Volume II

Systemic Bacteriology

and

Mycology

by

Medadteam

Dataology Page

Contents:

Chapter 1: Microbial Taxonomy

❖ **Definition of Taxonomy (taxon = group):**

- The science of biological classification.

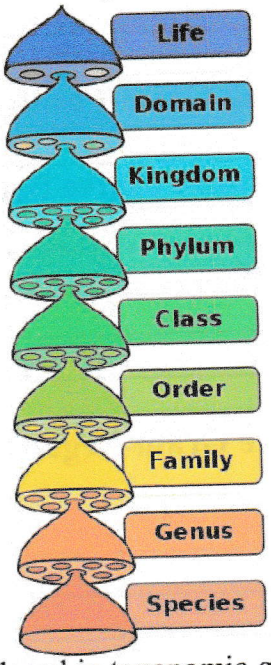
❖ **It consists of :**

A. **Classification** is the arrangement of organisms into groups.

B. **Nomenclature** is the assignment of names to taxonomic groups.

C. **Identification:** the determination of the particular taxon to which a particular isolate belongs.

❖ Taxonomic Groups



❖ Organisms except viruses are placed in taxonomic groups which include Domain, Kingdom, Phylum, Classes, Orders, Families, Genera, Species and strains in a descending order. Viruses are classified differently, so that **the family is the highest taxonomic group**.

❖ **The basic taxonomic group is the species.**

- Species are defined on the basis of phenotypic and genotypic differences among bacteria.
- **A bacterial species** is a collection of strains that share many stable properties and differ significantly from other groups of strains.
- **A strain** is an individual member within the species.
- **A genus** is a well-defined group of one or more species that is clearly separate from other genera.

❖ **The binomial system** is used for nomenclature of organisms (except viruses).

- It employs the genus and species names.
- The genus name is always capitalized (e.g. Escherichia) while the species name is never capitalized (e.g. coli); both terms are written in italic form (e.g. Escherichia coli). After first usage in a manuscript, the first name will often be abbreviated to the first letter (e.g. E. coli).

❖ Criteria for Classifying Bacteria:

1. Genetic homology:

- Is a similarity between the DNA or the RNA of organisms.
- It determined by base composition, nucleotide sequence or DNA hybridization rates.
- **Two organisms are considered to belong to the same species if they have:**

A. DNA homology of ≥ 70% , or

B. 16S rRNA sequences are > 97% identical

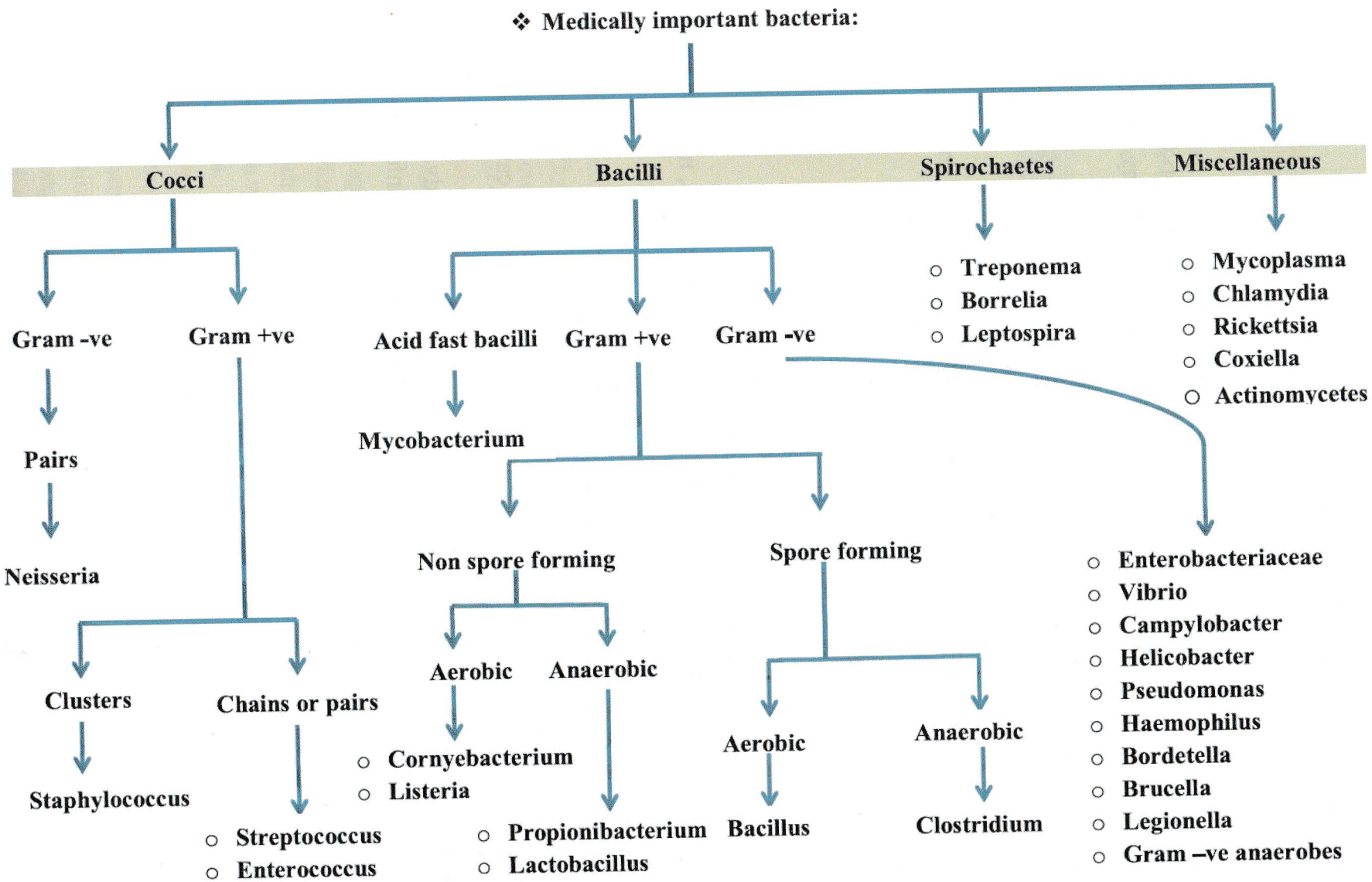
2. Phenotypic properties:

- E.g. biochemical reactions, chemical composition, cellular structures and immunological features are used in defining a bacterial species

❖ Systematic Microbiology

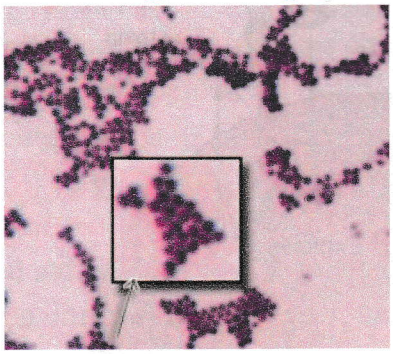
❖ The systematic study of microbes include basic knowledge of:

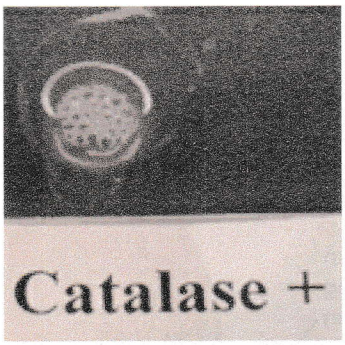
1. **Morphology** and structure of infectious microbial agents.
2. **Cultural characteristics** of the organism including O2 requirements, medium for primary isolation and incubation conditions.
3. **Biochemical reactions** of the microbe.
4. **Source** of the microbe in nature and **mode of transmission**.
5. **Diseases** caused by a particular infectious agent.
6. **Pathogenesis**, virulence factors and mechanisms by which microbes cause disease.
7. **Laboratory diagnosis** of the infectious disease.
8. **Antimicrobial agents** used to combat infectious agents in human body.
9. **Prophylactic measures** e.g. vaccination or by applying appropriate infection control measures.


❖ Medically important bacteria:
Cocci
Bacilli
Spirochaetes
Miscellaneous
Gram -ve
Gram +ve
Acid fast bacilli
Gram +ve
Gram -ve
Pairs
Neisseria
Mycobacterium
Clusters
Staphylococcus
Chains or pairs
Streptococcus
Enterococcus
Non spore forming
Spore forming
Aerobic
Anaerobic
Cornyebacterium
Listeria
Propionibacterium
Lactobacillus
Aerobic
Anaerobic
Bacillus
Clostridium
Treponema
Borrelia
Leptospira
Mycoplasma
Chlamydia
Rickettsia
Coxiella
Actinomycetes
Enterobacteriaceae
Vibrio
Campylobacter
Helicobacter
Pseudomonas
Haemophilus
Bordetella
Brucella
Legionella
Gram –ve anaerobes

Chapter 2: Staphylococcus

❖ Characters of the genus Staphylococcus:





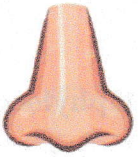
1. Gram-positive spherical cocci arranged in grape-like clusters.
2. Catalase positive.
3. Opaque pigmented colonies are usually produced on agar.

❖ The ability to produce staphylocoagulase divides the genus into two groups:

A. Coagulase-**positive** staphylococci: S. aureus has **the greatest pathogenic** potential and is the most medically important species.

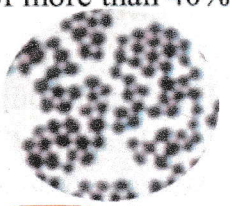
B. Coagulase-negative staphylococci: e.g., S. epidermidis and S. saprophyticus which are far less pathogenic.

Staphylococcus Aureus



❖ Staphylococci **inhabit** the **skin** (especially the perineum) and **mucosa**.

- The **nose** is the main habitat for S. aureus with a nasal carriage rate of more than 40% in adults.

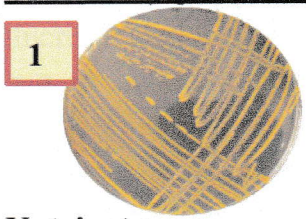


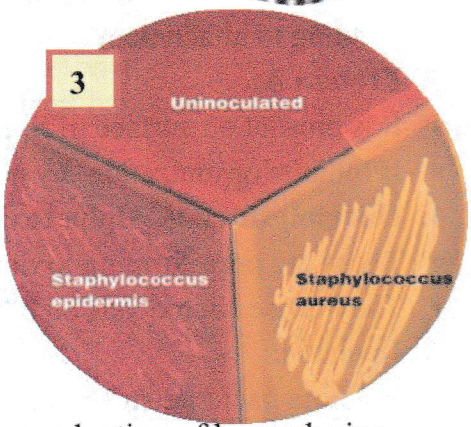
❖ Morphology:

- S.aureus strains are Gram-positive spherical cocci (about 1 μm in diameter) occurring in irregular grape-like clusters.

❖ Cultural characters:

- S. aureus is a facultative anaerobe.
- It usually produces **golden yellow endopigment.**
- <u>It is usually grown on:</u>

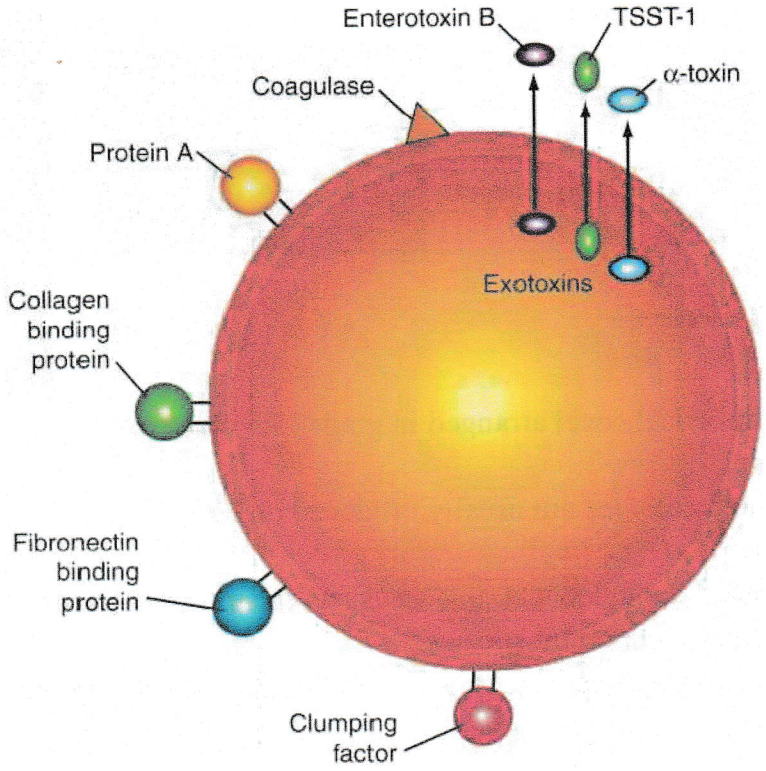




1) **Nutrient agar.**
2) **Blood agar**: producing complete (β-) haemolysis due to production of haemolysins.
3) **Mannitol salt agar** (selective indicator medium) producing yellow colonies due to mannitol fermentation :

☞ *This medium facilitates **isolation** of S. aureus (salt tolerant) from specimens contaminated by other bacteria.*

❖ Virulence factors and pathogenesis:



1. Staphylocoagulase:

- Coagulase is an extracellular protein that has the ability to convert plasma **fibrinogen to fibrin**.

☞ *A fibrin barrier is formed leading to:*

- Protection from phagocytic and immune defences.
- Localization of infection e.g.furuncles.

2. Adhesin: The clumping factor (fibrinogen-binding protein);:

- Leading to attachment of the organism to traumatized tissue and blood clots.

3. Protein A:

- It is present on surface of S. aureus.
- It Inhibits opsonization by non-specific binding to the Fc-portion of IgG .

4. Haemolysins e.g. Alpha toxin:

- These are pore-forming toxins that lyse host cell membranes.
- They cause haemolysis on blood agar.

5. Invasins:

- Promote bacterial spread in tissues e.g. Leucocidin, staphylokinase and hyaluronidase.

6. Exotoxins having superantigen mechanism :

a) **Enterotoxins** responsible for staphylococcal food poisoning

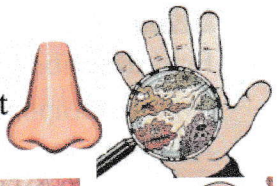
b) Toxic shock syndrome toxin-1 (TSST-1).

c) **Epidermolytic (exfoliatin) toxins** responsible for staphylococcal scalded skin syndrome (SSSS).

Staphylococcus Aureus Diseases

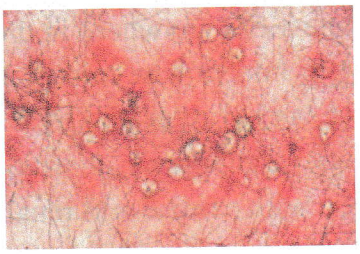
❖ **The chief sources of infection :** contact with

- Shedding human lesions, fomites contaminated from respiratory tract especially the nose and skin.
- Contaminated hands of the healthcare workers.

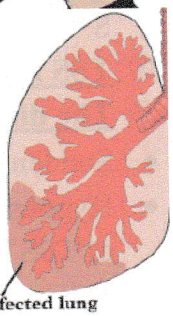


❖ **Staphylococcus Aureus Diseases**

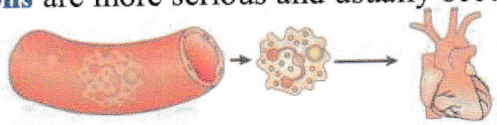
A. Pyogenic diseases:



1. **Localized skin infections, the most common:**
   a. Folliculitis, furuncles, carbuncles, or abscesses
   b. Surgical site infections.
   c. Traumatic wound infections following skin injury and burns.



2. **Staphylococcal pneumonia** is a complication of prior viral infections e.g. influenza.
3. **Invasive conditions** are more serious and usually occur in immunocompromised individuals:



- Invasion of bloodstream (**bacteraemia**) and spread to numerous body sites lead to **deep** infections e.g. osteomyelitis, endocarditis and meningitis.
- A resulting **septicaemia** may be rapidly fatal.

B. Toxin mediated diseases:

1. Staphylococcal food poisoning:

❀ **Incidence:**

- **It is the commonest type of bacterial food poisoning.**

❀ **Staphylococcal food poisoning is due to :**

- **Incubation period** is short: **1-6 hours** after ingestion of foods containing **preformed toxin**.

i. The source of food contamination may be:

- A carrier such as food handlers harbouring S. aureus on their hands or in the nose.
- A person with pyogenic staphylococcal infection e.g., furuncle.
- Incriminated foods include **protein** rich food like mayonnaise, milk and its products e.g. ice cream or **carbohydrate** rich food e.g. pasta, cake and koskosi.

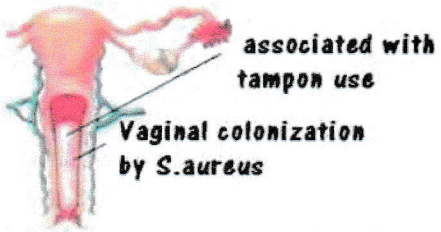
ii. Toxins produced by the organism use a superantigen mechanism:

- There are at least six antigenic types of **enterotoxins** (A, B, C, D, E and G) produced by 50% of S. aureus strains.
- Staphylococcal enterotoxins do **not change the characters** of the food regarding its taste, colour or odour, in addition, heating for 30 minutes of boiling may **kill the organism** but does **not destroy the toxin** since it is heat stable.

❀ **The disease is characterized by**

- Violent vomiting and diarrhea, usually without fever & usually self-limited.

2. Toxic shock syndrome (TSS):



❀ **Incidence:**

- First described in young menstruating females who use vaginal tampons that are left in place for extended period.
- Any individual suffering from TSST-1 producing S. aureus infections anywhere in the body.

❀ **TSS is due to** infection or colonization by **TSST1**-producing S. aureus.

❀ **The disease is characterized by**:

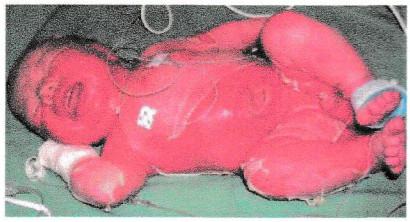
- Sudden onset of high fever, diarrhoea, vomiting and red rash.
- Hypotension with cardiac and renal failure may occur due to the **superantigen** action of TSST-1.
- The mortality rate may reach 10-15%.

3. Staphylococcal scalded skin syndrome (SSSS):

❀ **Incidence:** in neonates and children under 5 years of age.

❀ **SSS follows** infections caused by S. aureus that produces **exfoliatin** toxins.

❀ **The disease is characterized by**:



- Large bullae are formed under the epidermis, which rupture leaving moist, red, scalded dermis.
- Full recovery without scar formation is the rule

❖ **Laboratory diagnosis :**

1. **Specimens** may include pus, sputum, urine, CSF, blood in cases of bacteraemia, septicaemia and endocarditis.
2. **Direct detection** in Gram-stained smears:

- Gram-positive cocci are seen in clusters in association with pus cells.

☞ *Microscopy cannot discriminate staphylococcal species.*

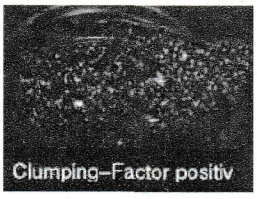
3. **Cultivation:**

- Specimens other than the blood should be plated directly onto blood agar and mannitol salt agar and incubated at 37°C.
- Blood samples should be cultivated by the blood culture technique.
- Subcultures are plated on blood agar and incubated as above.

4. **Identification:**

❀ After 24h incubation, the growth should be examined for colony merphology, Gram stain and catalase production. **S. aureus is identified as follows:**

- **On blood agar**: golden yellow colonies surrounded by complete haemolysis.
- **On mannitol salt agar**: yellow colonies.
- **Gram-stained film**: Gram positive cocci in clusters.
- **Coagulase test & Catalase test**: positive.
- **Clumping factor test**: positive.



☞ *Coagulase and clumping factor are the most important markers for identifying S. aureus in the laboratory.*

5. In case of food poisoning:

- **Specimens:** food remnants, vomitus and faeces should be tested for the causative S. aureus and its enterotoxin.
- **Isolation:** on selective media such as mannitol salt agar as the specimens are usually contaminated with other bacteria.
- **Detection of enterotoxin** production by the isolated strains or directly in the sample is done by ELISA.

6. In case of toxic shock syndrome:

- **Clinical findings.**
- **Isolation** of the organism from suspected sites e.g. wounds, vagina or from tampons by culture on mannitol salt agar.
- **Detection of TSST-1** in the blood by ELISA.

❖ **N.B.: Strain typing** is required in the epidemiologic studies of **outbreaks** of S. aureus diseases such as food poisoning and surgical site infections.

☞ ***Strain typing can be done by*** *colony morphology, biotype profiles, phage typing, plasmid analysis, ribotyping, chromosomal analysis and PCR.*

❖ Prevention and control:

- Improved hygiene especially hand hygiene and proper infection control practices & aseptic techniques in hospitals are the most effective methods of prevention.

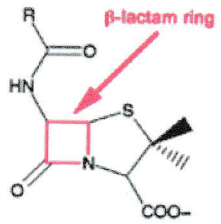
❖ Treatment and Antibiotic Susceptibilities:

- **Abscesses** may require surgical drainage and antibiotic therapy to prevent dissemination.
- **Systemic infections** require vigorous antibiotic treatment.

❖ Therapy is seriously faced with the following antibiotic-resistance patterns of S. aureus:

1. Penicillin resistant S. aureus:

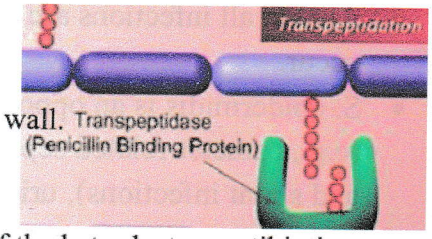
- 95% of S. aureus strains are resistant to penicillin due to **β-lactamase (penicillinase) production**.
- Resistant strains remain susceptible to the semi-synthetic penicillins (e.g. oxacillin and **methicillin**) and to cephalosporins.

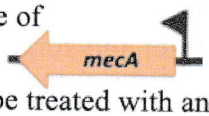


Penicillin

2. Methicillin resistant S. aureus (MRSA):

- It is a more serious type of resistance.
- There is a change in the **penicillin binding protein (PBP)** which is the binding site for the antibiotic on the organism's cell wall.
- This type of resistance is due to the presence of **mec-A gene** on the chromosome of MRSA.





- Infections caused by MRSA strains cannot be treated with any of the beta- lactam antibiotics. Also, MRSA isolates are often multiresistant to other antibiotics.
- **Vancomycin** is used as the drug of choice for treatment of MRSA infections.

3. Vancomycin resistant S. aureus:

- Some strains of MRSA displayed intermediate (VISA) or full resistance (VRSA) to vancomycin.
- The new antibiotics **linezolid and streptogramins** are used for treatment of infections not responding to vancomycin

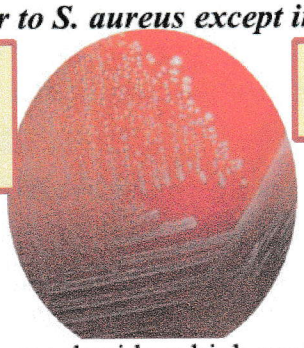
Coagulase Negative Staphylococci

I. S. epidermidis:

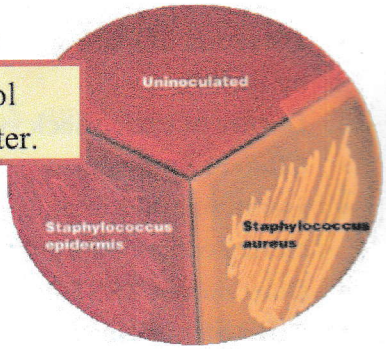
❖ **Morphology and Cultural Characteristics:**

☞ ***S. epidermidis is similar to S. aureus except in the following:***

2) It gives white **non** haemolytic colonies on blood agar.



1) It is mannitol **non** fermenter.

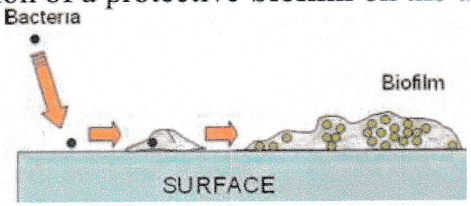


❖ **Virulence factors:**

Glycocalyx (or slime):

- It is an extracellular polysaccharide which enables the organism to colonize **prosthetic devices** and facilitates formation of a protective **biofilm** on the device surface.

☞ ***Biofilm:***



- It is an **aggregate of microorganisms** in which cells adhere to each other on a surface. These adherent cells are frequently embedded within a matrix of extracellular polysaccharide.
- Biofilms **protect bacteria** from host defences (e.g., antibodies), detergents and antibiotics.
- Biofilms also facilitate **exchange of genetic material** between bacterial cells leading to spread of antibiotic resistance among them.

❖ **Pathogenesis:**

- S. epidermidis is part of the normal **skin** flora and it is attached to the upper layer of the skin (epidermis) or mucosa (carriage rate 100%).
- Almost all infections are **endogenous** but person to person transmission by contact may occur.
- S. epidermidis is an opportunistic pathogen associated with **device related infections** (e.g., catheter related sepsis, prosthetic valve endocarditis, prosthetic joints and shunt infections), urinary tract and surgical wound infections.

❖ **Laboratory diagnosis:**

- S. epidermidis is diagnosed by its morphological and cultural characteristics.
- It is **sensitive to novobiocin**.

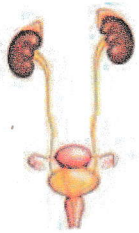
• **Sensitive to novobiocin**

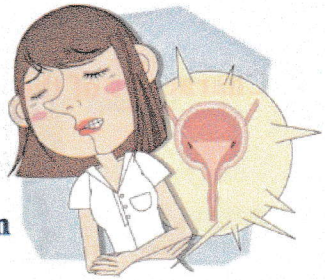
❖ **Treatment:**

☞ *S. epidermidis infections are difficult to treat. This is because:*

- The organism is often **multi resistant** to antibiotics.
- The infections usually occur in prosthetic devices where the bacteria can **sequester themselves** in a biofilm.

II. S. Saprophyticus:





❖ Morphology and cultural characteristics:

- S. saprophyticus is similar to S. epidermidis.

❖ Pathogenesis:

- S. saprophyticus may form part of the normal flora of human **skin** and mucosa of **genitourinary tract.**
- It may spread to urinary tract in colonized young sexually active women causing urinary tract infections (**honeymoon cystitis**) (**endogenous infection**).
- This is due to the ability of the organism to adhere to uroepithelial cells.

❖ Laboratory diagnosis:

- S. saprophyticus is similar to S. epidermidis except in being **novobiocin resistant.** →→→→

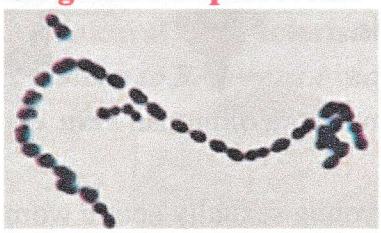
- **Novobiocin resistant**

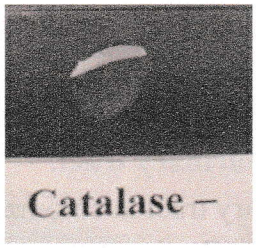
❖ Treatment:

- **Quinolones** are the drugs of choice.

Chapter 3: Streptococcus

❖ Characters of the genus Streptococcus:



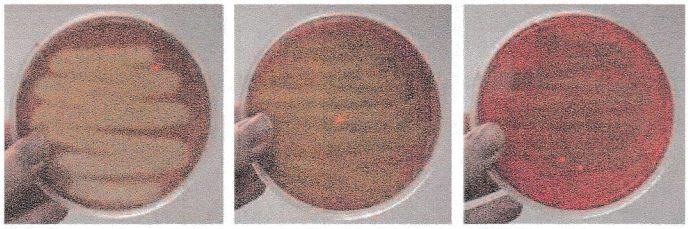


1. **Gram positive** ovoid cocci, arranged in chains or pairs.
2. **Catalase negative:** Catalase test is a key test for discriminating streptococci from the catalase positive staphylococci.
3. Growth requires **enriched media** containing blood or serum.

☞ *Streptococcus pyogenes, Streptococcus agalactiae and Streptococcus pneumoniae are the most important species.*

❖ The medically significant streptococci may be conveniently divided on the basis of:

1. **Haemolysis on blood agar :**



Beta Hemolysis | Alpha Hemolysis | Gamma Hemolysis

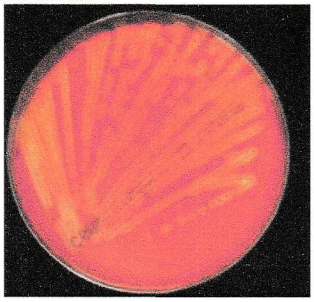
- Complete haemolysis: beta.
- Partial haemolysis: alpha.
- No haemolysis: gamma.

2. **Lancefield classification: A group specific carbohydrate antigen**

- According to which streptococci are classified into groups A to U.
- Antibodies against these group antigens are used for identification of streptococcal species.

Beta Haemolytic Streptococci

I. Streptococcus pyogenes (Group A Streptococcus):



❖ Morphology:

- S. pyogenes are Gram positive cocci in chains.

❖ Cultural characters:

- S. pyogenes produce **beta** haemolysis on blood agar.
- S. pyogenes growth is **inhibited by bacitracin**.

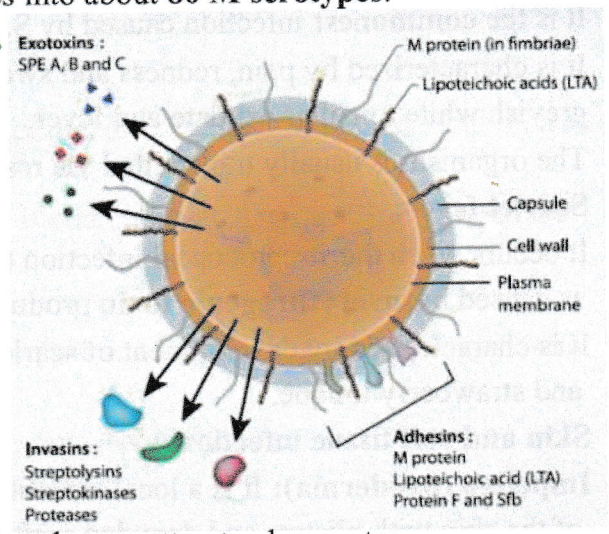
❖ Virulence factors and pathogenesis:

I. Factors that mediate adherence (colonization):

1. **M protein:** *It is the most important virulence factor.*

- It is a surface protein which enables the bacteria to **colonize skin** and to **escape phagocytosis**.
- It is **immunogenic** and divides S. pyogenes into about **80 M serotypes**.

2. **Fibronectin binding protein (Protein F).**
3. **Lipoteichoic acids.**



II. Factors that mediate invasion:

1. **Antiphagocytic factors:**

A. M protein.

B. C5a peptidase breaks down C5a so that it no longer attracts phagocytes.

C. Hyaluronic acid capsule:

- It is chemically **similar** to that of host connective tissue; therefore, it is **not immunogenic**.
- This allows the bacterium to **hide** its own antigens and to go **unrecognized** by its host.

2. **Invasins:**

A. Streptokinase (fibrinolysin):	**B. Streptolysins (haemolysins):**	**C. Streptococcal pyrogenic exotoxins (SPE- A, B & C):**
• It activates plasminogen of human plasma into plasmin that **digests fibrin and fibrinogen**. • It is ten times more potent than staphylokinase and is used for emergency **therapy of myocardial infarction** to remove blood clots.	• These are two pore forming toxins that lyse host cell membranes: ✌ Streptolysin **O** (oxygen labile) is a highly **immunogenic** protein and induces specific antibody formation (its detection is the basis for the anti-streptolysin O test). ✌ Streptolysin **S** (oxygen stable) is **non-**immunogenic.	• These toxins act as **superantigens** causing toxic shock syndrome, septicaemia, and necrotizing fasciitis. • In addition: ○ Toxin **A** is an **erythrogenic** toxin that is responsible for the red rash characteristic of *scarlet fever.* ○ Toxin **B** acts as a **protease** that contributes to the pathogenesis of *necrotizing fasciitis.*

D. Others:

- **E.g., hyaluronidase and nucleases** with streptokinase contribute to the **spreading** nature of streptococcal infections.

Streptococcus pyogenes Infections

❖ Streptococcus pyogenes Infections:

I. Localized infections:

1. Pharyngitis (sore throat, tonsillitis)

- It is the **commonest** infection caused by S. pyogenes.
- It is characterized by pain, redness and swelling of posterior pharynx, accompanied by greyish white tonsillar exudate and fever.
- The organism is usually transmitted via respiratory droplets.

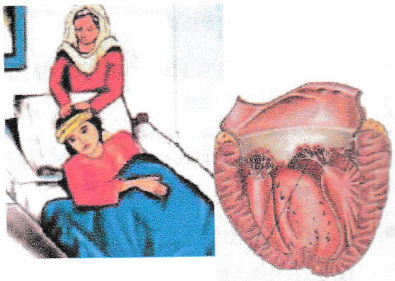
2. Scarlet fever:

- It occurs when the streptococcal infection (especially pharyngitis) is caused by an **erythrogenic toxin** producing S. pyogenes.
- It is characterized by development of scarlet red rash (sandpaper rash) and strawberry tongue.

3. Skin and soft tissue infections:

- **Impetigo (pyoderma):** It is a local infection of the superficial layers of the skin with blisters and denuded surface covered with crusts.
- **Cellulitis:** It is infection of the deep layers of the skin.
- **Erysipelas:** It is a form of cellulitis accompanied by fever and systemic toxicity.

II. Invasive infections:



1. Puerperal fever:

- This is a life threatening infection of the endometrium and surrounding structures complicating delivery or abortion.
- Septicaemia and toxic shock may occur.

2. Acute endocarditis:

- This fatal condition can occur in individuals with normal or damaged heart valves.

3. Necrotizing fasciitis:

- It is associated with severe tissue destruction particularly associated with **SPE-B (protease)**.
- The destructive nature of this condition led to the term "**flesh-eating bacteria**".

4. Toxic shock syndrome:

- This condition is mediated by the production of **SPE- A, B & C.**
- It often begins with skin wounds or minor traumas and rapidly deteriorates leading to necrotizing soft tissue infections.
- Shock, renal failure and acute respiratory distress syndrome are complications of the condition.

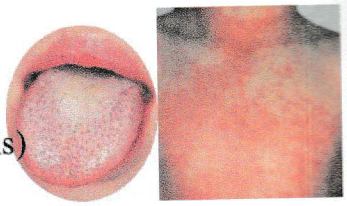
Streptococcus pyogenes Infections

❖ Streptococcus pyogenes Infections:

I. Localized infections:

1. Pharyngitis (sore throat, tonsillitis)

- It is the **commonest** infection caused by S. pyogenes.
- It is characterized by pain, redness and swelling of posterior pharynx, accompanied by greyish white tonsillar exudate and fever.
- The organism is usually transmitted via respiratory droplets.



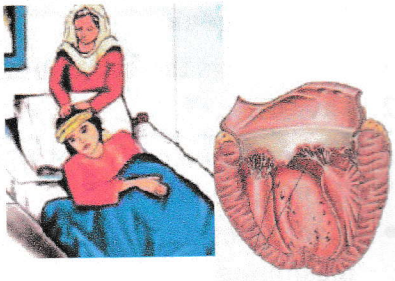
2. Scarlet fever:

- It occurs when the streptococcal infection (especially pharyngitis) is caused by an **erythrogenic toxin** producing S. pyogenes.
- It is characterized by development of scarlet red rash (sandpaper rash) and strawberry tongue.

3. Skin and soft tissue infections:

- **Impetigo (pyoderma):** It is a local infection of the superficial layers of the skin with blisters and denuded surface covered with crusts.
- **Cellulitis:** It is infection of the deep layers of the skin.
- **Erysipelas:** It is a form of cellulitis accompanied by fever and systemic toxicity.

II. Invasive infections:



1. Puerperal fever:

- This is a life threatening infection of the endometrium and surrounding structures complicating delivery or abortion.
- Septicaemia and toxic shock may occur.

2. Acute endocarditis:

- This fatal condition can occur in individuals with normal or damaged heart valves.

3. Necrotizing fasciitis:

- It is associated with severe tissue destruction particularly associated with **SPE-B (protease)**.
- The destructive nature of this condition led to the term "**flesh-eating bacteria**".

4. Toxic shock syndrome:

- This condition is mediated by the production of **SPE- A, B & C.**
- It often begins with skin wounds or minor traumas and rapidly deteriorates leading to necrotizing soft tissue infections.
- Shock, renal failure and acute respiratory distress syndrome are complications of the condition.

❖ Laboratory diagnosis:

A. Specimens include throat swab, pus, blood (in invasive infections)...etc.

B. Direct detection in clinical specimens:

1. **Gram-stained smears** are useful only in cases of skin and soft tissue infections since S. pyogenes cannot be visually distinguished from the normal oral streptococcal flora.
2. **Detection of Lancefield group A streptococcal antigen** in throat swab by an agglutination test using group A antibody.

C. Cultivation:

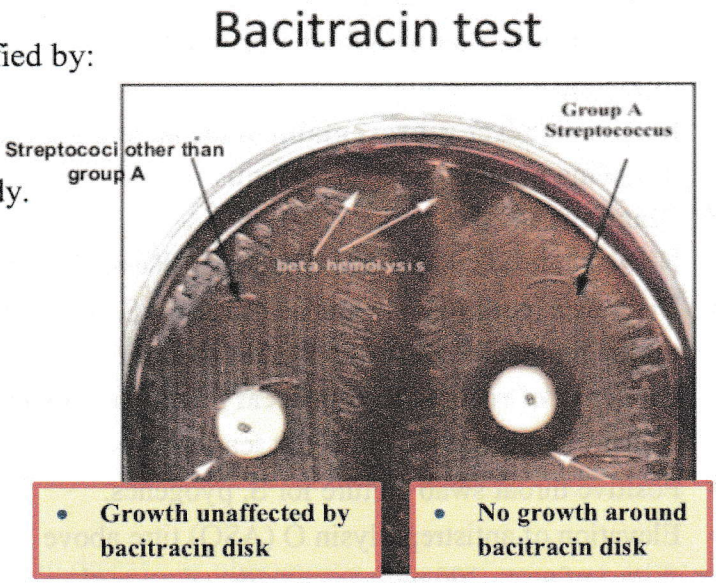
- Specimens other than the blood should be plated directly onto blood agar and incubated at 37°C.
- Blood samples should be cultivated by the blood culture technique. Subcultures are plated on blood agar.

D. Identification:

- After 24h incubation, the growth should be examined for colony morphology, Gram stain and catalase production:

1) On blood agar: Colonies are surrounded by beta-haemolysis.
2) Gram-stained film: Gram positive cocci in chains.
3) Catalase test: negative.

- S. pyogenes can further be identified by:
- Growth inhibition by bacitracin (i.e. it is bacitracin-sensitive).
- Agglutination by group A antibody.

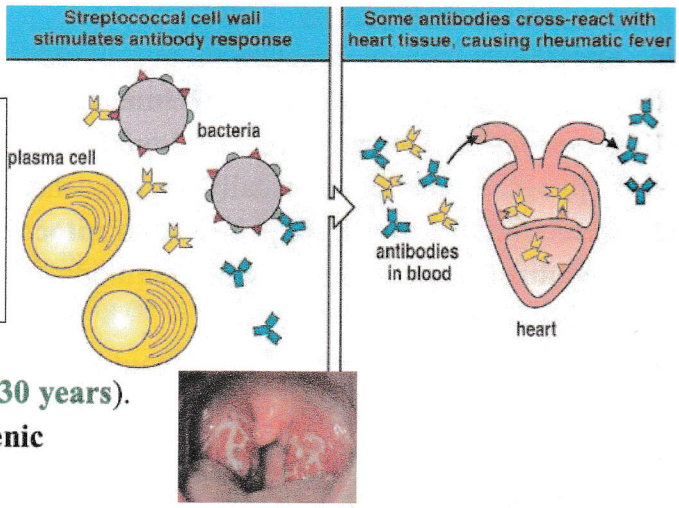


Post-Streptococcal Sequelae (Acute Rheumatic Fever and Acute Glomerulonephritis)

❖ Definition:

- These are **non**-suppurative inflammatory conditions which occur as a result of **immunologic response** to streptococcal antigens.
- They occur **weeks** following a local infection with S. pyogenes and affect an organ that was **not infected** by the streptococci.

