial infections of urinary or respiratory tracts.
- Implicated in bacteremic outbreaks in nurseries, cardiac surgery, and burn units.
- Fairly resistant to antibiotics.

**Species :**

- *Serratia marcescens* .
- *Serratia rubidaea* .
- *Serratia liquifaciens* .
- *Serratia odorifera* .

**Major characteristics:**

- Ferments lactose slowly.
- Produce characteristic pink pigment, especially when cultures are left at room temperature.
- *Serratia can be distinguished from other genera belonging to Enterobacteriaceae by its production of **three** special enzymes: **DNase, lipase, and gelatinase.***
- Some strains are hemolytic.



- Extracellular enzymes may be responsible for host tissue damage .
- Toxin resembling *Esch.coli* Verocytotoxin and heat-labile toxin have been described.

### **Biochemical Characteristics:**

- Non lactose fermenter .
- V-P: positive.
- Lysine: positive .
- Citrate: positive.
- ODC: positive.
- DNase : positive.
- Indole: Negative.
- TSI A/A: (NO gas).
- Motile.

### **Treatment :**

- *Serratia* strains are commonly resistant to cephalosporins.
- Resistant to ampicillin and gentamicin is variable ( many strains destroy this antibiotics enzymically .
- An aminoglycoside , such as gentamycin is usually the most reliable first-line choice.

## ***Enterobacter***

- Gram-negative **lactose fermenting-** motile rods.
- Found in soil, water, vegetable, sewage, the digestive tracts of animals and humans .
- **Opportunistic pathogens.**
- **Nosocomial infections** of immunocompromised patients.
- Difficult to treat due to **resistance to various antimicrobial drugs.**
- Medical important species is ***Enterobacter aerogens***.
- It produces mucoid colony resembling *klebsiella* on MacConkey Agar.
- Causes urinary tract infection, wound infection and septicaemia in immunocompromised.

### **Species:**

- ***Enterobacter sakazakii* . (*Ent. Sakazakii* ).**
- ***Enterobacter gergoviae* .**
- ***Enterobacter agglomerans*.**
- ***Enterobacter cloacae* .**

**Pathogenesis:**

- The normal habitat of *Enterobacter* spp. Is probably soil and water , but the organisms occasionally found in human faeces and the respiratory tract.
- *Enterobacter* spp. Are much less important cause of hospital infection than *Klebsiella* spp.
- Most infection are of the urinary tract .
- Although members of genus are an important cause of bacteraemia in some hospitals.
- Strains may produce a haemolysin resembling the alph haemolysin produced by strains of *Esch.coli*.
- An outer membrane protein termed OmpX , may be a pathogenic factor for some strains of *Enterobacter* .

**Treatment :**

- Highly resistant to penicillins and many cephalosporins ( strains with cephalosporinase ).
- Resistant to tetracyclines and streptomycin .
- Sensitive to gentamicin . polymyxins.

***Citrobacter :***

- Gram-negative.
- Lactose fermenting.
- Motile rods.
- Muroid forms sometimes occur.
- Opportunistic pathogen.

**Medical important species is:*****Citrobacter freundii.***

- Associated with urinary tract infection.
- Wound infection.
- Septicaemia in immunocompromised

*Citrobacter freundii*: Enterotoxigenic (the enterotoxin is similar to the ST enterotoxin of *E. coli*).

***Citrobacter diversus: (C. diversus ).***

- Neonatal meningitis.
- Brain abscesses.
- Neonatal septicemia.

***Citrobacter amalonaticus:***

Opportunistic pathogens can infect any body sites, particularly, the urinary tract.

**Treatment :**

Sensitive to aminoglycosides .

Sensitive to ampicillin , tetracycline and cephalosporins.

## B . Non-lactose fermenters:

- *Salmonella spp.*
- *Shigella spp.*
- *Proteus spp.*

### ***Salmonella spp.* :**

- There are well over 2000 different types of salmonella.
- Certain serotypes are a major cause of food-borne infection worldwide.
- *Salmonella* are typical member of the Enterobacteriaceae .
- Facultatively anaerobic.
- Gram-negative bacilli.
- Able to grow on a wide range of relatively simple media .
- Their normal habitat is animal intestine.

### **Species of medical importance are :**

- *S. typhi*. (typhoid fever)(the only host is human) .
- *S. paratyphi*.
- *S. enteritidis*. (gastroenteritis).

### **Pathogenesis :**

#### **1- Salmonellosis (Enteric fever) or Typhoid:**

- caused by *S. typhi* and *S. paratyphi*.
- Transmitted by fecal contaminated food and drinks (bacteria invasion of the bloodstream).
- Incubation period: 10-14 days-
- Bacteria can pass through the small intestines into the bloodstream and into the liver, spleen, bone marrow, and gall bladder.
- Infectious dose: High .
- **Mortality rate:** Untreated cases: 10-15%- **Treated cases:** < 1% .

**Gastroenteritis:**

- caused by *S. enteritidis* and *S. typhimurium*.
- Foodborne infection/intoxication.
- initial watery diarrhea,
- later bloody mucoid diarrhea associated with abdominal pain and tenesmus.

**Salmonella can cause any one of three types of Salmonellosis:**

1. Acute gastroenteritis or food infection type.
2. Septicemia or acute sepsis similar to pyogenic infection.
3. Enteric fevers:
  - Typhoid fever
  - Paratyphoid fever.

**Infective dose:**

- The median infective dose for most serotype , including Typhi has varied from  $10^6$  to  $10^9$  viable organism .
- However ,investigation of outbreaks suggests that in natural infection the infective dose might be below  $10^3$  viable organisms.
- Many factors are thought to influence the infective dose such as strain-to-strain variation.

**Virulence factors :**

1. **Surface antigens:** The ability of *Salmonella* to attach to host receptor cells and to survive intracellularly may be due to the O antigenic side chain or in case of typhi serotypes, the presence of Vi antigen.

Organisms containing Vi antigen are clearly more virulent than those lacking this antigen.

2. **Invasiveness:** Virulent *Salmonella* penetrate the epithelial lining of the small bowel, thus the brush border begins to degenerate.

After penetration the organism multiply and may spread to other body sites.

3. **Endotoxins:** Presumably, endotoxin is responsible for the fever production.

Endotoxin activation of the chemotactic properties of the complement system may cause the localization of leukocytes in the classic lesions seen in typhoid fever.

4. **Other factors:**

- **Enterotoxin:** Affects the small intestine.
- **Cytotoxin:** Associated with outer bacterial membrane which may mean that the toxin may be important in cellular invasion and cellular destruction.

**Classification of *salmonella*:**

The Kauffman-White system used to classify *Salmonella* is based on identifying the **O (somatic) and H (flagellar) antigens** possessed by the different serovars.

The detection of **Vi antigen** is also used in the identification of *S. typhi* and some other *Salmonella*.

**1.O Antigen:**

- *Salmonella* are grouped by their O antigens as groups A to Z.
- Many of the medically important *salmonella* belongs to groups A to G.

**2. H Antigen:**

- Many *Salmonella* are diphasic, that is, they can occur in two antigenic forms referred as phase I and Phase II.
- Phase I antigens are given alphabetical letters and phase II antigens either numbered or given a letter if known to occur in both phases.

**3. Vi Antigen:**

- This surface (K) antigen can be found in *S. typhi* and *S. paratyphi C* and few other *Salmonella*.
- It is associated with virulence and can be detected using Vi antiserum.

**Prevention**

- Public health = personal hygiene measures.
- Proper sewage treatment.
- Chlorination of water supplies.
- Detection of carriers.
- Pasteurization of milk and proper cooking of food.



**Vaccination (50-80% protection):**

Heat-killed , phenol-preserved whole-cell vaccines containing of mixture of culture of **Typhi** , **Paratyphi A** and **Parathphi B** ( **TAB** ) have been used for many years in countries with a high endemic level of typhoid fever.

