Manual of Medical Microbiology & Immunology

Volume II

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To My Family

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PREFACE

"Medical microbiology and immunology" volume II includes systematic medical bacteriology, mycology and virology as well as applied microbiology. This volume is a continuation of volume I, which includes general microbiology and immunology.

The properties of the common pathogenic microorganisms and the way by which they produce diseases have been presented in a simplified way. Recent trends for the elimination and diagnosis of tuberculosis, one of the most serious re-emerging diseases, are updated. Laboratory methods used in diagnosis, including those recently described, are clearly outlined. Recently approved vaccines in 2013 and their recommended immunization schedules are included. A short resume on treatment is also mentioned.

The section on virology is provided with 17 illustrations. Detailed laboratory techniques, photographs and illustrations for systematic bacteriology and mycology are included in the *"Manual of Practical Microbiology"* (by the same author) from which 16 coloured plates are included in this volume.

A section on applied microbiology, discussing the important clinical conditions their causes and diagnosis, is included and updated.

This edition has been revised and updated to include recent concepts and developments in pathogenesis, diagnosis, prophylaxis and treatment.

I would like to thank all my colleagues who participated in updating this edition. My special thanks are due to Dr. Abdel Fattah Attia and Dr. Eman El-Seidi for their valuable advice. My thanks are extended to Dr. Fakhry Ghobrial and Mr. Yehia El-Nabarawi for their valuable assistance in the production of the illustrations.

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AblaM.ElMishad

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PART I

SYSTEMATIC MEDICAL BACTERIOLOGY AND MYCOLOGY

CHAPTER **1**

STAPHYLOCOCCI

Staphylococci are gram positive cocci arranged in grape-like clusters. The genus Staphylococcus includes mainly 3 species that are human pathogens; *Staph, aureus, Staph, epidermidis* and *Staph, saprophyticus.* All are catalase positive. *Staph, aureus* is by far the most important pathogen.

Staph, aureus is distinguished from the others by being coagulase positive, mannitol positive and by causing haemolysis of RBCs *in vitro. Staph, epidermidis,* and *Staph, saprophyticus* are coagulase negative. They are normal human microbiota and sometimes cause infections, often associated with implanted appliances and devices.

Staphylococcus aureus

Staph, aureus causes pyogenic infections (e.g. endocarditis and osteomyelitis), food poisoning and toxic shock syndrome. It is one of the most common causes of hospital acquired pneumonia, septicaemia and surgical wound infections, as well as community acquired infections.

Morphology: Gram positive cocci arranged in grape-like clusters. It is non-motile and non-sporing.

Cultural characters: Facultative anaerobes; can grow on nutrient agar producing golden yellow colonies. They produce beta haemolytic colonies on blood agar. They produce yellow colonies on mannitol salt agar.

Pathogenesis and virulence factors:

Staph, aureus can produce disease both through its ability to multiply and invade tissues, by its cell wall components and through the production of extracellular enzymes and toxins:

I- Cell wall components that play a role in the pathogenesis of infection:

a- Protein A is a virulence factor because it binds to the Fc portion of IgG at the complement-binding site, thereby preventing complement activation. Thus, opsonization and phagocytosis of the organism is reduced. b-Teichoic acid mediates its adherence to mucosal surfaces, c- Peptidoglycan which has endotoxin-like properties, that explains the ability of *Staph, aureus* to cause septic shock without possessing an endotoxin.

d-Most strains possess polysaccharide microcapsule that is anti-phagocytic.

II- Enzymes & Toxins:

1- Coagulase which is an enzyme-like protein that causes coagulation of plasma with fibrin deposition around the lesions as well as on the surface of

staphylococci rendering them more resistant to phagocytosis. Coagulase production is considered synonymous with invasive pathogenic potential.

2- **Clumping factor** is a surface compound that is responsible for adherence (adhesin) of the organism to fibrinogen and fibrin in plasma leading to their aggregation and promoting their attachment to blood clots and traumatized tissues. Clumping factor is distinct from coagulase. It is immunogenic.