I. Acute rheumatic fever (ARF):



❖ Pathogenesis:

- It is due to **autoimmune reaction**: formation of antibodies to **streptococcal M protein**, which **cross reacts** with antigens of joints, heart and brain tissue.

❖ Age:

- ARF may affect **any age** (but mostly **4-30 years**).

❖ S.Pyogenes M serotype : Rheumatogenic

❖ Precipitating infection:

- It develops **2-3 weeks** following streptococcal **pharyngitis** (but not skin infection).

❖ The disease is characterized by:

- Fever, migrating poly-arthritis, carditis and chorea.

❖ Recurrence & Chemoprophylaxis:

- The immunological process is exacerbated by **recurrence** of streptococcal pharyngitis leading to valvular damage.
- Therefore, long-term antibiotic **prophylaxis** using penicillin is **recommended** following a single attack.
- Rheumatic fever is **preventable** if the patient is treated within the first 10 days following onset of acute pharyngitis.

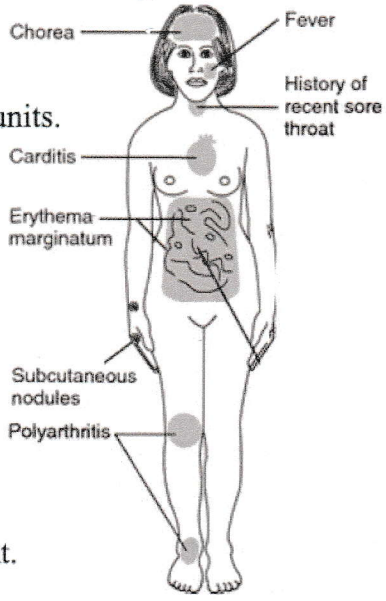
❖ Diagnosis of ARF:

- No single test is diagnostic. Diagnosis is usually based on the **modified Jones criteria.**
- Diagnosis requires an evidence of **recent** S. pyogenes infection **together with two major** criteria, **or one major** and **two minor** criteria.

A. Evidence of recent streptococcal infection: any of the following

- A history of acute tonsillitis (or scarlet fever).
- Positive throat swab culture for S. pyogenes.
- Elevation of antistreptolysin O (ASO) titre above 200 units.

☞ *A titre of up to 200 units is considered normal.*



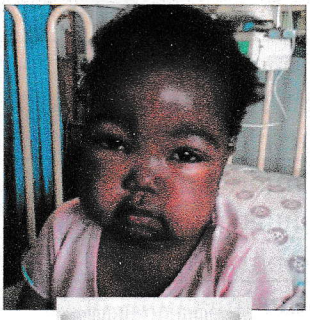
B. Major criteria:

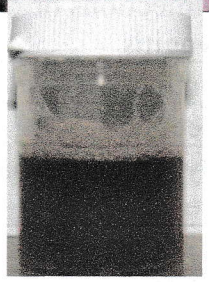
1) Carditis.
2) Migratory polyarthritis.
3) Erythema annulare.
4) Subcutaneous nodules.
5) Chorea.

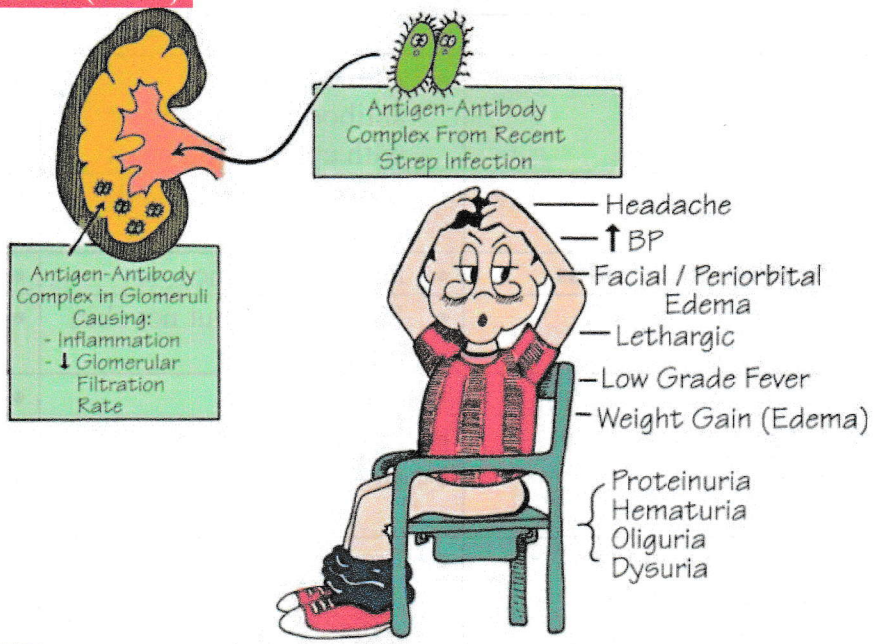
C. Minor criteria:

1) Elevated erythrocyte sedimentation rate, positive C-reactive protein or increased white cell count.
2) Fever. 3) Prior history of RF.

II. Acute glomerulonephritis (AGN):

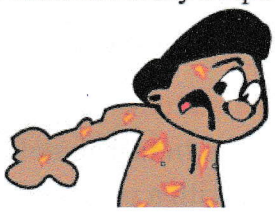






❖ **Pathogenesis:**

- It occurs due to **type III hypersensitivity:** deposition of antigen-antibody complexes on the glomerular basement membrane, provoking an inflammatory response that damages the kidney.



❖ **Age:**

- AGN affects **children** more than adults.

❖ **S.Pyogenes M serotype : nephritogenic**

❖ **Precipitating infection:**

- It develops **one week** mostly following **skin** infection rather than pharyngitis.

❖ **The disease is characterized by:**

- Hypertension, oedema of the face and ankles and smoky urine (due to RBCs in urine). Most patients recover completely and chronic renal failure rarely occurs.

❖ **Recurrence& Chemoprophylaxis:**

- Reinfection with streptococci **rarely** leads to **recurrence** of AGN and antibiotic **prophylaxis** is **unnecessary**.
- Treatment of the streptococcal skin disease or pharyngitis does **not prevent** AGN.

❖ **Diagnosis of AGN:**

- There are elevated antibody titres for **anti-DNase B.**
- **ASO titres :**
  - In AGN following pyoderma or **skin** infection, the ASO titres are generally **low**.
  - In AGN following **throat** infection (rare), the ASO titres may be **higher**.

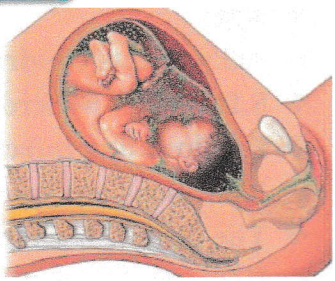
❖ **Treatment and Prevention:**

- **Penicillin** is still uniformly effective in treatment of S. pyogenes infections.
- **Long acting penicillin** is used as a chemoprophylactic agent against recurrent S. pyogenes infections to prevent repeated rheumatic attacks.
- **Erythromycin** is used as an alternative for penicillin-allergic patients.

❖ **Comparison between acute rheumatic fever & acute glomerulonephritis:**

	I. ARF	II. AGN
❖ **Pathogenesis**	• Anti-M protein antibodies cross- react with epitopes on heart	• Deposition of antigen-antibody complexes in the glomeruli
❖ **Age**	• Mostly between 4-30 years	• Children more than adults
❖ **S. pyogenes strains**	• Rheumatogenic	• Nephritogenic
❖ **Precipitating inf.**	• Pharyngitis (but not skin infection)	• Skin infections or pharyngitis
❖ **Recurrence**	• Common	• Uncommon
❖ **Chemoprophylaxis**	• Essential	• Unnecessary
❖ **Sequelae**	• Heart disease	• Rarely, chronic renal failure
❖ **Early treatment of precipitating inf.**	• Prevents the condition	• Does not prevent the condition
❖ **Serological test**	• ASO	• Anti-DNAase, ASO (doubtful)

II. Streptococcus agalactiae (Group B Streptococcus; GBS):



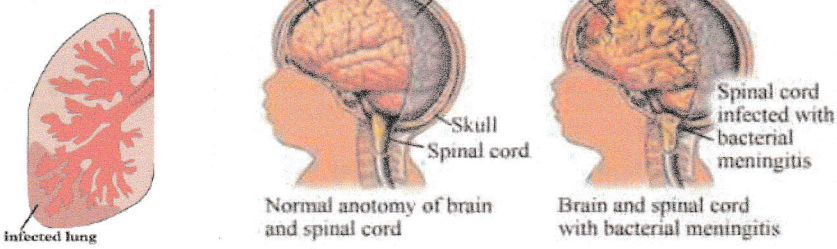
❖ **Culture characters:**

- S. agalactiae is **beta**-haemolytic, bacitracin-**resistant** streptococci with a polysaccharide capsule.

❖ **Pathogenesis:**

- About 25% of **pregnant** women are **vaginal** carriers for GBS.
- GBS infections are acquired by **neonates** at the time of birth; therefore, GBS are important aetiologic agents of infections during the first two months of life.

❖ **Diseases caused by S. agalactiae:**



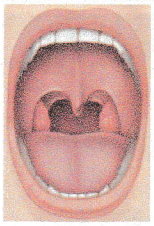
1) **Neonatal sepsis** which may manifest as pneumonia, septicaemia and **meningitis**.
2) Serious **infections in adults** e.g., pneumonia and endocarditis particularly in cancer and diabetic patients.

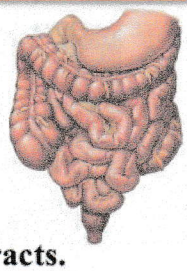
❖ **Prevention:**

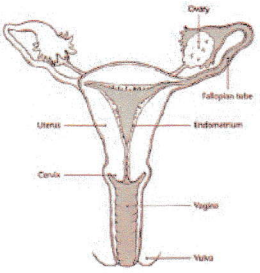
- Routine screening for S. agalactiae in pregnant women at the end of the 3rd trimester.
- Colonized mothers are given **ampicillin** during delivery (intrapartum) to reduce neonatal sepsis.

Alpha Haemolytic Streptococci

I. Viridans Streptococci:



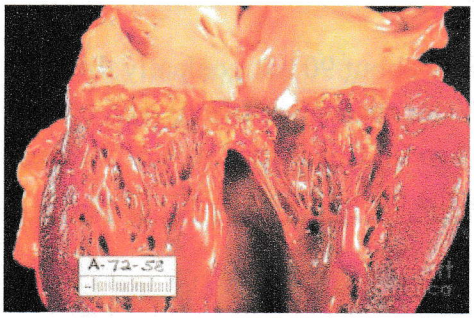




- They are **normal inhabitants** of the **oral cavity, gastrointestinal** and **female genital tracts.**
- **Viridans streptococci play a significant role in**

1) **Dental caries.**
2) **Subacute bacterial endocarditis (SBE)** (50% of all cases):

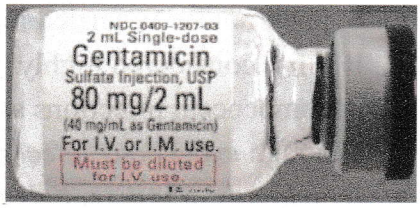
- SBE may occur when dental manipulations or trauma to mucosa of upper respiratory tract e.g. tonsillectomy, lead to bacteraemia.



- The organisms can **adhere** to cardiac valves, especially in people with underlying valvular disease (prosthetic valve, deformed heart valve e.g., rheumatic or congenital heart etc.) resulting in endocarditis.

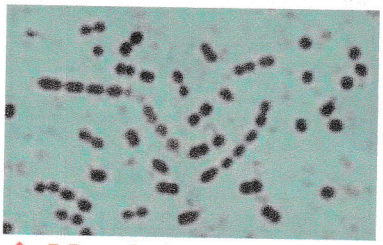
- **Laboratory diagnosis of SBE:**
- It is carried out by the **blood culture technique** with subculture on blood agar.
- The isolated organism should be discriminated from S. pneumoniae (see later).

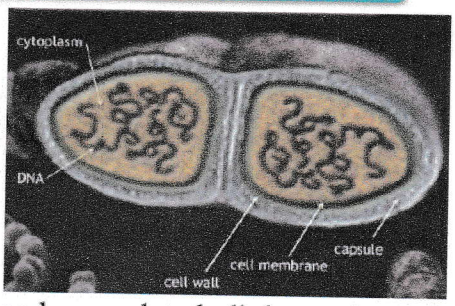
- **Treatment**
- Viridans streptococci are relatively **resistant to penicillin.**
- The use of the synergistic combination of **penicillin** and **gentamicin** in life- threatening infections, such as endocarditis, is essential.

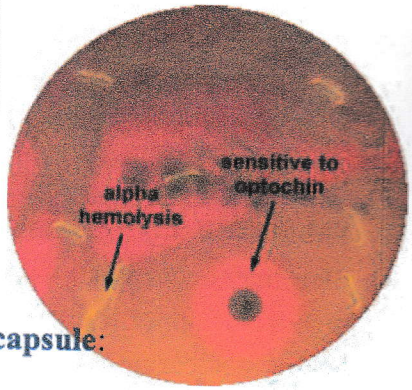


- **Prevention:**
- A single large dose of **ampicillin or amoxicillin** should be given to patients with abnormal heart valves prior to dental procedures or oral surgery to prevent endocarditis.

II. Streptococcus pneumoniae (Pneumococci):







❖ Morphology:

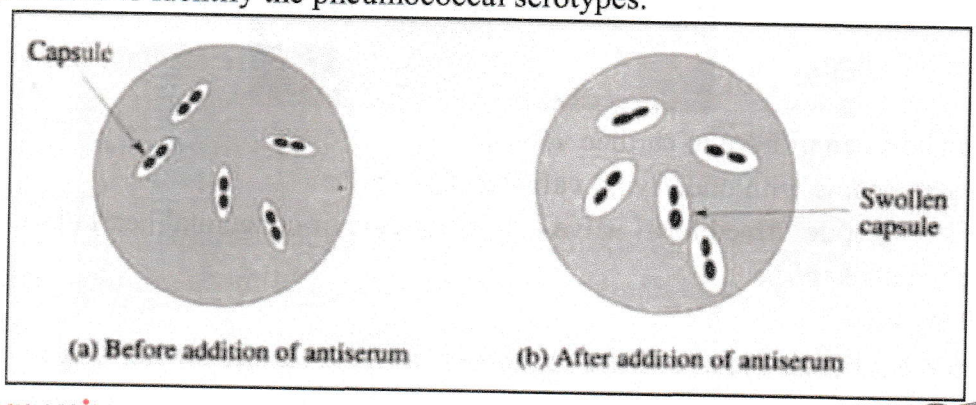
- Gram-positive, lancet-shaped, capsulated, diplococci.

❖ Culture:

- On blood agar, colonies show α haemolysis.

❖ Virulence factors::

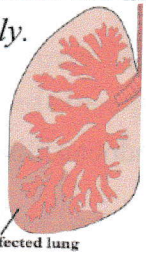
- The most important virulence factor is the **polysaccharide capsule**:
  - It is antiphagocytic.
  - It divides the organism into about 90 antigenically different serotypes.
  - The **capsule reacts with the specific antibody** and can be seen under the microscope to **swell**. This is the basis of the **quellung test** which is used to identify the pneumococcal serotypes.

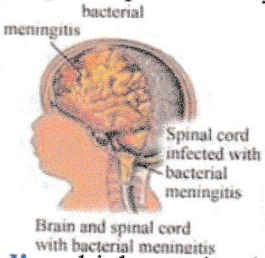


❖ Pathogenesis:

- S. pneumoniae is carried in the **pharynx** of about one third of adults.
- It is not considered highly communicable; therefore, pneumococcal infections are mostly **endogenous**, although *exogenous infections* by respiratory droplets may also occur.

- *Individuals at risk include the alcoholics, post-splenectomy, immunosuppressed, infants and the elderly.*
- Pneumococci cause:





  - **Pneumonia, meningitis and otitis media** which are the three major diseases.
  - *Sinusitis, conjunctivitis, endocarditis* and septic pericarditis may also occur.

❖ **Laboratory diagnosis:**

A. Specimens include sputum (characteristically blood-tinged 'rusty' sputum), CSF, ear or eye discharge and blood (in cases of bacteraemia accompanying pneumonia, meningitis and endocarditis).

B. Direct detection:

- **Gram-stained smear**: Gram-positive, capsulated, diplococci.
- The capsule appears as an unstained zone around the organism.
- **Quellung test.**
- **Detection of capsular polysaccharide antigen** in CSF by means of latex agglutination test.

C. Cultivation:

- Specimens other than the blood should be plated directly on blood agar and incubated at 37°C.
- Blood samples should be cultivated by the blood culture technique.

D. Identification:

- After 24 h incubation, the growth should be examined for colony morphology, catalase test and Gram stain.
- Colonies of viridans streptococci (non-pathogenic) are also encountered on blood agar while examining sputum for S. pneumoniae (pathogenic).
- Confusion occurs due to similarity in microscopic and colony morphology.
- **Discrimination between S. pneumoniae and viridans streptococci must be done according to :**

❖ Test	• S. pneumoniae	• Viridans strept.
❖ Growth inhibition by optochin	+	-
❖ Solubility of colonies in bile	+	-
❖ Capsular Ag detection (latex agglutination)	+	-
❖ Quellung test	+	-

❖ **Treatment:**

- **Third generation cephalosporins (e.g. ceftriaxone)** are the drugs of choice.
- Penicillin resistance in pneumococci has been reported.
- *Vancomycin* is the drug of choice for *penicillin resistant* pneumococci.

❖ **Prevention:**

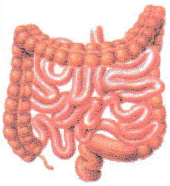
1) Capsular polysaccharide vaccine:

- It contains antigens from the most common 23 pneumococcal serotypes.
- **Anti-capsular antibody** confers type specific protection.
- The vaccine is used after splenectomy, in elderly and immunosuppressed patients.
- It is **not** effective in children less than 2 years of age who respond poorly to polysaccharide (thymus independent) antigen.

2) Protein conjugate vaccine:

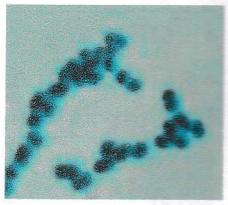
- It contains the capsular polysaccharide of the 13 most common pneumococccal serotypes conjugated to a **protein carrier** that makes the vaccine more effective in **children less than 2 years of age**.
- It is recommended to be given in 4 doses (at 2, 4, 6 months and 12-15 months)

Chapter 4: Enterococcus




- These organisms are **found normally** in the **human intestine** so they are used as an indicator of **faecal pollution of water**.
- The common species are **Enterococcus faecalis** and **Enterococcus faecium**.

❖ Characters of the genus Enterococcus:

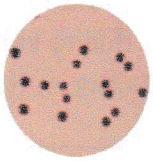


I. Enterococci are similar to streptococci in the following:

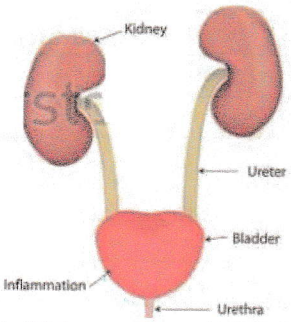
1) They are **Gram-positive cocci that occur in pairs or in short chains**.
2) They are **facultative anaerobes**.
3) They are **catalase negative.**
4) Most strains react with the streptococcal Lancefield group **D** antiserum.

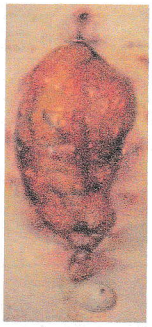
II. Enterococci differ from streptococci in being able to:

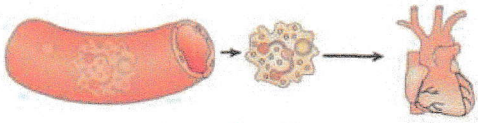
1) Grow at **45°C.**
2) **Salt tolerant**: Grow in broth containing 6.5% NaCl
3) **Tolerate bile salts**:
   - This allows survival of enterococci in bowel and gall bladder.
4) **Hydrolyze the polysaccharide esculin** producing black colonies on esculin- containing media.

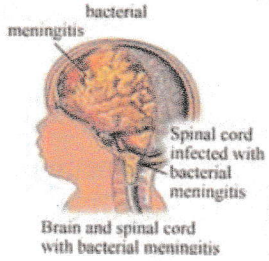


❖ Infections caused by enterococci:









1) Urinary tract infections are the most common.
2) Intra-abdominal or pelvic wound infections.
3) Bacteraemia.
4) Endocarditis.
5) Abscesses, meningitis, peritonitis, osteomyelitis and wound infection.

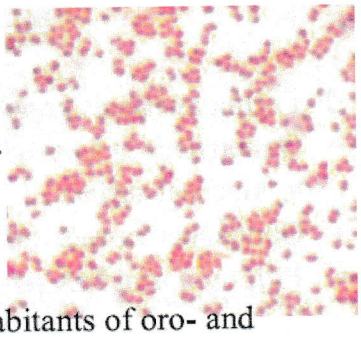
☞ *Enterococcus faecalis is most commonly isolated from healthcare-associated infections rather than community-acquired infections.*

❖ Treatment:

- Enterococci are frequently resistant to antibiotics.
- They are absolutely (intrinsically) **resistant to cephalosporins and clindamycin**.
- A synergic combination should be used for serious infections like bacteraemia, endocarditis or meningitis:

1) **Cell wall active drug** (penicillin, ampicillin, or vancomycin) **plus**
2) **Aminoglycoside** (gentamicin or streptomycin).

Chapter 5: Neisseria



❖ Characters of the genus Neisseria:

1. Gram-negative cocci arranged in pairs with the adjacent sides flattened to give the characteristic kidney or coffee bean shape.
2. Aerobic.
3. **Oxidase-positive (key test for the genus Neisseria).**

❖ The genus includes:

- Many commensals: e.g. *N. lactamica* and *N. sicca* that are inhabitants of oro- and nasopharynx of healthy individuals. They rarely cause diseases.
- Two important human pathogens:

✌ Neisseria gonorrhoeae (gonococci) & Neisseria meningitidis (meningococci).

I. Neisseria gonorrhoeae

❖ Morphology (mentioned above).

❖ Cultural characters:

- N. gonorrhoeae is **the most fastidious** neisseria species.
- It requires an enriched medium like **chocolate agar** but it does **not** grow on **blood** agar.
- **Modified Thayer-Martin (MTM) medium:**

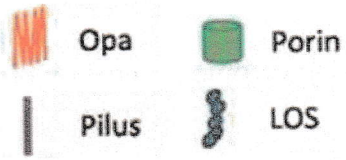
- Which is **chocolate** agar that is rendered **selective by** adding certain **antibiotics**, allows easier **isolation** of the organism from specimens contaminated by other microbes.

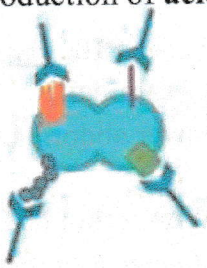
- Cultures should be incubated in a humid atmosphere with 5-10 % CO2 at 37°C.

❖ Biochemical reactions:

- N. gonorrhoeae is **oxidase positive.**
- N. gonorrhoeae utilizes **glucose only** with production of **acid only**.

❖ Virulence factors:





1. **Outer membrane proteins** which include:

A. Adherence proteins (Opa proteins): they get their name from the *opaque* appearance they give to colonies. They assist binding to epithelial cells.

B. Porin proteins: they promote intracellular **survival** inside phagocytes by:

- Preventing fusion of phagosomes and lysosomes.
- Suppressing the oxidative burst .

2. **IgA protease:**

- Inactivates secretory IgA leading to more adherence to and colonization of the mucosa.

3. **Pili:** mediate attachment to epithelial cells.
4. **Lipooligosaccharides (LOS):**

- A modified endotoxin that elicits an inflammatory response.

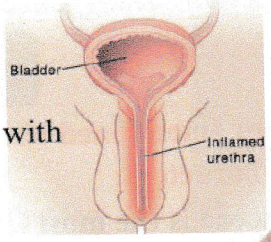
Diseases Caused by N. gonorrhoeae

1. Gonorrhoea:

❖ Pathogenesis of gonorrhoea:

- Gonorrhoea is a **sexually-acquired venereal disease** primarily localized to mucosal surface.
- Gonococci **cannot survive dryness**, so intimate contact is necessary for transmission.
- **Pili, Opa and IgA protease** allow adherence and colonization to mucosa.
- Endocytosis is followed by intracellular growth that causes destruction of epithelial cells.
- **Porin proteins** and catalase allow gonococci to survive inside polymorph- nuclear leucocytes.

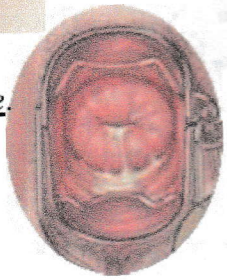
❖ Manifestations:



A. Men:

- Gonococci infect the urethra leading to acute *urethritis* with *dysuria* and purulent *discharge*.

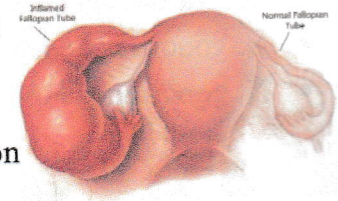
B. Women:



- Gonococci infect the cervix (**not vagina**), urethra, vulva and rectum leading to *cervicitis* with *dysuria* and with a purulent cervical *discharge*.
- About half of infections in women, however, are asymptomatic and may contribute to persistence and spread of gonorrhoea.

❖ Consequences of gonorrhoea:

A. Men:

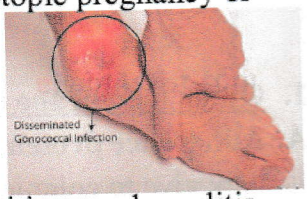


- The organism may *spread* to the prostate, bladder and epididymis causing inflammation and swelling.

B. Women:

- **Pelvic inflammatory disease; PID**: *spread* by ascending infection leading to endometritis and salpingitis.
- It manifests as fever and lower abdominal pain and may lead to ectopic pregnancy or sterility.

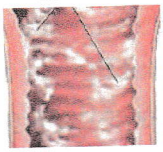
C. Disseminated gonococcal infection (DGI) or gonococcaemia:



- It occurs more in females (especially the pregnant) and individuals with defect of the **terminal** complement components.
- It may manifest as **arthritis** accompanied **with skin rash**, meningitis or endocarditis.
- DGI may result in disseminated intravascular coagulation (**DIC**) and shock due to the LOS endotoxin.

2. Neonatal conjunctivitis (ophthalmia neonatorum):

- It is an acute conjunctivitis in infants born to **mothers with gonorrhoea**.
- The eyes become infected at the time of delivery, and if untreated can lead to blindness.



3. Vulvovaginitis:

- Infection of the **vagina** and vulva in **young girls** due to sexual abuse.

❖ **Laboratory diagnosis of gonorrhea:**

A. Specimens:

- Urethral discharge from men and cervical and urethral discharge from women. Other specimens, e.g., cynovial fluid in case of arthritis may be examined.

B. Direct detection:

1. Gram-stained smears:

- **In men:**

- The finding of Gram-negative diplococci within some polymorph- nuclear leucocytes in urethral discharge is sufficient for diagnosis.

- **In women:**

- The use of the Gram-stained smear alone can be **difficult** to interpret.
- They can be **falsely positive** because of the presence of Gram- negative diplococci in the *normal* flora.
- And can be **falsely negative** because of the inability to see the *small number* of gonococci.

☞ ***Therefore, cultures of cervical specimens should be done.***

2. **A nucleic acid probe test** detects gonococcal nucleic acid in specimens.

C. Cultivation: The specimens are plated onto chocolate agar and MTM medium. Cultures should be incubated with 5-10% C02.

D. Identification:

1. Colonies should be examined by Gram stain and oxidase test. Colonies showing Gram-negative diplococci and positive oxidase test are considered Neisseria.
2. The organism is identified as N. gonorrhoeae by:

- Glucose utilization with production of acid.
- Nucleic acid probe.

❖ **Treatment:**

- **Third generation cephalosporins** (e.g. ceftriaxone) or **spectinomycin** is now recommended for treatment of gonococcal infections.
- Although N. gononfioeae is frequently resistant to **tetracycline**, this agent or azithromycin should be given due to the concurrent **Chlamydia** infection.

❖ **Prevention:**

- Chemoprophylaxis in the form of **erythromycin eye ointment** is essential to prevent gonococcal neonatal conjunctivitis.
- The use of **condoms** and the proper treatment of the symptomatic patients and their sexual partners are recommended.

❖ **N.B.: Repeated gonococcal infections** may occur although gonococcal antibodies are produced. This is due to:

1. High **antigenic variations** of gonococcal pili.
2. **Superficial** nature of the infection
3. Production of **IgA protease**

II. Neisseria Meningitidis

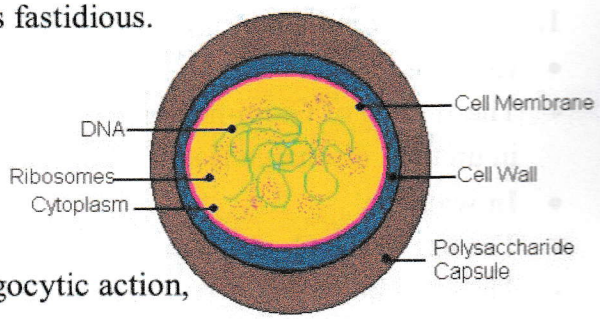
❖ **Morphology (as mentioned previously).**

❖ **Cultural characters:**

- N. meningitidis is similar in its cultural characters to N. gonorrhoeae except in its ability to grow on **blood agar** because it is less fastidious.

❖ **Biochemical reactions:**

- N. meningitidis is oxidase positive.
- N. meningitidis utilizes glucose and **maltose** with production of acid only.



❖ **Virulence factors:**

1. **The polysaccharide capsule**, with its antiphagocytic action, represents the most important virulence factor.
   - ☞ *The capsule classifies meningococci into at least 13 serogroups.*
2. **IgA protease** inactivates secretory IgA.
3. **Pili** are responsible for attachment to nasopharyngeal mucosal cells.
4. **LPS** is responsible for the endotoxin effect of meningococcal infections.

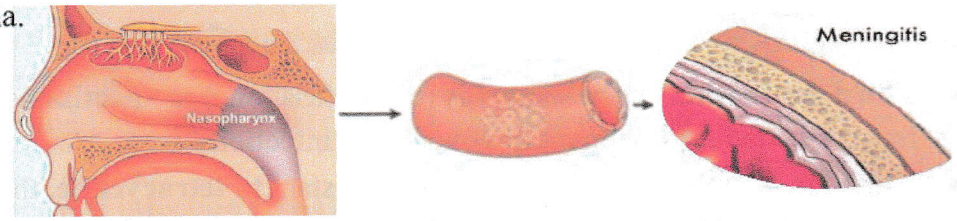
Diseases Caused By N. Meningitidis (Meningitis And Meningococcaemia)

❖ The organism causes 20% of all cases of meningitis which may occur sporadically or in epidemic form (**epidemic cerebrospinal meningitis**).

❖ The most common serogroups causing disease are A, B, C, Y, and W135.

❖ **Serogroup A** accounts for most meningococcal epidemics especially in Africa and some parts of Asia.

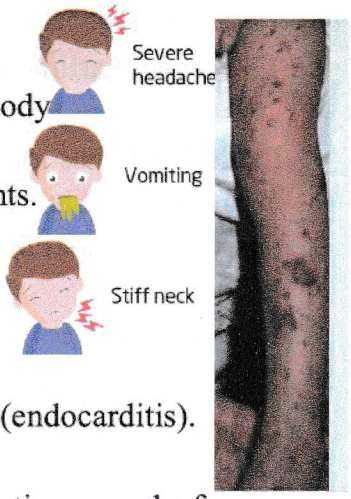
❖ **Pathogenesis:**



❀ N. meningitidis is **normally** carried in the **nasopharynx** in 5-30% of healthy population. This carrier state increases immediately before and during epidemics. Infection is transmitted by **droplets** from carriers and cases.

- **Pili** allow the attachment of the organism to the mucosal epithelium of the nasopharynx and together with **IgA protease** establish bacterial colonization.
- Endocytosis takes place and a slight local inflammation (sore throat) occurs.
- The virulence of meningococci is primarily due to the invasive capacity of the **capsulated** organism.
- The organism enters the bloodstream (**meningococcaemia**).
  From the blood, the organism may settle in different parts of the body.
  Localization of organisms in the meninges leads to meningitis and cerebral oedema.

❖ **Clinical manifestations:**



1. Susceptible hosts include:

A. Children under the age of 3 years because they fail to make antibody against the antiphagocytic capsule (**TI antigen**)

B. Individuals having defect in the **terminal** complement components.

2. Meningitis & cerebral oedema:
Sudden severe headache, projectile vomiting and stiff neck.

3. Meningococcaemia: spread to blood stream with **Skin rash** due to the **endotoxin** action of the LPS.

4. Localization of infection in the joints (arthritis) or endocardium (endocarditis).

5. Outcome :

- Coma and death or resolve with permanent neurological complications e.g. deafness.
- DIC In fulminant infections leading to shock and may end in death.

❖ **Laboratory diagnosis:**

☞ *Meningitis is a **medical emergency**, so diagnosis must be rapid and precise.*

A. Specimens: CSF, blood and aspirates from joint fluid.

- CSF examination: CSF shows characteristics of bacterial (septic) meningitis as follows:

– **Physically: CSF is cloudy and under tension.**

– **Cytologically: High leucocytic count (200-20,000/ml) with predominant neutrophils.**

– **Chemically: Reduced glucose level and elevated protein level.**

B. Direct detection:

1. Gram-stained smears prepared from CSF show Gram-negative intracellular diplococci.
2. Direct detection of meningococcal antigen in CSF by **latex agglutination or fluorescent antibody test.**

- The result is essential to direct the antimicrobial therapy and must be reported within one hour to the treating physician. Definitive diagnosis by culture is essential to apply the required infection control measures.

C. Cultivation:

1. Specimens other than the blood are plated onto chocolate agar, **blood agar** and MTM medium. Cultures should be incubated in a humid atmosphere with 10% C02 at 37°C.
2. Blood samples should be cultivated by the blood culture technique. Subcultures are plated on chocolate or blood agar and incubated as mentioned above.

D. Identification:

1. Colonies should be examined by Gram stain and oxidase test. Colonies showing Gram-negative diplococci and positive oxidase test are considered Neisseria.
2. The organism is identified as N. meningitidis by:

- Utilization of glucose and maltose with production of acid only.
- Detection of specific antigens by latex agglutination or immunofluorescence.
- Nucleic acid probe.

❖ **Treatment:**

- **Third-generation cephalosporins** such as ceftriaxone or cefotaxime are currently the drugs of choice for empiric therapy.
- **Penicillin G** has long been the treatment of choice for meningococcal infections because of its high anti-meningococcal activity and good penetration into CSF. Strains resistant to penicillin have rarely emerged.

❖ **Prevention:**

A. Vaccination:

- The vaccine provides 80-90% protection.
- Induced immunity lasts for about 3-5 years.
- Vaccination is important to groups at risk e.g. military recruits, school children, college students & travelers to certain parts of the world (e.g. pilgrims).

1. Capsular polysaccharide vaccines:

- Bivalent including serogroups A and C.
- Quadrivalent including serogroups A, C, Y & W-135.
  - ☞ *Group **B** capsular substance is **not** yet included in the vaccine. It is **poorly** immunogenic because of its **similarity to sialic acid** found in human tissues.*

2. **Protein conjugate vaccines** :

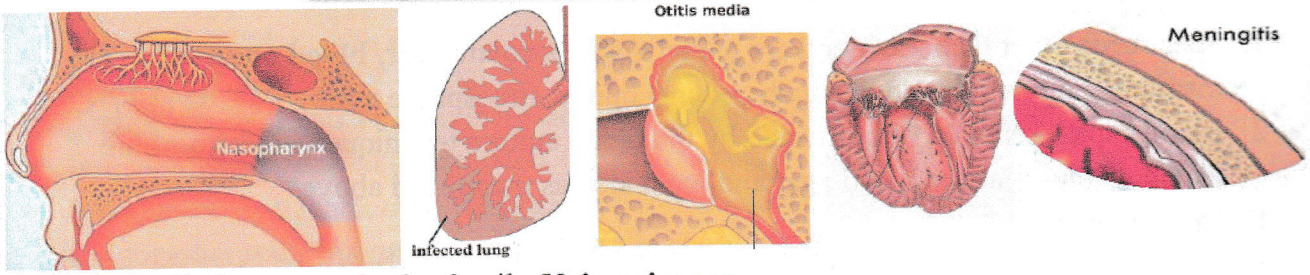
- Given to **children** less than two years old who respond poorly to polysaccharide (thymus independent) antigens.

B. Chemoprophylaxis:

- Recommended for close contacts e.g. healthcare and laboratory workers, household contacts and in outbreaks.
- The antibiotic recommended is either **rifampin** for 2 days orally or one **ceftriaxone** injection.

C. Droplet precautions including use of masks are recommended for the cases and contacts.

❖ Moraxella (Branhamella) Catarrhalis



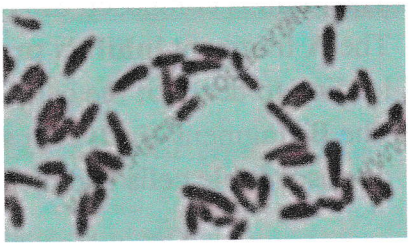
- Moraxella is a genus in the family **Neisseriaceae.**
- It is an **oxidase positive**, Gram **negative** organism that resembles neisseriae.
- It may occur as normal flora of the **nasopharynx** in humans.
- It causes **respiratory tract infections** including bronchitis, pneumonia, otitis media and sinusitis.
- It may cause **endocarditis and meningitis** in the elderly patients.

Chapter 6: Non Spore Forming Gram-Positive Bacilli

❖ **The Gram-positive non spore forming rods are subdivided into:**

- **Aerobic genera (Corynebacterium and Listeria).**
- **Anaerobic genera (Propionibacterium and Lactobacilli).**

Corynebacterium



❖ **Characters of the genus Corynebacterium:**

- Gram-positive bacilli with a characteristic club-shape, hence the genus name (club = Coryne).
- Aerobic.

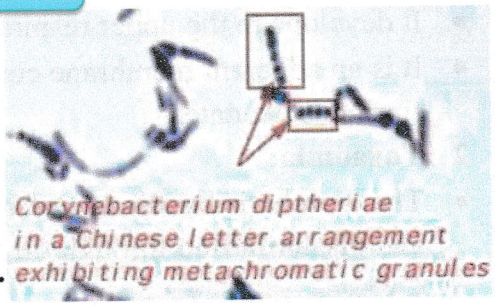
❖ **The genus comprises:**

- Corynebacterium diphtheriae, the causative agent of **diphtheria**.
- Many commensal species of skin and mucous membranes:
  - They are termed '**diphtheroids**' because they resemble C. diphtheriae in morphology.
  - They rarely cause disease in immunocompromised individuals.

Corynebacterium Diphtheriae

❖ **Morphology**

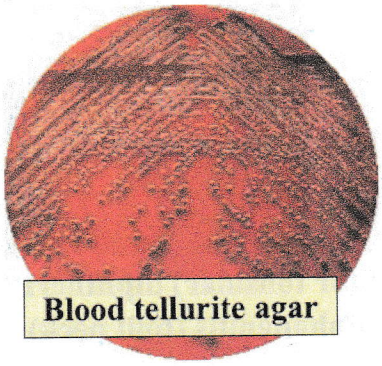
- Club-shaped pleomorphic Gram-positive bacilli.
- Arranged at acute angles or parallel to each other (**Chinese letters appearance**).
- The bacilli appear beaded when stained by **methylene blue** stain due to **metachromatic granules.**



Corynebacterium diptheriae in a Chinese letter arrangement exhibiting metachromatic granules

❖ **Cultural characters:** C.diphtheriae can grow on:

Loffler's Serum for *Corynebacterium diphtheriae*



Blood tellurite agar

- Enriched media e.g. **blood agar** and **Loeffler's** serum media.
- Selective differential media containing tellurite e.g. **: blood tellurite agar**
  - Used to isolate C. diphtheriae from sites where bacterial flora predominates. Corynebacterium colonies are gray/black on tellurite agar.