**Treatment :**

Type of an antibiotic which used to treatment depend on type of infection.

## *Shigella spp.*

The genus *shigella* is subdivided on biochemical serological ground into four species :

- *Shigella dysenteriae* – serogroup A. ( 13 serotypes ).
- *Sh.flexnei* – serogroup B ( 6 serotypes ).
- *Sh. boydii* – serogroup C ( 18 specific serotype ) .
- *Sh. sonnei* - serogroup D ( are serologically homogeneous ) .

1. Strains of *shigella* spp. are typical member of the Enterobacteriaceae and are closely related to the genus *Escherichia*.
2. Gram-negative bacilli .
3. Non-motile .
4. Non-capsulate.
5. *Shigella dysenteriae* type 1 produces **Shiga toxin** this differentiates it from other member of the genus.
6. There are 13 serotypes of *Shigella dysenteriae* .
7. Type 1 of *Shigella dysenteriae* is much the most important as a cause of sever bacillary dysentery.

**Pathogenesis :****Shigellosis (bacillary dysentery):**

- caused by *Sh. flexneri* and *Sh. dysenteriae*.
- Found in human large intestines( M cell ) as pathogen.
- Route of infection is fecal-oral route.
- **The infective dose is small** : 10<sup>3</sup> organisms (small)
- Produce a diarrhea.

**Toxins:**

**Endotoxin:** irritate the bowel wall .

**Exotoxin:** act as **Enterotoxin, cytotoxic and neurotoxin (Shiga toxin,** also called the **verotoxin)**.

**Complication:**

- Dehydration.

**High prevalence:**

- Poor sanitation.
- Poor personal hygiene.
- Polluted water supply.
- Young children are frequently affected.

**Treatment :**

- Most cases of Shigella dysentery ,especially those due to *Sh.sonnei* are mild and do not require antibiotic therapy.
- Treatment with a suitable antibiotic is necessary in the very young , the aged and debilitated and with those with severe infection .
- Oral ampicillin , co-trimoxazole or ciprofloxacin is appropriate.

***Proteus:***

- There is considerable morphological variation , but in agar-grown cultures the microscopical appearance is much like that of other coliform bacteria.
- A notable property of *Pr. mirabilis* and *Pr. vulgaris* strains is the ability to swarm on solid media : the bacterial growth spreads progressively from the edge of the colony and eventually covers the whole surface of the medium .
- Gram-negative.
- **Motile.**
- non-capsulated.
- non-lactose fermenting.
- found in the intestinal tract of humans and animals, soil, sewage and water.

**Species of medical importance:**

- *Pr. mirabilis* (Indole negative)
- *Pr. vulgaris* (Indole positive)

***Proteus mirabilis* :**

- is one of the common species of Enterobacteriaceae isolated in clinical pathogen in urinary tract infections and has been implicated in hospital outbreaks and cases of:
- cross-infection- laboratories- Septicemia.
- Abdominal and wound infection.
- Secondary invader of ulcer and burn.

***Pr. vulgaris:***

- Important nosocomial pathogen.
- Isolated in wound infection and urinary tract infection .

**Virulence factors of *Proteus* :**

- The invader *Pr. vulgaris* has numerous factors including fimbriae, flagella, outer membrane proteins lipopolysaccharide, capsule antigen, urease immunoglobulin A proteases, hemolysins, amino acid and deaminases .
- The severity of any infection caused by the members of the genus *Proteus* depends mainly on the availability of virulence factors that may include  $\beta$ - Lactamase, extended spectrum  $\beta$ -lactamase , Protease, Urease and hemolysin production .

**Treatment :**

Most strains of *Pr.mirabilis* do not produce  $\beta$ -lactamase ; they are consequently moderately sensitive to benzylpenicillin and fully sensitive to ampicillin , and most other  $\beta$ -lactam antibiotics.

***Yersinia:***

The organisms within these three genera are animal pathogens that , under certain conditions are transmissible to man , either directly or indirectly through food and water or via insect vectors.

- Pleomorphic microaerophilic or facultative anaerobic.
- Gram negative.
- Short coccobacillus.
- It occurs singly , in pairs or when in liquid culture in chains.
- Non motile .

**Three species of facultatively intracellular** bacteria that are pathogenic for humans:

1. *Y. pestis* (Pneumonic, bubonic and septicemic plague).
2. *Y. pseudotuberculosis* .
3. *Y. enterocolitica* .

These are primarily animal pathogens, and humans are accidental hosts for infection.

***Y. enterocolitica:***

- Human infection occurs by contaminated food and drinks from domestic animals or rodents.
- Causes inflammation of the intestinal tract.

**Gastroenteritis:**

- Diarrhea.
- Fever.
- Abdominal pain.

**Bubonic Plague (black Plague):**

**Bubo :** غدة لمفاوية متضخمة , تحمل الصديد , يحدث هذا في بعض الأمراض كالطاعون.

- Bites.
- Characterized by high fever.
- Enlarged lymph nodes, painful lymph nodes called buboes.
- Death rate ( 55%).

**Pneumonic Plague:**

- Rapidly.
- Infection of the lungs.
- High mortality rate (95%) during 24-36hrs.
- Painful in muscles.
- High fever.
- Enlarged liver and spleen.
- Bloody sputum.
- **Inoculum dose:** 10<sup>8</sup>-10<sup>9</sup> organism.
- **Incubation period (IP)** =5-10 days

**Diagnosis and treatment:**

must be rapid due to the fast progression and deadliness of the plague.

**Virulence Factors:**

- Antigenic change at 37c .
- Antiphagocytic capsule.
- Protein (V).
- Lipoprotein (W).
- Hemorrhagic lesions .
- Hemolysin.
- Coagulase.
- Fibrinolysin.

**Treatment :**

*Y. pestis* is sensitive to many antibiotics ,including aminoglycosides ,fluoroquinolones , chloramphenicol co-trimoxazole and tetracyclines but not penicillin.

*Unlike Y. pestis ,Y. enterocolitica* is usually sensitive **in vitro** to penicillins; it is also usually sensitive to aminoglycosides , chloramphenicol tetracyclines , cotrimoxazole and fluoroquinolone.

**Vaccination :**

Vaccines prepared from killed virulent strains of *Y. pestis* confer significant protection against bubonic but not pneumonic plague.



**Gram Negative Rods- Oxidase Positive :**

- Pseudomonadaceae .
- Vibrionaceae.

Pseudomonaceae.

Genus: Pseudomonas.

- Gram-negative bacilli .
- Motile.
- Aerobic rods .
- Non-fermentative.
- Versatile.
- Catalase ve + .
- Oxidase ve + .
- Most are saprophytes.

Found in water, soil, sewage, human and animal intestine.

**Medical importance species:**

*Ps. aeruginosa*

- Grows readily on a wide variety of culture media over a wide temperature range and emits a sweet grape-like odour that is easily recognized.
- Nosocomial pathogen.
- Opportunistic pathogens.
- very simple growth requirement.
- It is often observed "**growing in distilled water**", which is evidence of its minimal nutritional needs.
- Its optimum temperature for growth is 37 degrees, and it is able to grow at temperatures as **high as 42 degrees**.

**Most strains produce diffusible pigments:**

- **Pyocyanin** : greenish-blue ( due to production of soluble blue phenazine pigment ).
- **Fluorescein** or pyoverdin: yellow-green fluorescent pigment.
- **Pyorubrin** : red .
- **Melanin** : brown.

**Summary of the Virulence Determinants of Pathogenic *P. aeruginosa*****Adhesins :**

- Pili.
- Polysaccharide capsule.

**Invasins:**

- Elastase.
- Alkaline protease.
- Hemolysins (phospholipase and lecithinase).
- Cytotoxin (leukocidin).
- Iron uptake systems.
- Pyocyanin pigment.