3-Catalase degrades H_2O_2 into O_2 and H_2O. H_2O_2 is microbicidal and its degradation limits the ability of phagocytic cells to kill bacteria.

4-Exfoliative toxins; are two distinct proteins A and B that are **epidermolytic** and cause the desquamation seen in staphylococcal "scalded skin syndrome" in young children. They are **superantigens.**

5- **Toxic shock syndrome toxin** (TSST-1): This is produced by strains that cause the toxic shock syndrome which manifests by fever, diffuse macular rash, shock and multisystem involvement. It is a **superantigen** and causes toxic shock by stimulating the release of large amounts of cytokines from T cells and macrophages (see superantigens Vol I p.68).

6- Enterotoxins are multiple (A-E, G-J, K-R and U,V) they are produced by 50% *o f Staph, aureus* strains. The toxins are heat stable and resistant to the action of gut enzymes. They cause the diarrhoea and vomiting associated with staphylococcal food poisoning. They act as **superantigens.**

The exfoliative toxins, TSST-1 and the enterotoxins are all superantigens and the genes for these toxins are located on a chromosomal element called a pathogenicity island.

7- **Panton-Valentine leukocidin (PVL)** is an important virulence factor produced mainly by community-acquired **CA-** MRSA. It is encoded on a mobile phage. It lysis leukocytes, releasing inflammatory mediators leading to necrosis and sever inflammation.

8- Heamolysins toxins (a |3 y and 6) that attack mammalian cells membranes.

Diseases caused by *Staph, aureus:*

Staph, aureus causes infections of varying severity. The source of infection is a case or a healthy carrier harbouring the organism in the nose, throat, on the skin, under the nail and in the perineal area.

I -Pyogenic staphylococcal Diseases

a- Focal suppuration and abscess formation is characteristic of *Staph, aureus* lesions. This is due to coagulase which deposits fibrin around the lesions forming a wall, which is reinforced by inflammatory cells, e.g. **folliculitis, carbuncles, boils** and **abscesses.**

b- Invasive conditions; from any focus or after trauma, organisms may spread *via* the lymphatics or blood **(bacteraemia)** to other parts of the body

causing deep seated lesions e.g. **osteomyelitis, necrotizing pneumonia, empyema, endocarditis, meningitis, multiple abscesses in tissues** and **septicaemia.**

c- Outbreaks of **hospital acquired post-operative wound infections** commonly occur due to antibiotic resistant staphylococci. Foreign bodies, such as sutures and intravenous catheters predispose to these infections.

II. Toxigenic staphylococcal diseases:

1-Food poisoning: This results from ingestion of the preformed enterotoxin in contaminated food that is improperly cooked and kept unrefrigerated for some time.

The source of contamination of food is the hands or the nose of a cook or food handlers (carriers). The type of food involved in staphylococcal food poisoning is carbohydrate rich food e.g. cakes, pastry, koskosi, koshari (popular Egyptian food) as well as milk and milk products.

The incubation period is short (1-8 hrs) followed by nausea, vomiting, watery non-bloody diarrhoea and general malaise with no fever.

2-**Toxic shock syndrome** (TSS): This is associated with TSST-1. TSS was first described in menstruating women using tampons. The syndrome also occurs with wound or localized infections. TSS has an abrupt onset of fever, vomiting, diarrhoea, muscle pains and rash. Hypotension, heart failure and renal failure may occur in severe cases. TSST-1 can be detected in the blood by ELISA

3- Scalded skin syndrome: It is due to the exfoliative toxin. The syndrome occurs in babies and young children. It is characterized by large areas of desquamation of the skin and generalized bullae formation.

Diagnosis:

Specimens are; blood for blood cultures in septicaemia, bacteraemia and endocarditis, or swabs from lesions e.g. pus, sputum, CSF and urine.

1- Smears prepared directly from specimens and stained by gram stain show gram positive cocci in grape-like clusters among pus cells.