❖ Virulence factors:

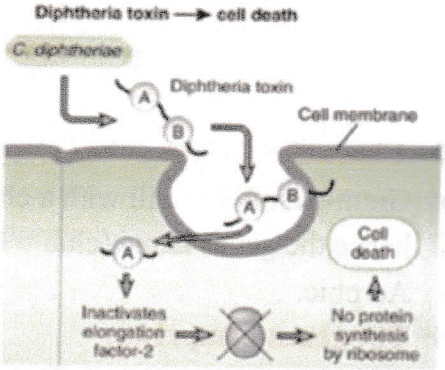
- The virulence factor of C. diphtheriae is **exotoxin.**
- All human cells are sensitive to the toxin especially the cardiac muscle and the cranial and peripheral nerves.
- Toxin production depends on:

1. The presence of a **lysogenic prophage** carrying the toxin gene in the bacterial chromosome.
2. **Low** extracellular concentration of **iron**.

- The toxin is composed of A and B subunits:

A. The **A** fragment **inhibits protein** synthesis leading to death of the cell.

B. The **B** fragment **binds to a specific receptor** on susceptible cells and mediates entry of fragment A.



Diphtheria

❖ Pathogenesis:

- Diphtheria is transmitted by **droplets**, from a case or a carrier.
- The incubation period of diphtheria is **2-5 days**.
- Diphtheria involves both local and systemic pathology:

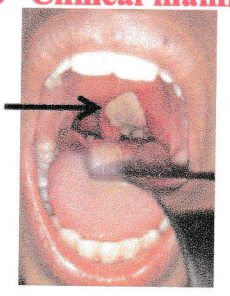
1. Local pseudomembrane:

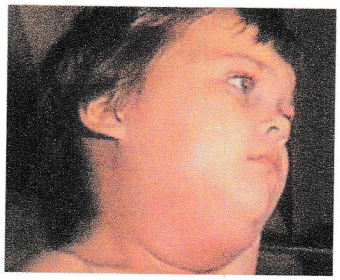
- It develops in the upper respiratory tract (tonsils, pharynx, larynx, and/or nose).
- It is an adherent membrane composed of mucosal cell debris, infection products and fibrinous exudates.

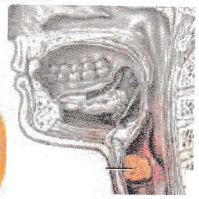
2. Toxaemia:

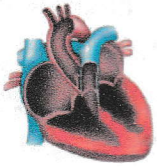
- The diphtheria bacilli do **not invade tissues**. They produce the **toxin** that is absorbed and **disseminated** through the blood to the susceptible tissues.

❖ Clinical manifestations









1) **Pharyngeal (or tonsillar) diphtheria** : The commonest form of diphtheria.
2) **Cervical lymphadenitis (bull neck)** is one of the initial symptoms.
3) **Severe toxaemia:** The patient appears quite toxic with low-grade fever.
4) **Complications:**

A. Extension of the membrane into the **larynx** causing airway obstruction.
B. Myocarditis leading to heart failure.
C. Nerve paralysis (especially cranial nerves).

❖ Laboratory diagnosis:

- The **diagnosis** of diphtheria is primarily **clinical**, and antitoxin therapy should be initiated **without waiting** laboratory diagnosis.
- Microbiological diagnosis serves to confirm the clinical diagnosis.
- This includes isolation of C. diphtheriae and detection of its toxigenicity.

A. Specimens: Throat swabs.

B. Direct detection in clinical specimens:

1. Gram-stained smears show club-shaped Gram-positive bacilli.
2. Methylene blue-stained smears show the characteristic beaded appearance.

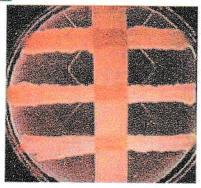
C. Cultivation:

- Culture is done on Loeffler's serum and blood tellurite media.
- A blood agar plate is also used for detection of **S. pyogenes for differential diagnosis**.

D. Identification:

- Diphtheriae is identified by colony morphology and microscopical examination of stained films.
- **Toxigenicity testing of the isolated C. diphtheriae strain is done by**:

1. **Elek's test:** The production of diphtheria toxin can be detected by the formation of **toxin-antitoxin precipitation bands** in the agar.
2. Detection of **toxin gene by PCR.**
3. Detection of toxin from **culture by ELISA.**



☞ *For diagnosis of carriers, throat or nasal swabs are subjected to the same procedures including toxigenicity testing.*

❖ Treatment

1. Diphtheria antitoxin:

- It must be given **immediately**, if diphtheria is clinically suspected.
- Antitoxin will **not neutralize** toxin that is already **fixed** to tissues, but will **neutralize the circulating (unbound) toxin**.
- The antitoxin may be given intramuscularly or intravenously **(IM or IV).**
- **Precautions** to avoid hypersensitivity reactions (anaphylactic shock or serum sickness): As diphtheria antitoxin is usually produced in horses.

2. Antibiotics:

- **Penicillin or erythromycin** is the drug of choice.
- Antibiotics inhibit growth of the organism, thus, reducing toxin production.
- Antibiotic therapy also decreases the incidence of chronic carriers.

3. Respiratory support and airway maintenance may be needed.

❖ Prevention:

A. Active immunization:

- Diphtheria is a **preventable disease** by routine active immunization with diphtheria toxoid.
- **Diphtheria toxoid** is prepared by treating the **exotoxin with formaldehyde**.

- Diphtheria toxoid may be **combined with**:

1) Tetanus toxoid (as pediatric DT or adult Td), or
2) With both tetanus toxoid and pertussis vaccine (DPT).

- Vaccine is given by intramuscular injection at 2, 4, 6, and 18 months of age with a booster dose at school entry (6 years).
- A booster dose of Td is then recommended every 10 years.

B. For close contacts:

- A booster of diphtheria **toxoid** with antibiotic **chemoprophylaxis** are recommended.

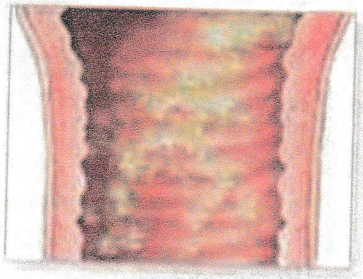
Gardnerella

❖ **Morphology:**

- Gardnerella vaginalis is a Gram **negative** or Gram-variable bacillus.
- G. vaginalis, formerly known as **Corynebacterium vaginalis**, is now classified as a separate genus termed Gardnerella.

❖ It is part of the **vaginal flora** in women, during the reproductive age.

❖ **Diseases:**



Bacterial Vaginosis

A. Gardnerella vaginalis is associated with **bacterial vaginosis** (BV):

- A nonspecific vaginitis that is accompanied by thin vaginal discharge with a bad odour.

B. Women with BV have a higher incidence of **preterm labour**.

❖ **Laboratory diagnosis is done on vaginal discharge:**

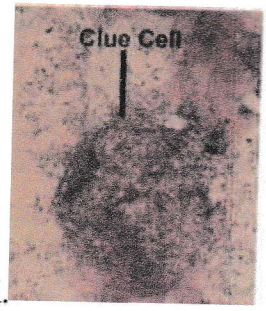
1) **Direct Gram-stained smear reveals** :

A. Presence of clue cells

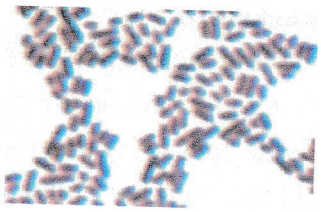
- Clue cells :vaginal epithelial cells covered with G. Vaginalis

B. Absence of lactobacilli.

2) An **increased pH (>5)** which is due to reduced number of lactobacilli.
3) **Whiff test:** addition of 10% KOH to the sample gives fishy odour.



Listeria



❖ Morphology:

- Short Gram positive, non spore forming rods, resembling corynebacteria except in being **motile**.

❖ Culture:

- Listeria grows on blood agar producing (**β-haemolysis**).
- Listeria grows well at cold temperature e.g. 4°C (**cold enrichment**).

❖ Pathogenesis:

1) Listeria species are found in a diversity of environmental sources including soil, water, food and faeces of humans and animals.

Listeria monocytogenes is the only species that infects humans.

- Listeria monocytogenes is **transmitted to humans by :**

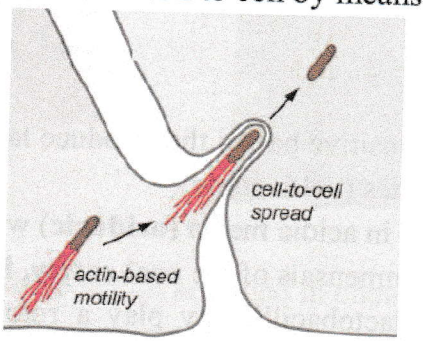
A. Contact with **domestic farm animals** or their faeces.
B. By **milk**
C. By contaminated vegetables (i.e. **food-borne**).

D. **Vertical transmission** can also occur transplacentally or during delivery.

2) **Virulence factor: listeriolysin-O**

- Being a facultative intracellular pathogen, L monocytogenes can *survive inside* the macrophages due to its ability to produce a membrane-damaging toxin called **listeriolysin-O**.
- This toxin allows the organism to *escape from phagocytic* vesicle to enter **the cytosole.**

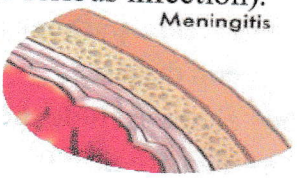
3) **Movement of L. monocyogenes** from cell to cell by means of **actin rockets**:



- A filament of actin that contracts and propels the bacteria through the membrane of one cell into another.
- The passage of the organism directly to a neighbouring cell allows *avoidance of the immune defensive mechanisms.*

❖ Diseases:

1) **Abortion,** premature delivery or sepsis during the peripartum period.
2) **Neonatal meningitis** (immunologic immaturity predisposes to serious infection).
3) **Septicaemia and meningitis** in immunocompromised adults.



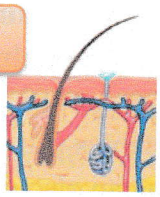
4) Food poisoning:

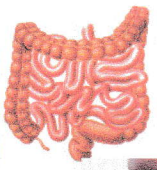
- L monocytogenes **resists** freezing, drying and heat:
  - *This enables the organism to survive food processing and cause food poisoning.*
- **Outbreaks** caused by *dairy products* (e.g., fresh soft cheese) and *under-cooked meat* (e.g., chicken and hot dogs).
- The organism multiplies and invades the intestinal epithelium.
- Incubation period ranges between **8-48 hours**.
- The disease is characterized by **watery diarrhoea**, fever and abdominal cramps but little vomiting.

❖ **Treatment:**

- **Ampicillin** is the drug of choice.

Propionibacterium





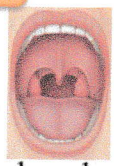
❖ They are anaerobic Gram-positive bacilli, having the typical morphology of *diphtheroides*.

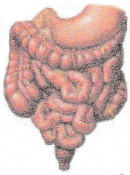
❖ Their metabolic products include **propionic acid** from which the genus name is derived.

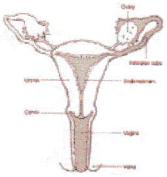
❖ P. acne is normally present in human **skin and GIT**.

❖ It is the major contributor to the complex pathogenesis of **acne vulgaris**.

Lactobacillus





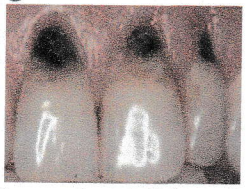


❖ They are anaerobic Gram positive bacilli that produce large quantities of **lactic acid** by fermentation of carbohydrates (**acidogenic**).

❖ They can survive and grow in acidic media (**aciduric**) with pH 4-5.

❖ Lactobacilli are normal commensals of the **oral cavity, intestine and vagina**.

❖ Acid production by oral lactobacilli may play a role in the progression of **dental caries**.



❖ **Benefits :**

A. **Protective effect in the vagina** due to acid production which inhibits colonization with pathogenic organisms.

B. **Treatment of intestinal disturbances** especially nosocomial diarrhoea in children by living cultures of lactobacilli ().

❖ **N.B.: Probiotics :**

- Are **live, non-pathogenic bacteria** that may be effective in the treatment or prevention of certain diseases.
- They either **exclude** the pathogen from **binding sites** on the mucosa or **enhance** the **immune response** against the pathogen.

Chapter 7: Spore Forming Gram-Positive Bacilli

❖ **The Gram-positive rod-shaped bacteria that form endo-spores, have two principal subdivisions:**

A. The aerobic or facultative anaerobic genus Bacillus.

B. The anaerobic genus Clostridium.

Bacillus

❖ **The genus Bacillus includes:**

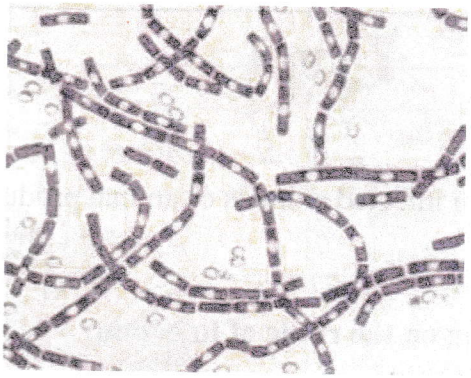
A. Two important pathogenic species of humans and animals:

- **Bacillus anthracis which causes anthrax.**
- **Bacillus cereus which causes food poisoning.**

B. **Harmless** saprophytes which are found everywhere in nature and are generally called **anthracoids.**

Bacillus anthracis

❖ **Morphology:**

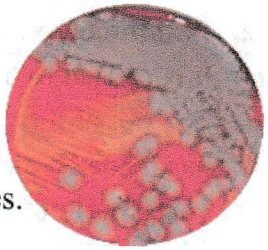


McFadyean reaction(polychrome MB stain)

- Large, rectangular, Gram-positive bacilli arranged in chains.
- They form oval central non-bulging spores which are stained with **modified Ziehl-Neelsen** (spore) stain.
- B. anthracis spores may survive in dry soil for years.
- They produce a **polypeptide capsule** which is detected in smears from infected tissues after staining by **polychrome methylene blue**:
  The organisms appear as blue rods in a purple/pink-stained capsular material (McFadyean reaction).

❖ **Cultural characters:**

- Anthracis can be cultivated in simple **nutrient** media under aerobic or anaerobic conditions.
- Anthracis grows well on **blood** agar, forming **non**-haemolytic colonies.



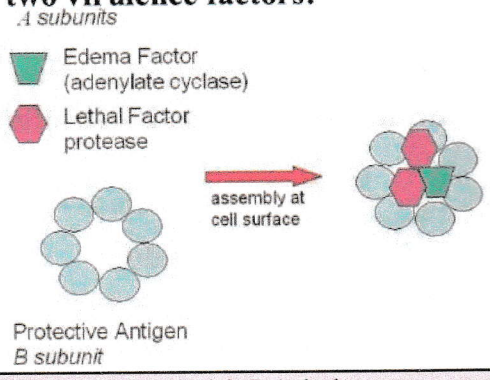
Anthrax

❖ Pathogenesis:

- **The pathogenicity of B. anthracis depends on two virulence factors:**

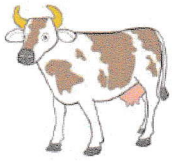
1. **Capsule:** that protects it from phagocytosis.
2. **Anthrax toxin:**

- The organism produces three distinct proteins:
  - ***Edema factor (EF),***
  - ***Lethal factor (LF),***
  - ***Protective antigen (PA).***

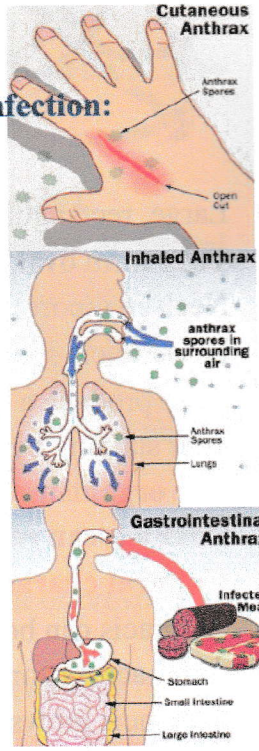


○ EF complexed with PA is known as **oedema toxin**, ○ The EF (the A or active subunit of the oedema toxin) has an **adenylate cyclase** activity resulting in severe oedema.	○ LF complexed with PA is known as **lethal toxin**. ○ The LF (the A or active subunit of the lethal toxin) has a **protease** activity which is responsible for tissue necrosis.
○ The PA (the B or binding subunit) mediates **binding** of each toxin to the target tissue. It is called the protective antigen because it has been used in producing a **protective vaccine.**	

❖ Clinical manifestations:



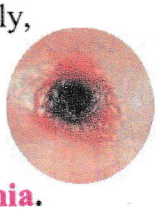
- **Mode of transmission:**
  - ○ Anthrax is primarily a disease of **farm animals**.
  - ○ Humans acquire anthrax as a result of contact with infected animals or animal products (**zoonotic disease**).
  - ○ There is **no** person-to-person transmission of anthrax.



- **The disease takes one of three forms, depending on the route of infection:**

1. **Cutaneous anthrax (malignant pustule):**

- It occurs from handling infected material.
- Spores from the soil or from an infected or dead animal enter through a skin cut or abrasion, usually on an exposed area.
- The spores germinate, and vegetative cells multiply locally, producing the anthrax toxin.



- The typical lesion is **malignant pustule**: a painless ulcer with a black eschar surrounded by marked oedema.
- Untreated cases may develop fatal fulminating **septicaemia**.

2. **Pulmonary anthrax:**

- It is caused by inhalation of spore-laden dust.
- The possibility of creating **aerosols** containing anthrax spores has made B. anthracis a chosen **weapon of bioterrorism**.

3. **Intestinal anthrax** occurs rarely through ingestion of infected meat.

❖ Diagnosis:

- The clinical diagnosis of anthrax is confirmed by *directly visualizing or culturing* the anthrax bacilli.
  - ☞ *Demonstration of capsulated B. anthracis from human and animal specimens, **even in low numbers**, confirms a clinical suspision of anthrax.*

❖ Treatment:

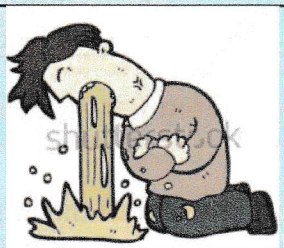
- **Ciprofloxacin** is the drug of choice.

❖ Prevention:

- People at high risk can be immunized by a **cell-free vaccine containing purified protective antigen** (PA).

Bacillus cereus

❖ Bacillus cereus causes two types of food poisoning:

	I. The emetic form	II. The diarrhoeal form
		
❖ **Incubation period**	• Short (1 to 6 hours)	• Long (8 to 16 hours)
❖ **Manifestations**	• Vomiting & abdominal cramps	• Diarrhoea & abdominal cramps
❖ **Aetiology**	• Preformed heat-stable enterotoxin (emetic toxin)	• Heat-labile enterotoxin (diarrhoeal toxin)
❖ **Action of toxin:**	• Irritation of gastric mucosa	• Activation of intestinal adenylate cyclase leading to intestinal fluid secretion.
❖ **Associated food**	 • Fried rice (especially from Chinese restaurants)	 • Meat dishes
❖ **Similar to food poisoning caused by**	• S. aureus	• C. perfringens

Clostridium

❖ Characters of the genus Clostridium:

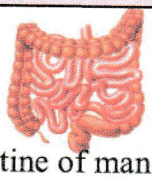
1. Large Gram-positive, spore-forming rods.
2. Anaerobic.
3. Most species are **motile**.
4. The natural habitat of Clostridium species is soil or the intestinal tract of animals and humans.

- The most important diseases caused by Clostridia species are gas gangrene, tetanus, botulism, pseudomembranous colitis and food poisoning.

❖ Clostridia Causing Gas Gangrene:

❖ The primary pathogen can be one or more of various clostridial species	
A. Saccharolytic Clostridia	**B. Proteolytic Clostridia (occasional)**
• C. perfringens (80%). • C. novyi. • C. septicum	• C.bifermentans. • C. histolyticum. • C. fallax

Clostridium Perfringens



❖ Clostridium perfringens is **normally present in** the large intestine of man and animals; therefore, it is considered one of the indicators of **faecal pollution of water**.

❖ Morphology:

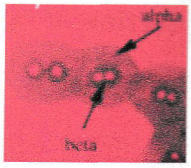
- Gram positive large rectangular bacilli, with oval sub-terminal spores.

❖ Culture:

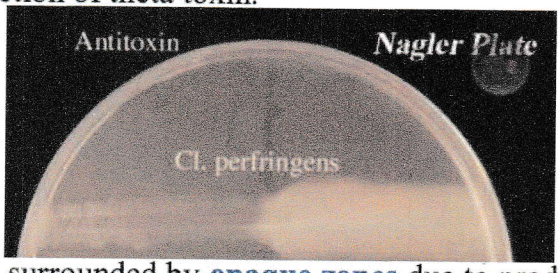
1. Anaerobic organism.
2. On blood agar, colonies are surrounded by a unique **double zone of haemolysis**:

A. α -haemolysis due to production of α-toxin

B. β-haemolysis due to production of theta toxin.



3. On **egg yolk agar**:



- C. perfringens colonies are surrounded by **opaque zones** due to production of α-toxin (**Nagler's reaction**).
- This effect can be inhibited specifically by antibody against α-toxin of C. perfringens.

❖ Virulence factors:

- C.perfringens elaborates at least 12 exotoxins, designated by Greek letters.
- These **exotoxins have haemolytic, cytotoxic and necrotic effects**.
- The most important of these is **α-toxin (lecithinase)** which lyses cell membrane lecithins, damaging cell membranes and causing cell death.

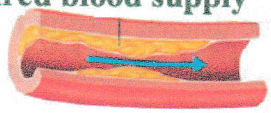
Gas Gangrene = Clostridial Myonecrosis

- ❖ It is an acute disease with a poor prognosis and often fatal outcome.
- ❖ The infection proceeds very rapidly and causes acute pain.

- ❖ **Pathogenesis and clinical findings:**

1) Clostridial wound infections:

- Are **polymicrobic** as the organism and its spores are found in the soil and in human and animal faeces.
- Occur in an **anaerobic tissue** environment caused by an **impaired blood supply** secondary to trauma, surgery, foreign bodies or malignancy.

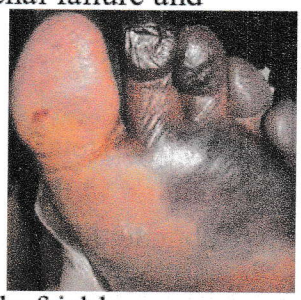


2) Spore access & Germination :

- The spores gain access to traumatized tissues by contamination from faeces or soil.
- **Lack of oxygenation** allows germination of the spores and growth of Clostridia.

3) Multiplication, toxin & spread:

- The organisms multiply in the subcutaneous tissues, producing **gas and an anaerobic cellulitis.**
- The organisms invade deeper into the muscle where they produce **exotoxins** especially **α-toxin** causing extensive cell death, and enzymes e.g., hyaluronidase and collagenase which facilitate the spread of infection.
- Spread of toxins to **bloodstream** causing massive haemolysis, renal failure and eventually death.



4) Muscle necrosis:

- **Fermentation** of tissue carbohydrates yields gas which rapidly accumulates and dissects along tissue planes.
  The gas bubbles can be felt in the tissues **(crepitations).**
- Infected muscle is discoloured, oedematous and produces a foul-smelling exudate, frankly **gangrenous**, black and extremely friable.

- ❖ **Diagnosis:**

- Diagnosis is based on **clinical** findings. Treatment must be promptly initiated on a clinical basis **without waiting** for laboratory confirmation because gas gangrene may spread and cause death within hours. Laboratory diagnosis is done as follows:

A. Specimen is taken from the depth of the wound and examined rapidly.

B. Gram-stained smear shows large Gram positive bacilli. There is typically an absence of PMNs due to the clostridial toxins.

C. Cultivation on **two blood agar plates**, one incubated anaerobically and the second incubated aerobically at 37°C for at least 48 hours.

D. Identification: Colonies of C, perfringens grow only anaerobically and are further identified by morphology, culture characters, Nagler's reaction and **sugar fermentation**.

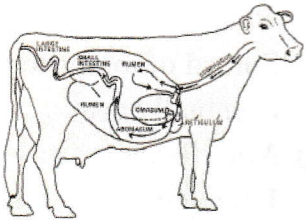
❖ **Treatment and prevention:**

- **Surgical** debridement and removal of foreign materials.
- **Antibiotics**: penicillin is the drug of choice.
- **Hyperbaric oxygen chamber**: to force oxygen into the wound.
- **Anti-alpha toxin antiserum**: may help if given early enough.

Clostridium Perfringens Food Poisoning

- C.perfringens is a major cause of food poisoning.
- The disease results from ingestion of a **large number** of organisms in contaminated food, usually meat or meat products. Following ingestion, C. perfringens produces an **enterotoxin** (in vivo).
- The incubation period is **8-24 hours** after ingestion of contaminated food.
- Symptoms include **watery diarrhoea**, cramps and abdominal pain.
- The disease lasts only about **24 hours**.
- **No specific treatment** is required, only fluid therapy is needed to correct the electrolyte imbalance.

Clostridium Tetani



❖ Clostridium tetani is the causative agent of **tetanus**.

❖ **It is found in** the **intestinal tracts and faeces of various animals** and hence its spores are abundant in soil, especially heavily manured soils.

❖ **Morphology:**

- C.tetani is a Gram-positive rod. It forms a bulging terminal spore that gives the organism a characteristic **drumstick appearance**.

❖ **Culture:**

- C.tetani is a **strict anaerobe**.
- It grows well on simple and **cooked meat media.**
- On blood agar, colonies are surrounded by zones of **β-haemolysis** due to the production of heamolysin.

❖ **Virulence factors: Tetanospasmin toxin (Neurotoxin):**

- It is of a **single** antigenic type although it is produced by different strains of C. tetani.
- It is among **the most toxic** substances known.
- The toxin is composed of **A** and **B** subunits

Tetanus

❖ Tetanus is a highly fatal disease; the mortality rates vary from 40% in adults to 90% in the neonates.

❖ **Pathogenesis:**

1) C.tetani infections :

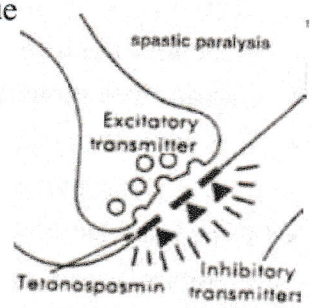
- Tetanus spores are wide-spread in soil and originate from the faeces of domestic animals.

2) Spore access & Germination :

- Most cases of tetanus result from lacerations or small puncture wounds (e.g. nail puncture) contaminated with C. tetani spores.
- Germination of spores is favoured by presence of **necrotic tissue and poor blood supply** in the wound.

3) Multiplication, toxin & spread:

- The vegetative cells of C. tetani grow locally in the necrotic tissue and elaborate **tetanospasmin toxin.**
- Tetanospasmin spreads to **central nervous system** by retrograde transport within the axon and probably haematogenously .

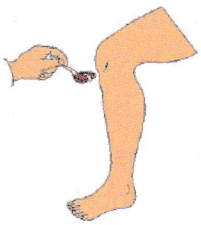


4) Spastic paralysis:

- **The B (binding) subunit** mediates binding to gangliosides and penetration of the A (active) subunit.
- **The A subunit blocks the release of the inhibitory mediators** (glycine and gamma-aminobutyric acid) at spinal synapses, thereby causing *severe prolonged muscle spasm* (spastic paralysis).

❖ **Clinical manifestations:**

1. Incubation period of tetanus varies from **4 days to several weeks**, depending on severity of the wound and proximity to the brain.
2. **Specific clinical features include:**



- **Lock jaw (trismus)** due to rigid contraction of the jaw muscles.
- **Arching of the back (opisthotonus)** due to spasm of strong extensors of the back.
- **Exaggerated reflexes** to any external stimulus (e.g., noise or light).

3. **Death** is usually the result of paralysis of the chest muscles leading to **respiratory failure.**

4. **Neonatal tetanus** is seen in newborns of **mothers lacking specific immunity**.

- It occurs as a result of contamination of the umbilical stump with C. tetani spores due to lack of aseptic technique during labour.

❖ **Diagnosis:**

- Diagnosis is a **clinical** one.
- Successful treatment depends on early diagnosis **before** a lethal amount of toxin becomes **fixed** to neural tissue.
- Laboratory diagnosis may be done for confirmation:
  - Specimens from the depth of the wound are examined.
  - The organism is identified by its morphology and culture characters (see before).

❖ **Treatment:**

1. **Human tetanus immunoglobulin (HTIG):**

- To neutralize the toxin in the blood, tetanus antitoxin is given rapidly.
- Human rather than horse antitoxin is used to avoid hypersensitivity reactions.

2. **Local debridement of the wound :**

- After the patient's spasms are controlled by sedatives and muscle relaxants.

3. **Metronidazole or penicillin:**

- Is usually administered to kill the bacteria and reduce further toxin production.

4. **Supportive measures:**

- E.g. respiratory assistance and intravenous fluids, are often critical to patient survival.

❖ **Prevention and control:**

- **Prophylactic immunization is the only way to control tetanus** because the spores of C. tetani are so widely disseminated in nature and cannot, therefore, be avoided.
- Prophylactic immunization provides the individual with neutralizing antibodies to tetanus toxin in the blood.
- The elaborated toxin is of a **single** antigenic type making immunization **effective**.
- This could be achieved by active immunization by **tetanus toxoid** (formaldehyde-inactivated toxin).
- **The toxoid is given to:**

A. Infants in the first year of life:

- The triple vaccine (DPT) is given at the age of 2, 4 and 6 months by intramuscular injection. Booster doses are given at 18 months and upon school entry.
- **N.B.:** Booster doses (Td) are given every 10 years to maintain immunity. More frequent boosters are unnecessary and may cause hypersensitivity reactions.

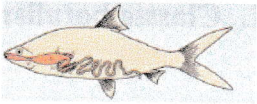
B. Wounded individuals:

- A booster dose of tetanus toxoid is given if last booster dose was >5 years ago.
- **N.B.: Antitetanic serum (HTIG)** for passive immediate protection should be given to:
  - Individuals with no history of vaccination
  - Last booster dose was >10 years ago
  - If the wound is grossly contaminated (passive/active immunization).

C. People at high risk e.g. military personnel.

D. Pregnant females to prevent tetanus neonatorum.

Clostridium Botulinum



- ❖ Clostridium botulinum is widely distributed in soil, sediments of lakes and ponds, and decaying vegetation.
- ❖ Hence, **the intestinal tracts of mammals, fish and birds** may contain the organism.
- ❖ **Morphology:**
- C.botulinum is a large Gram-positive bacillus that forms oval subterminat spores.
- ❖ **Virulence factors : Botulinum toxins (Neurotoxins):**
- There are several immunologically distinct botulinum toxins.
- **Types A, B and E cause human botulism.**
- **Type A toxin is the most potent toxin in existence** (1 gm would be enough to kill 14,000 people if ingested or 1.25 million people if inhaled!).
- The botulinum toxins are **similar** in structure **to the tetanus toxin** (A/B model).
- ❖ **Pathogenesis:**

1) C. botulinum infections :

- Spores, widespread in soil, contaminate vegetables and meat.
- The highest-risk foods are vegetables such as green beans and mushrooms, smoked and salted fish, and commercially canned salmon.

2) Spore access & Germination :

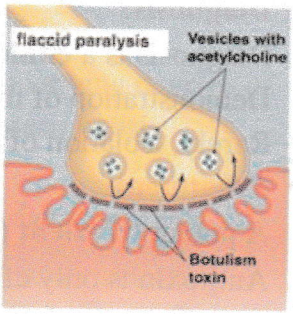
- When these foods are **canned without adequate sterilization**, spores survive and germinate in the **anaerobic** environment.

3) Multiplication, toxin & spread:

- Toxin is produced within the canned food and **ingested preformed**.
- The toxin is absorbed in the intestine and is transported systemically via the bloodstream to reach the **peripheral neuromuscular synapses**.

4) Flaccid paralysis :

- The toxin binds to the neurone and **prevents the release of acetylcholine** across the synaptic cleft. Thus, it produces paralysis of the motor system (flaccid paralysis).

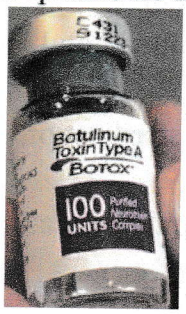


Neuromuscular junction with botulism toxin present

❖ N.B.: **Botox** is a commercial preparation of **exotoxin A** used to :

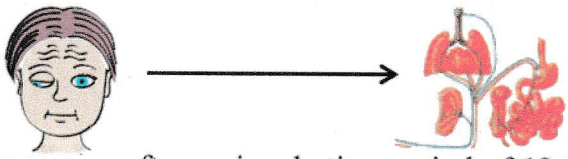
A. Remove wrinkles of the face.

B. Treatment of spasmodic muscle disorders e.g. torticollis.



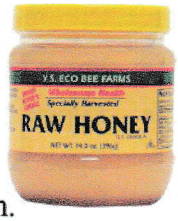
❖ Clinical forms:

1. Classic botulism:



- Manifestations start to appear after an incubation period of **12-36 hours**.
- The **cranial nerves** are affected first (causing blurred vision, inability to swallow and difficulty in speech), **followed by** a descending, symmetric paralysis of **motor nerves**.

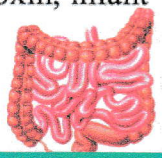
☞ *Nausea and vomiting are **not** usually prominent. **No** fever is apparent.*



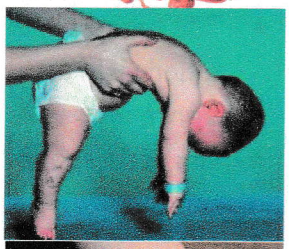
- Mortality rate is about 15% and is usually caused by respiratory failure.

2. Infant botulism:

- Ingestion of food supplements containing **raw honey** contaminated with botulinum spores has been implicated in transmission of infant botulism.
- In contrast to classic botulism, which is caused by ingestion of preformed toxin, infant botulism results from **germination** of spores **in the gastrointestinal tract** where vegetative cells replicate and release the botulinum toxin.



- C.botulinum causes the disease in infants between **2 weeks and 6 months** of age **before** the establishment of competing intestinal flora.
- The disease is characterized by constipation, weak sucking ability and generalized weakness **(floppy baby syndrome).**
- Almost all cases recover.



3. Wound Botulism:

- A rare form of botulism which occurs when a wound becomes contaminated with the organism and toxin is absorbed from that site.
- It may occur among **drug addicts** (skin popping with heroin).

❖ Diagnosis:

- Diagnosis is a **clinical** but may need laboratory confirmation as follows:
  - Demonstration of the toxin in serum, stools and food.
  - Rarely, isolation of the organism is done.

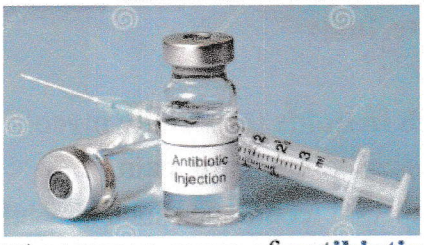
❖ Treatment:

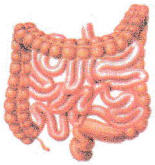
- A potent **trivalent (A, B, E) antitoxin** is available.
- Antitoxin is only effective if it binds to the toxin **before** the toxin binds the neuromuscular junction (**within 12 hours after ingestion**).
- Serum sickness may occur because the antitoxin is made in horses.
- There is no reason to give antibiotics except in infant and wound botulism.

❖ Prevention:

- The most important aspect of botulism prevention is proper sterilization of canned food.
- Because the toxin is heat-labile, **boiling for 10 minutes** or intense heating (cooking) of contaminated food will inactivate the toxin.
- Swollen cans must be discarded.

Clostridium Difficile





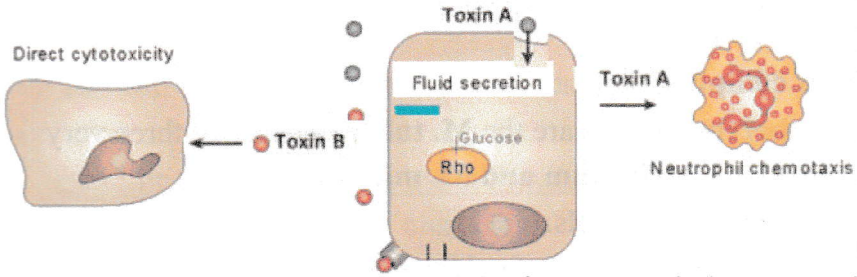
- ❖ C.difficile is the most common cause of **antibiotic-associated diarrhoea.**
- ❖ The organism is carried in the **intestinal tract** in approximately 3% of the general population and up to 30% of hospitalized patients.
- ❖ The hands of hospital personnels play an important role in **faeco-oral transmission.**

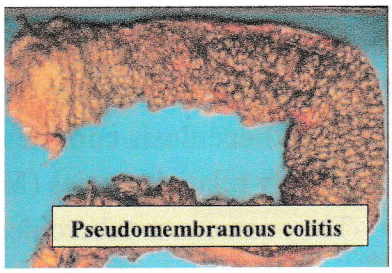
❖ Morphology:

- C.difficile is a spore-forming, Gram-positive bacillus.

❖ Virulence factors and pathogenesis:

- C.difficile is a minor component of the normal flora of the large intestine.
- When antimicrobial treatment suppresses more predominant, drug-sensitive members of the normal flora, C. difficile multiplies and produces **exotoxins A and B:**





A. Toxin **A** is mainly an **enterotoxin** that causes **fluid accumulation in the bowel**; it also stimulates an inflammatory response.

B. Toxin **B** is a potent **cytotoxin** that **kills colonic mucosal cells**.

- The severity of disease varies widely from **mild diarrhoea** through varying degrees of inflammation of the large intestine **to** a fulminant **pseudomembranous colitis.**

❖ Diagnosis:

- Pseudomembranous colitis can be differentiated from the transient diarrhoea that occurs as a side effect of many oral antibiotics by:

A. Detection of C. difficile toxins in stools by **ELISA.**

B. Detection of the toxins genes by **PCR.**

❖ Treatment:

1. **Withdrawal of the causative antibiotic.**
2. **Treatment with anti C. difficile drugs:**
   - **Oral vancomycin or metronidazole** may be required in an extended course to prevent recurrence (occurs in 15 to 20% of patients).
3. **Correction of** dehydration and electrolyte imbalance.
4. ***Antidiarrhoeal agents should NOT be taken.***
5. Restoration of the patient's colonic flora by **faecal enema** from a normal individual.

Chapter 8: Mycobacterium

❖ Characters of the genus Mycobacterium:

1. Acid-fastness:

- The cell wall of mycobacteria is **rich in lipids** (especially mycolic acid) that render them **resistant** to penetration by dyes used in **Gram** stain. explain esp. stain
- However, they can be stained with special techniques such as **Ziehl-Neelsen** method or **fluorochrome** staining method.
- In these methods, the organisms firmly **retain** the dye used and **resist decolourization even by acidic solutions**; therefore, they are termed '**acid-fast**'.

2. Slow rate of growth: explain

- Mycobacteria have a **longer generation time** and, consequently, a **slower** rate of **growth** than ordinary bacteria.
- This growth rate varies among different species so that it permits their grouping into:

A. Slow growers require more than 7 days to produce visible colonies on solid media

B. Rapid growers require less than 7 days. relatively

3. Obligate aerobe.

❖ Members of the genus Mycobacterium:

1. Mycobacterium tuberculosis complex:

- **M. tuberculosis complex = tubercle bacilli :** are the **M. tuberculosis** and three very closely related species (**M. bovis, M. africanum and M. microti**)
- They can cause tuberculosis in man and animals.