**Toxins:**

- Exdotoxin A (Cytotoxic by blocking protein synthesis poreforming protein) same mechanism of action as the diphtheria toxin.
- Toxin S: interfere with membrane permeability.
- Lipd A (Endotoxin): tissue necrosis.
- Enterotoxins : food poisoning .

**Motility:**

Spread through tissues .

**Fluorescent pigment** pyocyanin (impairs normal function of human nasal cilia, disrupts respiratory epithelium ).

**Disease and Treatment :**

- Urinary tract infection ( UTI) (introduced by catheter).
- Wound infection of burn sites.
- Septicaemia.
- Otitis externa (Malignant external ear infection in poorly treated diabetic patients).
- Pneumonia.
- Eye infection (injury or surgery).
- Endocarditis.
- Bacteremia.
- meningitis.
- Brain abscesses .

*Ps. aeruginosa* is normally resistant to many antimicrobial agents .

Aminoglycosides are often used in combination with a  $\beta$ -lactam antibiotic.

***Vibrionaceae:*****Curved Bacilli (Vibrio)**

- Gram -negative **curved rods**.
- Actively motile.
- Vibrios are distinguished from enterics by being **oxidase-positive** and motile by means of polar flagella.
- Vibrios are distinguished from pseudomonads by being fermentative and oxidative in their metabolism.
- Vibrios are one of the most common organisms in surface waters of the world.

**Medical importance species:**

- *V. cholerae* and *V. parahaemolyticus* are pathogens of humans .

Both produce **diarrhea**, but in different ways.

- *V. parahaemolyticus* is an invasive the colon.
- *V. cholerae* is noninvasive, affecting the small intestine through secretion of an enterotoxin.

***V. cholera* :**

- More than 130 different O serogroups have been described.
- Some of these strains can cause diarrhea in man .
- Found in fresh water.
- Shellfish and other sea food.
- Most often in communities with poor sewage and water treatment.
- **Grow in asparagine (as a sole source of carbon and nitrogen).**
- **Optimum pH growth range ( 8.5- 9.5)- Sensitive to acidic pH.**
- Causes **Cholera (epidemic cholera)** is a severe diarrheal disease.
- Transmission to humans is by contaminated water or food- Route of infection is (fecal-oral ).
- Incubation period 1-4 days .
- **Large inoculum** is required to cause disease because the bacteria are susceptible to acidic stomach environment .

**Cholera :**

- Is one of the most rapidly fatal illnesses known.
- A healthy person may become hypotensive within an hour of the onset of symptoms and may die within 2-3 hours if no treatment.
- More commonly, the disease progresses from the first liquid stool to shock in 4-12 hours, with death following in 18 hours to several days.
- Abrupt **watery diarrhea and vomiting ( Rice– water stool is characteristic)** contains enormous numbers of vibrios – **result in severe fluids and electrolytes loss- dehydration, can lead to coma and death.**
- The loss of potassium ions may result in cardiac complications and circulatory failure. Untreated cholera frequently results in high (50-60%) mortality.

**The bacterium:**

- Produces an invasins, neuraminidase, during the colonization stage which is degrade N-acetyl- neuraminic acid.
- **Cholera toxin (enterotoxin) is the most important virulence factor of *V. cholerae*.**
- Action on the mucosal epithelium- it is responsible for the characteristic diarrhea of cholera disease.
- **Cholera toxin activates the adenylate cyclase enzyme** of the intestinal mucosa leading to increased the secretion of H<sub>2</sub>O, Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, and HCO<sub>3</sub><sup>-</sup> into the lumen of the small intestine.

**Treatment:**

- Fluids and electrolytes replacement .
- Antibiotics are not important because they are lost in watery stool.
- Tetracycline shorten the duration of diarrhea and reduce fluid loss.

**Medically Important Gram-Positive Bacilli :**

Can be subdivided into three general groups, based on presence or absence of endospores and acid-fastness.

Three general groups:

1. Endospore-formers.
2. Non-endospore-formers.
3. Irregular shaped and staining properties.

**Spore-Forming Bacilli:**

Genus *Bacillus*.

Genus *Clostridium*.

Genus *Sporolactobacillus*.

**General Characteristics of the Genus *Bacillus*:**

- Gram-positive, endospore-forming, motile rods,
- Mostly saprobic.
- Aerobic and catalase positive.
- Versatile in degrading complex macromolecules.
- Source of antibiotics.
- Primary habitat is soil.
- **2 species of medical importance:**
  - *Bacillus anthracis*
  - *Bacillus cereus*

***Bacillus anthracis:***

- Large, block-shaped rods.
- Central spores that develop under all conditions except in the living body.
- **Virulence factors – polypeptide capsule and exotoxins.**
- 3 types of anthrax:
  - **Cutaneous** – spores enter through skin, black sore- eschar; least dangerous.
  - **Pulmonary** –inhalation of spores.
  - **Gastrointestinal** – ingested spores.

**Morphology:**

1. Large gram positive bacilli.
2. Non-motile.
3. Found singly, in pairs or in long chains.
4. Capsule could be demonstrated during growth in infected animals.
5. Spores are formed in culture, dead animal's tissue but not in the blood of infected animals.
6. Spores are oval and centrally located.

**Survival in Soil :**

- Spores remain viable in soil for decades.
- In World War II in Scotland spores were exploded.
- Survived for >40 years and were eradicated in 1987
- Changing environmental conditions (temp. rain etc.) help in survival and multiplication.



**Cultural Characteristics:**

1. Blood Agar and Nutrient Agar are commonly used to cultivate the bacilli.
2. Plates are incubated aerobically at 37 oC.
3. On blood agar plates, colonies have irregular borders and are *non-hemolytic*.
4. On nutrient agar: They are described as "*Medusa head*" or "*Comet tail*".

**Specimen Collection and Laboratory Diagnosis:**

CAUTION: Laboratory safety is very important when working with any materials suspected of containing *Bacillus anthracis*.

**Samples are collected depending on the site affected:**

1. Swab samples from cutaneous lesions and blood cultures.
2. Sputum and blood for pulmonary anthrax.
3. Gastric aspirate, feces and blood for enteric anthrax.

= Gram stained smears: Made from clinical samples, show large gram positive bacilli in long chains "Bamboo-like appearance".

= Giemsa stained smears: Purple bacilli with red capsule.

= Culture.

=Animal inoculation test: Experimental animals are injected intraperitoneally by a suspension of the test organism "Suspected *B. anthracis* culture".

*The animal dies in 48-96 hours due to respiratory failure.*

Large number of typical bacilli can be found in the blood and tissue of spleen of the infected animal.

**Biochemical Identification:**

1. Carbohydrate fermentation test:
2. Gelatine liqefaction test: Negative after 7 days. Growth has a characteristic appearance of an inverted pine tree.
3. Nitrate reduction test: Positive.
4. Starch hydrolysis test: Positive.
5. Voges-Proskauer test: Positive.
6. Sensitivity to penicillin: Sensitive.
7. Lysis by gamma phages: Positive. This test accurately differentiate *B.anthraxis* from other bacillus species.

**Pathogenesis:**

There are different clinical forms of anthrax:

- 1. CUTANEOUS ANTHRAX:** 95-98% of anthrax cases are of this type. Infection occur through wounds, burns, which may progress to toxemia and septicemia. The site of entry often produces a painless blister referred to as *Malignant pustule*.
- 2. ENTERIC "INTESTINAL" ANTHRAX:** Caused by the ingestion of infected meat. This form of the disease is severe and fatal.
- 3. PULMONARY ANTHRAX:** Caused by the inhalation of large number of *B. anthracis* spores. *It is usually fatal. This clinical form is commonly known as "wool sorter disease".*

### **Antigenic structure and pathogenic determinants ( virulence factors).**

1. The Capsular Polypeptide: Composed of poly peptide of a high molecular weight consisting of D-glutamic acid.
2. Polysaccharide Somatic Antigen: Composed of N acetulglucos-eamine and Dgalactose.
3. Complex Protein Toxin: This toxin appear to be responsible for signs and symptoms characteristic of anthrax. Accumulation of the toxin in tissue and its effect on the central nervous system results in death by respiratory failure and anoxia.