2- Cultures: *Staph, aureus* colonies will show complete haemolysis on blood agar and a golden yellow pigment better seen on nutrient agar. Mannitol salt agar is used to screen for nasal carriers of *Staph, aureus.*

3- Colonies are further identified by gram staining and tested for:

a- Catalase production: Staphylococci are catalase positive, which differentiates them from streptococci that are catalase negative. When a colony is placed in a drop of H_2O_2, bubbles are formed.

b-Coagulase production: Coagulase is the most important marker for identifying *Staph, aureus.* Coagulase production is tested for by adding few drops of a broth culture of the test organism to 0.5 ml of human or rabbit plasma; coagulation of plasma occurs within few hours.

c- Clumping factor can be tested for by mixing a drop of bacterial suspension with a drop of human plasma on a slide, clumping occurs.

4- Typing methods used for epidemiologic purposes include:-

a- Phage typing is used for epidemiological tracing of "outbreaks of wound infections" in hospitals. Staphylococci isolated from wounds, from nose and nail bed of attending personnel (doctors, nurses) and from fomites are identified by phage typing to reveal the source of wound infection.

It is also used to trace the source of contamination of food in "outbreaks of food poisoning"; the organisms isolated from food, vomitus, stools, and from the nose and nail bed of food handlers are phage typed.

b- Antibiotic susceptibility patterns are helpful in tracing sources of *Staph, aureus* infections.

c- Molecular typing methods (ribotyping or PCR) are used to document the spread of epidemic disease-producing strains of *Staph, aureus.*

PCR is used to detect the *mecA* gene. Other commercially available tests are use to detect its product i.e. PBP2a.

Treatment and drug resistance:

Staph, aureus shows marked ability to develop resistance to antibiotics specially in hospitals. Sensitivity testing is therefore essential for the choice of the appropriate antibiotic. About 90% of staphylococci are resistant to penicillin G, ampicillin and amoxicillin due to production of *B* lactamase (controlled by a plasmid) which breaks down the P lactame ring in penicillin. These were treated by the p* lactamase resistant antibiotics such as methicillin, nafcillin and oxacillin. However resistance to the latter drugs occurred in about 65% of staphylococci due to acquisition of a *mecA* gene that codes for a penicillin-binding protein (PBP2a) not affected by these drugs. These strains are mainly found in hospitals and are called hospital-acquired methicillin resistant *Staph, aureus* HA-MRSA. The drugs of choice for these organisms are the glycopeptides, i.e. vancomycin and teicoplanin, *Staph, aureus* with intermediate resistance (VISA) or complete resistance to vancomycin (VRSA) were reported. The mechanism of resistance to vancomycin is alteration in cell wall structure. These strains are susceptible to linezolid, daptomycin, and a combination of two streptogramins, quinupristin / dalfopristin, which inhibit bacterial protein synthesis. Mupirocin is used as a topical antibiotic to

reduce nasal carriage of the organism in hospital personnel and in patients with recurrent staphylococcal skin infections.

Differenceses between CA-MRSA and HA-MRSA

CA-MRSA infections are those acquired by persons who have not been recently (within the past year) hospitalized or undergone invasive medical procedure (e.g. dialysis, surgery, catheters) i.e. not hospital acquired.

It may cause serious skin and soft tissue infections including necrotizing fasciitis, necrotizing pneumonia and septicaemia it is more virulent than HA-MRSA due to production of its potent PVL toxin.

CA-MRSA affects specific populations of healthy adults in groups sharing personal items e.g. athletes; prisoners, military recruits and children. It is more transmissible than HA-MRSA and causes outbreaks in these groups.

DNA analysis showed that it is genetically distinguishable from HA-MRSA.

CA-MRSA is less resistant than HA-MRSA and is more susceptible to several antibiotic classes e.g. clindamycin, ciprofloxacin. Some are even sensitive to erythromycin, gentamycin, tetracycline and sulfamethoxazole.

Prevention: There are no vaccines. Measures taken to control spread of staphylococcal infections including:
-Proper hygiene measures including, frequent hand washing.
- Aseptic management of lesions and proper disposal of bandages. -Avoidance of sharing personal items such as towels and razors.
- Shared exercise equipment should be wiped down between users. -Treatment of nasal carriers or their removal from high risk areas, e.g. operating rooms, intensive care units and newborn nurseries...etc.