☞ *Humans are the only reservoir for M. tuberculosis whereas both cows and humans are reservoirs for M. bovis.*

2. Nontubercutous mycobacteria (NTM) e.g. M. avium and M. fortuitum.

3. Mycobacterium leprae: the causative organism of leprosy.

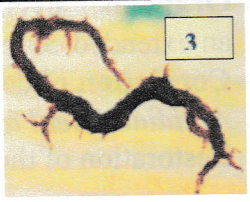
4. Saprophytic mycobacteria: rarely incriminated in human diseases.

Mycobacterium Tuberculosis

❖ Morphology:

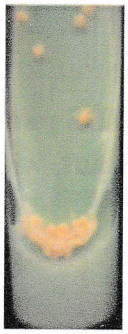
- M. tuberculosis are slender acid-fast bacilli.

1. When stained with the **Ziehl-Neelsen or Kinyoun** method: They appear pink in a blue background.
2. When stained with **the auramine-rhodamine dye**: AFB fluoresce orange yellow in a black background.
3. Smears prepared from cultures (especially fluid media) may reveal bacilli arranged in a characteristic **serpentine cords** like pattern due to **cord factor** associated with virulent strains.

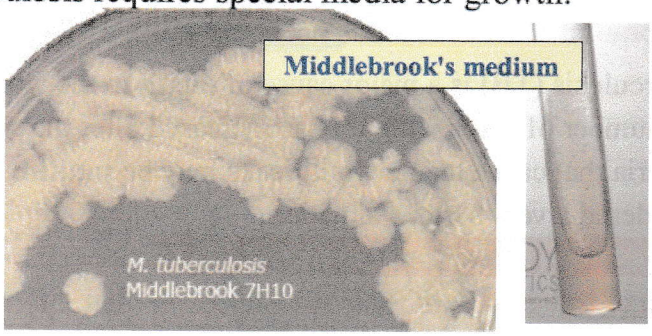


❖ **Cultural characters:** M.tuberculosis requires special media for growth:

Lowenstein-Jensen medium



Middlebrook's medium



1. **Lowenstein-Jensen (L-J) medium** which is an egg-based medium that is rendered selective by addition of **malachite green dye**.
2. **Middlebrook's medium** which is an enriched medium that can be rendered selective by addition of **antibiotics.** It may be fluid (broth) or solid (agar-based).

☞ *Colonies appear after about 2-8 weeks of incubation at 37°C.*

❖ **Biochemical reactions:**

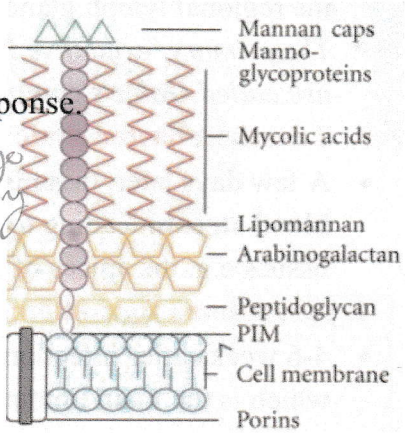
- To differentiate M. tuberculosis from other members of the genus.
- M. tuberculosis is positive for **niacin** production, **nitrate** reduction and production of heat-sensitive **catalase**.

❖ **Cell wall structure:**

- The cell wall of the tubercle bacilli is unique to the Mycobacterium species.
- It has a **peptidoglycan layer** which is similar to that of Gram-positive bacteria.
- In addition, it comprises the following **lipids**:

1. **Mycolic acids:** are long chain fatty acids containing 60 to 90 carbons.
2. **Cord factor:** inhibits leucocyte migration and disrupts mitochondrial respiration.
3. **Mycobacterial sulfolipids:** inhibit phagolysosome formation.

- The high concentration of lipids in the cell wall of M. tuberculosis has been associated with:



1. Ability to **survive** inside macrophages and induce CMI response.
2. **Impermeability** to stains and dyes.
3. ***Resistance to*** drying.
4. ***Resistance to*** many antibiotics.
5. ***Resistance to*** killing by acidic and alkaline compounds.
6. ***Resistance to*** osmotic lysis via complement deposition.

❖ **Susceptibility to physical and chemical agents:**

1. **Tubercle bacilli are killed by:**

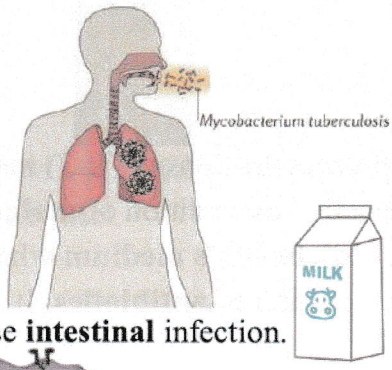
- Heating at 55°C for 1 h, autoclaving, pasteurization and sunlight.
- Intermediate level disinfectants such as ethyl and isopropyl alcohols and chlorine.

2. They **survive** for many weeks when dried in sputum smeared on clothing and in dust.
3. They are **resistant to** acids and alkalis, an important feature, which is used in cultivation procedures from contaminated specimens.

Human Tuberculosis

- Tuberculosis (TB) is a major cause of **death** in the world.
- The number of cases with TB has increased after the start of **AIDS pandemic**.
- Bacteria that cause the new outbreaks may be **multidrug-resistant**, which is considered a very serious situation.

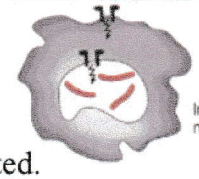
Pathogenesis and clinical manifestations



1. Mode of infection:

- **Aerosol (airborne) infection**:
  - is the commonest mode of infection due to inhalation of droplet nuclei carried by air from a patient with **open pulmonary tuberculosis**.
- **Ingestion of milk** contaminated with **M. bovis** may cause **intestinal** infection.

2. Local lesion:

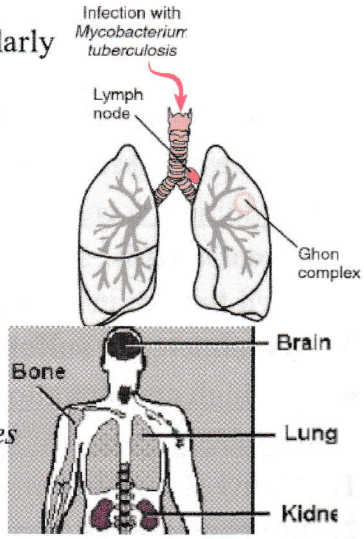


- The droplet nuclei reach the terminal alveoli where they are engulfed by alveolar macrophages:

A. The majority of these bacilli are destroyed or inhibited.

B. The remaining bacilli are able to survive and multiply intracellularly by inhibiting fusion of phagosomes and lysosomes allowing **intracellular survival** (even if fusion occurs, the fatty nature of cell wall reduces killing effect).

☞ *Its capacity to multiply **both** inside **and** outside cells, makes M. tuberculosis a facultative intracellular pathogen.*

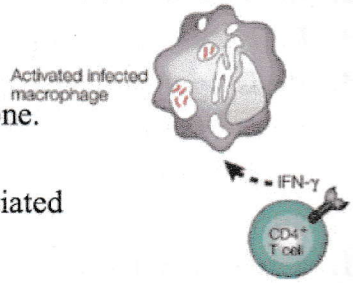


- The accumulating bacilli kill the initial alveolar macrophages.
- Two weeks later the bacilli are transported via lymphatics to the regional lymph glands:

☞ *The primary, exudative lung lesion plus the draining lymph nodes are called **Gohn's complex.***

3. Transient bacteraemic (bacillaemic) phase.

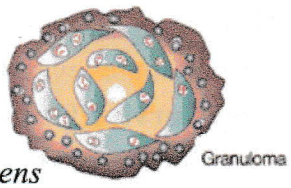
- A few days later, organisms leave the lymph glands to the **blood stream** leading to **dissemination** of organisms to distant tissues e.g. the apices of the lungs, the kidneys, the brain, and bone.



4. Granuloma formation:

- 4-6 weeks after infection: **Cell-mediated immune response** initiated which is the main immune mechanism against mycobacteria.
- Specific **Th1** cells recognize infected macrophages and release cytokines (particularly **IFN-γ**) that activate macrophages:

☞ *When mycobacteria **resist** the microbicidal effect of the activated macrophages, a characteristic localized inflammatory response called a **granuloma (tubercle)** develops to **isolate** pathogens that resist intracellular destruction.*



- Within these tubercles, M. tuberculosis can survive in small numbers in a relatively **dormant** state (**latent tuberculosis infection**). This situation is due to a **balanced** state of host-parasite relationship.

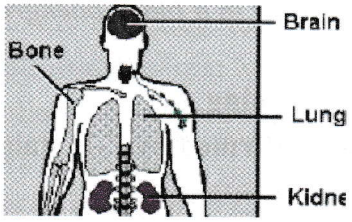
5. Outcome of primary infection: Primary tuberculosis may follow one of 2 courses:

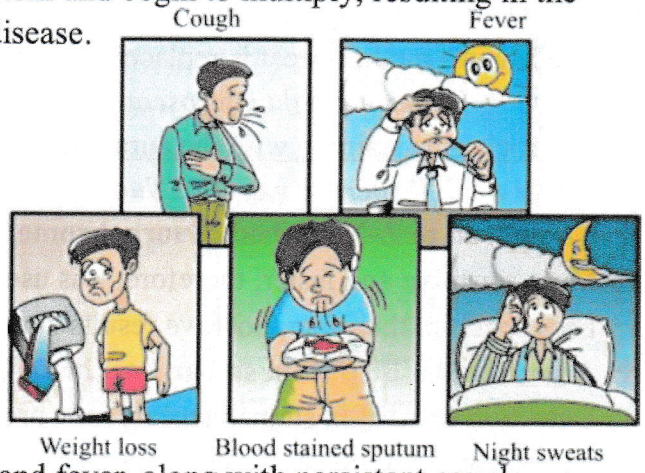
A. Latent tuberculosis infection:

- This occurs in about **90%** of the infected people.
- The above mentioned events can all occur **without** development of **symptoms**.
- Within 4-6 weeks after initial contact with the organism, these individuals become **tuberculin and Quantiferon positive (seroconversion).**

B. Tuberculosis disease:

- In the remaining **10%** of people.
- **TB bacilli overcome the immune system** and begin to multiply, resulting in the progression from TB infection to TB disease.
- **Symptoms of disease include :**

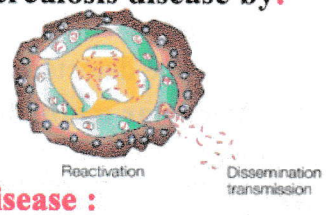




- General malaise, fatigue, night sweats and fever, along with persistent cough.
- Bloody sputum may occur as a late manifestation.
- Extrapulmonary TB : Tuberculous meningitis, lymphadenitis, renal and intestinal tuberculosis.

6. Reactivation of latent tuberculosis and development of tuberculosis disease by:

- Immunosuppression, disturbance of the host- parasite balance.
- Reactivation usually occurs within **2 years** after initial infection in about **5%** of individuals with latent TB infection.
- The most common site of reactivation is the **apex of the lung**.



❖ Comparison between Latent tuberculosis infection & TB disease :

❖ Characters	A. Latent TB infection	B. Pulmonary TB disease
❖ M. tuberculosis present	• Yes	• Yes
❖ Tuberculin (or Quantiferon) test	• Positive	• Positive
❖ Sputum smears and cultures	• Negative	• Positive
❖ Symptoms	• No symptoms	• Cough, fever, weight loss
❖ Infectivity	• Not infectious	• Often infectious
❖ Case definition	• Not a case of TB	• A case of TB

❖ **Diagnosis :**

I. Laboratory diagnosis of open pulmonary tuberculosis:

- The definitive laboratory diagnosis of tuberculosis disease depends on the detection and isolation of M. tuberculosis from clinical specimens.

A. Specimens:

- Include sputum, bronchoalveolar lavage.
- **Three early morning sputum** specimens collected on consecutive days, from a deep productive cough, give the best results.

B. Direct detection:

1. **Smears** prepared directly from sputum are subjected to one of the following **acid-fast staining methods**:

a) Using the carbol-fuchsin dye :

- In **Ziehl-Neelsen (Z-N)** method, heat is used to help penetration of carbol-fuchsin.
- In **Kinyoun** method, heat is replaced with phenol.

☞ *Under the light microscope, AFB appear pink in a blue background.*

b) Fluorochrome staining with the auramine-rhodamine stain:

☞ *Under the UV microscope, AFB fluoresce orange yellow in a black background.*

❖ **Compared to Z-N method, fluorochrome staining is:**

- More sensitive and faster; therefore, it is used for **screening**.
- Less specific; therefore, positive results **should be confirmed by** Z-N stain.

❖ **A positive AFB sputum smear has the following advantages:**

- Detection of AFB in stained smears may provide **early presumptive diagnosis** which allows rapid patient care. The result should be reported to the clinician within hours.
- **Monitoring** the infectivity of the patient and the response to anti-tuberculosis therapy.

❖ **Drawbacks of AFB smear:**

- A positive AFB smear **cannot differentiate** between M. tuberculosis and other mycobacteria.
- A negative AFB smear **does not exclude** TB infection because organisms may be too few to be detected microscopically.

2. **Molecular methods:** **PCR** can be used for **rapid** (same day) detection of M. tuberculosis in sputum samples.

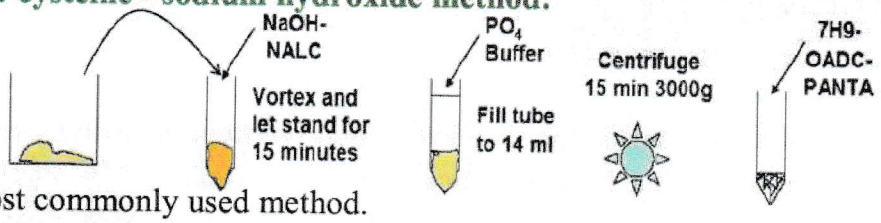
C. Processing of sputum by liquefaction, concentration & decontamination:

❖ This procedure is applied to sputum & other specimens contaminated with normal flora **to prepare** such specimens for culture, smear examination and DNA detection.

❖ **It enhances detection of the AFB because:**

- **Liquefaction** of sputum **releases trapped mycobacteria** from tenacious specimens.
- The mycobacteria in the liquefied specimen can then be **concentrated** in a small sediment by **centrifugation**.
- **Decontamination** kills normal flora allowing **obvious growth of** the slowly growing **mycobacteria.**

❖ **N-acetyl-L-cysteine - sodium hydroxide method:**



- It is the most commonly used method.
- It is based on the ability of **NaOH to inhibit organisms** other than mycobacteria.
- The mucolytic agent N-acetyl-L-cysteine (**NALC**) is combined with **NaOH** to help **liquefaction** of sputum.

NaoH → inhibit liquefaction

D. Cultivation:

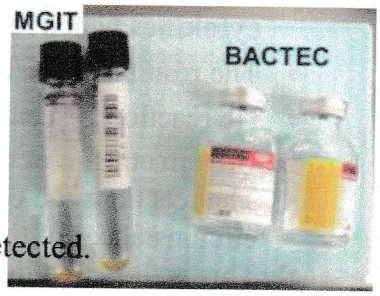
☞ ***Definitive diagnosis of TB disease is achieved only by a positive culture.***

❖ <u>One of the following culture media can be inoculated by sediment of processed sputum and incubated in 5 to 10% C02 at 37°C:</u>

1. Conventional culture media (L-J and Middlebrook's agar media):

- Non-pigmented, rough colonies appear after about 2-8 weeks of incubation.
- Colonies of M. tuberculosis are identified *microscopically* by Z-N stain and *biochemically* by nitrate reduction, niacin production and catalase tests.

2. Fluid medium systems: Middlebrook broth is used as the base for the following systems:



a) Bactec AFB system:

- Bactec medium contains radio-labelled palmitate as the sole carbon source.
- As M. tuberculosis multiplies, it **breaks down the palmitate and liberates c^{14} labelled CO2** that can be detected.

b) Mycobacteria Growth Indicator Tube (MGIT):

- In this selective medium, bacterial growth will result in **oxygen depletion.**
- This can be detected by a **fluorescence** sensor that fluoresces upon exposure to UV light.
- The major advantage of culture on these fluid systems is that they allow detection of growth in 4 to 14 days.

E. Tuberculin and Quantiferon tests .

II. Laboratory diagnosis of extrapulmonary tuberculosis:

- Diagnosis of extrapulmonary TB such as tuberculous meningitis, lymphadenitis, renal and intestinal tuberculosis is performed **as mentioned for pulmonary TB** but by collecting the **appropriate specimens**.

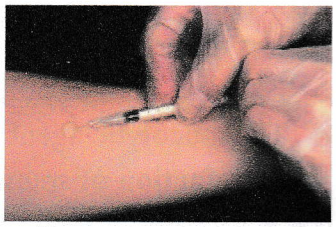
☞ *N.B. specimens collected from normally sterile sites, e.g. **CSF do not** require decontamination.*

III. Laboratory diagnosis of latent tuberculosis:

1. Tuberculin Skin Test (TST):

A. Principle:

- The tuberculin test is a skin test that detects **delayed hypersensitivity** response to previous exposure of the host to tubercle bacilli.



☞ *Therefore, a positive TST indicates **previous infection** by the organism but **not necessarily active** disease.*

B. Technique:

- **Purified protein derivative (PPD),** which is prepared from culture filtrate of the organism, is the antigen used in the tuberculin test.
- **Mantoux method:** 0.1 ml of PPD containing 5 tuberculin units (TU) is injected **intradermally** in the skin of the anterior aspect of the forearm.
- The result is read after 48-72 h by palpating for the presence of **induration.**
- The diameter of the induration (*NOT the erythema*) is measured in **millimeters.**

C. Underlying mechanism:

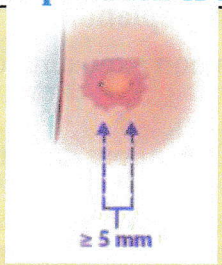
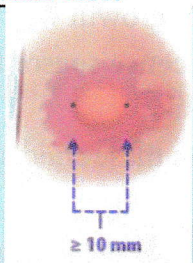
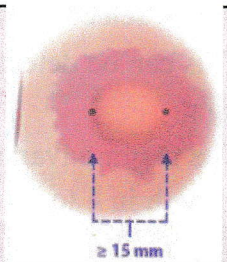
- As a result of previous exposure of the host to tubercle bacilli **Th1 cells become sensitized.**
- In positive reactors, the injected PPD stimulates the presensitized Th1 cells to **secrete cytokines** which recruit inflammatory cells particularly macrophages.
- The result is a **raised, indurated** area around the site of injection.
- **No** reaction is seen in people who have **not** been **sensitized** to TB.

D. Interpretation of the tuberculin test:

❖ The reactions are categorized by different criteria (risk factors) depending on the circumstances of the patient.

❖ This is the so-called **'5-10-15 millimeter system'.**

❖ **Interpretation of the tuberculin skin test:**

		
A. An induration of 5 or more millimeters	**B. An induration of 10 or more millimeters**	**C. An induration of 15 or more millimeters**
❖ Considered positive for: • People with past history of TB • Close contacts of infectious TB patients • People with HIV infection	❖ Considered positive for: • People in endemic areas where TB is common • People with certain medical conditions such as diabetes • Unvaccinated children younger than 4 years old	❖ Considered positive even in absence of any risk factor for TB.

❖ **False reactions:**

i. **False negative reactions:**

- **Anergy:**
  - The inability to react to TST may occur as a result of a **weakened immune system**, e.g. severe TB disease, HIV infection, or cancer.
- **Recent TB infection:**
  - After exposure, it takes 4 to 6 weeks for tuberculin test to become positive.

ii. **False positive reactions:**

- Infection with **nontuberculous mycobacteria** (NTM) due to cross-reaction with M. tuberculosis antigens. Cannot differentiate
- Vaccination with **bacille Calmette-Guerin (BCG)**: After BCG vaccination, tuberculin skin test remains positive for up to 5 years.

2. Quantiferon TB (IFN-γ release assay): specific & can differentiate

❖ **Principle:**

- Measurement of the **amount of IFN-γ** released from the patient's sensitized T-lymphocytes after exposure to specific M. tuberculosis antigens in cell culture.

❖ Compared to TST, this test is **specific for diagnosing latent** tuberculosis infection because the antigens used are **not** present in NTM or BCG vaccine strains.

❖ **Treatment regimens of tuberculosis:**

☞ ***To be successful, the treatment should fulfill the following requirements:***

1. **Combination therapy:**

- Combination of **4 drugs or more** is essential to reduce the drug toxicity and to prevent the emergence of drug resistant mutants.

2. **Prolonged therapy:**

❖ **This is because of:**

I. The **intracellular** location of the organism
II. Blocking of drug penetration by **caseous** material
III. The **slow** rate of **growth** of the organism
IV. The presence of the **persisters** (metabolically inactive bacilli) within the lesion.

❖ Although therapy is given for **at least 6 months**, the patient's sputum becomes non-infectious within 2-3 weeks.

❖ Because of patients non-compliance, a regimen recommended by WHO known as **directly observed therapy short course (DOTS)** is thought to be effective for such patients.

❖ **Anti-tuberculous drugs include:**

1) **First-line drugs:**

- Isoniazid (INH), rifampin, pyrazinamide, ethambutol and streptomycin.

2) **Second-line drugs:**

☞ *These drugs are only used if **resistance** occurs during administration of first- line drugs.*

- Fluoroquinolones, para-aminosalicylic acid (PASA), ethionamide, cycloserine, capreomycin, kanamycin, amikacin and rifabutin

❖ **Resistance patterns:**

1) Multidrug-resistance (MDR) means :

- Resistance of M. tuberculosis to **both isoniazid and rifampin**.
- It is a serious situation that presents difficulty in treatment.

2) Extensive (extreme) drug resistance (XPR) means :

- **MDR plus** resistance to a **fluoroquinolone and at least one additional drug**

☞ *M. tuberculosis isolates should be tested for drug resistance as early as possible in order to ensure appropriate treatment*

❖ **Prevention and Control:**

1. General measures:

- Early case finding and effective treatment
- Applying proper Infection control measures in hospitals (e.g. use of N95 masks),
- Avoid overcrowding.
- Better housing and nutrition to improve host resistance
- Pasteurization or boiling of milk

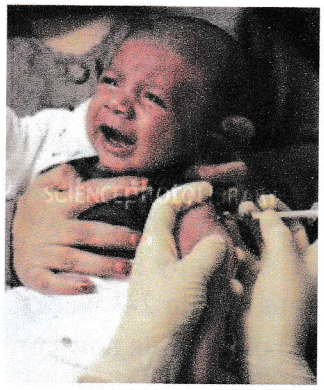
2. Treatment of latent Infections :

- To reduce the risk of progression to active tuberculosis, **isoniazid (INH) is given for 6-9 months** to:

A. Recent converters: Individuals who show recent conversion to a positive tuberculin or quantiferon test.

B. Tuberculin or Quantiferon positive individuals who are subject to immunosuppression.

3. BCG vaccine:



- It is a live attenuated vaccine prepared from **M.bovis** and attenuated by repeated subcultures.
- It is routinely given to **newborns intradermally in the left deltoid region**
- The protective efficacy of BCG vaccine in preventing TB infection is controversial.
- It proved to be effective in protection against childhood tuberculous **meningitis** and **disseminated** tuberculosis.

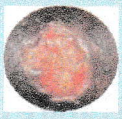
Non-Tuberculous Mycobacteria

- Nontuberculous mycobacteria **(NTM)** include those Mycobacterium species that are **not members** of the Mycobacterium tuberculosis complex, hence the use of the terms "nontuberculous mycobacteria" or "mycobacteria other than tuberculosis' (**MOTT**).
- They are wide-spread in the environment and have been recovered from **tap water, water of haemodialysis units and drinking water distribution systems**

General features of NTM:

1) Most NTM are **morphologically indistinguishable** from M tuberculosis
2) Most NTM grow well on **media** used to grow **M tuberculosis.**
3) Growth characteristics (**rate of growth and pigment production**) classify NTM into **4 groups (I-IV)**
4) NTM generally cause **opportunistic infections** particularly in immunocompromised patients, e.g. AIDS patients.
5) Transmission is from **environmental** sources. Person to person transfer does **not** take place.
6) NTM are generally **more resistant to antituberculous** drugs. Combinations may have to be used (rifabutin, clarithromycin and azithromycin).
7) NTM show **high resistance** to a wide range of **disinfectants**.

Examples of important NTM and the diseases they produce

A. Slow Growers			B. Rapid growers	
Microorganism:				
○ M. avium-intracellulara complex (MAC)	○ M. kansasii	○ M. ulcerans	○ M. fortuitum-chelonae complex	○ M. abscessus
Disease:				
• Pulmonary TB-like disease, particularly in AIDS patients	• Chronic lung disease resembling classical TB Ulcerating	• Infections of the skin **(Buruli ulcer)** 	• Skin and soft tissue infections	• Chronic lung disease (which can disseminate to skin, bone and joints)

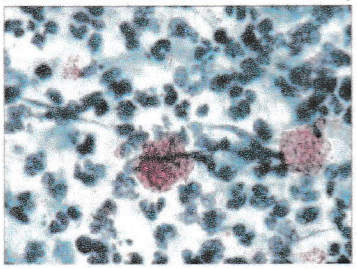
Mycobacterium Leprae

❖ **Morphology:**

- M. leprae closely resembles M. tuberculosis in size and shape.
- It occurs chiefly in bundles (globi) within the infected cells.
- M. leprae is **less acid-fast** than M. tuberculosis:

☞ *Therefore, the use of the modified Ziehl-Neelsen method, in which the decolourizing agent is **modified**, is necessary **to avoid over**decolourizing the AFB.*

Mycobacterium leprae (Acid Fast bacilli with modified Z-N stain)



❖ **Culture:**

1) In vitro:

- M. leprae has **not** yet been successfully cultured in vitro.
- It is considered an **obligate intracellular** pathogen.

2) In vivo:

A. The nine-banded armadillo:

- The nine-banded armadillo is a natural reservoir for M leprae.
- This animal has become the main source of M. leprae for biochemical and immunological research including development of a vaccine.

B. Mice:

- Inoculation of the specimen into the hind foot-pads of mice is used to test the sensitivity of the bacilli to new drugs.

Leprosy

❖ **Leprosy (Hansen's disease)** is a chronic infectious disease that has historically been more feared than any other infectious disease.

❖ **Pathogenesis and clinical manifestations:**

- The exact mode of transmission is **not certain**.

☞ *However, infection requires prolonged and close contact with patients.*

- Infection may be transmitted by **contact with skin lesions or aerosol inhalation**.
- The clinical disease may develop years after initial contact with the organism; this is due to:

A. Low infectivity of M. leprae

B. Long generation time (slow rate of growth).

- Leprosy bacilli grow best at **low temperature** (optimally 30°C); therefore, the skin and superficial nerves are preferentially affected.
- The pathogenesis of leprosy appears to derive from:

a) The ability of M. leprae to **survive and replicate within** macrophages, nerve cells and other host cells, and

b) The consequent **immune response** to the organism.

❖ **Forms of the disease:**

I. Tuberculoid leprosy (TL):

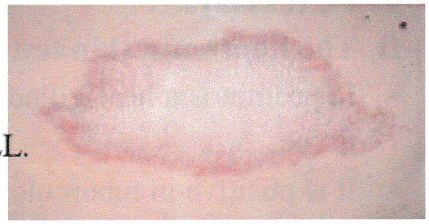
1. **Immune response ,T helper-type response & Number of AFB in tissues:**

- Cell-mediated immune response mediated by **Th1 cells** predominates and forms granulomas, resulting in the destruction of most of the mycobacteria.

☞ ***So only few AFB remain in the tissues (paucibacillary leprosy).***

2. **Lesions :**

- Few and mainly in the form of **hypopigmented maculo-anaesthetic skin lesions.**



3. **Lepromin skin test** is **positive.**
4. **Disease progress & Prognosis:** :

- Although skin and peripheral nerves are damaged, TL progresses slowly, and carries a better prognosis than LL.
- In some patients it is self-limiting, but in others TL may progress across the spectrum towards LL.

II. Lepromatous leprosy (LL):

1. **Immune response ,T helper-type response & Number of AFB in tissues:**

- Cell-mediated immune response is **depressed.**
- Although humoral response mediated by **Th2 cells** is predominating, it is **not protective** as the organism is intracellular.

☞ ***The lesions usually contain large numbers of AFB (multibacillary leprosy).***

2. **Lesions :**

- Mainly **nodular** and may form on the face & the nose may collapse giving the characteristic **lionine facies**.
- There is a marked sensory loss due to extensive nerve damage.

3. **Lepromin test** is **negative.**
4. **Disease progress & Prognosis:**

- LL is the more severe form and progresses rapidly.

III. Intermediate forms inbetween TL & LL.

❖ **Comparison between tuberculoid leprosy and lepromatous leprosy:**

	A. Tuberculoid leprosy	B. Lepromatous leprosy
❖ **Immune response**	o Predominant CMI	o Predominant AMI
❖ **T helper-type response**	o Th1	o Th2
❖ **Lepromin test**	o Positive	o Negative
❖ **Cytokine profile**	o IL-2, IFN-γ and TNF-β	o IL-4, IL-5 and IL-10
❖ **Number of AFB in tissues**	o Few	o Abundant
❖ **Lesions**	o Macular skin lesions	o Nodular skin lesions
❖ **Disease progress**	o Slow	o Aggressive
❖ **Prognosis**	o Good	o Bad

❖ **Laboratory diagnosis:**

- The diagnosis of leprosy is essentially a **clinical** one; laboratory diagnosis is done for confirmation:

A. Specimen: Slit skin smears, skin biopsy, or scrapings from the nasal mucosa.

B. Direct detection:

- Smears stained with **modified Z-N method** show intracellular AFB in bunches.
- A positive smear is **sufficient** for diagnosis.

C. PCR assays.

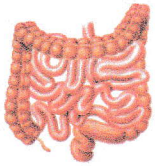
D. The Lepromin skin test:

- Lepromin is a heat-killed suspension of M. leprae prepared from infected armadillo tissue.
- It is positive in tuberculoid leprosy and negative in lepromatous leprosy.
- The test is of **no diagnostic**, but of **prognostic value**.

❖ **Treatment:**

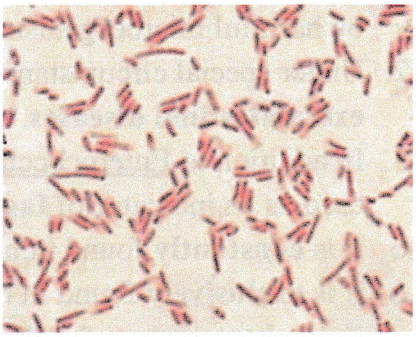
- **Prolonged multidrug therapy** is recommended to reduce development of resistance.
- The drugs used are **rifampicin, clofazimine and dapsone.**
- Among these, rifampicin is the most important anti-leprosy drug and is, therefore, included in the treatment of both forms of leprosy.
- In **LL, triple** therapy is recommended for a minimum of **2 years**.
- In **TL** a combination of **dapsone and rifampicin** is used for **6 months.**
- Nerve damage and deformities are *irreversible.*

Chapter 9: Enterobacteriaceae



❖ **The Enterobacteriaceae family** is a large group of **facultative anaerobic, non-spore- forming, Gram-negative bacilli.**

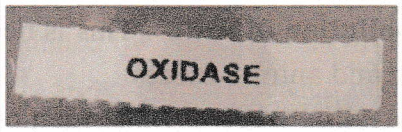
- The natural habitat is the **intestinal tract** of humans and animals.
- Some members are widely distributed in the environment in water and soil, and on plants.



❖ **General features:**

- Members of Enterobacteriaceae share features that help **differentiating** them from other Gram-negative bacilli:

1. **Facultative anaerobes.**
2. **Oxidase negative.**
3. **Fermentation:**
   - **Ferment glucose.**
   - **Lactose fermentation** is an important differential character.
   - Fermentation of other sugars is variable.
4. **Reduce nitrate to nitrite.**



Oxidase negative

❖ **Differential diagnosis :**

- **Pseudomonas aeruginosa and Bacteroides** species which are Gram-negative bacilli normally found in human intestines are considered **non**-Enterobacteriaceae:
- This is because they do **not meet all the criteria** of the Enterobacteriaceae family:
  - P. aeruginosa is an obligate aerobe, oxidase positive, does not ferment glucose or reduce nitrate.
  - Bacteroides is a strict anaerobe.

❖ The family is classified into many genera and species on the basis of biochemical reactions, especially **fermentation of carbohydrates & DNA-DNA hybridization**.

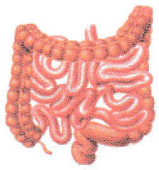
❖ **According to their medical importance, they are classified into:**

1. **Opportunistic pathogens:**
   - Most Enterobacteriaceae e.g. Escherichia, Klebsiella, Enterobacter, Citrobacter, Serratia and Proteus genera, are commensals and may cause opportunistic infections.
2. **Enteric pathogens:**
   - Salmonella, Shigella, and Yersinia genera, and certain strains of Escherichia coli primarily cause intestinal infections and systemic diseases (e.g. typhoid fever caused by Salmonella Typhi).

❖ Many strains of these enteric Gram-negative rods are **highly antibiotic resistant**, because of the production of **β-lactamases** and other drug-modifying enzymes.

- **Extended spectrum β-lactamases (ESBL)** are plasmid mediated enzymes that are produced by several enteric bacteria e.g. E. coli, Klebsiella, Enterobacter and Proteus. ESBL render the bacteria resistant to all β-lactam antibiotics (penicillins, cephalosporins and monobactam).

Escherichia coli



❖ Escherichia coli is the most predominant **facultative anaerobe** in the **large intestine of man.**

❖ **It has the following medical importance:**

1. Being part of the normal bowel flora, E. coli provides **protection against colonization** by harmful microorganisms.
2. Under special circumstances, certain pathogenic strains cause **intestinal and extraintestinal diseases**.
3. In addition to Enterococcus faecalis and C. perfringens, E. coli is used as an indicator of **faecal pollution of water**. This is because:

- It is **constantly** found in human and animal **faeces**.
- It is **exclusively** found in the **intestine** whereas other members of Enterobacteriaceae family are found in the environment as well.

❖ **Morphology:**

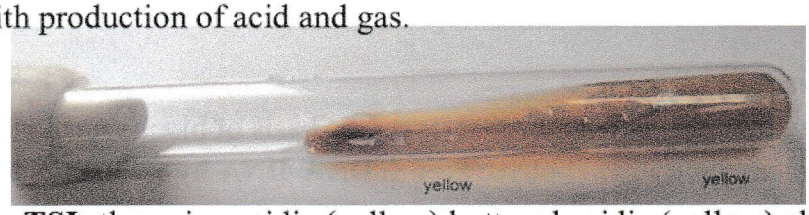
- Gram-negative bacilli, usually **motile** and some are capsulated.

❖ **Cultural characters:**

- E.coli grows on **MacConkey agar** producing rose pink colonies due to lactose fermentation.

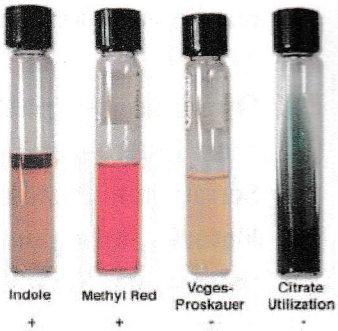
❖ **Biochemical reactions:**

1. E.coli **ferments** glucose, lactose, maltose, mannite and sucrose with production of acid and gas.



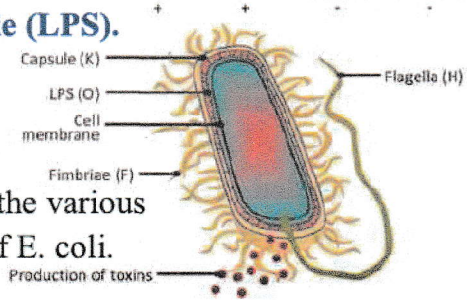
- **On TSI:** they give acidic (yellow) butt and acidic (yellow) slant with cracking of the agar due to gas production.

2. **(IMVC + + –) :I**ndole-positive, **M**ethyl red (MR) positive, **V**oges-Proskauer (VP) negative, and **C**itrate-negative



❖ **Antigenic structure:**

- **Serological classification of E. coli strains is based on:**
  - **O (somatic) antigen of the cell wall lipopolysaccharide (LPS).**
  - **H (flagellar) antigen.**
  - **K (capsular) antigen in capsulated strains**.



- Because there are many different O, H, and K antigens, the various combinations result in more than 1000 antigenic types of E. coli.
- **Specific** serotypes are associated with **certain** diseases e.g., O55 and O111 cause outbreaks of neonatal diarrhoea.

❖ **Virulence factors:**

A. Uropathogenic E. coli have:

1. **Fimbrial adhesins:** **bind** to specific receptors on the urinary tract epithelium.
2. **Capsular (K) antigens**:

- Interfere with **phagocytosis**, thereby enhancing the organism's ability to cause pyelonephritis.

3. **Haemolysins:** act as **membrane**-damaging toxins that are linked with kidney damage.

B. Diarrhoeagenic E. coli have:

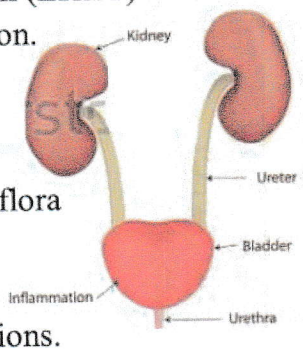
1. **Pili (colonization factors).**
2. **Enterotoxins:** Two types,

- Heat-labile (LT) and heat-stable (ST) are produced by entero**toxigenic** E. coli (ETEC).

3. **The shiga toxins:** are produced by the entero**haemorrhagic** E. coli (EHEC).

C. The LPS causes **endotoxic shock** when released into the circulation.

Diseases Caused by E. coli



1. **Urinary tract infection (UTI):**

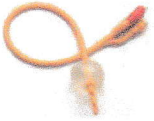
- The source of E. coli that causes UTI is the patient's own colonic flora that colonizes the uro-genital area.

A. Community-acquired UTI:

- E. coli is **the commonest cause** and accounts for **> 80%** of infections.
- The organisms **ascend** from the periurethral region into urethra (urethritis), bladder (cystitis), ureters, renal pelvis (pyelitis) and renal parenchyma (pyelonephritis).

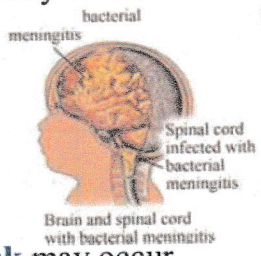
B. Hospital-acquired UTI:

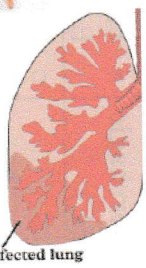
- It is usually associated with urinary catheters and is mostly caused by multi-resistant strains.



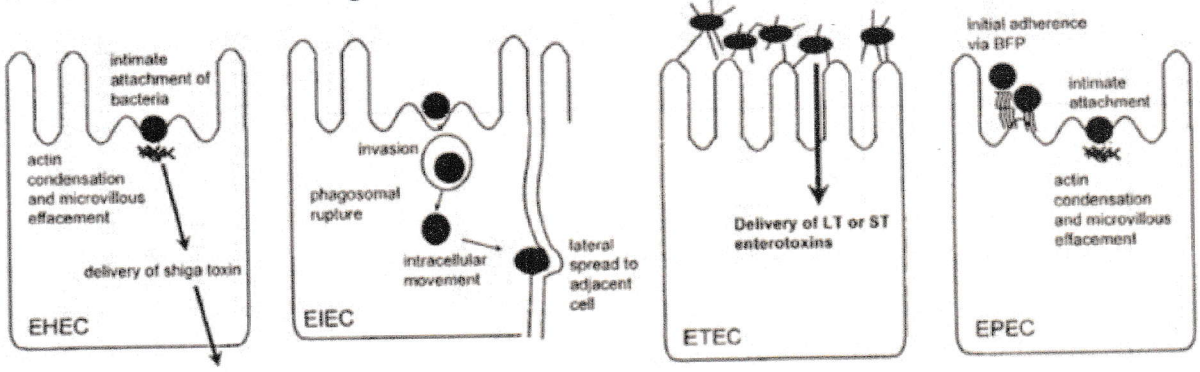
2. **Neonatal meningitis:**

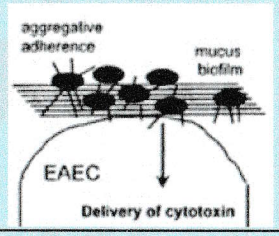
- **E. coli K1** is a common cause of neonatal meningitis.
- The source of the organism is the mother's birth canal, where the infection is acquired during birth.