### ***B. anthracis* virulence factors**

#### **It has two major determinants of virulence:**

1. The formation of a **poly-D-glutamyl capsule**, which mediates the invasive stage of the infection.
2. The production of the multi component **anthrax toxin** (Edema factor, Lethal factor, Protective factor.) which mediates the toxigenic stage .

#### Control and Treatment:

- Treated with penicillin, tetracycline, or ciprofloxacin.
- Vaccines :
  - Live spores and toxoid to protect livestock.
  - Purified toxoid; for high risk occupations , military personnel.

#### **TREATMENT:**

Penicillin is the drug of choice.

For penicillin-sensitive patients, tetracycline, erythromycin, chloramphenicol and streptomycin may be given as alternative drugs.

***Bacillus cereus* :**

Biochemical Reaction :

- a-) catalase positive.
- b-) Voges-Proskauer positive.
- c-) DNase positive.
- d-) resistant to polymyxin B antibiotics .

**Types of toxin produced by *B.cereus*.**

**They are two types :**

A-) Emetic toxin (ETE ).

B-) Enterotoxin .

**Three types of enterotoxin :**

**a-) hemolytic enterotoxin (HBL).**

cause intestinal fluid secretion.

**b-) non-hemolytic enterotoxin (Nhe ).**

responsible for diarrhea in *B.cereus* of food poisoning.

**c-) Entk.**

is a single component protein that has not been shown to be involved in food poisoning .

***Bacillus cereus* Food Poisoning**

Normal inhabitant of the soil, but it can be regularly isolated from foods such as grains and spices (**cause fried rice syndrome**). *B. cereus* produces one emetic toxin (ETE) and 3 different enterotoxins: HBL, Nhe and EntK. *Bacillus cereus* produce  $\beta$ -hemolysis on blood agar.

It is frequently isolated from milk and dairy products. In milk, *B. cereus* causes a defect known as 'bitty' cream or sweet curdling. It is found in rice, rice products, oriental dishes and ingredients .

- 1) *B. cereus* produces a large number of secreted cytotoxins and enzymes that may contribute to diarrhoeal disease.
- 2) The identity of the enterotoxin(s) is still a controversial topic.
- 3) The three cytotoxins are currently considered the aetiological agents of *B. cereus* diarrhoeal foodborne disease :
  - haemolysin BL (Hbl).
  - nonhaemolytic enterotoxin (Nhe) .
  - cytotoxin K.
- 4) Hbl and Nhe are related three-component toxins, while the single-component CytK (Cytotoxin K (**EntK**) ) belongs to the family of  $\beta$ -barrel pore-forming toxins.
- 5) In addition, several other protein cytotoxins, haemolysins and degradative enzymes have been described that may potentially contribute to the pathogenicity of *B. cereus* diarrhoeal disease.
- 6) These include cereolysin O, haemolysin II, haemolysin III, InhA2 (metalloprotease) and three phospholipases C .
- 7) Phospholipase and Sphingomyelinase were known to be toxic, but now they have been demonstrated to be nontoxic, and some of the hemolysins associated with them are marginally toxic.

***B. cereus* causes two types of food-borne illnesses :**

**1- short-incubation" or emetic form:** is characterized by nausea and vomiting and abdominal cramps and has an incubation period of 1 to 6 hours. It resembles *Staph. aureus* food poisoning in its symptoms and incubation period. It is caused by heat-stable **emetic toxin, ETE** .

**2- long-incubation" or diarrheal form:** manifested primarily by abdominal cramps and diarrhea following an incubation period of 8 to 16 hours. Diarrhea may be a small volume or profuse and watery. And it resembles food poisoning caused by *Clostridium perfringens*. It is mediated by the heat-labile diarrheagenic **enterotoxin Nhe** and/or **hemolytic enterotoxin HBL** .

**Pathogenesis:**

Disease caused by *B.cereus*

**A-) Diarrhea syndrome**

characterized by :

- 1-) abdominal pain.
- 2-) watery diarrhea.
- 3-) symptoms appear within 8-16 hours after meal.
- 4-) the diarrheagenic toxin has high molecular weight, and is heat labile that could be inactivated by heating for 5 minutes at 56°C .

**B-) Emetic syndrome**

characterized by :

- 1-) vomiting .
- 2-) nausea.
- 3-) symptoms appear within 1-5 hours after meal.
- 4-) emetic toxin has low molecular weight and is very heat stable.

**Pathogenesis & clinical features**

- ° Spores are found on most raw foods like rice.
- ° Spores are heat-resistant & survive rapid frying .
- ° Produce enterotoxin – ingested → food poisoning.
- ° Short IP – 4-6 hours – similar to Staphylococcal food poisoning (vomiting & diarrhoea).

**TREATMENT**

- Symptomatic -fluid replacement .
- Penicillin.

## Genus *Clostridium*.

- Gram-positive, spore-forming rods.
- Anaerobic and catalase negative.
- 120 species.
- Oval or spherical spores produced only under anaerobic conditions.
- Synthesize organic acids, alcohols, and exotoxins.
- Cause wound infections, tissue infections, and food intoxications.

### Gas Gangrene :

- *Clostridium perfringens* most frequent clostridia involved in soft tissue and wound infections – **myonecrosis**.
- Spores found in soil, human skin, intestine, and vagina.
- Predisposing factors – surgical incisions, compound fractures, diabetic ulcers, septic abortions, puncture wounds, gunshot wounds.

### **Virulence Factors:**

- Toxins
  - Alpha toxin – causes RBC rupture, edema, and tissue destruction
- Collagenase
- Hyaluronidase
- DNase

### Pathology :

- Not highly invasive; requires damaged and dead tissue and anaerobic conditions.
- Conditions stimulate spore germination, vegetative growth and release of exotoxins, and other virulence factors.
- Fermentation of muscle carbohydrates results in the formation of gas and further destruction of tissue.

**Treatment and Prevention :**

- Immediate cleansing of dirty wounds, deep wounds, compound fractures, and infected incisions.
- Debridement of diseased tissue.
- Large doses of cephalosporin or penicillin.
- Hyperbaric oxygen therapy .
- No vaccines available.

***Clostridium Difficile*-Associated Disease (CDAD):**

- Normal resident of colon, in low numbers.
- Causes antibiotic-associated colitis:
  - Relatively non-invasive; treatment with broad-spectrum antibiotics kills the other bacteria, allowing *C. difficile* to overgrow
- Produces enterotoxins that damage intestines.
- Major cause of diarrhea in hospitals.
- Increasingly more common in community-acquired diarrhea.

**Treatment and Prevention :**

- Mild uncomplicated cases respond to fluid and electrolyte replacement and withdrawal of antimicrobials.
- **Severe infections** treated with oral vancomycin or metronidazole and replacement cultures.
- Increased precautions to prevent spread.

**Tetanus :**

- *Clostridium tetani*.
- Common resident of soil and GI tracts of animals .
- Causes tetanus or lockjaw, a neuromuscular disease.
- Most commonly among geriatric patients and **intravenous** (IV) drug abusers; neonates in developing countries.



**Pathology :**

- Spores usually enter through accidental puncture wounds, burns, umbilical stumps, frostbite, and crushed body parts.
- Anaerobic environment is required for vegetative cells to grow and release toxin.
- **Tetanospasmin** – neurotoxin causes paralysis by binding to motor nerve endings; blocking the release of neurotransmitter for muscular contraction inhibition; muscles contract uncontrollably .
- Death most often due to paralysis of respiratory muscles.