COAGULASE NEGATIVE STAPHYLOCOCCI *Staph, epidermidis,* present on normal skin and mucous membranes, usually produces non-pigmented colonies. It causes infections on top of prosthetic devices e.g. prosthetic valves or artificial joints and intravenous catheters. A surface protein (adhesins) and an exopolysaccharide (glycocalyx and slime) produced by the organism, participate in its adherence and lead to formation of a multi-layered biofilm on the surface of devices and valves, causing infections that are resistant to antibiotics. Most infections are hospital acquired affecting immunosuppressed patients. It is also a major cause of sepsis in neonates. It is highly resistant to antibiotics. Most strains produce P-lactamase and many are methicillin/nafcillin resistant. The drug of choice is vancomycin to which gentamic in is added.

Staph, saprophyticus is an opportunistic pathogen, second to *E. coli* as a cause of urinary tract infection in sexually active young women. Infection usually occurs 24 hr after intercourse. It produces urease which may play a role in its invasiveness in urinary tract infections. It is novobiocin-resistant while *Staph, epidermidis* is novobiocin-sensitive.

Staph. Lugdunensis has emerged as a virulent organism causing disease similar to *Staph, aureus.*

CHAPTER 2

STREPTOCOCCI

Streptococci are gram positive cocci arranged in chains or pairs. All strepococci are **catalase negative,** grow on enriched media and are facultative anaerobes. They are widely distributed in nature; some are commensals in the throat, intestine...etc., while others cause human disease.

One of the important characters for identification of streptococci is the type of haemolysis they produce on blood agar:

- Beta haemolytic streptococci produce complete haemolysis with a clear zone around the colonies on blood agar due to the haemolysin they produce e.g. *Str. pyogenes* and *Str. agalactiae.*

-Alpha haemolytic streptococci produce greenish discolouration of blood agar, due to production of H_2O_2, that changes haemoglobin to met-haemoglobin e.g. viridans streptococci and *Str. pneumoniae.*

-Some streptococci are non-haemolytic e.g. *Str. bovis.*

A **carbohydrate antigen** present in the cell wall of streptococci is used to divide streptococci into several serologic groups (Lancefield groups A-H and K-U). Typing is generally done only for groups A, B, C, F, and G. **Group** A or ***Str. pyogenes*** is the most important as it causes several human diseases.

Classification of streptococci based on DNA-DNA hybridization and 16S rRNA sequencing, describes 6 groups:

1-**Pyogenic group** which includes *Str. pyogenes* and *Str. agalactiae.* These are P-haemolytic.
2-**Mitis group** which includes *Str. pneumoniae, Str. mitis, Str. oralis* and *Str. sanguis.* These are a-haemolytic.
3-**Anginosus group** includes *Str. anginosus, Str. milleri* and *Str. intermedius.These* are a or non-haemolytic.
4-Salivarius group includes *Str. salivarius.* These are a or non-haemolytic.
5-**Mutans group** includes *Str. mutans* and *Str. sobrinus.* These are a or non-haemolytic.
6- Bovis group includes *Str. bovis.* These are a or non-haemolytic.They cause UTI and endocarditis in presence of colon cancer.

Alpha haemolytic species in these groups (except *Str. pneumoniae)* are called **viridans streptococci.**

Streptococci of **medical importance** include ***Str. pyogenes, Str. agalactiae, Str. pneumoniae,*** **viridans streptococci.** ***Str. face alls*** now called ***Enterococcus faecalls*** is placed in a separate genus Enterococcus chapter 3.

Streptococcus pyogenes

These are group A beta haemolytic streptococci and are the most important human pathogens in the genus Streptococci.

Morphology: Gram positive cocci arranged in chains. Some strains are capsulated.

Cultural characters: Facultative anaerobes, grow on blood agar producing clear zones of haemolysis (P-haemolysis) around the colonies. They are catalase negative. CO_2 5-10 % enhances their growth.