3. **Pneumonia, sepsis, septicaemia, and endotoxic shock** may occur particularly in neonates.
4. **Diarrhoea:** Diarrhoeagenic E. coli include several types: see the next table.

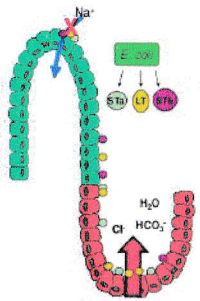


Types of diarrhoeagenic E. coli:					
❖ **Type**	• Entero**haemorrhagic** E. coli (EHEC)	• Entero**invasive** E. coli (EIEC)	• Entero**pathogenic** E. coli (EPEC)	• Entero**aggregative** E. coli (E**agg** EC)	• Entero**toxigenic** E. coli (ETEC)
❖ **Transmission**	○ **Bovine** faecal contamination of: – Raw or undercooked meat (e.g. hamburger). – Unpasteurized milk. – Raw vegetables.	○ Faeco-oral ○	○ Faeco-oral	○ Faeco-oral 	○ Faeco-oral
❖ **Pathogenesis**	○ Production of **verotoxin (shiga-like toxin)** most commonly by E. coli serotype **O157:H7**	○ Invasion of colon mucosa, **without** toxin production.	○ Tight **adherence** to intestinal mucosa and interference with **water** absorption.	○ Adherence to intestinal mucosa by **aggregative fimbria**. ○ Production of ***enterotoxin.***	○ Production of ***enterotoxins*** (LT and ST; see below)
❖ **Clinical presentation**	○ **Bloody** diarrhea. ○ Possible complications: – Haemorrhagic **colitis**. – Haemolytic **uraemic** syndrome (HUS); potentially fatal.	○ **Bloody** diarrhoea (dysentery-like syndrome).	○ **Watery** diarrhoea in infants (infantile diarrhoea).	○ **Watery** diarrhoea (persistant) in children.	○ Severe **watery** diarrhoea in infants and children. ○ ***Traveller's*** diarrhoea in adults

❖ **Enterotoxigenic E. coli pathogensis:**

- **LT activates adenylate cyclase** resulting in elevation of **c-AMP**. This induces active **secretion of chloride & water** by intestinal mucosal cells and inhibits Na+ reabsorption. The intestinal lumen becomes full of fluids, resulting in *watery diarrhoea*. LT is similar in structure (A/B subunits) and function to **cholera toxin.**
- **ST activates guanylate cyclase** resulting in formation of **c-GMP**, leading to loss of fluids from the intestine.

❖ Clinical presentation of Entero**haemorrhagic** E. coli is similar to that caused by **Shigella dysenteriae.**



❖ **Laboratory diagnosis:**

A. Specimens:

- Urine, wound swabs, respiratory secretions, blood and CSF (in case of extra- intestinal infections).
- Faeces (in case of diarrhoea).

B. Direct detection:

- **Gram-stained smears** are useful only in specimens normally devoid of flora, e.g. CSF.
- E.coli **K1 antigen** is detected in CSF in neonatal meningitis by latex agglutination.

C. Cultivation:

- Specimens are plated onto MacConkey agar as well as blood agar and incubated at 37°C.
- Urine should be quantitatively cultured to determine bacteruria .
- Blood samples should be cultivated by the blood culture technique in cases of septicaemia and meningitis. Subcultures are plated on MacConkey agar and blood agar and incubated as above.

D. Identification:

1. After 24h incubation, colonies should be examined regarding morphology, Gram stain and oxidase test.
2. Colonies showing Gram-negative bacilli and are oxidase negative should be considered Enterobacteriaceae.
3. E.coli produces rose pink colonies on MacConkey agar.
4. Colonies should be tested biochemically (see above).

❖ **N.B.:**

- Because E. coli is part of the normal intestinal flora, **strains isolated from faeces** of patients with diarrhoea should be further tested to confirm their responsibility for the disease:

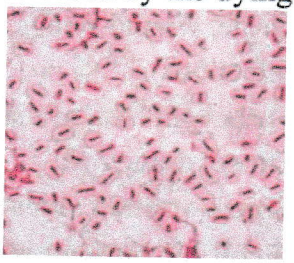
☞ ***This may be done as follows:***

1. **Culture on sorbitol-MacConkey agar:** Unlike other strains of E. coli, EHEC does **not** ferment **sorbitol**.
2. **Slide agglutination:** using specific antisera (EPEC, EHEC).
3. **ELISA:** to test for *toxin* production *(ETEC, EHEC).*
4. **DNA probe or PCR:** to detect genes of *toxin* production *(ETEC, EHEC).*
5. **Tissue culture:** to detect *toxin* production *(ETEC, EHEC),* invasiveness (EIEC) or adherence (EPEC, EaggEC).

❖ **Treatment:**

- In cases of diarrhea: treatment depends on correction of dehydration and electrolyte imbalance.
- Antibiotic therapy of **extra-intestinal E. coli** infections should be guided by **in vitro** susceptibility testing because of the wide spread of **resistant** strains especially ESBL-producing strains.

- Antibiotics may be useful **except in cases of EHEC**; in such cases antibiotics may increase the risk of developing **HUS** by increasing **shiga toxin** released by the dying bacteria.



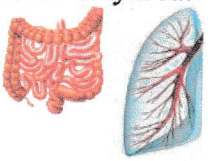
Klebsiella

❖ **Klebsiellae occur in two common habitats:**

- The environment: in surface water, sewage, soil and on plants.
- Mucosal surfaces of intestinal and respiratory tracts.

❖ **The genus includes** K. pneumoniae, K. ozaenae, K. rhinoscleromatis and K. oxytoca.

☞ *K. pneumoniae is the medically most important species.*



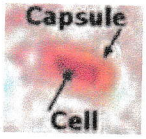
❖ **Morphology:**

- Klebsiella organisms are **non-motile** capsulated Gram-negative bacilli.

❖ **Culture:**

- Klebsiella grows on **MacConkey agar** producing rose pink colonies due to lactose fermentation.

- The colonies are usually **mucoid** due to **capsular** material.



❖ **Biochemical reactions:**

1. Klebsiella ferments glucose, lactose, maltose, mannite and sucrose with production of acid and gas.

- On TSI, they give acidic (yellow) butt and acidic (yellow) slant with cracking of the agar due to gas production.

2. **IMVC of K. pneumoniae is - - + +.**

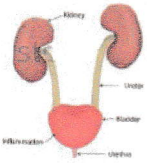
❖ **Pathogenesis:**

☞ ***The capsule is the most important virulence factor.***

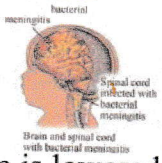
- Klebsiella infections are either community- or hospital-acquired.

1. **K. pneumoniae and much less frequently K. oxytoca may cause:**

- **Urinary tract infection**: It is the most common infection.



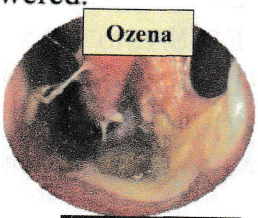
- **Pneumonia:**



– Klebsiella is carried in the respiratory tract of about 10% of healthy people who are prone to pneumonia if host defence is lowered.

- **Wound and bloodstream infections.**
- **Neonatal sepsis: Septicaemia or meningitis**.

2. **Klebsiella ozaenae** is associated with **atrophic rhinitis (ozena):** a fetid progressive atrophy of nasal mucosa.



3. **Klebsiella rhinoscleromatis** is associated with **rhinoscleroma**: a destructive granuloma of the nose and pharynx.

❖ **Treatment:**

- Emerging antimicrobial resistance among Klebsiella spp. is increasing.
- Resistance is mediated by β-lactamases (especially ESBL). Therefore, routine in vitro susceptibility testing is required.

Citrobacter, Enterobacter And Serratia

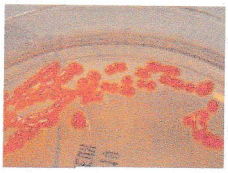
- Organisms of these genera are **motile** Gram-negative bacilli.
- **Found in** soil, water and occasionally in the human respiratory and intestinal tracts and animal intestine.
- **They cause opportunistic infections** in humans especially *pneumonia* and *urinary tract infection.*

A. **Citrobacter:** It is similar to **E. coli except** in being **citrate-positive**.

B. **Enterobacter**: It is similar to **Klebsiella except** in being **motile.**

C. **Serratia:**

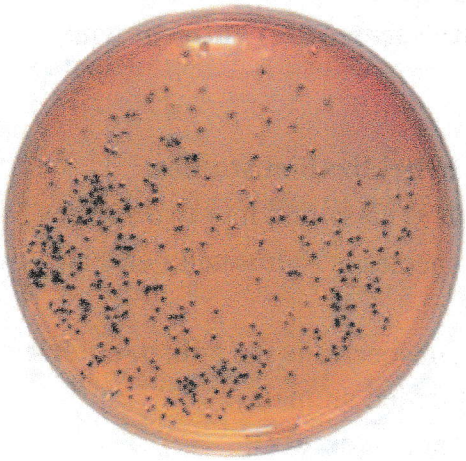
- Some strains produce **red non-diffusible endo-pigment.**
- Used for testing efficiency of **bacterial filters**.



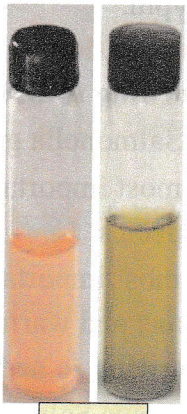
Salmonella

- **Salmonellae are commonly found in** the intestinal tract of mammals, birds (particularly poultry) and reptiles.
- **Morphology:**
- Salmonellae are Gram-negative bacilli and almost all are **motile**.
- **Culture:**

1



2



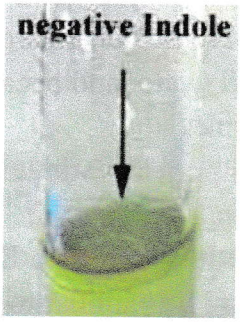
3

1. Salmonella grows as **pale** lactose-**non**fermenting colonies on **MacConkey** and desoxycholate citrate agar (**DCA**) media.
2. **Salmonelta-Shigella (SS) agar** is superior to MacConkey and DCA media in detecting **H2S** producing Salmonella species that give **black colonies**.
3. **Selenite and tetrathionate broth** (enrichment media) are used to isolate Salmonella from stools.

❖ **Biochemical reactions**

1. **Fermentation of** glucose, maltose and mannite with production of acid and **gas.**

☞ *Salmonella Typhi produces acid only.*



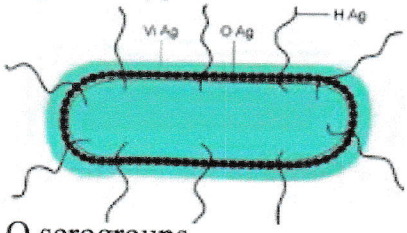
2. **Urease-negative and indole-negative.**
3. Most species produce **H2S.**

❖ **N.B.: On TSI:**

- Salmonellae give acidic (yellow) butt and alkaline (red) slant with cracking of the agar ***except in case of S. Typhi.***
- Black colour develops in case of H2S production.

❖ **Antigenic structure:**

- The antigens used to define groups and types of salmonellae include:



1. **The O (somatic) antigens:**

- Which divide Salmonella into O serogroups.
- The O antigen may be **shared** between different groups.

☞ *Therefore, **cross reactions** may occur between these groups during serologic testing.*

2. **The H (flagellar) antigens:** which divide the serogroups into **serotypes.**
3. **The Vi (capsular polysaccharide) antigen**: which may be present in some strains of S. Typhi.

❖ **Classification**

1. **According to DNA hybridization studies:**

- The Salmonella genus is classified into two species.
- The most important species is **Salmonella enterica**, which is further classified into six subspecies.
- The most important subspecies is the **subsp. enterica** whose strains are isolated from humans and warm-blooded animals.

2. **According to antigenic structure:**

- The genus Salmonella is classified into serogroups and serotypes (~ 2400 serotypes).

3. **Clinically:** Salmonella species are categorized into:

i. **Typhoidal species:**

- Salmonella Typhi that causes typhoid fever.
- S. Paratyphi A, S. Paratyphi B and S. Paratyphi C that cause paratyphoid fever.

ii. **Nontyphoidal species:**

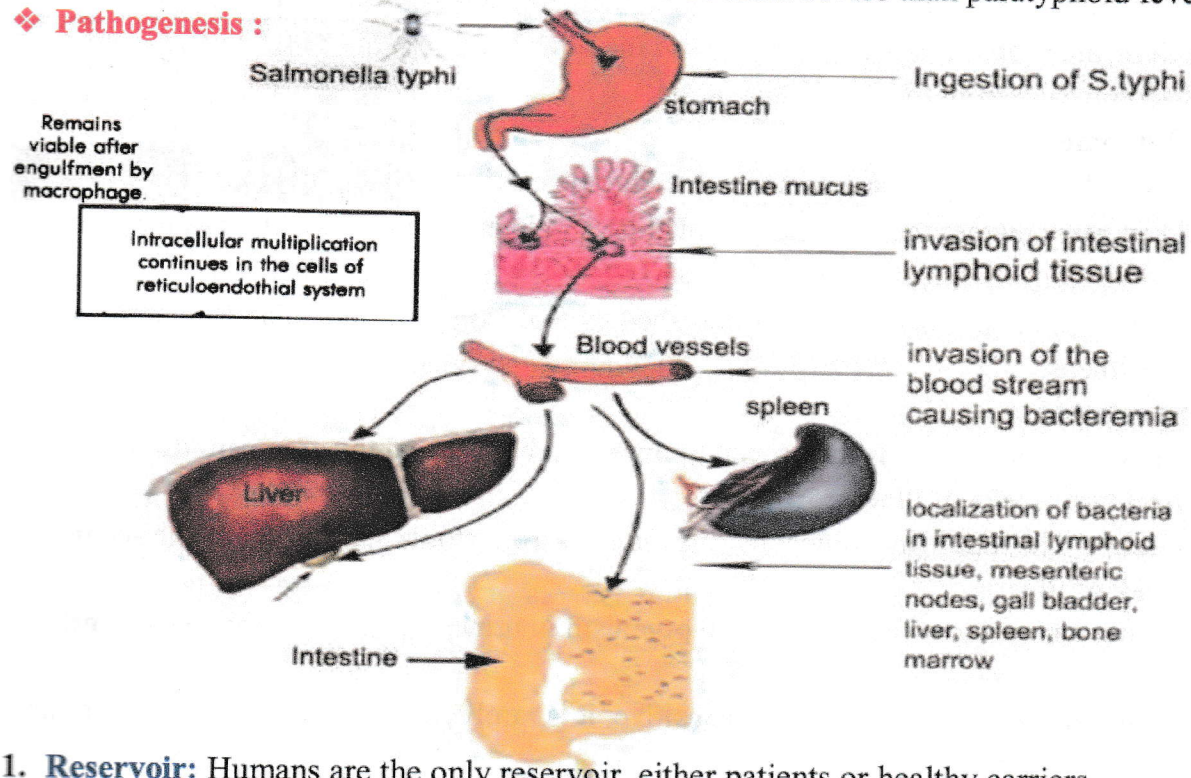
- S. Enteritidis and S. Typhimurium that cause enterocolitis .
- S. Choleraesuis that causes septicaemia and metastatic infections.

Typhoid and Paratyphoid Fever (Enteric Fever)

❖ **Enteric fever is a** generalized infection of the **reticuloendothelial system** and **intestinal lymphoid tissue** accompanied by **bacteraemia and sustained fever**.

- **Typhoid fever** is more common and tends to be more severe than paratyphoid fever.

❖ **Pathogenesis :**



1. **Reservoir:** Humans are the only reservoir, either patients or healthy carriers.
2. **Mode of transmission :** faecally contaminated food and water.
3. **The incubation period** is usually 10-14 days
4. **Transient primary bacteraemia:**

- After ingestion, the organisms **adhere** to the mucosa of the *small intestine* then **invade** to the submucosal layer.
- The organisms are taken up by **macrophages** of the Royer's patches where they replicate intracellularly.
- They are transported in the macrophages to the mesenteric **lymph nodes** and via the thoracic duct to the **bloodstream** (transient primary bacteraemia).
- The organisms reach **organs** where cells of the reticuloendothelial system are concentrated (i.e. the spleen, bone marrow, liver and Peyer's patches).

5. **Secondary heavier bacteraemia:**

- The organisms **multiply** in these organs and then **re**invade the blood causing a secondary heavier bacteraemia (**onset of fever**).

6. **Spread:**

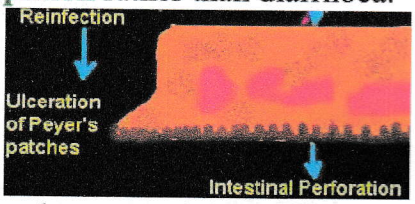
- From the blood, the organisms can reach other organs e.g. kidney and gall bladder.
- From the gall bladder, salmonellae enter the *intestine for a second time* in much larger numbers causing a strong inflammatory response.

7. Excretion :

- The organism is excreted with **faeces** (**second week of the disease**).
- In about 25% of cases, the organism is excreted in the urine.

8. Symptoms & complications:

- **Symptoms:** fever, headache, abdominal pain, and **constipation** rather than diarrhoea.
- **Complications:** relapse, perforation of the bowel and haemorrhage from the bowel ulceration.



9. Outcome:

- A small percentage of patients become chronic carriers.
- They carry the organism in the gall bladder and intermittently excrete it in the stools.

❖ Laboratory diagnosis of enteric fever

- Definitive diagnosis is only accomplished by isolation of salmonellae from clinical specimens.

A. Specimens

1. **Blood:** Blood culture is the procedure most likely to reveal the organism **during the first two weeks** of illness.
2. **Faeces:** The organism is most readily isolated from stools starting **from the second week** of illness.
3. **Urine:** Intermittent excretion of the organism in urine may occur **after the second week** of illness.
4. **Bone marrow:** Although highly sensitive, it is rarely indicated because it requires an invasive technique.

B. Direct detection by microscopy is useless.

C. Cultivation

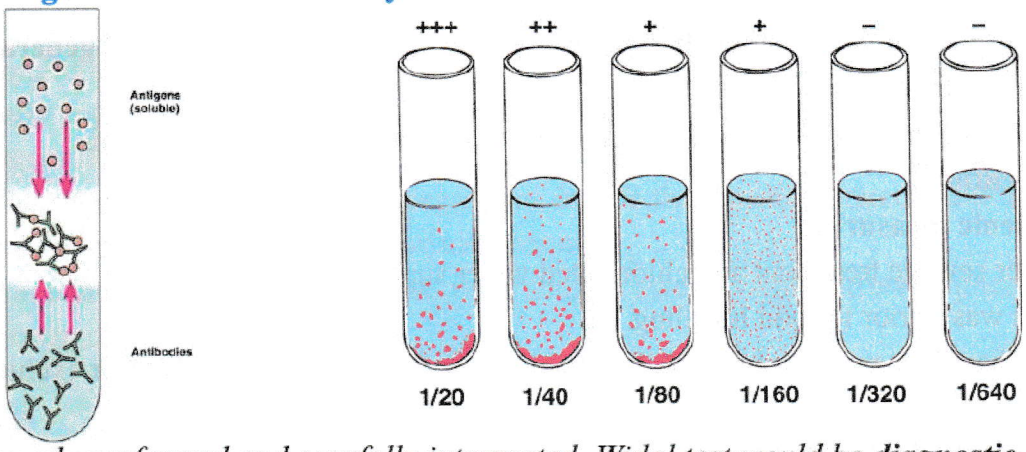
1) **Blood or bone marrow:** Samples should be cultivated by the blood culture technique. Subcultures are plated on MacConkey agar.
2) **Stools:** Faecal specimens should be inoculated directly on **both:**

- Differential selective solid media such as MacConkey, DCA or Salmonella-Shigella (SS) agar.
- Enrichment (tetrathionate or selenite) broth. Subcultures on solid media are done after overnight incubation.

D. Identification

1) After 24h incubation, colonies should be examined regarding morphology, Gram stain and oxidase test.
2) Colonies showing Gram-negative bacilli and are oxidase negative are considered Enterobacteriaceae.
3) Salmonella produces pale (lactose-nonfermenting) colonies on MacConkey agar and DCA, and black colonies on SS agar.
4) Salmonella is identified by testing colonies for biochemical reactions (see before).
5) Confirmation is done by slide agglutination using O serogroup antibodies.

E. Serodiagnosis of enteric fever by Widal test:



☞ *If properly performed and carefully interpreted, Widal test would be* ***diagnostic****.*

❖ Widal test is a tube agglutination test that measures **agglutinating antibodies to** the O and H **antigens** of Salmonella Typhi and Paratyphi A and B.

- Timing: Antibodies can be detected **at the beginning of the second week onwards**.
- It is done by mixing serial (twofold) dilutions of the **patient's serum with** O and H **antigens** from representative salmonellae.
  - ☞ *The antibody titre is the highest serum dilution showing agglutination.*
- The results are reported by giving the titre for both O and H antibodies.
- **Rising of antibody titre** is detected by testing 2 serum samples obtained at an interval of 7-10 days.
- **O antibody** appears early and its presence signifies **active infection**. It disappears faster than H antibody, the presence of which determines the type of infecting organism.

❖ **Interpretation of Widal Test:**

- High titre of O **and** H antibodies (**≥1:160**) or **rising titre** (fourfold or greater) suggest **active infection.**
- High titre of **H** antibody **alone** (**≥1:160**) indicates **past immunization or past infection**.

A. False positive results:

1) The presence of **cross-reacting antibodies**: e.g. infection with other related members of Enterobacteriaceae or autoimmune diseases.
2) **Endemicity of the disease**: Healthy people have antibody titre due to subclinical infection. In Egypt, titres up to 1:80 are considered insignificant.
3) **Vaccination:** Vaccinated people have :

- **Both** O and H antibodies if **recently** immunized.
- **H** antibody alone in cases of **past** vaccination.

B. False negative results:

1. Performance of the test during the **first week** of illness (before appearance of antibodies).
2. **Early antibiotic** treatment.

❖ **Treatment**

- The drug of choice is either **fluoroquinolones** (e.g. ciprofloxacin) or **3rd generation cephalosporins** (e.g. ceftriaxone).
- *Ampicillin or ciprofloxacin* should be used in *chronic carriers* of S. Typhi.

❖ **Prevention**

A. Hygienic measures:

1. Proper sewage treatment and chlorinated water supply.
2. Hand wash prior to food handling.
3. Food handlers are examined to diagnose carriers, who should be treated or excluded from handling food.

B. Immunological measures:

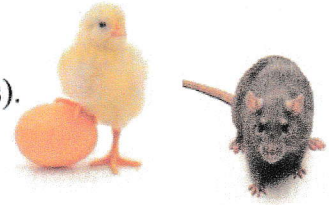
1. **Oral typhoid vaccine**: A live avirulent mutant strain of S. Typhi.
2. **Vi capsular polysaccharide vaccine** of S. Typhi. It is given *intramuscularly*.
3. **TAB vaccine:** A heat killed vaccine containing S. Typhi, S. Paratyphi A and S. Paratyphi B. The vaccine is given *subcutaneously*.

Salmonella Food Poisoning (Gastroenteritis or Enterocolitis)

❖ It is a worldwide infection caused by **S. Enteritidis and S. Typhimurium.**

❖ **Pathogenesis :**

❁ **Mode of transmission :**



- Food of animal and poultry origins (particularly raw eggs).
- Water or food contaminated with rat excreta.

❁ **The incubation period is 8-48 hours.**

- The organism invades and replicates in the epithelial cells of small and large intestines (**not in macrophages**) leading to inflammatory lesions and diarrhoea.
- There is **no toxin** production.

❁ **Symptoms:**

- Fever, nausea, vomiting, **severe diarrhoea**, and abdominal cramps.
- The condition is usually self-limited, lasting only for few days.

❖ **Diagnosis by** isolation of the organism from stools.

❖ **Treatment :**

- Correction of dehydration and electrolyte imbalance.
- Antibiotic therapy is not needed **except in immunocompromised** individuals.

❖ **Prevention:** The disease is prevented by

- Avoiding contamination of food and water by rodent excreta.
- Proper cooking of poultry and eggs.

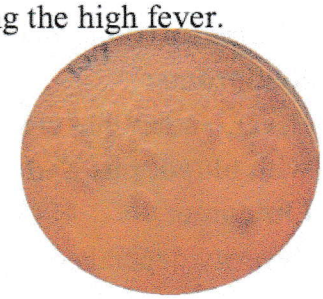
Septicaemia

- The condition is commonly caused by **S. Choleraesuis.**
- It usually occurs in **immunocompromised** individuals.
- **Pathogenesis :**

- After ingestion of salmonellae, the organisms invade the intestinal mucosa and invade the bloodstream early **without intestinal lesions**.
- Bacteraemia results in metastatic infections such as osteomyelitis, arthritis, pneumonia and meningitis.

- **Diagnosis:** Blood culture is usually positive particularly during the high fever.

Shigella



- Shigella species cause **bacillary dysentery** in man.
- **Morphology:**

- Shigellae are ***non*-motile** Gram-negative bacilli.

- **Culture:**

- Shigellae grow as pale lactose-nonfermenting colonies on MacConkey and DCA media.

- **Biochemical reactions:**

- Shigellae ferment glucose, with production of **acid only.**
- They are lactose-nonfermenters.
- All shigellae are **urease and H2S *negative*.**
- On TSI, they give acidic (yellow) butt and alkaline (red) slant with ***no*** gas and ***no*** H2S production.

- **Antigenic structure:**

- Shigellae have **O antigens** which divide the genus into four serogroups (species):
  - **A. Group A: S. dysenteriae** (13 serotypes).
  - **B. Group B: S. flexneri** (8 serotypes).
  - **C. Group C: S. boydii** (18 serotypes).
  - **D. Group D: S. sonnei** (1 serotype).

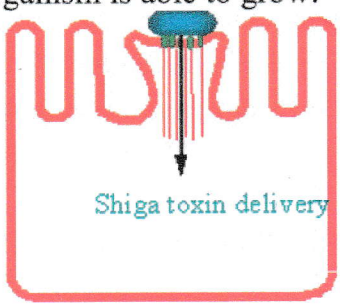
- **Virulence factors:**

A. Invasiveness:

- The essential pathogenic process of bacillary dysentery is invasion of mucosal epithelium of the terminal ileum and large intestine where the organism is able to grow.
- The organism **does not invade into the blood.**

B. Shiga toxin:

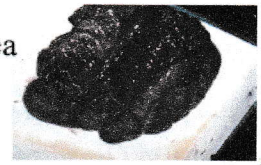
- Shiga toxin is an **exotoxin produced by S. dysenteriae type 1.**
- <u>The toxin can act as:</u>
  - **Enterotoxin**
  - **Cytotoxin** (that may contribute to mucosal damage)



- **Neurotoxin** (that may cause meningismus and coma).
- The toxin may cause **haemorrhagic colitis and hdemolytic uraemic syndrome (HUS).**

❖ **Pathogenesis**

- **Reservoir:** Man is the only reservoir for shigellosis.
- **Mode of transmission:**
- By food or water contaminated with human faeces.
- Ingestion of few organisms (~ 100 organisms) is able to cause disease, so person-to-person transmission can occur.
- **The incubation period** is **1-4 days.**
- **Symptoms:**
- Fever, abdominal cramps, tenesmus and diarrhoea with **blood, pus and mucus in stools**.



- **Outcome:**
- Severity depends on age of patients and the strain.
- **S. dysenteriae type 1** causes the severest form of the disease due to shiga toxin production.

❖ **Laboratory diagnosis**

A. Specimens: Fresh stools.

B. Direct detection:

- Direct microscopy is done to differentiate bacillary from **amoebic dysentery**.
- In bacillary dysentery, **large number of PMNLs and some erythrocytes** are seen under the microscope.

C. Cultivation: Faecal specimens should be initially inoculated onto **both:**

- Solid media such as MacConkey, DCA or SS agar.
- Enrichment broth (selenite broth). Subcultures on solid media are done after overnight incubation.

D. Identification:

- After 24h incubation, colonies should be examined regarding morphology, motility, Gram stain and oxidase test.
- Colonies showing Gram-negative bacilli and are oxidase negative are considered Enterobacteriaceae.
- Shigella produces pale (lactose-nonfermenting) colonies on MacConkey agar and DCA.
- Shigella is identified by testing the colonies for biochemical reactions (see above).
- Confirmation is done by slide agglutination test using O serogroup antibodies.

❖ **N.B.: Shigella is distinguished from Salmonella by 3 criteria:**

1. **Non**-motile.
2. **No** H_2S production.
3. **No** gas production from glucose fermentation.

❖ **Treatment:**

- Fluid and electrolyte replacement for mild cases.
- Antibiotics are recommended for treatment of severe Shigella infection (**fluoroquinolones** e.g. ciprofloxacin, and **trimethoprime/ sulfamethoxazole**).

❖ **Prevention and control**

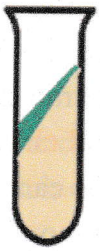
- Public health measures are recommended for control of shigellosis.

Proteus, Providencia and Morganella

❖ These organisms occur in the environment as well as in human and animal intestines.

❖ Important species include **Pr. mirabilis, Pr. vulgaris, Providencia rettgeri and M. morganii.**

❖ **These 3 genera have the following common diagnostic features:**

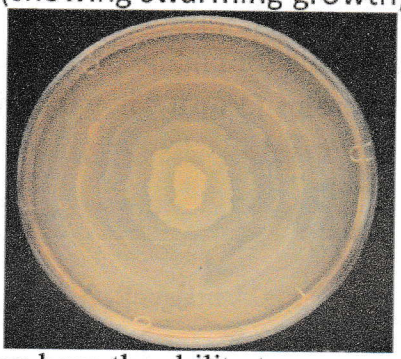

Phenylalanine +

1. **Motile** pleomorphic Gram-negative bacilli.
2. **Lactose-nonfermenter (LNF).**
3. **Urease-positive.**
4. **Phenylalanine deaminase-positive**:

- This distinguishes them from other members of Enterobacteriaceae).

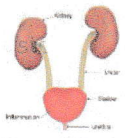
❖ **Proteus can be differentiated from:**

Proteus on nutrient agar (showing swarming growth)



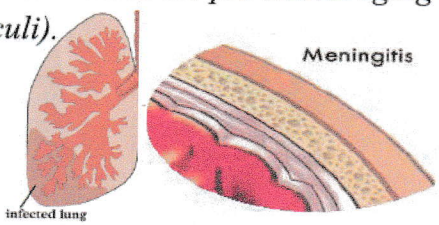
1. **Providencia and Morganella**, as proteus have the ability to **swarm on nutrient agar**.
2. **Salmonella** in stools (both are LNF and H2S positive), as proteus have the ability to produce **urease.**

❖ **Diseases**



1. **Urinary tract infection** especially caused by Proteus.

☞ *Urease hydrolyses the urea in urine to form ammonia which raises the pH encouraging the formation of stones (calculi).*



2. **Wound infections and abscess formation**.
3. **Respiratory infections**: Otitis media and pneumonia.
4. **Septicaemia and meningitis**.

❖ **Treatment:**

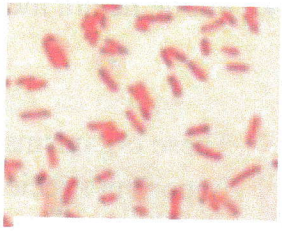
- Antibiotic susceptibility testing should be performed because of the high frequency of resistance to antibiotics.

Yersinia

❖ **The genus Yersinia includes 3 important human pathogenic species:**

- **Y. pestis:** the aetiologic agent of plague (black death).
- The enteropathogenic species: **Y. pseudotuberculosis and Y. enterocolitica.**

Yersinia Pestis



❖ Y. pestis is one of the most virulent bacteria known; 1-10 organisms are capable of causing disease.

❖ **Plague** (black death) is a **zoonotic disease** primarily affecting rodents which act as reservoirs.

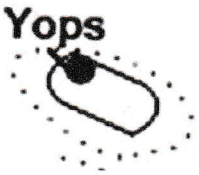
❖ **Morphology:**

- Y. pestis are **non**-motile Gram-negative coccobacilli that have characteristic bipolar staining, producing a **closed safety pin** appearance.

❖ **Cultural characteristics:**

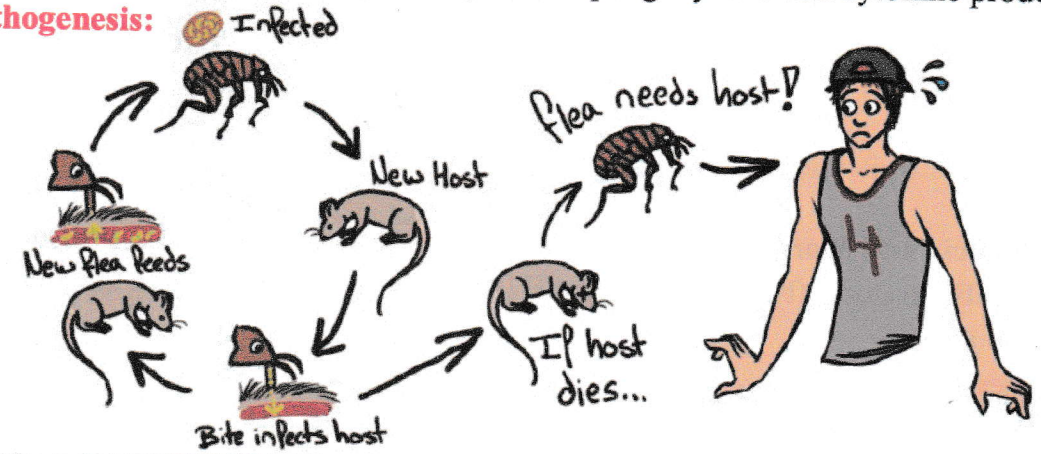
- Y. pestis can be cultured on blood agar and MacConkey agar forming small pinpoint (lactose-nonfermenting) colonies after 24 h incubation at **25°C.**

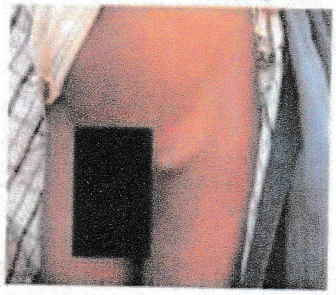
❖ **Virulence factors:**



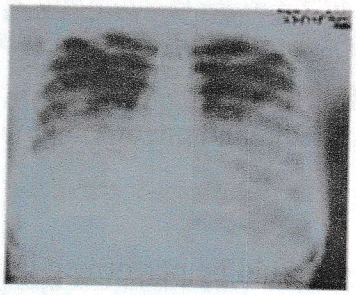
1. **Fraction 1 (F1) antigen:** It is an antiphagocytic capsular-like envelope of polysaccharide-protein complex.
2. **Endotoxin:** responsible for endotoxic shock.
3. **Yersinia outer proteins (Yop):** They inhibit phagocytosis and cytokine production.

❖ **Pathogenesis:**





Bubonic plague



Pneumonic plague

Septicemic plague

❖ **Steps of Pathogenesis:**

A. Transmission of plague between rodents is accomplished by the rat flea (**Xenopsylla cheopis**).

- The flea acquires Y. pestis from an infected blood meal from a bacteraemic rodent.

B. Man can be accidently infected by one of the following ways:

1. Flea-borne from:

- Infected rodents through the bite of the flea.
- This results in **bubonic plague** which is characterized by pain and swelling of the lymph nodes draining the site of the flea bite.

2. Inhalation of respiratory droplets from :

- Humans or animals with **pneumonic plague**.
- Aerosol created during handling yersinia culture in the laboratory.

- Pneumonic plague is characterized by fever, chills, cough and difficulty in breathing; shock and death occur eventually if not treated early.

C. In either cases, **septicaemic plague** may occur as a result of spread of the organism to the bloodstream. Through haematogenous spread, the organism may reach other organs.

❖ **N.B.: In bioterrorism,** the organism may be delivered by aerosol to cause pneumonic plague, or by using infected fleas to cause bubonic plague.

❖ **Laboratory diagnosis:**

A. Specimens: Lymph node aspirates, sputum or blood.

B. Direct detection:

- Gram or **Giemsa** stain reveal the characteristic bipolar staining of coccobacilli.
- Fluorescent-antibody (FA) test to detect F1 antigen.

C. Cultivation and Identification:

- Although hazardous, culture may be done on blood and MacConkey's agar, and the colonies are identified by FA test.

❖ **Treatment: Streptomycin** is the drug of choice.

❖ **Prevention:**

- Anti-rat and anti-flea measures & Avoidance of sick or dead animals.

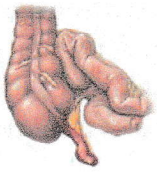
Yersinia enterocolitica and Y. pseudotuberculosis

❖ Y. enterocolitica and Y. pseudotuberculosis are rare causes of **enterocolitis.**

❖ They are transmitted to humans by **food** contaminated with **excreta of domestic** animals.

❖ **Refrigerated foods** are potential vehicles because Y. enterocolitica can grow at refrigeration temperatures.

❖ Disease (yersiniosis) can range from mild diarrhoea to what appears to be acute appendicitis.



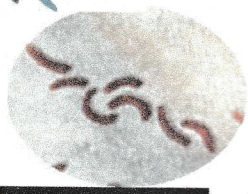
Chapter 10: Vibrio

❖ Vibrios are one of the most common organisms in both **sea and fresh water** habitats and in association with **aquatic animals**.

❖ **Species :**

A. Vibrio cholerae is the most clinically important species.

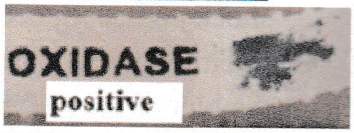
B. Other important species are **V. parahaemolyticus and V. vulnificus.**



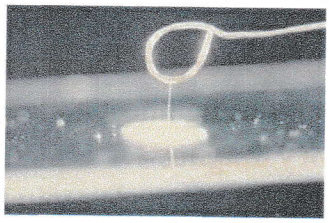
❖ **Characteristic features:**

- Members of the genus Vibrio are characterized by the following:

1. **Gram-negative curved bacilli.**
2. **Darting motility**: Highly motile with a **single** polar flagellum.
3. **Facultative anaerobes** (preferring oxygenated environment).
4. Tolerant to **alkaline** conditions and **destroyed by low pH.**
5. Ferment sugars **without** gas production and reduce nitrates.
6. **Oxidase-positive** (therefore, not classified as Enterobacteriaceae).
7. **String-positive.**



❖ **String test:**



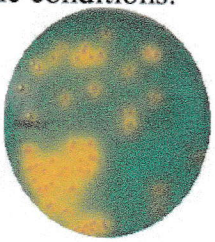
- The string test differentiates vibrios (string-positive) from Aeromonas (string- negative).
- The test is done by emulsifying a large colony in a small drop of 0.5% sodium desoxycholate.
- Within 60 seconds, the cells lyse and DNA strings can be observed when a loopful is lifted from the slide **up to 3 cm**.

Vibrio Cholerae

❖ **Morphology (mentioned above)**

❖ **Cultural characters**

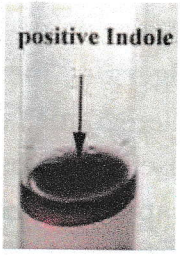
- V. cholerae is a facultative anaerobe and grows best under alkaline conditions.
- It can be grown on:



1. **Alkaline peptone water (pH 8.5):**
   - A **surface pellicle**, containing the organism, is formed after an incubation period of 6 to 8 h at 37°C.
2. **Nutrient agar** at slightly alkaline pH.
3. **Thiosulphate citrate bile sucrose (TCBS) agar** (a selective indicator medium):
   - V. cholerae produces *yellow* colonies on TCBS owing to *sucrose fermentation*.