**Treatment and Prevention :**

- Treatment aimed at deterring degree of toxemia and infection and maintaining homeostasis.
- Antitoxin therapy with human tetanus immune globulin; inactivates circulating toxin but does not counteract that which is already bound.
- Control infection with penicillin or tetracycline; and muscle relaxants.
- Vaccine available; booster needed every 10 years.

**Clostridial Food Poisoning :**

- *Clostridium botulinum* – rare but severe intoxication usually from home canned food.
- *Clostridium perfringens* – mild intestinal illness; second most common form of food poisoning worldwide.

**Botulinum Food Poisoning:**

- Botulism – intoxication associated with inadequate food preservation.
- *Clostridium botulinum* – spore-forming anaerobe; commonly inhabits soil and water.

**Pathogenesis:**

- Spores are present on food when gathered and processed.
- If reliable temperature and pressure are not achieved air will be evacuated but spores will remain.
- Anaerobic conditions favor spore germination and vegetative growth.
- Potent toxin, **botulin**, is released.
- Toxin is carried to neuromuscular junctions and blocks the release of acetylcholine, necessary for muscle contraction to occur.
- Double or blurred vision, difficulty swallowing, neuromuscular symptoms .

**Infant and Wound Botulism:**

- Infant botulism – caused by ingested spores that germinate and release toxin; flaccid paralysis.
- Wound botulism – spores enter wound and cause food poisoning symptoms.

**Treatment and Prevention:**

- Determine presence of toxin in food, intestinal contents or feces.
- Administer antitoxin; cardiac and respiratory support.
- Infectious botulism treated with penicillin.
- Practice proper methods of preserving and handling canned foods; addition of preservatives.

**Clostridial Gastroenteritis:**

- *Clostridium perfringens*.
- Spores contaminate food that has not been cooked thoroughly enough to destroy spores.
- Spores germinate and multiply (especially if unrefrigerated).
- When consumed, toxin is produced in the intestine; acts on epithelial cells, acute abdominal pain, diarrhea, and nausea.
- Rapid recovery.

***Mycobacterium* : ( fungus-bacterium ).**

- Mycobacteria are slender rod bacteria that are stained with special differential stains (Ziehl-Neelsen).
- Once the staining has taken, they cannot be destained with dilute acids, hence the designation acid-fast.
- In terms of human disease, the most important mycobacteria are the tuberculosis bacteria (TB).
- *M. tuberculosis* and *M. bovis* and the leprosy pathogen (LB) *M. leprae*.
- TB can be grown on lipid-rich culture mediums.
- Their generation time is 12–18 hours.
- Initial droplet infection results in primary tuberculosis, localized mainly in the apices of the lungs.
- Ninety percent of primary infection foci remain clinically silent.
- In 10% of persons infected, primary tuberculosis progresses to the secondary stage (reactivation or organ tuberculosis) after a few months or even years, which is characterized by extensive tissue necrosis.

**Medically important:**

- *M. tuberculosis* is cause agent of tuberculosis in humans.

**Tuberculosis (TB)** is the leading cause of death in the world. Most people with TB infection have a positive reaction to the **tuberculin skin test** (purified protein derivative).

- *M. bovis* is the agent of TB in cows and rarely in humans (Both cows & humans can serve as reservoirs)- Humans can infected by the consumption of unpasteurized milk.
- *M. leprae*, the causative agent of **leprosy**.

The cytoplasm of young cultures is homogeneous, while that of old cultures is granular.

*M tuberculosis* is acid-fast due to the fact that it contains mycolic acid and lipids.

The lipids of *M. tuberculosis* consist of three fractions:

- (1) phosphatide which is soluble in ether.
- (2) fat which is soluble in ether and acetone.
- (3) wax which is soluble in chloroform and ether.

### **Chemical composition.**

The fact that as much as 40% of the dry weight of mycobacteria may consist of lipid undoubtedly accounts for many of their unusual growth and staining characteristics. A comprehensive discussion of mycobacterial lipids is beyond the scope of this text, but one class of lipids, **the mycosides, is unique to acid-fast organisms and is involved in some manner with the pathogenicity of the mycobacteria.**

### **Toxin production.**

- *M. tuberculosis* does not produce an exotoxin.
- It contains toxic substances which are liberated when the cell decomposes.
- In 1890 R. Koch isolated from the tubercle bacillus a substance known as **tuberculin**.
- A **tuberculin** has been derived from the bovine variety of *M. tuberculosis*, which contains protein substances, fatty acids, lipids, neutral fats, and crystalline alcohol.
- There is also a **tuberculin** free of waste substances and designated PPD (purified protein derivative) or PT (purificatum tuberculinum).
- **Tuberculin** is toxic for guinea pigs which are affected with tuberculosis (injection of 0.1 ml of the standard preparation is fatal for 50 percent of experimental animals). Small doses of tuberculin produce no changes in healthy guinea pigs.
- **Virulent mycobacteria differ from the non-virulent organisms in that they contain a great number of lipopolysaccharide components.**

- The lipid fraction (cord factor) responsible for adhesion of mycobacteria and their growth in cords and strands is marked by high toxicity.
- The cord factor of *M. tuberculosis* destroys the mitochondria of the cells of the infected body and causes disorders in respiration and phosphorylation.

**The high concentration of lipids in the cell wall of *M. tuberculosis* properties :**

- Impermeability to stains and dyes.
- Resistance to many antibiotics .
- Resistance to killing by acidic and alkaline compounds .
- Resistance to osmotic lysis by complement.
- Resistance to lethal oxidations and survival inside of macrophages.

**Pathogenicity for animals :**

Tuberculosis is an infection which is wide-spread among cattle, chickens, turkeys, etc. Pigs, sheep and goats contract the disease less frequently.

Cattle, sheep and goats are quite resistant to the human type of tubercle mycobacteria. Guinea pigs are highly susceptible to the human type, and their infection results in a generalized pathological condition and death. Infection of rabbits produces chronic tuberculosis.

**Pathogenesis and disease in man:**

- It has been shown that tuberculosis in man is caused by several types of mycobacteria — the human type (*M. tuberculosis*), the bovine type (*M. bovis*), etc.
- The share of atypical mycobacteria which cause a variety of clinical forms of tuberculosis among humans has recently grown to 50 percent .
- Infection with tuberculosis takes place through the respiratory tract by the droplets and dust, and, sometimes, through contaminated foodstuffs, and through the skin and mucous membranes.
- Intrauterine infection via the placenta may also occur.

## Stages of the Tuberculosis Disease:

### Disease progression depends on :

Strain of MTB.

Prior exposure.

Vaccination.

Infectious dose.

Immune status of the host.

**Stage 1: Droplet nuclei (Primary nodule) ( tubercle ):** inhaled. One droplet nuclei contains no more than 3 bacilli.

Droplet nuclei are so small that they can remain air-borne for extended periods of time.

**Stage 2: (Tissue necrosis)** Begins 7-21 days after initial infection. MTB multiplies within macrophages until the macrophages burst.

**Stage 3: (Consolidation)** the individual becomes tuberculin-positive. The host developing a cell mediated immune response. An antibody mediated immune will not control of a MTB infection because MTB is intracellular and if extracellular, it is resistant to complement killing due to the high lipid concentration in its cell wall. at this stage that **tubercle formation** begins. The center of the tubercle is characterized by semi-solid or "cheesy" necrosis". MTB cannot multiply within these tubercles because of the low pH. MTB can, however, persist within these tubercles for extended periods. ( more contagious).

**Stage 4: (Calcification)** MTB uses macrophages to replicate, and the tubercle grows. The growing tubercle may invade a bronchus. If this happens, MTB infection can spread to other parts of the lung.