Pathogenesis and virulence factors:

1- M protein: *Str. pyogenes* has been divided into over 150 distinct M types based on serologic difference in the M protein, which protrudes from the outer cell wall in hairy like projections. It is the most important virulence factor as it is antiphagocytic. Antibodies to M protein are protective. The M protein and perhaps other cell wall antigens play an important role in the pathogenesis of rheumatic fever. Antibodies to these components react with cardiac muscle tissue. Some M types are rheumatogenic, while other types are nephritogenic. Some M types produce serum opacity factor. However, rheumatogenic strains do not produce such factor. Two other proteins T and R have no role in virulence but are used in serotyping.

2- Although M protein is the main antiphagocytic component, some strains also have a **hyaluronic acid capsule** that plays a role in retarding phagocytosis. It is not immunogenic.

3- **Lipoteichoic acid;** is another surface component that covers the pili and mediates adherence of the organism to mucosal surfaces.

4- F protein (fibronectin-bindin protein) is a structure expressed on the surface of *Str. pyogenes* that interacts with host cell fibronectin and mediates adherence and internalization of the bacteria into the host cell

5- **Toxins and enzymes:** Many extracellular products, that are immunogenic, are produced by *Str. pyogenes* including:

a- Streptokinase (fibrinolysin): It activates plasminogen to formplasmin, which can dissolve fibrin in clots, thrombi and emboli. Streptokinase has been given intravenously for treatment of pulmonary emboli and coronary artery and venous thrombi.

b- DNase (Streptodornase); depolymerizes DNA in exudates or necrotic tissue. It has 4 types (A-D). Antibodies to type **B** -which is the predominant one- develop during pyoderma and can be used for diagnostic purposes.

Both streptokinase and streptodoraase are responsible for the spreading nature of streptococcal infections. A mixture of streptokinase and streptodornase is used therapeutically to liquefy thick exudates, to break fibrin adhesions in serous cavities and to dissolve thrombi.

c- Hyaluronidase; destroys host hyaluronic acid, the cement substance of connective tissue, and helps spreading of skin infections (cellulitis).

d- Streptolysins (haemolysins): There are 2 types:
-Streptolysin "O" (oxygen labile); is immunogenic, and antibodies to it (ASO) develop in *Str. pyogenes* infections. -Streptolysin "S" (oxygen stable) is not immunogenic and is responsible for the haemolysis produced on blood agar. **e-Pyrogenic exotoxins** ; There are three antigenic types ***Pyrogenic exotoxin** A **(erythrogenic toxin);** is produced by certain streptococci lysogenized by a bacteriophage carrying the gene for the toxin. It has the same mode of action as TSST of *Staph, aureus.* It is a **superantigen** that causes the release of a large amount of cytokines from helper T cells and macrophages (Vol. I, p. 68). It is associated with streptococcal toxic shock syndrome (STSS) and scarlet fever.

***Pyrogenic exotoxin** B; is a protease that rapidly destroys tissues and is produced in large amounts by the strains of *Str. pyogenes* that cause necrotizing fasciitis. These strains are referred to as "flesh eating bacteria".

***Pyrogenic exotoxin** C is also described and contributes to STSS.

f- Others include **C5a peptidase,** neuraminidase and serum opacity factor.

Diseases caused by *Str.* ***pyogenes:***

I. Pyogenic local infections:

1- Streptococcal sore throat or **pharyngitis** are the most common infections caused by *Str. pyogenes* which is transmitted by inhalation of droplets. They are characterized by pharyngitis, enlarged tonsils with purulent exudate, high fever and enlarged cervical lymph nodes. They may be followed by rheumatic fever or acute glomerulonephritis.

2- Pyoderma (impetigo): A local infection of the skin characterized by formation of blisters which break leaving a denuded surface covered with pus or crusts. It is usually caused by M types 49, 57 and 59-61 and may be followed by acute glomerulonephritis.