❖ **Biochemical reactions:**

- Vibrio cholerae ferments glucose, maltose, mannite and sucrose **without** gas production.
- On TSI, it gives acidic (yellow) butt and acidic (yellow) slant with **no cracking** of the agar.
- V. choierae is **oxidase-positive and indole-positive.**



❖ **Serological characters and biotypes of V. cholera:**

- O.antigens are specific and distinguish strains of V. cholerae into **serogroups** designated in numerals (O1, O2, O3 etc).

O1 { Classical { Ogawa, Inaba, Hikojima
El Tor

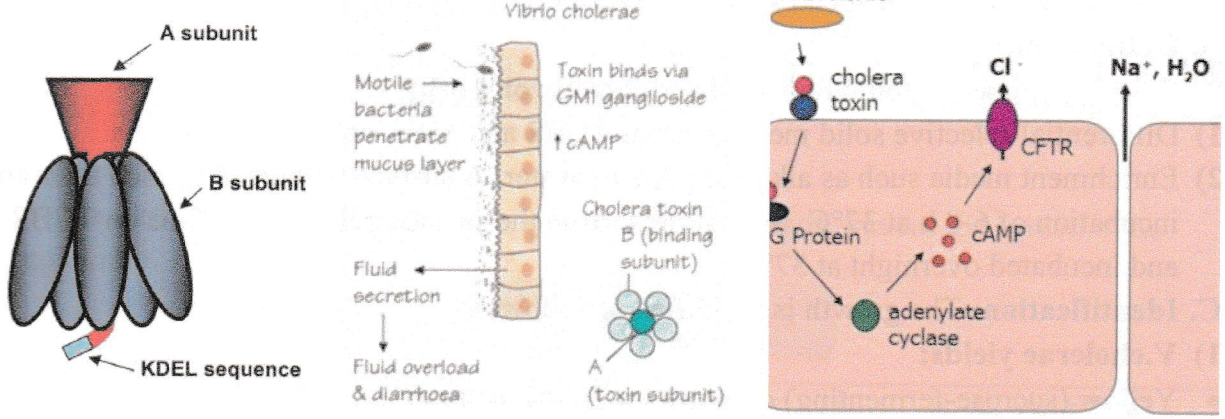
- The serogroup **O1** has three distinct serotypes (**Ogawa, Inaba and Hikojima**) based on antigenic differences.
- Each serotype may display the **classical or El Tor** biotype based on differences in biochemical reactions.
- V.cholerae serogroups **O1 and O139** are the causative agents of **epidemic** cholera.
- Other serogroups (**non O1/O139**) are involved in **sporadic** forms of cholera-like diarrhoeal disease, but not in epidemics.

❖ **N.B.: The flagellar (H) antigens** are shared with **all** vibrios and are, therefore, of **no** use in distinguishing strains.

Cholera

❖ Cholera is a severe diarrhoeal disease caused by V. cholerae serogroups O1 or O139.

❖ **Pathogenesis:**



❀ **Reservoir:** humans and the aquatic environment.

❀ **Mode of transmission:** faeco-oral route, through contaminated water or food.

❀ **Access, multiplication, toxin:**

- Vibrios are sensitive to acid and most **die in the stomach**:

↳ *A high infectivity dose* (10^7-10^{12}) *is, therefore, required and this is why direct person-to-person spread is not common.*

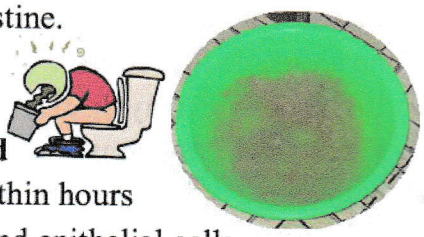
↳ *Achlorhydria or antacids reduce the infective dose.*

- Bacteria that passed the stomach **penetrate** the mucous layer of the small intestine and adhere to its mucosa by fimbriae and other colonization factors.
- V. cholera will then **multiply** and secrete the potent **cholera enterotoxin (choleragen)**.

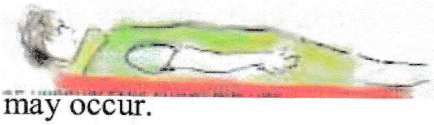
❖ **Cholera toxin:**

- It is composed of **5 binding (B) subunits and an active (A) subunit**.
- The toxin binds through its B subunits to specific receptors on the intestinal epithelial cells.
- The enzymatically active (A) subunit enters the cells→ activates the adenylate cyclase enzyme → rise in cAMP production → massive secretion of electrolytes (Na+, K+, Cl-, and HCO3-) and water into the lumen of the small intestine.

❖ **Clinical Manifestations:**



1) **Incubation period ranges** from **1-4 days**.
2) The disease begins with an abrupt onset of **vomiting and massive watery diarrhea** up to several litres of fluid within hours
- The watery diarrhoea is speckled with flakes of mucus and epithelial cells (**rice-water stools**) and contains enormous numbers of vibrios.
3) The disease runs its course in **2 to 7 days**; the outcome depends upon the extent of water and electrolyte loss and the efficiency of treatment.



4) **Death** may occur from hypovolaemic shock.
5) **Chronic carriers** are rare although convalescent carriers may occur.

❖ **Diagnosis:**

- The diagnosis is suggested by the characteristic clinical picture.

A. Specimens: Stools (or rectal swabs for carriers).

B. Cultivation:

- Faecal specimens should be inoculated directly on **both:**

1) Differential selective solid media such as TCBS and MacConkey's agar media.
2) Enrichment media such as alkaline peptone water. A surface pellicle is formed after an incubation of 6-8 h at 37°C. Subcultures, from the surface pellicle, are done on TCBS and incubated overnight at 37°C.

C. Identification: The growth is identified as follows:

1) **V.cholerae yields:**

- Yellow (sucrose-fermenting) colonies on TCBS medium.
- Pale (lactose-nonfermenting) colonies on MacConkey's agar.

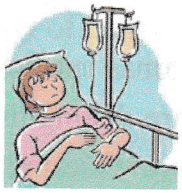
2) **Microscopic examination of:**

- A wet mount: shows rods with darting motility.
- A Gram-stained smear: shows small, Gram-negative curved rods.
- Biochemical reactions: (see before).
- String test: positive (see before).
- Confirmation: is done by a **slide agglutination test** with specific anti-O1 and anti-O139 antisera.

❖ **N.B.: During an epidemic:**

- Diagnosis depends mainly on **clinical** judgment, and there is little need for the laboratory.

- Laboratory confirmation may be done by **microscopical examination** of a wet mount of liquid stools to detect the characteristic darting motility of vibrios which is stopped by specific antibody.



❖ **Treatment:**

- Treatment of cholera consists **essentially of replacing fluid and electrolytes** either intravenously or orally.
- Antibiotics such as **tetracycline or ciprofloxacin** are useful in treatment:
  - They shorten the duration of diarrhoea and reduce the time of excretion of the organisms.

❖ **Control:**

1) Sanitation:

- Application of sanitary principles that protect drinking water and food from contamination with human faeces.

2) Vaccination: Three Cholera vaccines are available:

❖ **Nature of vaccine**	**1. Killed cholera vaccine**	**2. Live-attenuated vaccine**	**3. Whole cell/B subunit vaccine**
❖ **Route**	○ Intramuscular	○ Oral	○ Oral
		↳ *Oral vaccines provide better protection*	
❖ **Protection duration**	○ 3-6 months	○ At least 6 months	○ 2 years

☞ *An inexpensive, effective cholera vaccine that provides long-term protection is **not** yet available.*

3) Chemoprophylaxis:

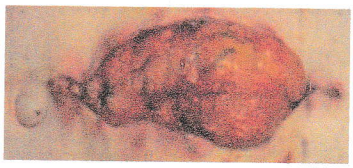
- **Tetracycline** may be given to close contacts but it **cannot** prevent the spread of an epidemic.

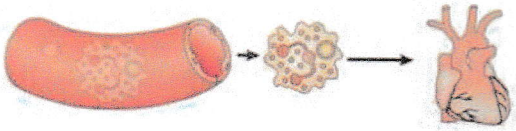
Other Vibrio Species

❖ **Species**	**A. Vibrio para- haemolyticus**	**B. V. vulnificus**
❖ **Reservoir**	○ Sea water; halophilic (salt loving) organisms.	
❖ **Disease**	○ Gastro enteritis	
		○ Cellulitis
❖ **Transmission**	○ Consumption of undercooked or raw seafood	
		○ Swimming in sea water
❖ **Manifestations**	○ Watery diarrhoea with abdominal cramps	
		○ Rapidly spreading lesions
❖ **Treatment**	○ Self- limiting	
		○ Tetracycline, 3rd generation cephalosporins

Aeromonas

- Aeromonas species are morphologically **similar to** vibrios.
- They are distinguished from vibrios by being **string-negative**.
- They are widespread inhabitant in **water and soil**.
- **Aeromonas hydrophila** is the most important species. It causes:

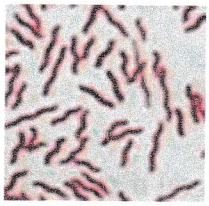


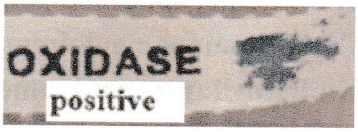


1) **Wound infections.**
2) **Diarrhoea resembling cholera.**
3) **Bacteraemia and septicaemia in immunocompromised patients.**

Chapter 11: Campylobacter

- Campylobacters are carried in the **intestinal tract** of a wide variety of **birds** (especially poultry) and domestic **animals** (e.g. cattles, dogs and cats).
- **Characteristic features:**





1. They are **curved or S-shaped, slender, Gram-negative rods.**
2. They have **single** polar flagella contributing to the characteristic **darting motility.**
3. Most campylobacters are **microaerophilic** (requiring 5% O2 and 10% CO2).
4. They are **oxidase positive.**

- The genus comprises many species, the most important of which is C. jejuni which accounts for about 90% of the Campylobacter diseases, especially in children.

Campylobacter Jejuni

- **Morphology (mentioned above)**
- **Cultural characters:** C. jejuni can be grown on:

- Enriched media e.g. **blood or chocolate** agar.
- Selective media e.g. **Skirrow's medium** which is blood agar that is rendered selective by addition of antibiotics.

☞ *Cultures are incubated in **microaerophilic** atmosphere at42°C (**thermophilic**) for 2 days.*

Campylobacter Enteritis

- Campylobacter jejuni causes enteritis that affects **mainly children under 5 years and young adults.**
- **Pathogenesis:**

- C.jejuni *adheres* to intestinal epithelial cells or overlying mucus.
- It *invades* and destroys epithelial cells in the jejunum, ileum and colon.
- <u>Some strains of C. jejuni produce an :</u>

  A. Enterotoxin (similar to cholera toxin) that causes **watery diarrhea.**

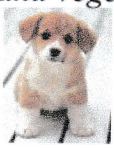
  B. Cytotoxin that causes **bloody diarrhoea.**




- **Transmission :**

1. C. jejuni is transmitted to humans primarily **faeco-orally**, through consumption of food and water contaminated with animal faeces.

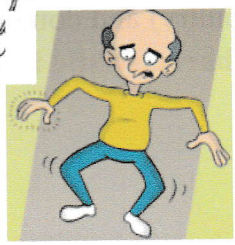
   ☞ *Common foods include poultry, raw milk, meat, fruits and vegetables.*
2. Person-to-person transmission may rarely occur
3. Exposure to **sick kittens and puppies** has also been associated with outbreaks especially in children.

❖ **Clinical manifestation:**

❁ **The incubation period is 2-5 days.**

❁ **Symptoms :**



- **Watery** diarrhoea followed by **bloody** stools accompanied by fever and abdominal cramps.

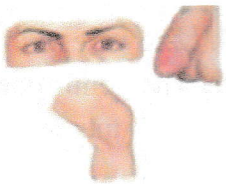
❖ **Complications :** autoimmune diseases:

1. **Guillain-Barre syndrome:**

- It is an acute paralytic disease of the peripheral nervous system.
- It is attributed to antibodies against C. jejuni, that **cross react** with antigens on neurons.

2. **Reactive arthritis.**

3. **Reiter's disease:**



- It is characterized by the triad of arthritis, conjunctivitis and urethritis.
- Most patients are men who are **HLA-B27-positive**.

❁ **Outcome:**

- Most infections are self-limited and illness generally lasts 7-10 days.

❖ **Laboratory diagnosis:**

A. Specimens; Stools.

B. Direct detection: Presumptive diagnosis can be made by finding curved organisms with rapid darting motility in a wet mount of faeces.

C. Cultivation: C. jejuni can be isolated from faecal specimens by using:

1. **A filtration method and a non-selective culture medium:**

- A bacterial filter with a certain pore size is used.
- It permits passage of the small slender campylobacters and excludes larger faecal organisms.
- The filtrate is then inoculated on a non-selective medium e.g. chocolate agar.

2. **A selective medium (e.g. Skirrow's medium).**

☞ *In either method, cultures are incubated in a microaerophilic atmosphere at 42°C for 2 days.*

D. Identification: Growth of C. jejuni is identified biochemically.

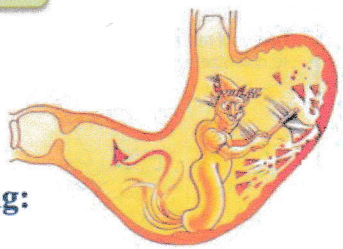
❖ **Treatment:**

- Replacement of fluids and electrolytes is the main line of therapy.
- **Erythromycin** is the antibiotic of choice.

❖ **Prevention:**

- Proper cooking of chicken, pasteurization of milk and chlorination of drinking water.
- Proper sewage disposal.
- Personal hygiene especially hand washing.

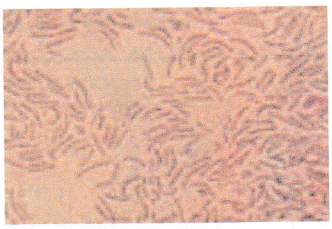
Chapter 12: Helicobacter

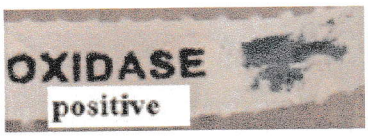


❖ Helicobacters (particularly Helicobacter pylori) are the only organisms which are able to colonize the **stomach**.

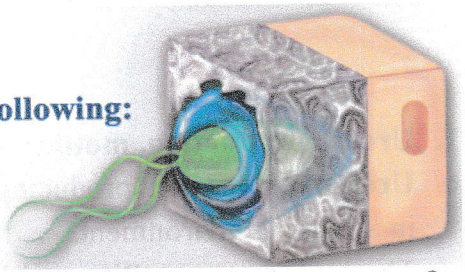
❖ **Characteristic features:**

- **Helicobacters are similar to campylobacters in the following:**





1. Spiral or curved Gram-negative bacilli.
2. Able to grow on the same media (e.g. Skirrow's medium).
3. Microaerophilic.
4. Oxidase-positive.



- **Helicobacters differ from campylobacters in the following:**

1. They have **multiple** polar flagella contributing to the characteristic **corkscrew** movement.
2. They can grow at **37°C** but not at 42°C.

- H.pylori is the most important Helicobacter species since it is the causative agent of **peptic ulcer**. The latter is now approached as an infectious disease.

Helicobacter Pylori

❖ **Morphology (mentioned above).**

❖ **Cultural characters:** H. pylori can be grown on:

- Enriched media e.g. blood or serum agar.
- Selective media e.g. Skirrow's medium.
- The organism requires **microaerophilic** condition, high humidity and incubation at 37°C for 3-6 days.

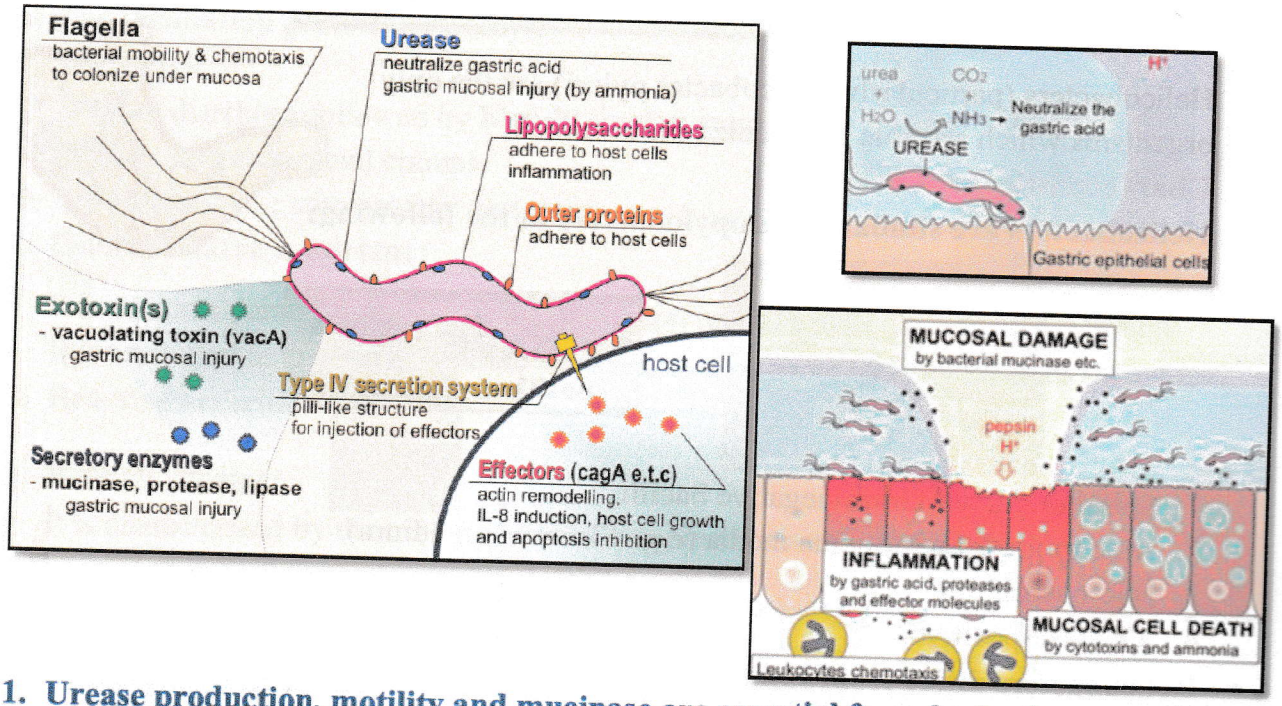
❖ **Biochemical reactions:**

- All helicobacters are oxidase positive.
- H. pylori produces large quantities of **urease.**

❖ **Epidemiology and transmission:**

- H. pylori has been found in the **stomach** of humans in all parts of the world.
- There appears to be no reservoir of H. pylori aside from the human stomach.
- Transmission of H. pylori is thought to be from **person to person** (faeco-oral or oral-oral) as evidenced by the following:
  - There is clustering of infection within **families**.
  - The organism has **not** been isolated from **food or water**.

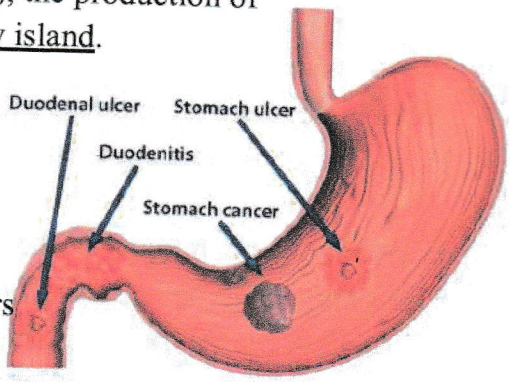
❖ Pathogenesis:



1. **Urease production, motility and mucinase are essential for colonization:**
   - **Urease** cleaves urea producing **ammonium cloud**, which permits H. pylori to survive in an acidic environment.
   - The **corkscrew motility** enables the organism to penetrate (pore) through the viscous gastric mucus.
   - **Mucinase** helps in penetration of mucous layer by the organism allowing it to reach the stomach lining, where the pH is neutral.
2. **The organism binds to the mucosal cells by adhesins.**
3. **Mucosal damage occurs as a result of:**
   - Release of **ammonia.**
   - Production of **vacuolating cytotoxin (VacA).**
   - Recruitment and activation of **inflammatory cells**
   - This is mediated by chemokines (e.g. interleukin-8), the production of which is controlled by genes within a pathogenicity island.

❖ Clinical outcomes:

1. **Chronic gastritis:**
   - It develops in colonized people and may be associated with epigastric discomfort.
2. **Duodenal or gastric ulcers:**
   - H. pylori is associated with >90% of duodenal ulcers and 70-80% of gastric ulcers.
3. **Stomach cancer:**
   - Gastric carcinoma or **gastric lymphoma** may follow the chronic gastritis. H. pylori is now classed by the WHO as **type I carcinogen.**



❖ Diagnosis:

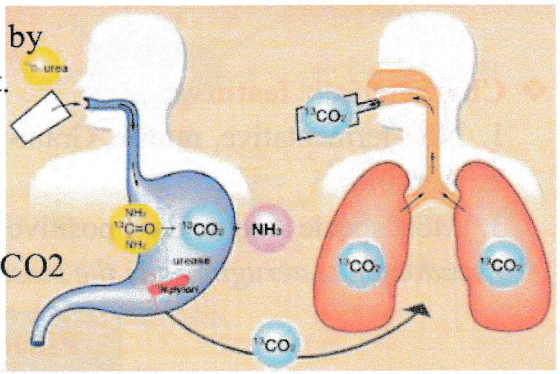
I. Non invasive methods:

1. Antigen detection:

- Detection of H. pylori antigens in stools is done by ELISA for diagnosis and follow up of treatment.

2. Urea breath test:

- Radiolabeled urea is ingested.
- If the organism is present, urease will cleave the ingested urea, releasing radiolabeled CO2 which can be detected in the breath.



3. Serology:

- The presence of **IgG** antibodies in the patient's serum can be used as evidence of infection.

II. Invasive methods:

A. Specimens: Gastric biopsies obtained by endoscopy.

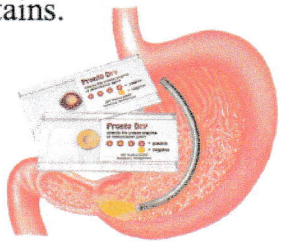
B. Direct detection:

1. Histologic examination:

- H. pylori can be visualized in the biopsy sections using special stains.

2. Biopsy urease tests:

- The gastric biopsy is placed onto a urea-containing medium with a pH indicator which changes its colour upon release of ammonia.



3. Molecular methods:

- DNA probes and PCR can also be used to detect pylori nucleic acid.

C. Cultivation (mentioned above).

D. Identification:

- H. pylori is oxidase positive and strong urease producer.
- It can also be identified by DNA probes and PCR.

❖ Treatment:

- Elimination of H. pylori requires combination therapy with **2 antibiotics and an antacid**.
- A typical regimen includes **amoxicillin** plus **clarithromycin** plus a **proton-pump-inhibitor.**

Chapter 13: Non-Fermentative Gram-Negative Bacilli

Pseudomonas

❖ Characteristic features:

1. Non-fermentative, **motile**, Gram-negative bacilli.
2. Aerobic.
3. Most species are oxidase positive.

☞ *Pseudomonas aeruginosa is the most important species.*

Pseudomonas Aeruginosa

❖ P. aeruginosa is worldwide in distribution preferring moist environment:

- It is found in water, soil and on plants.
- It may be part of the normal microbial flora, particularly in the **gastrointestinal tract** and **moist** body sites.
- It favours persistence in the **hospital** environment due to:

a) It is able to grow in water containing only **traces of nutrients** e.g. tap water.

b) It is ability to **resist disinfectants**.

❖ Morphology (mentioned above).

❖ Cultural characters: P. aeruginosa grows on:

1. Nutrient agar:

- It usually produces diffusible **exopigments.**
- When the yellow pyoverdin pigment combines with the blue pyocyanin pigment, the bright **green** colour characteristic of P. aeruginosa colonies is created.

2. Blood agar: It causes **complete** haemolysis.

3. MacConkey agar: It produces **pale** lactose non-fermenting colonies.

4. TSI: It gives red (alkaline) butt and red (alkaline) slant.

- It has a grape-like (fruity) odour.

❖ Biochemical activities:

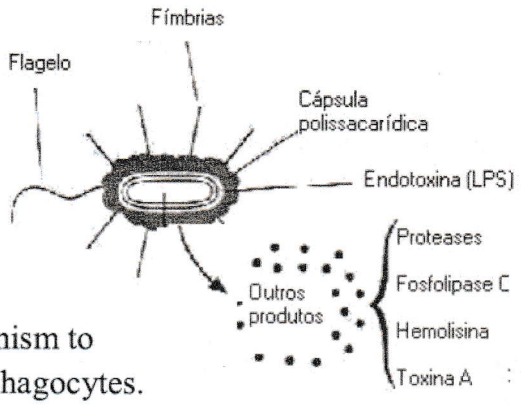
- P. aeruginosa is oxidase-positive.
- It is carbohydrate non-fermenter; **acid** is produced from glucose only oxidatively.

❖ Pathogenesis:

- P. aeruginosa is a significant human pathogen particularly in immunocompromised patients.
- It is a major cause of **hospital-acquired (nosocomial) infections.**

- The organism is considered invasive and toxigenic and may cause pyogenic infections.

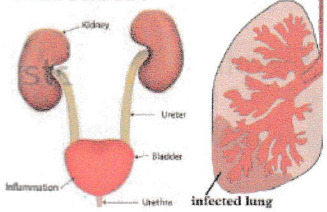
❖ **Virulence factors include:**

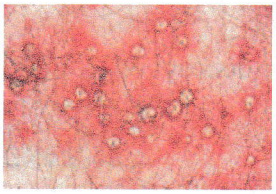


1. **Pili.**
2. **Exotoxin A**: It causes tissue necrosis by the same mechanism as diphtheria exotoxin.
3. **Endotoxin.**
4. **Enzymes:** Proteases and elastases.
5. **Capsule:**

- The polysaccharide material allows the organism to live in a **biofilm** away from antibodies and phagocytes.
- Capsulated strains predominate in patients with **cystic fibrosis.**

❖ **Diseases:**

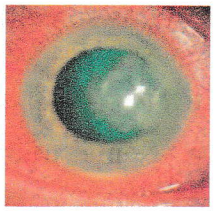




1. **Urinary tract infections (UTI).**
2. **Respiratory tract infections (RTI):**

- Especially in intubated patients or in those with cystic fibrosis.

☞ *UTI, RTI and wound infections predominate in hospitals.*



3. **Wound infections (especially burns).**
4. **Folliculitis.**
5. **External ear infections:** in swimmers (swimmer's ear), diabetics and the elderly.
6. **Eye infections:** frequently associated with the use of contact lens.

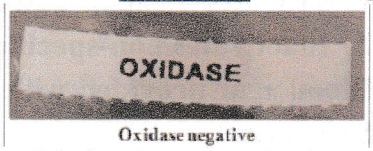
❖ **Treatment:**

- P. aeruginosa is resistant to many antibiotics.
- Combined antibiotic treatment is usually indicated in serious infections :
- Anti-pseudomonal **β- lactam** e.g. piperacillin plus an **aminoglycoside** e.g. gentamycin

Acinetobacter

- Acinetobacter species are similar to P. aeruginosa in being non-femnentative, aerobic, Gram-negative bacilli.

- They differ from P. aeruginosa in being **non-motile, and oxidase-negative.**
- **They are found in :**
  - Free-living saprophytes found in soil, water and foods.
  - Commensals on the skin.



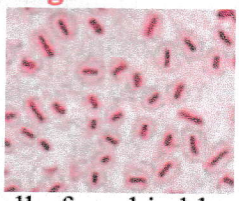
Oxidase negative

- They are opportunistic pathogens causing **hospital-acquired infections**, particularly in ICUs.
- Antibiotic resistant strains may be responsible for **outbreaks** of pneumonia, UTI and wound infections that may progress to septicaemia.

Chapter 14: Haemophilus

❖ The genus name is derived from the Greek words meaning **blood-loving.**

❖ **Characteristic features:**

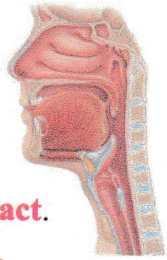


1. Gram-negative bacilli.
2. Pleomorphic, ranging from coccobacilli to long slender filaments.
3. Facultative anaerobes.
4. Require one or both of the following growth factors which are normally found in blood:

- **X factor (haemin):** is available from non-haemolysed **&** haemolysed RBCs.
- **V factor (NAD):** is liberated from **lysed** RBCs.

❖ **Medically important Haemophilus species:**

- H. influenza, H. ducreyi & H. aegyptius (H. influenzae biotype aegyptius).



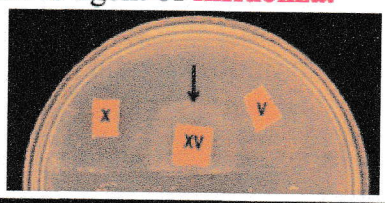
Haemophilus Influenzae

❖ H.influenzae occurs as part of the normal flora of the **upper respiratory tract.**

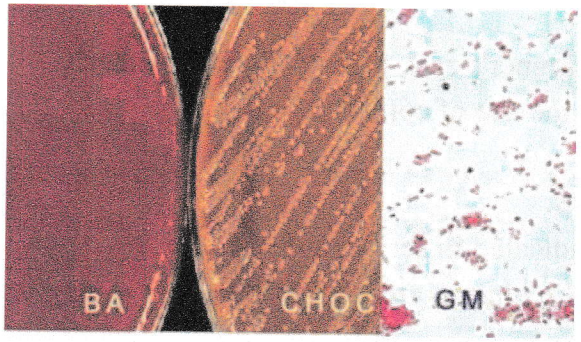
❖ It is so-called because it was thought to be the causative agent of **influenza.**

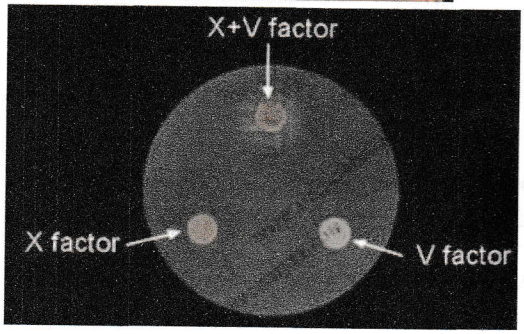
❖ **Morphology:**

- Pleomorphic Gram-negative bacilli.
- Some strains have a **polysaccharide capsule.**



❖ **Cultural characters:**





1) H. influenzae requires both X and V factors for growth:
Therefore, it can be cultured on:

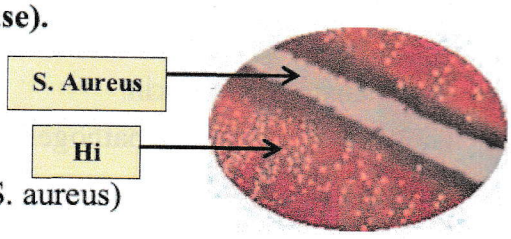
A. Chocolate agar (heat-lysed blood):

☞ *The heat used in preparing chocolate agar:*

1. **Provides V factor and extra X factor from RBCs into the medium.**
2. **Inactivates non-specific inhibitors (e.g. NADase).**

B. Blood agar provided with free V factor.

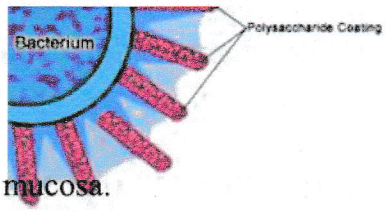
2) Satellatism:



- Growth of H. influenzae on blood agar **around colonies of a haemolytic organism** (e.g., S. aureus) that liberates V factor from lysed RBCs.

3) Growth is enhanced by **5% CO2.**

❖ **Virulence factors and clinical significance:**



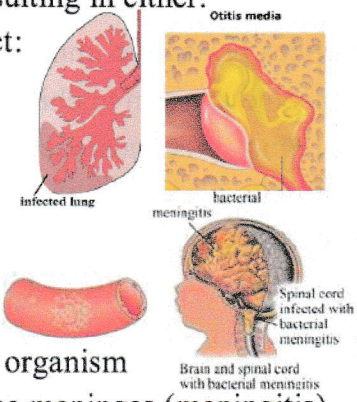
1. The major virulence factor is the **polysaccharide capsule**, according to which influenzae is divided into six serotypes a-f.

☞ ***Type b (Hib) causes most of the severe invasive diseases.***

2. **IgA protease** helps **colonization** of the upper respiratory tract mucosa.

❖ **Pathogenesis:**

1) H. influenzae is transmitted by **respiratory droplets**, resulting in either:



A. **Asymptomatic** colonization of the upper respiratory tract:

- Colonization is facilitated by Ig A protease production.

B. **Infections** (mainly by **non**-capsulated strains) :

- Otitis media, sinusitis, epiglottitis or pneumonia.
- Pneumonia occurs mainly following viral infection or in patients with chronic lung disease.

2) **Blood stream invasion** (mainly by **capsulated** strains):

- After being established in the upper respiratory tract, the organism can enter the blood stream (bacteraemia) and spread to the meninges (meningitis).

3) Most infections occur in children between the ages of **6 months and 6 years**.

- This is attributed to the **decline of maternal IgG** together with the **inability** of the child **to generate antibodies** against the polysaccharide capsular **(TI) antigen.**

❖ **Laboratory diagnosis**

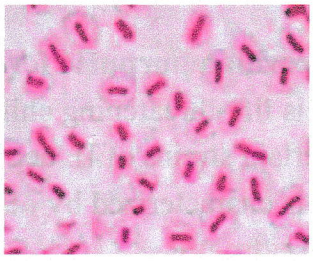
A. Specimens include CSF, blood, pus and respiratory secretions

B. Direct detection:

1. Gram-stained smear: Gram-negative coccobacilli associated with PMNLs.
2. **Quellung reaction.** 
3. Detection of type b capsular antigen in CSF by latex agglutination or fluorescent antibody test.
4. PCR.

C. Cultivation:

1. Specimens other than the blood should be plated directly onto chocolate agar and incubated at 37°C with 5% CO2.
2. Blood samples should be cultivated by the blood culture technique. Subcultures are plated on chocolate agar and incubated as mentioned above.



D. Identification:

1. **Colony morphology:**

a) **Non-capsulated strains produce small flat colonies**

b) **Capsulated strains produce larger mucoid colonies.**

2. Gram-stained film: Gram-negative pleomorphic coccobacilli.
3. Serological identification by latex agglutination or Quellung reaction.
4. DNA probes.

❖ **Treatment**

- **Third generation cephalosporins** (e.g., cefotaxime or ceftriaxone) are the antibiotics of choice.

❖ **Prophylaxis**

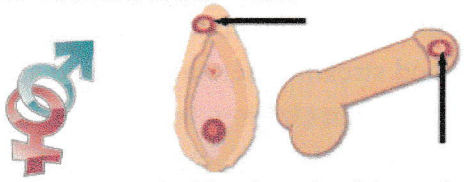
1. **H. influenzae type b (Hib) vaccine:**

- It is a capsular polysaccharide **conjugated to a carrier protein** (such as diphtheria toxoid) to elicit a T-dependent immune response.
- It provides protective immunity even in children younger than 24 months.
- Hib vaccine is given to children at the age of **2, 4, 6, and 15 months.**
- It has reduced the incidence of serious infections caused by this organism.

2. **Rifampicin** is used for chemoprophylaxis of unvaccinated close contacts of cases of **Hib meningitis.**

Haemophilus Ducreyi

❖ **H. ducreyi causes** :



- **Chancroid (soft sore)** :

- A sexually transmitted disease
- Manifest as **painful, soft** genital ulcer accompanied by inguinal lymphadenopathy

☞ *N.B.: syphilitic chancre is painless and hard.*

❖ **Diagnosis** is usually **clinical** because this highly fastidious organism is difficult to be grown in the laboratory.

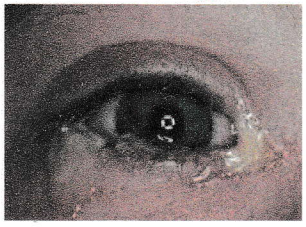
❖ **Treatment: Ceftriaxone and erythromycin** are effective.

Haemophilus Aegyptius

- **H.aegyptius (H. influenzae biogroup aegyptius) causes the following infections:**

1. **Acute mucopurulent conjunctivitis:**

- H. aegyptius is the commonest bacterial cause of this condition, which is known as **pink eye.**



2. **Brazilian purpuric fever**:

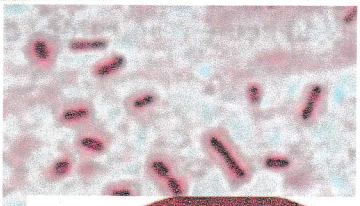
- It is life-threatening childhood illness caused by certain strains of H. aegyptius.
- It is characterized by **purpura and shock.**

❖ **Treatment:**

- Topical **tetracycline and chloramphenicol** are effective for treatment.

Chapter 15: Bordetella

❖ Characteristic features:



1) Small, encapsulated Gram-negative coccobacilli.
2) Strict aerobes.

☞ *B.pertussis is the most important species of the genus and causes whooping cough (pertussis).*

Bordetella Pertussis

❖ Morphology (mentioned above).

❖ Cultural characters:

1) Bordetella pertussis can be grown on:

- **Bordet-Gengou medium**

  (blood agar supplemented with special growth factors).

- Charcoal blood agar.

2) Growth is **slow** and requires incubation for **5-7 days at 37°C**.

3) Colonies have characteristic **mercury-droplet** appearance.

❖ Virulence Factors

I. Factors mediating attachment (colonization):

1) Filamentous haemagglutinin (FHA): It is a filamentous protein on the cell surface.

2) Pertussis toxin (PTx): It occurs in 2 forms:

- Cell-bound form acting as an adhesin.
- Secreted form acting as a toxin (see below).

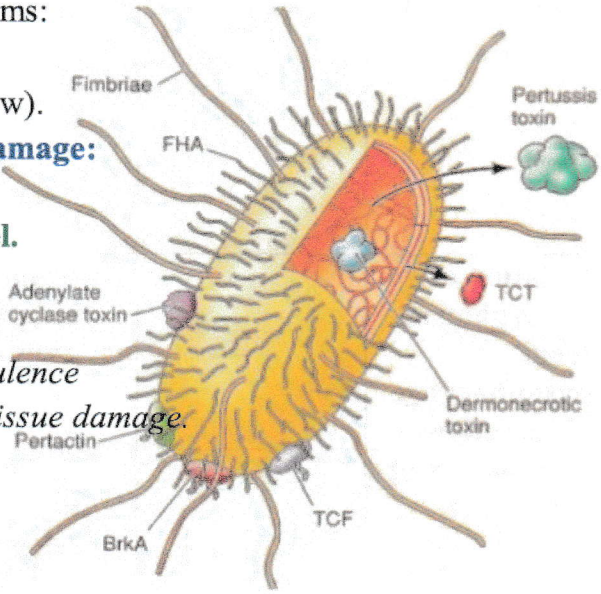
II. Factors (toxins) mediating tissue damage:

1) Pertussis toxin (PTx):

It has an A-B structure/function model.

- It causes a striking lymphocytosis.
- It increases sensitivity to histamine.

☞ *Thus, pertussis toxin is an important virulence factor mediating both colonization and tissue damage.*



2) Adenyl cyclase toxin:

- It impairs leucocyte chemotaxis: thus inhibiting phagocytosis.
- It causes local oedema.

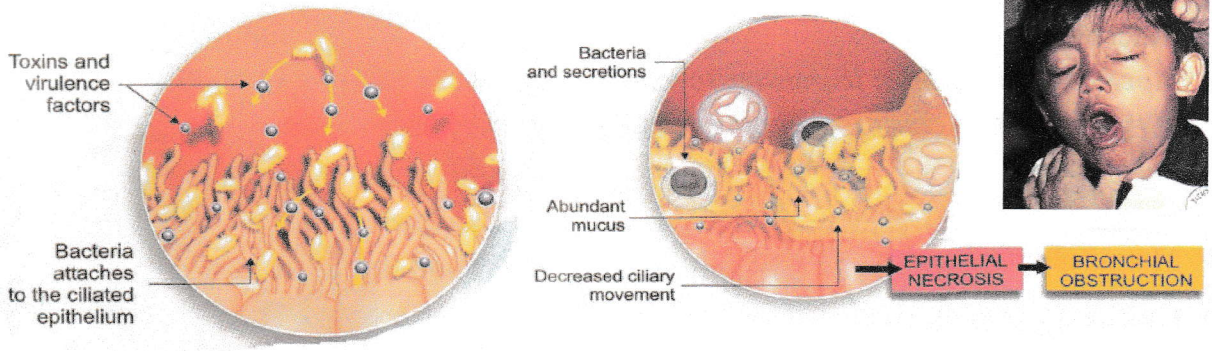
3) Tracheal cytotoxin:

- It interferes with ciliary movement.
- It kills ciliated respiratory cells.