### Treatment :

is accomplished with antibacterial preparations. They include derivatives of isonicotinic acid hydrazide (tubazide, phthivazide, etc.), streptomycin, and PAS — preparations of the first series. Preparations of the second series (cycloserine, kanamycin, biomycin, etc) are used to enhance the therapeutic effect. The isolated *M. tuberculosis* are tested for sensitivity to drugs which are added to fluid or solid media indifferent concentrations.

## Pathogenic Gram-Negative Cocci:

*Neisseriae* :

**The genus *Neisseriae* contains tow important human pathogens:**

- *Neisseriae meningitidis* ( meningococcus ).
- *Neisseriae gonorrhoeae* ( gonococcus ).
- Gram – negative
- Oval cocci occurring in pairs (diplococcic ,kidney or coffee bean shaped ).
- Obligate human pathogen.
- Oxidase positive.
- Aerobic coccus.
- Non motile.
- Ferment carbohydrate producing acid but not gas
- *Neisseriae meningitidis* and *Neisseriae gonorrhoeae* are very similar in their morphological and cultural characters.
- Meningococci and gonococci do not grow on plain nutrient agar or room temperature . They grow on enriched media (**chocolate blood agar**) The selective medium is **Thayer Martin medium** (Chocolate blood agar+ VCN antibiotic inhibitor) for primary *Neisseriae* isolation , ( non-pathogenic *neisseriae* grow on ordinary nutrient media ).
- They require extra CO<sub>2</sub> for grow the specially up on primary isolation.
- Many normal individuals may harbor *N. meningitidis* in the upper respiratory tract, but *N. gonorrhoeae* is never part of the normal flora and is only found after sexual contact with an infected person (or direct contact, in the case of infections in the newborn).
- Both pathogens produce **IgA proteases** which promote virulence.



- The only distinguishing structural feature between *N. meningitidis* and *N. gonorrhoeae* is the presence of a **polysaccharide capsule** in the former.
- The capsule is antiphagocytic and is an important virulence factor.

***N. meningitidis*:**

- Meningococci are divisible into 13 serogroups , based on antigenic variation in their capsular polysaccharides .
- Most common are serogroups A , B , C and W-135.
- Other serogroups that are rarely associated with disease include serogroups D , H , I , K , L 29E , X , Y and Z .
- The serogroups is usually determined by a slid agglutination test with absorbed group-specific antisera.

**Site of infection:**

- Meninges.

**Biochemical reaction:**

- Oxidase positive.
- Ferment glucose & maltose.

**Disease:**

- Meningococca.
- Meningococemia.

**virulence factor:**

- The major virulence factor is **antiphagocytic capsule**.
- **lipooligosaccharide**, is endotoxic (highly toxic).
- Attachment is mediated by **fimbriae** and possibly by other outer membrane components .
- The organism is extremely susceptible to temperatures above or below 37 °c
- The healthy human nasopharynx is the only known reservoir of *N. meningitidis* .
- Meningococci are spread via respiratory droplets & transmission requires aspiration of infective particles .

**Meningitis:**

- Refers to the inflammation the meninges of the brain or spinal cord. **Meninges** are any of the three membranes that envelope the brain and spinal cord.
- The disease **meningitis** is caused by a number of different bacteria viruses.
- Bacterial causes include *Haemophilus influenzae*, *E. coli*, *Strep. pneumoniae*, *Strep. pyogenes*, *S. aureus*, and *N. meningitidis*.

***N. gonorrhoeae*:**

- The organism tends to occur **intracellularly** in the **cytoplasm of neutrophils** which are attracted to the site of inflammation in the meninges, so this type of infection is called **pyogenic (pus-forming)**.
- Cause lifethreating disease when the bacteria invade the blood or CFS.
- Most common cause of meningitis in individual under 20.

**Site of infection:**

Urethra.

Cervix.

**Biochemical reaction:**

- Oxidase positive.
- Ferment glucose only.

**Disease:**

- **Gonococcal.**

**Virulence factors:**

- leukocyte association factor.
- pili.
- endotoxin.

**Treatment :**

Intravenous Penicillin, cefotaxime or ceftriaxone are the drug of choice.

**Campylobacter :**

- Gram-negative bacteria.
- Small, delicate, spirally curved.
- Motile bacteria with single polar flagellum.
- Stricly microaerophilic bacteria requiring 5-10% O<sub>2</sub> and 10% CO<sub>2</sub> enriched environment.
- Oxidase and catalase positive.
- One of the most common gastroenteritis in the developing world.
- Animals are reservoir of the bacteria, causes sepsis, abortion or enteritis.
- Human infected by contaminated food, milk or water.
- Infections produce bloody diarrhea that is self-limiting .

**Medical importance species :**

- *Campylobacter jejuni*
- *Campylobacter coli*
- *Campylobacter fetus*

***Campylobacter jejuni* and *Campylobacter coli*:**

- Small , spiral , Gram-negative rod with a single flagellum al on or both poles.
- Requires selective media like skirrow's and Butzler's media for isolation of the bacteria from fecal specimen. ( Colonies are colourless or grey (teardrop) ).
- *C. jejuni* and *C. coli* found in animal feces, most common causes of human Campylobacter enteritis.

**Campylobacter enteritis** : manifests with fever, headache, abdominal pain and bloody mucoid diarrhea, and usually self-limited enteritis in a week period.

**Source of infection:**

- Contaminated food.
- Contaminated drinks.
- Unpasteurized milk.

**Biochemical reaction:**

- *C. jejuni* ... hydrolyzes hippurate.
- *C. coli* ... does not hydrolyze hippurate.

**Treatment:**

- Erythromycin is effective if given early in the disease.
- Ciprofloxacin and other fluoroquinolones are also effective.
- Many strains are sensitive to metronidazole.

***Helicobacter pylori* :**

- Gram negative.
- Spiral-shaped.
- Strictly micro-aerophilic.
- Requires carbon dioxide for growth.
- vibrio-like organism.
- Highly motile rods with polar flagella.
- Colonizes the stomach of hosts.
- Catalase positive.
- Oxidase positive.
- Urease positive.

**Route of entry:** Ingestion of contaminated food and drinks.

**Virulence factor:**

- Vacuolating toxin ( VacA).
- Cytotoxin ( CagA).
- Urease. ( powerful urease ).
- Protease.
- Flagella for adhesions.
- Toxins (inhibit stomach acid production).
- LPS.

**Disease and Treatment :****Two unequivocal indication for treatment are:**

- Peptic ulcer disease (gastric and duodenal ulcer) .
- Gastric MALT lymphoma.

*H.pylori* is sensitive to most  $\beta$ -lactam antibiotics , macrolides , tetracyclines and nitroimidazoles.

## ***Legionella***

### ***L. pneumophila***

- Gram negative
- Fastidious.
- Aerobic.
- Intracellular rods.
- Non- spore forming.
- Ubiquitous in warm moist environment.( natural habitat is water ).

**Growth on BCYE** (buffered charcoal yeast extract) media with gray-white colonies.

#### **Virulence Factors:**

- Proteases.
- Phosphatases.
- Lipases.
- Dnase.
- RNase.
- Major secretory protein (Metalloprotease): Possess cytotoxic and hemolytic property.

**Route of transmission:** Inhalation of aerosols from contaminated cooling towers, tap water and potable water following chlorination .

#### **Disease and Treatment :**

1. Legionnaires disease: causes Pneumonia (infection of the lung).
2. Pontiac fever: mild respiratory illness without pneumonia which resembles acute influenza.

#### **Treatment:**

- Erythromycin is the standard therapy in legionella pneumonia.
- Rifampin.



***Borrelia:******Borellia recurrentis:***

Causative agent of **relapsing fever- (transmitted by lice, one relapse) .**

Highly flexible irregular spiral organism, and move by rotation and twisting.