II. Invasive diseases: These are diffuse rapidly spreading infections that involve the lymphatics (with minimal local suppuration) and can extend to the blood stream causing **bacteraemia** or **septicaemia.**

1- **Erysipelas:** It is a skin infection characterized by redness, oedema and a rapidly advancing margin, often on the face.
2- **Cellulitis** is an acute spreading infection of the skin and subcutaneous tissues. It follows infections associated with mild trauma, burns, wounds or surgical incisions. It differs from erysipelas by being flat and the line between the involved and uninvolved tissue is indistinct.
3- **Puerperal sepsis:** Infection of the uterus after delivery or abortion leading to endometritis associated with septicaemia and toxic shock.
4- **Acute bacterial endocarditis:** The organism reaches the heart valve through the blood stream as a complication of any of the primary lesions mentioned above. The presence of a deformed or rheumatically affected valve encourages the condition.

Ill-1 ovigenic diseases:

1- Fulminant infections and streptococcus toxic shock syndrome:

Str. pyogenes may cause fulminant invasive infections with toxic shock syndrome manifestations i.e. shock, bacteraemia, respiratory and multiorgan failure. Infection follows minor trauma and presents with soft tissue infections including **necrotizing fasciitis** (progressive subcutaneous tissue infection, destruction of fascia and fat and myositis). Such severe infections are associated with group **A** streptococci of the M type 1 and 3 (and types 12 & 28) that produce pyrogenic exotoxin **A** and B. Death occurs in about 30% of patients.

2- Scarlet fever: It is a disease of children characterized by pharyngitis and an erythematous rash. It occurs due to infection with a streptococcal strain that produces erythrogenic toxin **(A-C)** in susceptible individuals (i.e. have no antitoxin). Scarlet fever and toxic shock syndrome are clinically overlapping diseases.

IY-Post-streptococcal immunologic diseases:

1- Acute glomerulonephritis (AGN): This sometimes develops 1-4 weeks after throat or skin infection with nephritogenic strains of streptococci (M types 2, 4, 12, 25, 42, 49, 56, 57 and 60).This disease is characterized by oedema, urea nitrogen retention, high blood pressure and low serum complement levels. Blood, alburnin and granular casts are present in urine. The condition is due to antigen antibody complex deposition on the glomerular basement membrane (type III hypersensitivity reaction). The majority of patients recover completely. However, few may die or pass to chronic glomerulonephritis and renal failure.

2-Acute rheumatic fever (ARF): This is the most serious complication of streptococcal throat infection since it may result in damage of the heart valves and muscle. The onset follows 1-4 weeks after throat infection with group A streptococci M types 1, 3, 5, 6, 18 and others.

Rheumatic fever is a more **common complication** and has more tendency to recur than glomerulonephritis. This is because rheumatic fever can result from infection by many serotypes of ***Str. pyogenes*** whereas glomerulonephritis is associated with only a limited number of serotypes.

The most accepted theory for the pathogenesis of ARF is an autoimmune disease. Streptococci have M proteins immunologically similar to proteins present in the heart tissues (myosin and sarcolemmal membrane proteins), so antibodies produced against certain streptococcal M proteins can react with the heart (i.e. cross reactivity) causing damage to the heart valves.

ARF is characterized by fever, migrating polyarthritis and carditis. Recurrence of rheumatic activity occurs due to repeated streptococcal infections and every attack adds to the cardiac damage. This can be prevented by prophylactic long acting penicillin administration. RF is more common in tropical countries e.g. Egypt (Table p. 184).

Diagnosis of Streptococcal Diseases:

1-Specimens: Swabs from throat or other lesions, pus, or blood in case of bacteraemia or septicaemia.

2-Direct smears stained by gram show gram positive cocci in chains. These are useful only in specimens where streptococci do not exist as normal flora e.g. CSF or pus from wounds.

3-**Antigen detection tests:** Several kits are available for rapid detection of group A streptococcal antigens in throat swabs. Antigens are extracted from the swab with certain enzymes, and reacted with antibodies to these antigens using ELISA or agglutination tests for their detection.