4) Endotoxin.

Whooping Cough (Pertussis)

❖ It is an **acute respiratory disease** of childhood transmitted by droplet.



❖ Classical pertussis has 3 stages: the catarrhal, paroxysmal and convalescent stages.

	• **Incubation period**	1) **Catarrhal stage**	2) **Paroxysmal stage**	3) **Convalescent stage**
❖ **Duration (weeks)**	○ 1-2	○ 1-2	○ ~3	○ ~3
❖ **Virulence factors involved**	○ FHA ○ PTx	○ FHA ○ PTx	○ PTx, ○ Adenyl cyclase ○ Tracheal cytotoxin & endotoxin	—
❖ **Manifestations**	○ None	○ Runny nose, malaise, fever.	○ **Repetitive cough with whoops, ending in vomiting** ○ Leucocytosis	○ Diminished paroxysmal cough ○ Complications: pneumonia, encephalopathy & seizures.
❖ **Isolation of the organism from nasopharyngeal secretions**	—	○ **Isolated in large numbers.**	○ Rarely isolated	—
❖ **Antimicrobial treatment**	—	○ Reduces the severity and duration	○ Has no effect on the progress of the disease	—

❖ **N.B.:**

1) Whoops is a characteristic sound produced by a rapid inspiratory gasp of air.
2) The patient is highly **contagious** during the **catarrhal** stage.

❖ **Laboratory diagnosis:**

A. Specimens:

- Nasopharyngeal secretions obtained by per-nasal swabs, aspiration or cough plate.

B. Direct detection in nasopharyngeal secretions by:

- The direct fluorescent-antibody (DFA) test.
- Nucleic acid detection by PCR.

☞ *N.B.: Direct Gram stain is useless.*

C. Cultivation:

- The organism requires the special media Bordet-Gengou agar and charcoal blood agar for growth.
- Growth is slow and requires incubation for 5-7 days at 37°C.

D. Identification

- Colony morphology: Colonies have mercury-droplet appearance.
- The organism is identified morphologically, serologically (by direct fluorescent antibody test or agglutination), or by PCR.

E. Serology:

- It is of little help because antibodies are not detectable until the **third week** of illness.

❖ **Treatment:**

- Antibiotic treatment is highly effective if given early.
- It helps eradication of the organism, reduces infectivity and decreases the risk of secondary complications.
- **Erythromycin** is the drug of choice.
- **Trimethoprim-sulfamethoxazole** is an alternative.

❖ **Prevention:**

I. Whooping cough vaccines:

1) Killed whole cell vaccine:

- It is a part of the **DPT vaccine** (The P in DPT stands for pertussis cells).
- Side effects : in 20% of the children:
  - Irreversible brain damage (**encephalopathy**) may occur especially if given **after 6 years of age.**

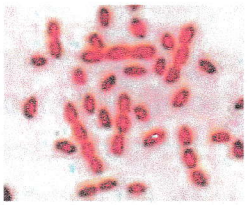
2) Acellular vaccine:

- It is a combination of **pertussis toxoid** (genetically inactivated PTx), **filamentous haemagglutinin** and other virulence factors.
- The acellular pertussis vaccine has fewer side effects than the whole cell vaccine and is currently recommended for use combined with diphtheria and tetanus toxoids as **DTaP**.
- Immunity wanes within 5-7 years. Accordingly, a booster is recommended after the age of 10 years.

II. Chemoprophylaxis:

- **Erythromycin** may be used as chemoprophylaxis for household contacts.

Chapter 16: Brucella



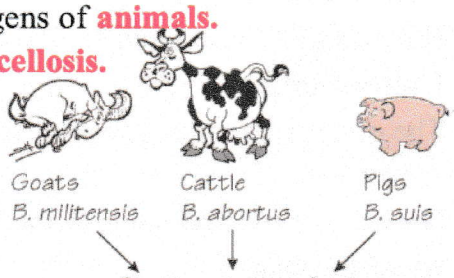
❖ **Characteristic features:**

1) Short Gram negative bacilli (coccobacilli)
2) Aerobic
3) **Facultative intracellular pathogens**

❖ Members of the genus Brucella are primarily pathogens of **animals.**

❖ Three major species can infect humans causing **brucellosis.**

❖ They are named after their animal hosts as follows:



1. **B. abortus infects cattle causing abortion.**
2. **B. melitensis infects sheep and goats.**
3. **B. suis infects pigs.**

❖ **Morphology (mentioned above).**

❖ **Culture:**

- Brucella can be grown on enriched media.
- B.abortus requires 10% CO2.

❖ **Biochemical reactions:**

- Different biochemical reactions help in identification of the genus Brucella and in distinguishing the different Brucella species.

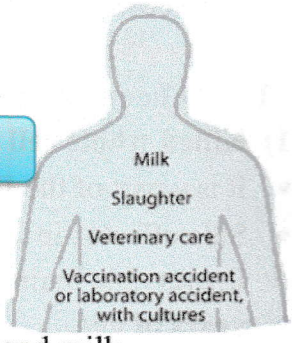
❖ **Antigenic structure:**

- The Brucella species have two distinguishing **antigens (A and M)** associated with the LPS of Brucella cell wall:

1. The **A** antigen predominates in B. **A**bortus whereas
2. The **M** antigen predominates in B. **M**elitensis.

- They can be detected by agglutination tests using specific antisera.

Brucellosis (Undulant Fever or Malta Fever)



❖ **Brucellosis is a zoonotic disease**

❖ **Mode of transmission:**

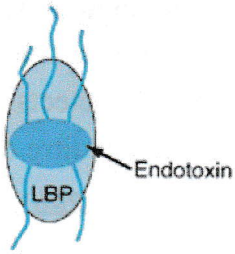
- Brucellae localize in the **placenta and mammary glands** of animals where they are shed in large numbers in uterine discharge and milk.
- The organisms are also shed in faeces and urine of infected animals.
- Transmission occurs by:

1. **Ingestion** of contaminated unpasteurized **milk** or milk products.
2. **Direct contact through skin abrasions** during handling of infected animals or their discharges.

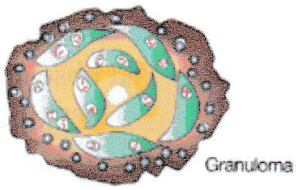
☞ *Therefore, brucellosis is an **occupational disease** affecting mostly butchers, farmers and veterinarians.*

3. **Inhalation** of infected aerosol during handling of infected animals or Brucella cultures in the **laboratories**.

❖ **Pathogenesis:**

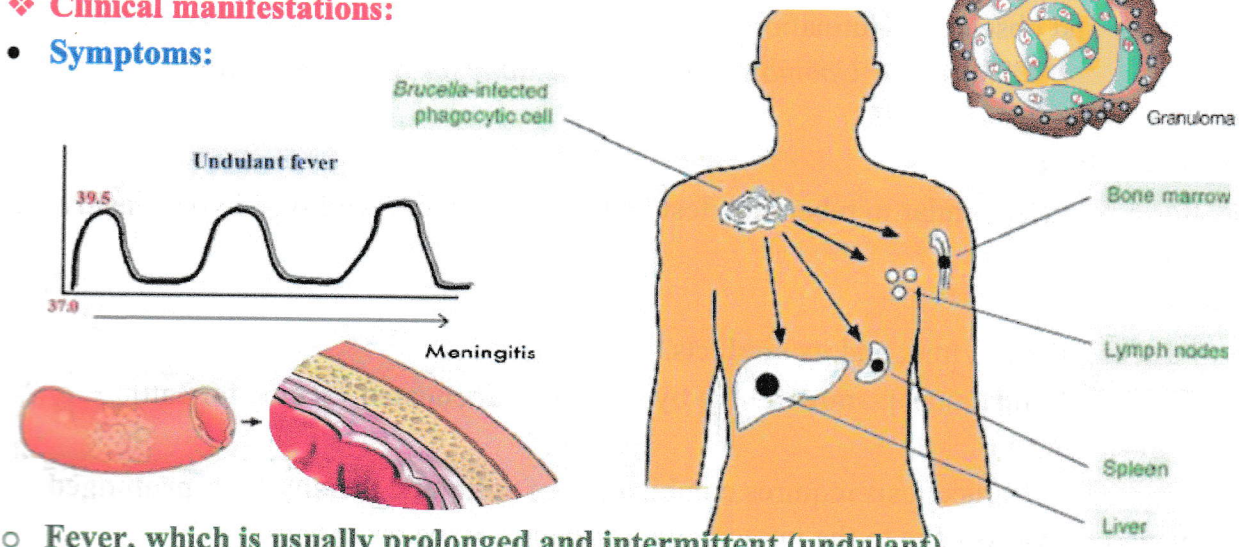


- The **endotoxin (LPS)** is the major virulence factor.
- The organism passes from the site of entry to the regional lymph nodes, the thoracic duct, and thus into the blood (**septicaemia**).
- The organism localizes in the **reticulo-endothelial system**, namely lymph nodes, liver, spleen and bone marrow.
- Being a facultative intracellular parasite, it can survive and multiply within phagocytic cells. This results in **granuloma** formation (CMI response).



❖ **Clinical manifestations:**

- **Symptoms:**



  - **Fever, which is usually prolonged and intermittent (undulant).**
  - Chills, weakness, malaise, body aches, sweating and headache.
- **Examination:** Enlarged lymph nodes, liver and spleen are frequently found.
- **Complications:** osteomyelitis, endocarditis and meningitis.
- **Outcome:** Recovery usually occurs after a few weeks or months, but chronic stage (more than 1 year's illness) can develop.

❖ **Laboratory diagnosis:**

- Because the non-specific symptoms may not point to diagnosis of brucellosis, a detailed **history** is often crucial, including the patient's occupation and exposure to animals.

A. Specimens:

- Blood or bone marrow during the acute illness.
- Other samples may be needed according to the affected sites.

B. Cultivation:

☞ *Isolation of the organism by the blood culture technique is **rarely done because:***

- There is a high risk to laboratory personnel to acquire **infection by inhalation**.
- The culture requires a **long** incubation (**6-7 weeks**).
- Being **fastidious**, the organism needs special growth requirements which are not always available.

C. Serologic diagnosis: It is the most frequently used diagnostic method:

1. Standard tube agglutination test (STAT):

- STAT detects antibodies (IgM + IgG) to the three Brucella spp.
- A single titre of ≥ 160 or a fourfold rise in titre or greater is considered significant.
- ***False negative results may be due to:***

i. The **prozone effect** that is commonly encountered in cases of brucellosis.

☞ *Therefore wide range of serum dilutions (up to 1:5120) should be done.*

ii. The presence of **incomplete or blocking antibodies**, which are produced in certain cases and obscure agglutination.

☞ *Therefore, Coomb's test should be done for negative results.*

2. ELISA for IgG or IgM.

D. Brucellin test:

- It is a skin test similar to tuberculin test and is based on **delayed** type hypersensitivity.
- It is rarely used.

❖ **Prevention:**

- Pasteurization of milk and its products.
- Control of infections in animals, e.g. by using live attenuated vaccine for cattle.

❖ **Treatment:**

- Treatment of brucellosis requires combination of antibiotic therapy for a **prolonged period (6 weeks)** due to the intracellular residence of the organisms.
- **Doxycyclines and rifampin** are the combination of choice

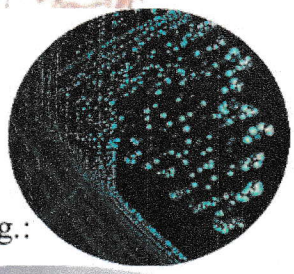
Chapter 17: Legionella

- The genus Legionella contains many species that normally live in **water.**
- L. pneumophila is the major pathogenic species.
- It causes Legionnaire's disease (atypical pneumonia) and Pontiac fever (mild flu-like illness without pneumonia).

Legionella pneumophila

Morphology:

- L. pneumophila is a **motile**, faintly stained Gram-negative bacillus.

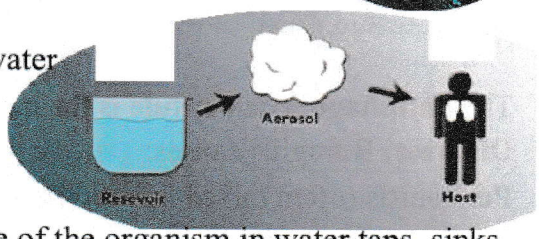


Culture:

- L. pneumophila is an aerobic, highly fastidious organism.
- It can be grown on **buffered charcoal yeast extract** (BCYE) agar.

Reservoir: L pneumophila is frequently found in **water sources** e.g.:

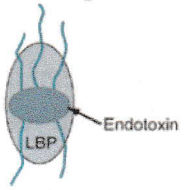
1. Water in cooling tanks and in air conditioners
2. Within free-living amoebae in environmental water
3. Within environmental biofilms.



Mode of transmission:

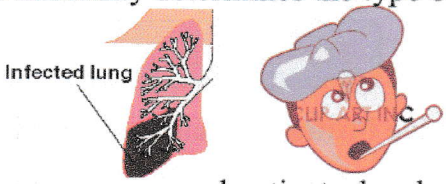
- **Inhalation** of contaminated water aerosols.
- **Outbreaks** have been attributed to the presence of the organism in water taps, sinks, showers and air-conditioners.

☞ *N.B.: There is no person-to-person transmission.*



Pathogenesis:

- Aside from **endotoxin**, no other virulence factors are known.
- The organism is **facultative intracellular**: replicates intracellularly within alveolar macrophages.
- Accordingly, cell-mediated immunity determines the type of **clinical presentation:**



1. **Legionnaire's disease:** Immunosuppressed patients develop atypical pneumonia.
2. **Pontiac fever:** Healthy individuals develop mild flu-like illness without pneumonia.

Diagnosis:

- Culture of respiratory secretions on BCYE agar.
- Detection of antigen in urine provides rapid diagnosis.
- Detection of rising antibody titre in patient's serum.

Treatment:

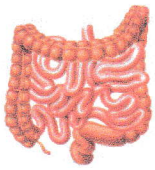
- **Azithromycin or erythromycin with rifampin** for immunocompromised patients.

Prevention:

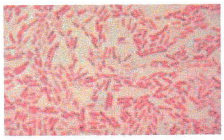
- Routine decontamination of water tanks by high temperature and hyperchlorination.

Chapter 18: Gram-Negative Anaerobic Bacilli

Bacteroides

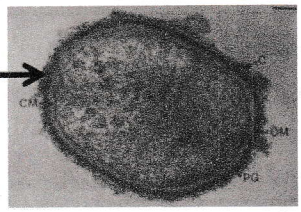


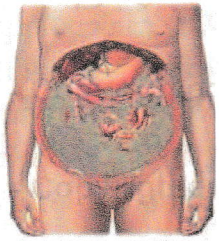
- Bacteroides are the predominant organisms found in the **human colon**.
- **B. fragilis** is the commonest pathogenic species.
- **Morphology:**



- Gram-negative capsulated bacilli.
- **Transmission:**
- Bacteroides are transmitted via gut contents to the blood or peritoneum during abdominal surgery or following trauma (i.e. **endogenous infection**).
- **Virulence factors:**

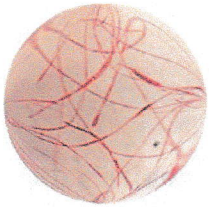
Capsule →



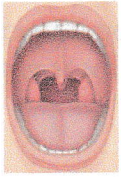


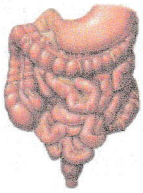
- The **antiphagocytic capsule** is the most important virulence factor.
- **Diseases:** B.fragilis causes:
- **Peritonitis** (often mixed infection), **abdominal abscess.**
- Bacteraemia which may occasionally lead to **infection of the head and neck**.
- **Diagnosis:**
- The organism can be cultured anaerobically on blood agar, and is identified by biochemical reactions and gas chromatography.
- **Treatment:**
- Most strains are resistant to penicillin but sensitive to **metronidazole, clindamycin** and **chloramphenicol** (especially for brain abscess).
- Prophylactic antibiotics may be used for bowel surgery.

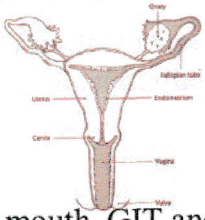
Fusobacterium



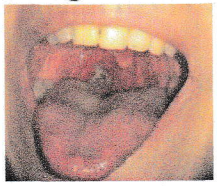
- They are large Gram-negative **fusiform or cigar**-shaped bacilli.



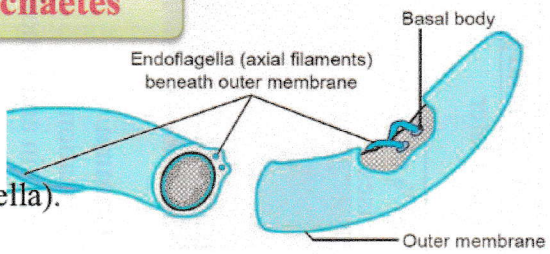




- They are part of normal microbial flora in the mouth, GIT and female genital tract.
- Most infections with these organisms are of **endogenous origin**.
- In association with **spirochaetes**, they cause **fusospirochaetal disease** in the oral cavity **(Vincent's angina).**



Chapter 19: Spirochaetes



❖ **Characteristic features:**

- Spirochaetes are slender, flexible, spiral rods.
- They have a characteristic **corkscrew motility** due to the presence of axial filaments (endoflagella).
- They have a Gram-negative cell wall structure.
- Spirochaetes include anaerobic, microaerophilic and aerobic species.

❖ **Three genera of spirochaetes cause human infections:**

1) Treponema. 2) Borrelia. 3) Leptospira

Treponema

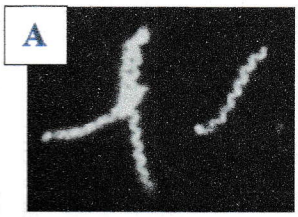
❖ The most important species is **T. pallidum**, which causes **syphilis** (a sexually transmitted disease).

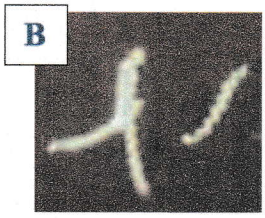
❖ T. pallidum is a **human parasite.**

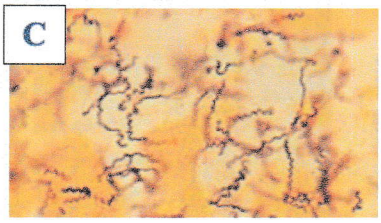
❖ It has **no** animal or environmental reservoirs *because of its rapid death outside the host.*

❖ **Morphology:**

1. T. pallidum has regular coils with pointed ends.
2. The organism is **very thin** (1/5 the diameter of E. coli). **It can only be seen by:**





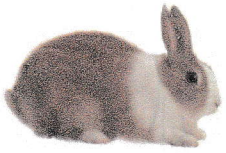


A. Dark-field microscopy: in wet unstained preparations

B. Direct immunofluorescence

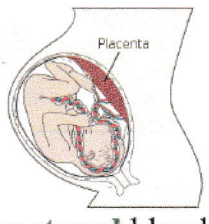
C. Light microscopy after impregnation (thickening) by silver staining e.g. Fontana stain.

❖ **Culture:**



- In vitro: T. pallidum **cannot be grown** on artificial culture media.
- In vivo: It can be maintained in the laboratory by inoculation of positive specimens into **rabbit testicles**.

Syphilis

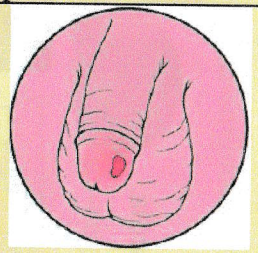
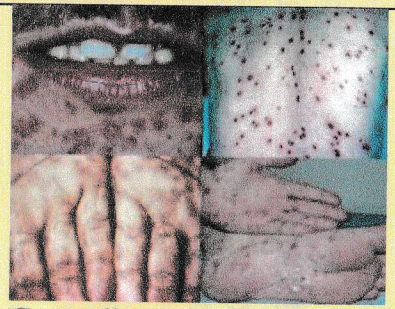
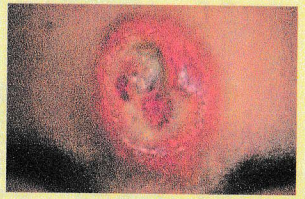
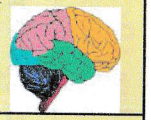


❖ **Mode of transmission:**

1. **Sexually:** leading to venereal syphilis.
2. **Transplacentally:** leading to congenital syphilis.
3. **Fresh blood transfusion:** T. pallidum is **not** transmitted by **stored** blood because it dies when stored at 4°C within 3-5 days.

❖ **Clinical forms:**

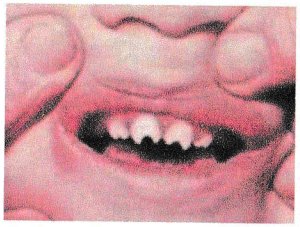
- Classically, untreated syphilis occurs in the following stages : see the following table

❖ Stage	1. Primary	2. Secondary	3. Latent	4. Tertiary
❖ Time	○ 2-10 weeks after exposure	○ 1 -3 months late	○ May last for years	○ Years later
❖ Clinical manifestations	 ○ **Chancre: Hard painless** genital or oral ulcer. ○ Contagious (infectious) ○ Heals spontaneously (within 3-6 months)	 1. Generalized **lymphadenopathy** 2. Generalized maculopapular **skin rash** 3. Mucous patches in mouth 4. **Chondyloma lata** around the genitals or anus ○ **Highly infectious** ○ Spontaneous healing (within 3 months) 	○ None	○ 30% of untreated cases  1. **Gumma** formation (in skin and bones) 2. Cardiovascular syphilis 3. Neurosyphilis  
❖ Diagnosis	○ Direct detection of the organism ○ Serologic tests: reactive only late	○ Direct detection of the organism ○ Serologic tests	○ Serologic tests only	○ Serologic tests only

❖ **Congenital Syphilis:**

- In utero infections can lead to:

1. **Abortion or stillbirth**
2. **Congenital abnormalities**, which may be obvious at birth.
3. **Silent infection**, in which manifestations may not be apparent until about **2 years of age (facial and tooth deformities).**



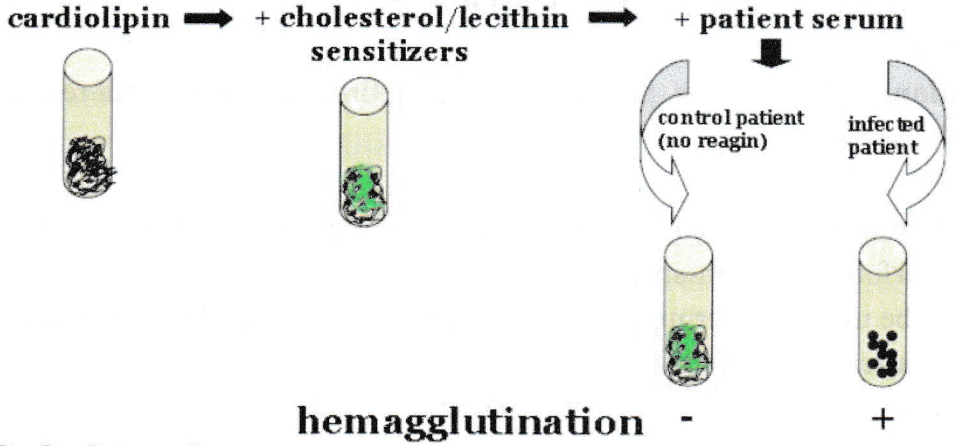
❖ Laboratory diagnosis

A. Direct detection of the organism from chancre, mucous patches or chondyloma lata by:

- Dark-field microscopy of wet unstained preparation, which detects the characteristic motility.
- Direct immunofluorescence: after staining with fluorescein-labelled anti- treponemal antibodies. This method is highly sensitive and specific.

B. Serologic tests of syphilis: They include:

I. Non-treponemal tests; non-specific tests:



1. Antibody detected:

- These tests detect non-treponemal antibodies known as **reagin (heterophil antibodies).**
- These antibodies are produced in response to **lipoidal material** released from damaged host cells.

2. Antigen used:

- The antigen used is called **cardiolipin**, which is an extract from beef heart muscle with added lipids.

3. Tests:

- The venereal disease research laboratory **(VDRL)** test in which the flocculation is seen by microscopic examination.
- The rapid plasma reagin **(RPR)** test in which the flocculation is seen with the naked eye.

4. Applications:

i. **Screening:** As these tests are inexpensive, rapid and simple, they are used for screening.

- However, being non-specific, they may give positive results in other conditions like: **autoimmune diseases, pregnancy, leprosy, viral infections and immunization**.

☞ *Therefore, positive results should be confirmed by one of the specific treponemal tests.*

ii. **Follow up:** Since reactivity declines **within 6-18 months** after successful treatment, these tests are used for follow up.

II. Treponemal tests; specific tests:

1. Antibody detected:

- These tests detect specific **treponemal antibodies**.

2. Antigen used:

- The antigen used is **T. pallidum antigen**.

3. Tests:

- Fluorescent treponemal antibody **(FTA)** test.
- T. pallidum haemagglutination assay **(TPHA).**
- Enzyme immune-assay **(EIA)** and **Western blot.**

4. Applications:

- **Confirmation:** Being specific, they are used in confirming or ruling out reactive non-treponemal test results.

❖ **N.B:**

- Treponemal tests are **not used for screening**, as they are more expensive and more difficult to perform than non-specific tests.
- They cannot be used to determine the response to treatment, as they **remain reactive for life** even after effective treatment.

❖ **Comparison between non-treponemal and treponemal tests:**

	A. Non-treponemal tests	B. Treponemal tests
❖ **Specificity**	○ Non-specific	○ Specific
❖ **Antibody detected**	○ Reagin ab	○ Anti-treponemal Ab
❖ **Antigen**	○ Cardiolipin	○ Treponemal antigens
❖ **Tests**	○ VDRL, RPR	○ FTA, TPHA, EIA
❖ **Cost & performance**	○ Inexpensive, rapid, simple	○ Expensive, time consuming, difficult to perform
❖ **Uses**	○ Screening & follow up of treatment	○ Confirmation
❖ **Reactivity**	○ 6-18 months after treatment	○ For life

❖ **Diagnosis of congenital syphilis:**

- It is done by detection of treponemal **IgM** antibodies in newborn's serum by EIA.

❖ **Treatment**

- **Penicillin** is the drug of choice for treatment of syphilis. No resistance to penicillin has been recorded.
- **Tetracycline, erythromycin and chloramphenicol** can be used as alternative antibiotics for patients allergic to penicillin.
- Only **penicillin or chloramphenicol** can be used for patients with **neurosyphilis.**
- Syphilitic pregnant mothers should be adequately treated to prevent congenital syphilis.

Borrelia

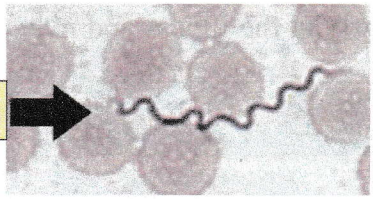
❖ Borrelia can be pathogenic for **humans, domestic animals and rodents.**

❖ In humans they cause relapsing fever and Lyme disease.

❖ **Ticks** transmit all known species of Borrelia **except B. recurrentis** which is transmitted by the human body **louse.**

❖ **Morphology:**

Giemsa stain →



- Borreliae are highly motile spirochaetes with irregular loose coils.
- They are best visualized with **Giemsa stain**. Some are Gram negative.

❖ **Cultural characteristics**

- Borreliae are microaerophilic, slowly growing spirochaetes.
- They grow on highly enriched media containing serum and tissue extract.

Relapsing Fever

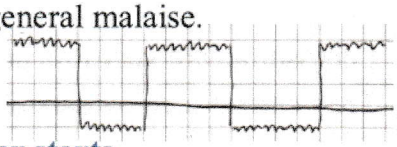
❖ Relapsing fever is a febrile, septicaemic disease in which several relapses may occur.

❖ **Transmission:** There are two forms of relapsing fever:

1. **Tick-borne (endemic) relapsing fever:** 	2. **Louse-borne (epidemic) relapsing fever**: 
❖ **Caused by:**	
• **B. hermsii.**	• **B. recurrentis.**
❖ **The reservoir:**	
• **Rodents and small animals**	• **Man is the only reservoir**
❖ **Transmission by :**	
• **Zoonotic disease**: transmitted from animals to man.	• **Not** a zoonotic disease: transmitted from man to man by human body **louse**.

❖ **Clinical Manifestations:**

1. **Incubation period** of 3 to 10 days.
2. **Symptoms:** sudden onset of fever, severe headache and general malaise.
3. **Fever :**



- **Lasts for 3 to 5 days, followed by an afebrile period which lasts about a week before a second attack of fever starts.**
- These relapses may be repeated 3 to 7 times.

4. **Relapses** occur as a result of **antigenic variations** in the causative Borrelia spp. As antibodies develop against antigens of the existing organism, new antigenic variants emerge and produce relapses of the illness.

❖ **Laboratory diagnosis:**

- Diagnosis is usually made during the **febrile stage,** where abundant spirochaetes are present in the blood.
- This is done by detecting loosely coiled spirochaetes in blood films stained by Giemsa stain.

❖ **Treatment:**

- **Tetracycline** may be beneficial early in the illness and may prevent relapses.

Lyme Disease

❖ The disease is caused by **B. burgdorferi,** which is transmitted by **ticks** bite.

❖ Although caused by different spirochaetes, the general similarities in the progression (stages) of Lyme disease and syphilis are striking.

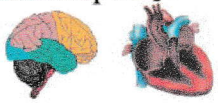
❖ **The clinical findings have been divided into 3 stages:**

1. **Stage 1: erythema migrans** :

- A characteristic spreading circular red lesion with a clear center occurs at the site of tick bite.

2. **Stage 2:**

- The organism **disseminates** via blood resulting in muscle and joint pain (arthralgia), secondary skin lesions and lymphadenopathy.



3. **Latent stage.**
4. **Stage 3:**

- This stage is characterized by arthritis (specially large joints), **CNS and cardiac** dysfunction.

❖ **Diagnosis:** serologically by ELISA, to be confirmed by Western blot.

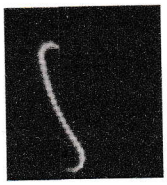
Leptospira

❖ Leptospirae may be free-living or may live in association with human or animal hosts.

❖ **Leptospira interrogans** is the causative agent of leptospirosis (Weil's disease), which is a **zoonotic disease.**

❖ **Morphology:**

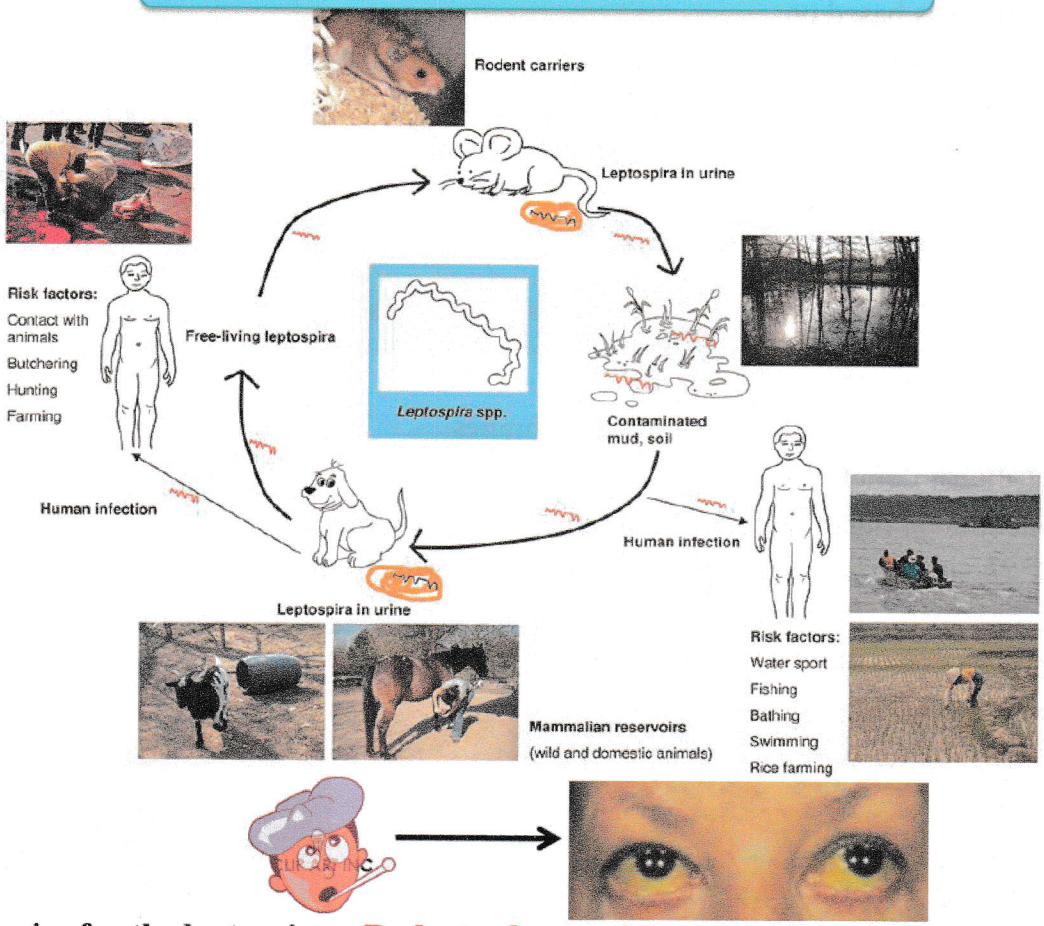
- Leptospirae are very thin and tightly coiled motile rods with hooked ends.
- They can be seen by dark-field microscopy.



❖ **Culture:**

- Leptospira interrogans is an obligate aerobe.
- It can be grown on serum-containing media.
- Growth usually occurs within 1-2 weeks.

Leptospirosis (Weil's Disease; Infectious Jaundice)



- **Reservoirs for the leptospirae: Rodents, dogs, swine and cattle.**
- Infected animals excrete large numbers of the organism in urine resulting in **contamination of water and soil** where leptospirae remain viable for several weeks.
- **Mode of transmission:**

1. Contact with **contaminated water** where the organism enters through small skin abrasions. This may occur;

- As an occupational hazard, affecting mainly farmers, sewage workers and miners.
- During practicing water sports, e.g., swimming.

2. Consumption of **contaminated food or drink** where the organism penetrates the mucous membrane.

- **Clinical manifestations**

- The incubation period is usually **7 to 14 days.**
- Leptospirosis is typically a **biphasic illness** with a quiescent period inbetween:

1. **The first phase** :

- Characterized by a febrile influenza-like illness due to blood invasion (**septicaemia**).

2. **The second phase** :

- Characterized by dysfunction of liver (**jaundice**), kidney (**uraemia**) and CNS (**aseptic meningitis**) due to invasion of these organs by leptospirae from the blood stream.

❖ Laboratory Diagnosis

- Diagnosis is based on:

1. History of possible exposure, together with suggestive clinical signs.
2. Marked rise in specific **IgM** (highly sensitive).
3. Occasional isolation of the organism from blood, urine or CSF.

❖ Treatment

- **Penicillin or doxycycline** are the drugs of choice

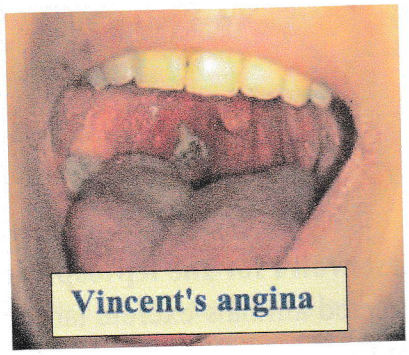
Fusospirochaetal Disease

❖ Under certain circumstances:

- Particularly injury to oral mucous membranes.
- Nutritional deficiency.
- Concomitant infection (e.g. With herpes simplex virus),
  - ☞ *The normal spirochaetes of the mouth, together with anaerobic fusiform bacilli (fusobacteria) find suitable conditions for multiplication.*

❖ They increase in numbers causing:


Trench mouth


Vincent's angina

1. Trench mouth:

- A condition of **acute necrotizing ulcerative gingivitis** (ANUG).

2. Vincent's angina:

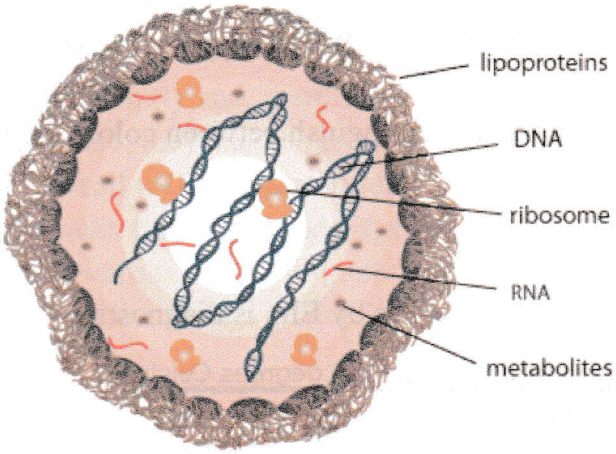
- Fusospirochaetal infection of the **pharynx with pseudomembrane** formation, similar to diphtheria and follicular tonsillitis.

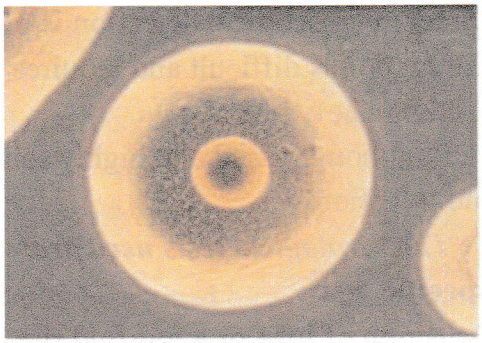
❖ Laboratory diagnosis

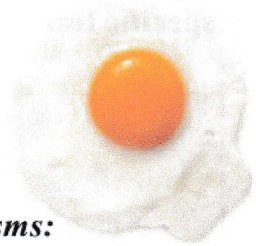
- Gram-stained smear from the pseudomembrane shows large number of Gram- negative fusiform bacilli and spirochaetes in association with pus cells and other commensals

Chapter 20: Mycoplasma

❖ Characteristic Features:







1. Mycoplasmas are **bacteria without cell wall.**

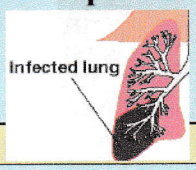
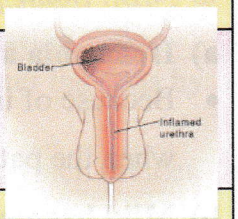
☞ ***The lack of a cell wall renders these organisms:***

a) **Resistant to antibiotics** which inhibit cell wall synthesis (e.g., beta-lactam antibiotics),
b) **Unstainable by the Gram stain**
c) Variable in shape (**pleomorphic**).

2. Their contents are **enveloped** by a cell membrane.
3. They are the only bacteria that contain **sterol** (in the form of cholesterol) in the cell membrane.
4. They are **the smallest bacteria** that can be grown on cell-free media.

- They require serum-enriched medium containing **cholesterol.**
- After several days of incubation, mycoplasmas produce minute colonies which have a characteristic **fried-egg** appearance that can only be visualized microscopically.