Cultured in complex serum-rich artificial media and embryonated eggs.

Famous in antigenic variation.

- Transmitted person-to-person by human body lice (vectors) from infected human .
- Infect host only when louse is injured, e.g., during scratching .
- Lice leave host that develops a fever and seek normal temperature host.

***Borellia hermsii:***

**Tick-borne borreliosis: Relapsing Fever (transmitted by ticks, three relapses).**

- Sporadic cases .
- Transmitted by soft body ticks (vectors) from small mammal reservoir .

Ticks can multiply and infect new human hosts.

بوريليا هي نوع من البكتيريا من شعبة البكتيريا الملتوية تسبب داء لايم وهو مرض منقول عن طريق الناقلات وهي هنا منقولة في المقام الأول عن طريق القراد وبعض القمل بالاعتماد على النوع. يوجد 36 نوع معروف من البورلية .

**Virulence Factors:**

- Antigenic Variation.
- Outer Surface Proteins.
- OspA.
- OspC.
- Dbp ( Decorin-binding protein).
- Erps ( Osp E/F-related proteins) and CRASPs(Complement acquiring surface proteins).
- cp32.

**Epidemiology of *Borrelia* Infections:***Borrelia recurrentis.**Borrelia hermsii.**Borrelia burgdorferi.*

<i>Borrelia</i> species .	Infection	Reservoir	Vector
<i>Borrelia recurrentis.</i>	Relapsing fever Epidemic ( louse-borne).	Humans.	Body louse.
<i>Borrelia hermsii.</i>	Relapsing fever Epidemic ( tick-borne ).	Rodents , soft-shelled ticks.	Soft-shelled tick.
<i>Borrelia burgdorferi.</i>	Lyme disease . <u><b>Borreliosis:</b></u> First recognized in 1977 among children in Lyme, Conn. believed to be suffering from rheumatoid arthritis.	Rodents, deer ,domestic pets , hard-shelled icks.	Hard-shelled tick.

**Lyme Disease :**

**Laboratory Diagnosis :**

Specimen used: Blood, biopsy specimen, joint and cerebrospinal fluid .

**Treatment :**

Doxycycline, amoxicillin, parenteral and broad spectrum cephalosporins .

**Vaccine :**

LYME rixvaccine.

**Leptospira:**

*L. interrogans* : cause leptospirosis.

- Tightly coiled, thin, flexible spiraled spirochetes forming one polared **hooked ends** .
- Grow best in semisolid (Fletcher's) media under aerobic condition at 28-30C .
- Obligate aerobes .
- Can survive for weeks in **alkaline PH water** .
- Fatty acid oxidation is major source of energy.
- Essentially zoonotic infection and humans are accidental host.
- Source of infection is contaminated food and water .

***Brucella* :**

Infect a wide range of mammals ranging from rodents to killer whales .  
The zoonotic and economic as a cause of highly transmissible disease in cattle ,sheep , pigs and goats.

- **Zoonotic.**
- Gram-negative coccobacilli.
- Or short bacilli .
- Non -motile.
- Non-capsulate.
- Non- sporing.
- Obligate **intracellular** aerobic.
- All strains grow best at 37° C in a medium enriched with animal serum and glucose.

**The mine human pathogen are :**

- *B. abortus* ( Cattle ).
- *B. melitensis* ( Goat / Sheep ).
- *Brucella suis* (Swine).

**Disease and Treatment :**

**Brucellosis (Undulant fever)** is a zoonotic disease transmitted to human by direct contact with infected tissue via skin and mucus membrane, or ingestion of infected milk via intestinal tract .

**Brucella infection respond to a combination of:**

- Streptomycin or gentamicin and tetracycline .
- Or respond to rifampicin and doxycycline.
- Tetracycline alone is often adequate in mild cases.

**No vaccine to humans.**

## ***Francisella***

### ***Francisella tularensis*:**

- Small coccobacillus.
- facultative **intracellular**.
- Gram-negative.
- Non-motile.
- Non-sporing.
- Capsulate
- Grow in blood-**cysteine** agar.

*Francisella tularensis* produces tularemia in man and certain small mammals.

1. Type A (*Francisella tularensis tularensis* ) is highly pathogenic.
2. Type B (*Francisella tularensis palaeartica* ) is much less virulent.

### **Disease and Treatment :**

- **Tularemia ( Rabbit Fever)** is a major **zoonotic** disease and transmitted to human by
- 1- Biting arthropods (rabbit).
  - 2- Direct contact with infected animal tissue.
  - 3- Inhalation of aerosols.
  - 4- Ingestion of contaminated food and water.
  - 5- By fly or Ticks.
- Ulceroglandular tularemia
  - Oculoglandular tularemia.
  - Pneumonic tularemia.
  - Typhoidal tulsremia.

### **Little is known about mechanisms of pathogenicity :**

- A carbohydrate capsule is essential for virulence.
- A smooth type LPS is also present in the outer membrane , but apparently has low endotoxin activity.

### ***Francisella tularensis* is:**

- sensitive to aminoglycosides , chloramphenicol , fluoroquinolones and tetracyclines .
- Resistant to most  $\beta$ -lactam antibiotic .

## ***Pasteurella***

- Gram-negative.
- Small .
- Non-motile.
- Non-sporing.
- Capsulate
- Aerobic or facultative anaerobic.
- Coccobacilli or rods shaped .
- Grow in ordinary media .
- Catalase positive.
- Oxidase positive.
- Primarily parasites of domestic and wild animals and birds.
- Five capsular antigens A,B,D,E and F ( **C is not Valid** ),and at least 11 somatic LPS antigens have been identified.

### ***Pasteurella multocida*** (Shipping fever and cat bite fever):

- Commensal or Opportunist pathogen of many species of domestic and wild animals and birds.
- Human beings occasionally become infected especially following animal bites ( cat and dogs ) .
- Occur in gastrointestinal and respiratory tract of many domestic and wild animals.

### **Treatment :**

- Infection usually respond to penicillin .
- Tetracycline erythromycin or co-trimoxazole are alternatives.

**Order Spirochaetales :**

Classification Spirochetes are members of the order Spirochaetales which contains 2 families :

- Spirochaetaceae – contains 2 medically important genera :
  - *Treponema*
  - *Borrelia*
- Leptospiraceae – 1 medically important genus :
  - *Leptospira*.

**Characteristics:**

- Helical single cells, spiral or cork-screw-shaped.
- Extremely thin and can be very long.
- Gram negative.
- Aerobic to strict anaerobic.
- Free or strict parasites.
- Motile, move by bending and rotating body movements.
- Spirochete consist of protoplasmic cylinder bounded by a cell wall and outer membrane.
- There is an **axial filament or endoflagella (preiplasmic flagella)** between the cell wall and outer membrane.



**Medical importance:****1- Treponema :**

- *T. pallidum* ( cause Syphilis).
- *T. peritenuis* ( cause Yaws- 3 stages- (granulomatous disease).
- *T. carateum* ( cause Pinta (primarily restricted to skin- tropical area).

**2- Borrelia :**

- *B. recurrentis* (cause relapsing fever ).

**3- Leptospira :**

- *L. interrogans* ( cause Leptospirosis ).

**None of the pathogenic *Treponemes* have been successfully cultured on artificial media .**

- *T. pallidum* is usually cultured in the testes of rabbits, although it has been grown in tissue culture for short periods of time.
- *T. pallidum* does not survive for long outside the host.
- Visualized by darkfield microscopy or iron staining .

***Borrelia*** may be grown on a complex media called Kelly's media, but this is not usually done in diagnostic labs.

***Leptospira*** can be grown on semi-solid media containing peptone, beef extract supplemented with rabbit serum or bovine serum albumin and tween 80. **Incubation** may be for as long as 28 days.