❖ Mycoplasma species, diseases and modes of transmission in humans:

❖ Species:	
• **Mycoplasma pneumoniae** 	• **Genital mycoplasmas:** 1. M. hominis 2. M. genitalium 3. Ureaplasma urealyticum 
❖ Disease:	
1. Atypical pneumonia (20% of pneumonia cases) 2. Pharyngitis, bronchitis & otitis media	**1. Nongonococcal urethritis** 2. Pelvic inflammatory disease (PID) 3. Post-abortal or postpartum fever
❖ Transmission:	
• Inhalation of respiratory droplets	• Sexual 

❖ **Laboratory diagnosis:**

I. Diagnosis of Mycoplasma infection is based on serology because:

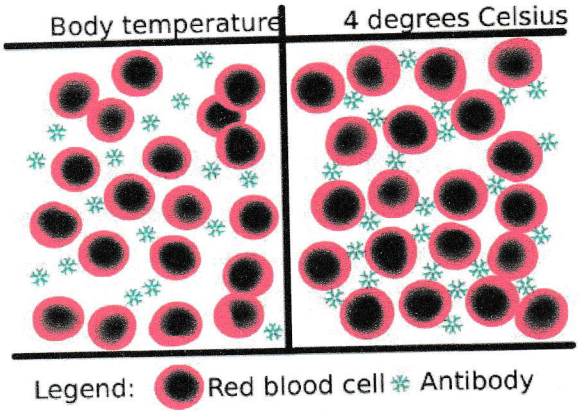
1. Direct microscopical detection is of limited value.
2. Cultivation is difficult and requires prolonged incubation (up to 3 weeks in case of M. pneumoniae).
3. PCR, although rapid and highly sensitive, does not distinguish between colonization and infection.

II. The serological tests used are:

1) Specific tests:

- Detection of Ig**M** or a fourfold rise in Ig**G** antibody titre by EIA is diagnostic.

2) Nonspecific test:



- Detection of **cold agglutinins:**
  - Which are IgM antibodies that agglutinate red cells at **4°C** but not at 37°C.
  - A titre of **≥ 1:128** of cold agglutinins is indicative of **recent** infection.

❀ **Other results:**

a. Positive results may occur in:

- Viral infections, malaria, haemolytic anaemia and liver disorders.
- Most of these diseases have symptoms that easily distinguish them from those of primary atypical pneumonia.

b. Negative results may occur in:

- About 50% of cases of M. pneumoniae infections.

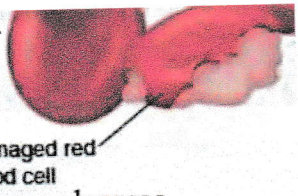
❀ ***N.B.: Some patients develop very high titres of cold agglutinins. This may result in:***

a) Ischaemia and even necrosis of hands and feet

- Because of in vivo clumping of red blood cells when the patient is exposed to cold temperature.

b) Mild haemolytic anaemia:

- Due to in vivo destruction of red blood cells throughout the body.



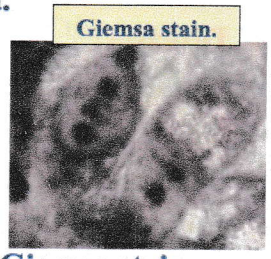
❖ **Treatment:**

- **Erythromycin and tetracycline** are the drugs of choice for all mycoplasmas.

Chapter 21: Chlamydia

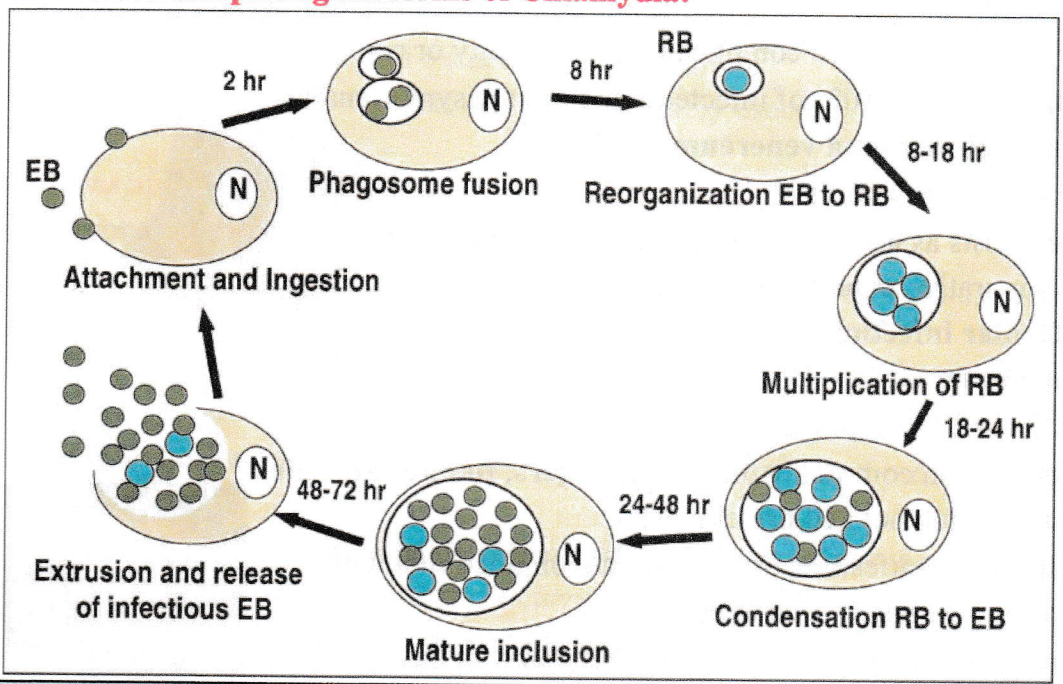
❖ Characteristic Features:

1. Chlamydia is a genus of **very small obligate intracellular bacteria.**
   - They lack the ability to produce sufficient energy to grow independently and, therefore, can grow only inside host cells.
2. In the lab, they can be grown on **tissue culture.**
3. Their cell wall structure resembles that of Gram-negative bacteria but **lacks typical peptidoglycan layer.**
4. Gram stain is not useful; but the organism can readily be stained by **Giemsa stain.**
5. Chlamydiae have a unique **biphasic** life cycle.



❖ Life cycle:

- **There are two morphological forms of Chlamydia:**



1. Elementary bodies (EBs):	2. Reticulate bodies (RBs):
• They are **small, extra**cellular, metabolically **inert** particles.	• They are **larger, intra**cellular, metabolically **active** particles.
• They represent the **infectious** form.	• They represent the **replicating** form.

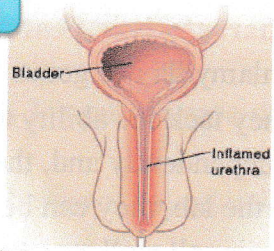
- The EBs bind to specific host cell receptors by **protein adhesins** and enter the cells by endocytosis.
- Once inside the cell, the EB reorganizes within hours into the larger RB which divides repeatedly by **binary fission** and produces new infectious EBs.
- The EBs are then released by **lysis** of the host cell.

☞ N.B.: The site of replication appears as an **intra-cytoplasmic inclusion body**, which can be stained and visualized microscopically.

❖ Chlamydiae that infect humans are divided into three species :

1) Chlamydia trachomatis. 2) Chlamydophila pneumoniae. 3) Chlamydophila psittaci.

Chlamydia Trachomatis

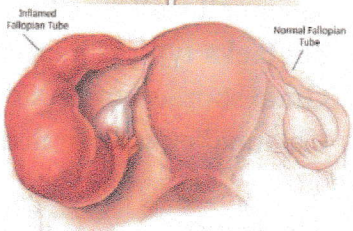


❖ Diseases:

I. Genital tract infections:

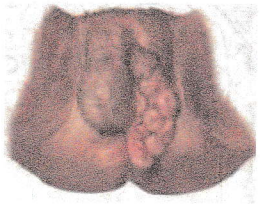
- **These are sexually transmitted diseases.**

1. Nongonococcal urethritis:



- It is caused by serotypes **D-K.**
- Males present with urethritis, which may progress to epididymitis, prostatitis or proctitis.
- Females may present with urethritis and/or cervicitis, which may progress to salpingitis and pelvic inflammatory disease (PID). The condition may be complicated by infertility or ectopic pregnancy.
- N.B.: More than 50% of infected females are asymptomatic.

2. Lymphogranuloma venereum (LGV):



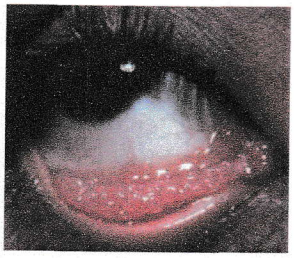
- It is caused by serotypes **L1, L2 or L3.**
- It manifests as an initial genital papule accompanied by suppurative inguinal lymphadenopathy.

II. Ocular infections:

1) Trachoma:

- It is caused by serotypes **A, B or C.**
- Transmission commonly occurs by **fingers, fomites and flies** mostly during hot and dry climates.
- Trachoma manifests as chronic keratoconjunctivitis with scarring which may lead to blindness.
- Trachoma is an ancient disease which was well described in **Egyptian** writings around 3800 B.C.

2) Inclusion conjunctivitis:

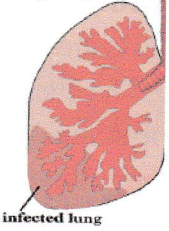


- It is caused by serotypes **D-K.**
- It is the most common cause of neonatal conjunctivitis.
- Transmission occurs:

a) In neonates: during passage through an infected **birth canal**

b) In adults: as a result of transfer of organisms from the genitals to the eye (as an **auto-infection**) or through a vehicle (e.g., **swimming pools**).

III. Neonatal pneumonia:



- It is caused by serotypes **D-K.**
- Infection occurs during passage through an infected **birth canal.**

❖ **Laboratory diagnosis:**

A. Specimens are taken from urethra, cervix, conjunctiva, etc.

B. Direct detection:

1. **Microscopic examination of Giemsa-stained smears** for detection of the intra-cytoplasmic basophilic inclusion bodies.
- This method is insensitive, nonspecific and requires good experience.

2. **Antigen detection by immunofluorescence or by ELISA.**
- This method is more specific, reliable and rapid.

3. **Nucleic acid detection by DNA probes or PCR.**

C. Cultivation and identification:

- Specimens are inoculated onto tissue culture.
- Intra-cytoplasmic inclusion bodies can be detected by Giemsa stain or immunofluorescence.

D. Serological tests are **not** helpful except in LGV infection.

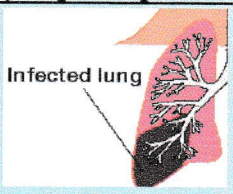
E. Frei skin test: It is similar to tuberculin test. It is rarely used for diagnosis of LGV infection.

❖ **Treatment and prevention:**

- The antibiotic of choice is **erythromycin or tetracycline** in adults.
- Erythromycin is recommended for neonates and pregnant women because of the effect of tetracycline on teeth and bones.
- Chlamydiae are **not sensitive to β-lactam antibiotics** *due to lack of typical peptidoglycan layer.*
- Detection and treatment of asymptomatic individuals are important preventive measures.

☞ *N.B.: It is recommended that patients receiving treatment for **gonorrhoea** also be treated with **tetracycline** for possible concurrent **chlamydial** infection.*

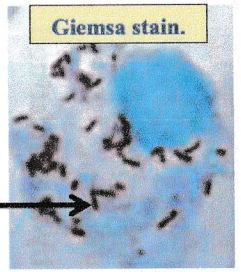
Chlamydophila

1. Chlamydophila pneumoniae	2. Chlamydophila psittaci
 • This organism is transmitted from **person to person** by aerosols. • It causes **a**typical pneumonia. • It has been associated with atherosclerosis and **a**sthma.	 • C.psittaci is transmitted via inhalation of the organism in dried **bird** faeces or respiratory secretions. ☞ *Therefore, people handling birds regularly are particularly at risk.* • It causes **psittacosis (zoonotic disease)** which is an atypical pneumonia

❖ **N.B.:** Although **chlamidiae** are similar to viruses in being small obligate intracellular organisms, they are considered **bacteria** **because of the differences mentioned in the table:**

❖ Species	❖ Disease	❖ Serotype	❖ Transmission
1. Chlamydia trachomatis	**A. Genital infections:** ○ Non gonococcal urethritis ○ Lymphogranuloma venereum	○ D-K ○ L1.L2.L3	○ Sexual ○ Sexual
	B. Ocular infections: ○ Trachoma ○ Inclusion conjunctivitis: - in neonates - in adults	○ A,B,C ○ D-K	○ Fingers, flies, fomite ○ During birth ○ Swimming pool
	C. Neonatal pneumonia	○ D-K	○ During birth
2. Chlamydophila pneumoniae	○ Atypical pneumonia ○ Atherosclerosis ○ Asthma	-----	○ Inhalation of respiratory droplets from humans
3. Chlamydophila psittaci	○ Psittacosis = atypical pneumonia	-----	○ Inhalation of particles contaminated from dried bird faeces or respiratory secretions

Chapter 22: Rickettsia



Rickettsia inside the host cell

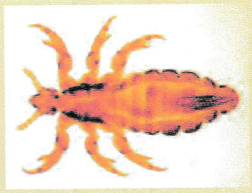
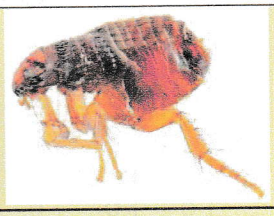
❖ **Characteristic Features:**

1. Rickettsia species are **small obligate intracellular bacilli.**
   - They are unable to make sufficient ATP for independent life.
2. They have a Gram-negative cell wall structure.
   - Rickettsiae are best stained by **Giemsa stain.**
3. They cannot grow on artificial laboratory media, but can be isolated by inoculation of laboratory animals or tissue cultures.
4. Organisms are maintained in nature by **arthropods transmission**.

❖ **Common Rickettsia species:**

	I. Typhus group			II. Spotted Fever group		
❖ **Organism**	○ **R. prowazekii**	○ **R. typhi**	○ **Orientia tsutsugamushi**	○ **R. rickettsii**	○ **R. akari**	○ **R. conorii**
❖ **Disease**	○ Epidemic typhus	○ Endemic typhus	○ Scrub typhus	○ Rocky Mountain spotted fever	○ Rickettsial pox	○ Mediterranean Spotted fever
❖ **Arthropod vector**	○ Louse	○ Flea	○ Mite	○ Tick	○ Mite	○ Tick
❖ **Mammalian reservoir**	○ Human	○ Rodents	○ Rodents	○ Dogs, rodents	○ Mice	○ Dogs.
❖ **Geographic distribution**	○ South America, Africa	○ Worldwide	○ Far East	○ Rocky Mountain states, Eastern USA	○ Asia, Far East, USA	○ Mediterranean
❖ **Clinical severity**	++	-	++	+	-	+

❖ **Mode of transmission:**

			
○ Louse	○ Flea	○ Tick	○ Mite

1. Transmission of infection by:

a. **Infected louse or flea** occurs by rubbing faeces of the vector into broken skin.

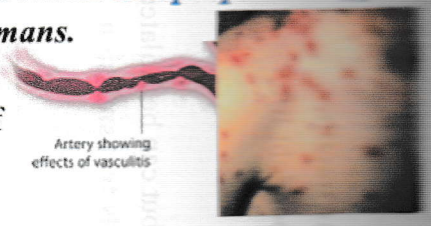
b. **Infected tick or mite** occurs via vector bite.

2. In case of epidemic typhus, the **infected lice** are **eventually killed by the infecting bacteria.**

☞ *Accordingly, this disease is **not** maintained in the lice population, i.e., there is **no louse-to-louse** transmission and **human infection is an obligatory stage** in the cycle.*

3. Ticks and mites can transmit the organism **transovarially** to their progeny and thereby, the organism can be maintained in the tick or mite population without **mammalian host for many years.**

☞ ***N.B.: All rickettsial diseases are considered to be zoonosis except epidemic typhus which occurs only in humans.***



❖ **Pathogenesis and clinical manifestations:**

1. **Vasculitis:** Rickettsiae infect the endothelial lining of the blood vessels of the skin leading to vasculitis.

2. **Symptoms :**

a. Fever, headache and characteristic skin rash and in oedema and haemorrhage.

b. Rickettsial encephalitis with coma, convulsions and pulmonary oedema

3. **Brill-Zinsser disease:**

- In some cases of epidemic typhus, rickettsiae are **not eliminated** from the body on clinical recovery and remain in the lymph nodes.
- As much as 50 years later, the infection can be **reactivated** to cause Brill-Zinsser disease (i.e., **endogenous infection**), and the patient once again acts as a source of infection for any louse that may be present.

❖ **Laboratory diagnosis:**

- **Serologic diagnosis**: indirect immunofluorescent test is used to demonstrate anti-rickettsial antibodies.

❖ **Treatment:**

- **Tetracycline** is the drug of choice and **chloramphenicol** is an alternative.

❖ **Prevention:**

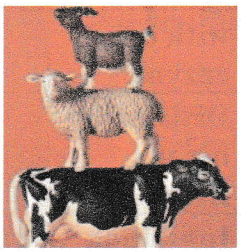
- Prevention is based on reducing exposure to the arthropod vector (e.g., delousing in case of epidemic typhus) and personal hygiene.

Chapter 23: Coxiella

❖ Coxiella was formerly classified as Rickettsia.

☞ ***However, it differs from Rickettsia in the following:***

1. It is **not transmitted to humans by arthropods.**
2. It is **extremely resistant** to heat, drying and sunlight, hence can persist **outside** the host for a long period.
3. It mainly affects the **lungs** rather than vascular endothelium, so that infection is **not** accompanied by **skin rash.**



❖ **Coxiella burnetii** is the only species of the genus Coxiella:

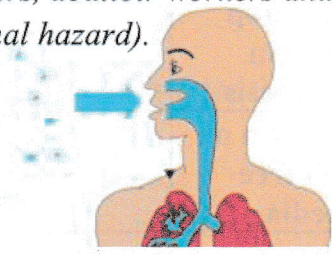
- It causes Q fever where Q stands for "**Query**" as the cause of fever was unknown for several years.

Q Fever

❖ Q fever is a **zoonotic disease.**

❖ The important reservoirs for human infection are **cattle, sheep and goats**;

☞ *Therefore, people handling infected animals (e.g., farmers, abattoir workers and veterinarians) are at high risk (occupational hazard).*

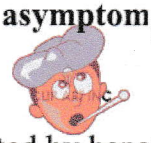


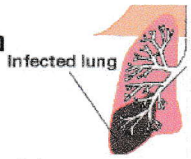
❖ **Mode of transmission:**

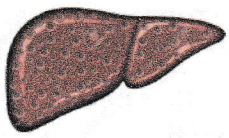
1. **Inhalation** of infected material especially from faeces, placenta or urine of infected animals.
2. **Ingestion** of milk from infected animals.

❖ **Clinical presentation:**

1. In the majority of cases, infection remains **asymptom**
2. Clinical illness may be in the form of:







a. Fever and influenza-like symptoms.
b. Atypical pneumonia that may be complicated by hepatitis.

☞ ***The combination of pneumonia and hepatitis should suggest Q fever.***

3. Chronic Q fever is rare and usually manifests as **endocarditis.**

❖ **Laboratory diagnosis:**

- Diagnosis of Q fever depends upon serology because culture and molecular techniques have low sensitivity and are available only in reference laboratories.

❖ **Treatment:**

- **Doxycycline** is the drug of choice for treatment.

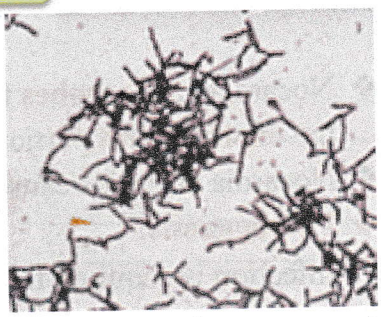
❖ **Prevention:**

A. Vaccination:

- Q fever vaccine used in humans consists of **killed C. burnetii whole cells**.
- It is usually given to people at risk

B. Pasteurization of milk.

Chapter 24: Actinomycetes



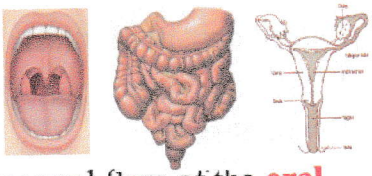
❖ **Characteristic Features**

- Actinomycetes are **true bacteria** but they form **long branching filaments** that resemble hyphae of fungi.
- They are Gram positive, but some are also *weakly acid-fast.*

❖ **Members of medical importance:**

1. **Streptomyces : producers of antimicrobial agents.**
2. **Nocardia brasiliensis,** cause mycetoma (actinomycetoma or actinomycotic mycetoma)
3. **Actinomyces israelii and Nocardia asteroides.**

Actinomyces Israelii

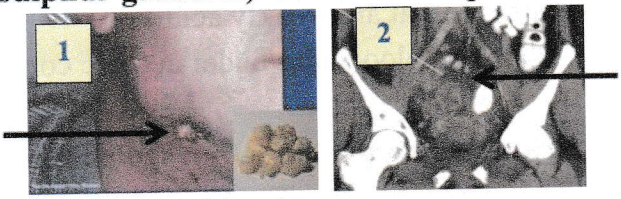


❖ Actinomyces israelii is an **anaerobe** that forms part of the normal flora of the **oral cavity, intestinal tract and vagina.**

❖ It causes **actinomycosis.**

❖ **Pathogenesis:**

- Infection is **endogenous** following local trauma where the organism invades the tissues.
- A hard painless swelling develops, which drains pus through sinus tracts.
- The **pus contains hard yellow granules (sulphur granules)** which are composed of a mass of bacterial filaments.

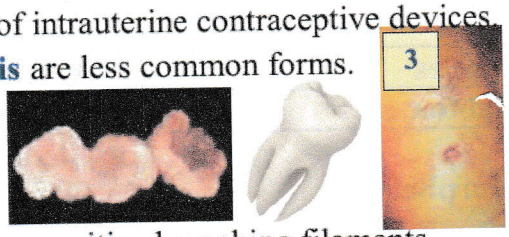


❖ **Clinical forms:**

1. **Cervicofacial actinomycosis :**

- The face, neck and mandible are affected.
- It is usually related to poor dental hygiene and may be initiated by tooth extraction or some other trauma to the mouth or jaw.

2. **Pelvic actinomycosis** associated with the use of intrauterine contraceptive devices.
3. **Thoracic, abdominal and CNS actinomycosis** are less common forms.



❖ **Laboratory diagnosis:**

⊛ **Specimen:** Pus containing sulphur granules.

- The granules are crushed and subjected to:

1. **Microscopic examination**, which reveals Gram-positive branching filaments.
2. **Culture** on blood agar under **anaerobic** conditions for about 2 weeks:

- Which yields colonies with **molar tooth** appearance.
- The colonies are identified by morphology and immunofluorescence staining.

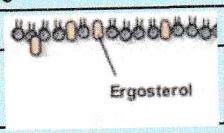
❖ **Treatment:**

- Surgical drainage of pus.
- Prolonged administration of **penicillin,** which is the drug of choice.

Chapter 25: Mycology

❖ Mycology is the study of **fungi.**

❖ **Fungi differ from bacteria in the following :**

❖ Feature	A. Fungi	B. Bacteria
❖ Size	○ Larger	○ Smaller
❖ Nucleus	○ Eukaryotic	○ Prokaryotic
❖ Mitochondria	○ Present	○ Absent
❖ Ergosterol in cytoplasmic membrane	○ Present 	○ Absent
❖ Cell wall content	○ Chitin	○ Peptidoglycan
❖ Spores	○ For reproduction	○ For survival
❖ Metabolism	○ Heterotrophic ○ No obligate anaerobes	○ Hetero and autotrophic ○ Many obligate anaerobes

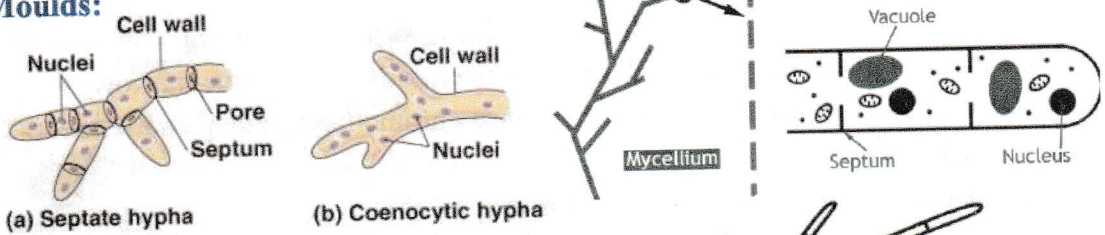
❖ **N.B.:**

○ **Mycoplasma is the only bacteria that contains cholesterol in the cytoplasmic.**

○ **Fungi** reproduction may be by **both** sexual (meiotic) or asexual (mitotic) spores

❖ **Morphological forms:**

1. Moulds:



- **They consist of long filaments (hyphae) which may be:**

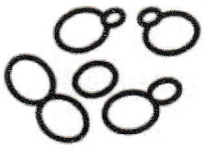
A. Septate (with cross walls)

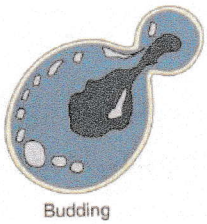
B. Non-septate (without cross walls).

Hyphae

- They grow by branching and tip elongation forming a mass called **mycelium.**
- Examples include **Aspergillus, Penicillum and the dermatophytes**.

2. Yeasts:





Pseudohyphae

- They grow as single cells (round or oval).
- They reproduce by **budding** and may form **pseudohyphae** (hyphae with constrictions; sausage-like chain).
- Examples include **Candida and Cryptococcus.**

❖ **N.B.: Dimorphic fungi** e.g., **Histoplasma** :

- Are those that can **switch between the previous two forms** depending on the **temperature:**

a. At room temperature: they grow as moulds (hyphae).

b. At body temperature: they grow as yeasts.

❖ **Clinical classification:**

A. Mycotic infections:

1. Superficial mycoses:

- Affecting the keratinized layer of the skin e.g. **Pityriasis versicolor.**

2. Cutaneous mycoses:

- Affecting the deep layers of the skin, e.g. **Candida and dermatophytes.**

3. Subcutaneous mycoses:

- In which fungi present in the soil are implanted in the subcutaneous tissue by trauma, e.g. **mycetoma.**

4. Deep (systemic) mycoses:

- Affecting internal organs. These fungi fall in two groups:

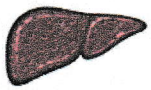
i. True pathogens:

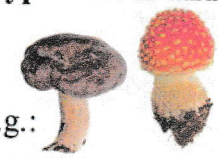
- Infecting *normal healthy* individuals, e.g., **Histoplasma and Blastomyces**.

ii. Opportunistic pathogens:

- Infecting *immunocompromised* individuals e.g., **Pneumocystis, Cryptococcus and Candida.**

B. Mycotoxicosis:





- It is produced by consumption of **food containing fungal toxins** e.g.:
  - i. **Mushroom poisoning** causes damage to liver, kidney and bone marrow.
  - ii. **Aflatoxin of Aspergillus flavus** may cause chronic liver damage and cancer.

C. Allergic disorders:

- Spores of free-living fungi as **Aspergillus** may be the allergen in some cases of atopy (asthma, hay fever, urticaria ... etc.).

❖ **Pathogenesis:**

- Infection with certain systemic fungi (e.g., **Histoplasma**) elicits a **granulomatous** host defense response (composed of macrophages and helper T cells).
- Infection with other fungi (notably **Aspergillus**) elicits a **pyogenic response** (composed of neutrophils).

❖ **Diagnosis of fungal infections:**

A. Specimens are collected according to the site of infection such as skin scales, nail clippings, hair, respiratory secretions, biopsies, blood...etc.

B. Direct detection:

1. Microscopical examination:

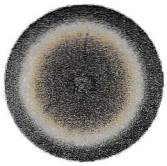
i. Unstained (wet) preparations:

- Are examined to demonstrate hyphae, spores or yeast cells.
- In case of superficial and cutaneous mycoses, the specimen (skin scales, nail clippings or hair) is first mounted with 10% KOH to dissolve keratin, allowing visualization of the fungus.

ii. Stained preparations:

- Different stains are used, e.g. Gram's stain, India ink and silver stains.

2. Antigen detection in the specimen (e.g. Cryptococcus antigens in CSF).



C. Cultivation:

- **Sabouraud's dextrose agar (SDA)** is the most commonly used medium.
- Growth of most fungi is better at 25-30°C.
- Growth may take several weeks.
- If deep mycotic infection is suspected (which is usually caused by dimorphic fungi), enriched media are inoculated and incubated at 37°C to allow growth of the yeasty phase of the fungus.

D. Identification of culture growth by:

1. **Colony morphology** including the surface and reverse views of the growth is the principal way for identifying fungi.
2. **Microscopic examination** of wet and stained smears to distinguish the different types of hyphae and conidia (spores).
3. **Biochemical tests.**
4. **DNA probes** can be used to identify fungi growing in culture at earlier stage when the colony is much smaller.

E. Serodiagnosis:

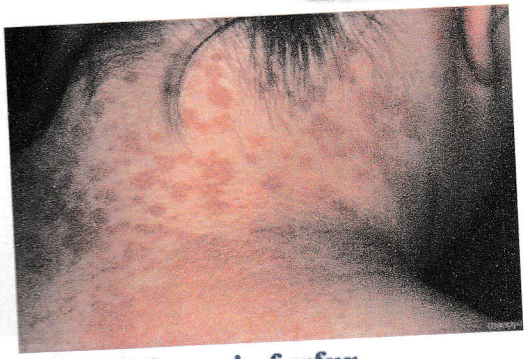
- Detection of specific antibody may help in the diagnosis of systemic mycoses.

F. Skin testing (delayed type hypersensitivity).

❖ **Antifungal Drugs:**

- Because fungi are eukaryotes, the range of non-toxic systemically active antifungal drugs is still limited.
- The selective toxicity of antifungal drugs is based on the presence of **ergosterol** in fungal cell membranes, in contrast to the cholesterol found in human cell membranes and the absence of sterols in bacterial cell membranes.
- The most commonly used drugs are **amphotericin B, mycostatin (nystatin) and azole drugs (e.g., fluconazole, ketoconazole and itraconazole).**

Pityriasis Versicolor



- It is caused by **Malassezia furfur.**
- It is a common fungus infection of the horny layer of the skin.
- It affects the upper part of the trunk and sides of the neck.
- It appears as scaly macules, either hyper- or hypopigmented.

Dermatophytes

❖ Three genera of dermatophytes infect man: **Microsporum, Trichophyton and Epidermophyton.**

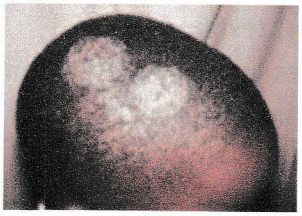
- They affect the keratinized tissue (hair, nail and skin).
- The sources of infection may be man, animals as dogs and cats, and soil.
- Infection is transmitted by direct or indirect contact.

❖ **The disease is characterized by :**

- **Superficial, extends radially and heals at the centre to form a circular lesion called ringworm which is usually referred to as tinea.**

❖ **According to the site, there are several types of ringworm infections:**

1. **Tinea capitis (ringworm of the scalp).**
2. Tinea pedis (athlete's foot).
3. Tinea unguinum (ringworm of nails).
4. Tinea circinata (ringworm of non-hairy skin).
5. Tinea cruris (ringworm of the skin of the groin).
6. Tinea barbae (ringworm of the skin of beard).



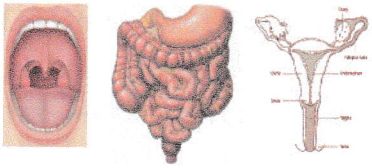
Candidiasis

❖ **Candidiasis (moniliasis)** is most frequently caused by **Candida albicans** and rarely due to infection by other species, e.g. **C. stellatoidea, C. krusei**...etc.

❖ Candida albicans is present as normal flora in the oral cavity, vagina and intestine.

❖ **Predisposing factors to candidiasis include:**

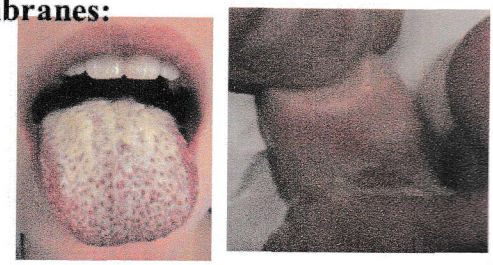
1. Diabetes.
2. Broad-spectrum antibiotic treatment (superinfection).
3. Steroid therapy.
4. Immunosuppression.
5. Prolonged exposure to water, pregnancy and old age.



❖ **Clinical features of candidiasis:**

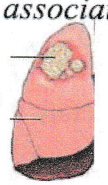
1. **Cutaneous: Affecting skin and mucous membranes:**

- **Oral thrush.**
- Vulvovaginitis.
- Paronychia (nail infection).
- **Interdigital.**
- Intertrigo (between skin folds) e.g. diaper rash.
- *Chronic mucocutaneous candidiasis is usually associated with T-cell deficiency.*



2. **Systemic: Affecting the lung or kidney.**

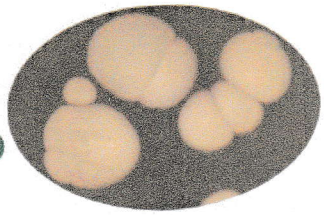
- It usually complicates an underlying disease as TB, malignancy or immunosuppression.



❖ **Laboratory diagnosis:**

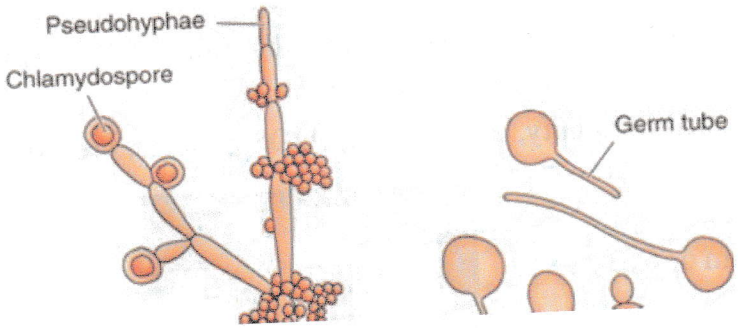
A. Specimens from skin, mouth, vagina, sputum...etc.

B. Microscopic examination of a Gram-stained smear for the presence of Gram-positive, oval, budding yeast cells and **pseudohyphae.**



C. Cultivation:

- C.albicans can grow on most culture media at 37°C.
- The selective medium is **Sabouraud's dextrose agar (SDA) containing chloramphenicol**.
- After 1-2 days, yeast colonies appear *creamy white, pasty with yeasty odour,*
- **C.albicans is differentiated from other Candida species by:**

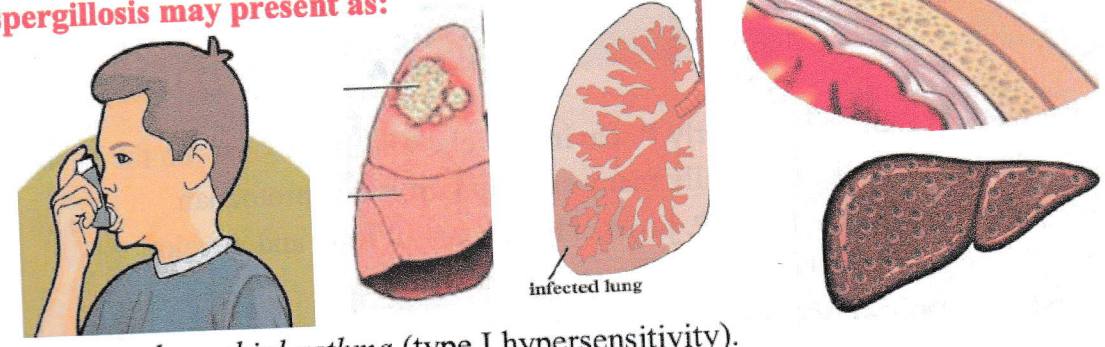


A. Formation of **germ tubes** (true hyphae with no constrictions) in serum at 37°C within 1-2 hours.

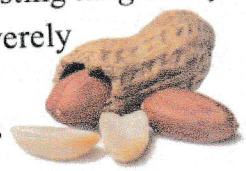
B. Formation of **chlamydospores** (thick-walled resting spores) on corn meal agar.

Aspergillosis

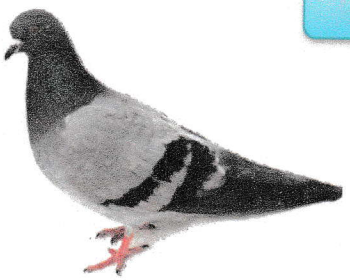
- It is caused by **Aspergillus fumigatus**, rarely by **Aspergillus niger** and **A. flavus**.
- The spores are found in **air** and are continuously inhaled
- **Aspergillosis may present as:**

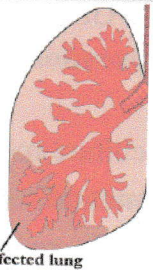


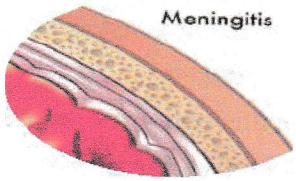
1. **Allergy** e.g. *bronchial asthma* (type I hypersensitivity).
2. **Non-invasive infections** e.g. aspergilloma (fungus ball) in a preexisting *lung cavity*.
3. **Invasive infections** e.g. *pneumonia or meningitis* may occur in severely immunocompromised patients especially those with neutropenia.
4. **Mycotoxicosis**: **Aflatoxin of A.flavus** ingested with spoiled grains or peanut is *hepatotoxic and carcinogenic.*



Cryptococcosis



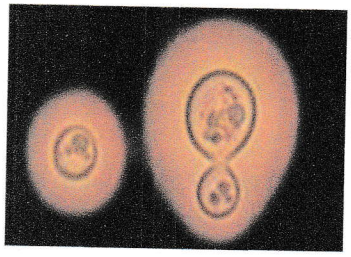




- It is caused by **Cryptococcus neoformans.**
- The organism is present in the soil contaminated with excreta of birds particularly **pigeons.**
- Infection occurs by inhalation which may result in cryptococcus **pneumonia.**
- Spread to the central nervous system leads to **meningitis.**
- Reduced cell-mediated immunity, especially in AIDS patients, predisposes to severe disease.

❖ **Laboratory diagnosis:**

1. **Specimens:** Sputum or CSF.
2. **Direct detection:**

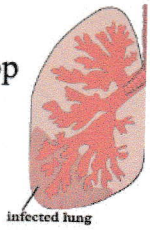


- A smear stained with **India ink** will demonstrate Cryptococcus neoformans as budding yeast cells surrounded by a **large gelatinous capsule.**
- Detection of capsular antigen in the CSF, by latex agglutination test.

3. **Culture:** on SDA, the organism gives mucoid colonies.
4. **Identification:** by characteristic morphology and urease production.

Pneumocystis

- **Pneumocystis jiroveci** (formerly **P. carinii**) is classified as **a yeast** on the basis of molecular analysis, but medically many still think of it as a protozoan.
- Most people are infected with P. jiroveci by the **age of four** and develop no symptoms, unless they are immunocompromised.
- It causes **interstitial pneumonia** especially in association with HIV infection.



- The organism **cannot** be cultivated. It can be detected in clinical specimens (e.g. lung biopsy) after staining with **silver stain.**

Mycetoma (Madura Foot or Maduromycosis)

- Mycetoma is a **localized** infection that involves **cutaneous and subcutaneous tissue, fascia and bone.**
- It usually affects the **foot** and rarely the hands and buttocks.
- The organisms involved are present in the **soil** and are implanted, by trauma, into the tissues especially in bare footed people. Therefore, lesions are localized at the site of the trauma.
- It is a clinical syndrome characterized by granuloma, multiple abscesses, draining sinuses and pus with granules.
- **According to causative organisms there are two types of mycetoma :**

	A. Eumycotic mycetoma	B. Actinomycotic mycetoma
Causative agent	• ***True Fungal infection;*** ○ e.g. Madurella mycetomatis, Madurella grisea and Allescheria boydii	• ***Bacterial ;*** ○ e.g., Actinomadura madurae, Nocardia brasiliensis and Streptomyces somaliensis
Colour of granules	○ **Mainly black and white**	○ Mainly yellow (sulphur)
Microscopic exam	○ Thick hyphae and spores	○ Thin fragmented filaments
Culture	○ On SDA ○ aerobic ○ at 25 - 30°C	○ On blood agar ○ aerobic and anaerobic ○ at 37 °C
Chemotherapy	○ No or poor response ○ Needs **surgical** treatment and may be amputation	○ Effective

- It is important to know whether the mycetoma is caused by fungi or actinomycetes because actinomycotic mycetoma will respond to **antibiotics** while fungal infection will not. The latter needs **surgical treatment** up to amputation.