**Virulence factors :*****Treponema:***

- Molecular mimicry – the outer sheath contains molecules that resemble the molecules commonly found on the surface of human cells , this allows the organism to resist host defenses.
- Hyaluronidase.

Outer membrane proteins promote adherence .

Hyaluronidase may facilitate perivascular infiltration .

Antiphagocytic coating of fibronectin .

***Borrelia:***

- Antigenic variation.

***Leptospira:***

- Unknown.

## Clinical significance

### Treponema

- *T. pallidum* causes venereal (transmitted by sexual contact) and non-venereal (transmitted by directly by non-sexual contact, and indirectly by common usage of eating and drinking utensils) syphilis.
- In venereal syphilis the primary lesion is on the genitals.
- In non-venereal syphilis it is on oral mucous membranes.
- The normal untreated course of the disease occurs in several stages:

#### *Primary Syphilis:*

- disease process involves invasion of mucus membranes, rapid multiplication & wide dissemination through lymphatics and systemic circulation.
- 10-90 days (usually 3-4 weeks)
- inflammatory response at the site of infection resulting in the hallmark syphilitic lesion, called the **chancre (usually painless)**
- Chancre changes from hard to ulcerative with profuse shedding of spirochetes
- Swelling lymph nodes
- Primary lesion heals spontaneously two months, leading to false sense of relief

#### *Secondary Syphilis:*

- 2-10 weeks after primary lesion.
- Widely disseminated mucocutaneous rash
- Secondary lesions of the skin and mucus membranes are highly contagious.
- Generalized immunological response .

#### *Latent Stage Syphilis:*

- Following secondary disease, host enters latent period.
- First 4 years = early latent.
- About 40% of late latent patients progress to late tertiary syphilitic disease.

***Tertiary Syphilis:***

- characterized by localized granulomatous dermal lesions in which few organisms are present.
- Granulomas reflect containment by the immunologic reaction of the host to chronic infection.
- Late neurosyphilis develops in untreated cases, usually more than 5 years after initial infection.
- Central nervous system and spinal cord .
- Dementia, wasting, etc.
- Cardiovascular involvement appears 10-40 years after initial infection with resulting myocardial insufficiency and death.

**Congenital syphilis :**

**Route of transmission:** Mother-to-child during gestation (transplacental infection).

**Diagnosis :****Direct:**

- Motile spirochetes in dark field microscope.
- Immunofluorescence stain (Staining with anti-treponemal antibodies labeled with fluorescent dyes).

**Indirect:**Serological tests for syphilis (STS):**Two** classes of serological test:

- **Nonspecific test** : Non-treponemal antigen test (detect antibodies to nonspecific antigen)
- **Specific tests**: Treponemal antigen tests (detect antibodies against specific *T. palladium* antigens).

**Treatment:**

- Penicillin, Tetracycline and Erythromycin.
- Remain positive for years despite treatment.

Mycoplasma, Chlamydia and Rickettsia :

## **Mycoplasma:**

**Mycoplasma** are :

- The smallest prokaryotes organisms (too small to seen under light microscope).
- That can grow in cell-free culture. ( capable of self-replication). Do not have cell wall (Don't stain with a Gram's stain).
- It has a high content of sterols to prevent osmotic lysis.
- Found in man , animals , plants , insects , soil and sewage.
- Completely resistant to penicillin and cephalosporin and vancomycin.
- Part of normal flora of human genital tract or oral cavity of healthy adults.
- Grow on media enriched with serum (need cholesterol).
- Ureaplasmas were known originally as T strains or T mycoplasmas ( T for " tiny " ) to describe the small size of the colonies in comparison with those produced by other mycoplasmas .
- **Fourteen** *Mycoplasma* species , **two** *Acholeplasma* species , and **two** *Ureaplasma* species have been isolated from man .

***Mycoplasma pneumoniae* :**

- Have specialized structures at one or both ends by which they attach to respiratory or genital tract mucosal surfaces.

**Route of transmission:**

- Infected respiratory secretion.
- Grows in 5-14 days
- It is a major cause of pneumonia in young age groups (5-20 yrs.)

**Treatment :**

*Mycoplasma pneumoniae* is sensitive to the tetracyclines and erythromycin in vitro and these antibiotics have been used widely in clinical practice , where they have sometime proved less effective for treating pneumonia than in planned trials , probably because disease is often well established before treatment begins.

**Chlamydia:**

- Obligate intracellular bacterial pathogens of eukaryotic cell.
- Variable cocci,
- Gram-negative.
- Do not have peptidoglycan (muramic acid).

**Widely distributed in nature and tow genera :**

- *Chlamydia* and *Chlamydophila*.

**Dimorphic growth cycle quite distinct from that of other bacteria.**

- Infection is initiated by environmentally resistant metabolically inert infectious structures called Elementary bodies.
- Whereas the larger more fragile Reticulate bodies .

***Chlamydia:***

Infect a wide spectrum of hosts: birds, mammals, and humans.

Human infections include:

- Trachoma .
- Adult inclusion Conjunctivitis .
- Various urogenital tract infections of males and females.
- Infant pneumonia.

***Chlamydia trachomatis:***

- Genital tract infection.
- Trachoma may cause of blindness.

***Chlamydia pneumonia:***

- **Humans are the only host.**
- stain tissues with Giemsa or use a direct fluorescent antibody technique



**Treatment:**

- The antibiotic of choice is doxycycline or azithromycin in adults and erythromycin in babies .
- Penicillin inhibit but do not kill .
- Long term therapy is necessary.

***Rickettsia and Orientia***

- Include organisms responsible for numerous diseases in many parts of the world.
- Rickettsial aetiology of Rocky Mountain spotted fever.
- Several other diseases including epidemic and murine typhus , were later shown to be rickettsial infections .
- Small .
- Gram – negative bacilli .
- **Obligate intracellular bacteria.**
- Have a small genome ( approximately 1 Mb ).
- Pleomorphic.

**Two cell types designated :**

- Large and small cell variants (LCV and SCV).
- Both types are infectious.

**Grow in :**

- Yolk sac of embryonated eggs.
- Cell culture and laboratory animals.

**Treatment :**

- The drug of choice for treating rickettsias infection of all types and in all age groups is doxycycline.
- Chloramphenicol is an alternative.
- Both drugs are rickettsiostatic and allow the patient's immune system time to respond and control the infection.

**Ehrlichieae:***Ehrlichia* and *Anaplasma*:

- Gram negative cocci.
- Obligate intracellular parasite.
- Classified in the Rickettsiales.
- Cause (ehrlichiosis).
- A noncontagious disease known to be transmitted by a tick.

*Ehrlichia* and *Anaplasma*:

Species can establish prolonged even persistent infection in vivo and some species, including *E.chaffensis* ,kill heavily infected cell in vitro.

**Pathogenesis:**

- Human monocytic ehrlichiosis .
- Human granulocytic anaplasmosis.

**Treatment :**

- Doxycycline is very effective in shortening the course of infection and reduces mortality.

***Bartonella* ( Bartonellosis)**

- Small polymorphic.
- Motile.
- Very small Gram negative bacilli .
- Facultative intracellular parasite.
- They range in shape from small coccoid and ring-shaped structures to long chains or clusters.
- Parasites of the erythrocytes of human (adhered to RBCs ) where they appear as short rods.
- Transmitted by insect vectors such as ticks, fleas.
- *Unlike rickettsiae Bartonella* bacteria can be grown on artificial media.

**Pathogenesis:**

- Oroya fever or Carrion's disease and verruga peruana , spread by sandflies.
- Louse-borne trench fever.
- Cat scratch disease, can transmitted by fleas and possibly ticks.

**Treatment :**

- Chloramphenicol can drastically reduce the mortality rate in Oroya fever.
- Penicillin , streptomycin , tetracyclines , fluoroquinolones and newer macrolides such as clarithromycin may also be effective in uncomplicated cases.