4-Cultures are done on blood agar incubated at 37°C. Growth and haemolysis are enhanced by incubation at 5-10% **CO_2.** Colonies producing complete haemolysis, catalase negative and bacitracin sensitive can be ***Str. pyogenes.*** They can be identified also by specific fluorescent antibodies.

Bacitracin sensitivity test is done by placing a bacitracin disc on the inoculum of the organism on blood agar. A zone of inhibition around the disc is observed in case of group A streptococci.

5- Commercially available kits can be used for serogrouping when needed.

6- Blood cultures are done for infections associated with bacteraemia or septicaemia e.g. bacterial endocarditis and puerperal sepsis. In the latter case, it is more valuable in diagnosis than the uterine swab which is often contaminated with normal flora.

Diagnosis of post-streptococcal diseases:

1- A recent history of *Str. pyogenes* throat infection, as proved by culture or by direct antigen detection tests favours the diagnosis of ARF or AGN.

2- A recent history of *Str. pyogenes* skin infection e.g. scarlet fever or pyoderma favours the diagnosis of AGN.

3- Serologic tests

a- Antistreptolysin O: ASO antibody titres are high soon after group A streptococcal infections. An elevated ASO titre (more than 200 units) indicates recent streptococcal infection. ASO is the most commonly used test.

b- Anti-DNase B antibodies are high (80 units or more) in group A streptococcal skin infections and serve as an indication of recent streptococcal infections in patients suspected of having AGN.

c- Antihyalurinidase, antistreptokinase tests may be used for diagnosis.

d-Other less specific laboratory tests include; high erythrocyte sedimentation rate (ESR) and positive C-reactive protein (CRP).

4- The clinical criteria for diagnosis of ARF called Jones criteria are: 1- Major criteria that include; carditis, migrating polyarthritis, erythema marginatum, subcutaneous nodules and chorea. 2- Minor criteria; fever, tachycardia and the less specific laboratory tests. Two major criteria or one major and two minor criteria in presence of a recent history of *Str. pyogenes* infection are necessary for the diagnosis of ARF. A committee on rheumatic fever is planning to change these criteria because of the change in the clinical picture of ARF.

Treatment and Chemoprophylaxis:

Penicillin G is the drug of choice for treatment of streptococcal diseases. In penicillin allergic patients, erythromycin, clindamycin or azithromycin is used. Prompt treatment protects against ARF. Long acting penicillin (as a monthly injection for several years) should be given as a chemoprophylactic measure to children who had an attack of ARF to prevent recurrence.

Streptococcus agalactiae

These belong to Lancefield group **B** and are β-haemolytic but bacitracin resistant. They possess a polysaccharide **capsule,** which is antiphagocytic. They hydrolyze hippurate and are CAMP factor positive.

They inhabit about 25% of the normal adult vagina. They are acquired by the infant from the birth canal during labour and cause **neonatal septicaemia, meningitis** and **pneumonia.** The main predisposing factor is delayed delivery after rupture of membranes -longer than 18 hours. Premature babies are at higher risk. Women identified as carriers should receive intravenous ampicillin at least 4 hours prior to delivery.

A rapid test for detection of group **B** streptococci in vaginal samples is available. It detects the DNA of the organism and gives results in one hour.

In adults *Str. agalactiae* may cause pneumonia, endocarditis, postpartum endometritis and osteomyelitis. Diabetes is the main predisposing factor.

CAMP test is used for identification of ***Str. agalactiae*** cultures: Single straight streaks of the streptococcus to be tested and a p-lysin-producing *Staph, aureus* strain are made perpendicular to each other, and 4 mm apart on the surface of a sheep blood agar plate. After 24-48 hrs incubation at 35°C, a positive test result will appear as an arrow head-shaped zone of complete haemolysis in the area into which both staphylococcal P-lysin and CAMP factor have diffused. The haemolysin is enhanced in the vicinity of the P-lysin producing *Staph, aureus.* (See *Manual of Practical Microbiology* by the same author p. 85).

Viridans streptococci

The group includes *Str. mutans, Str. salivarius, Str. mitis, Str. sanguis* and others. They are gram positive cocci arranged in chains, typically they are a-haemolytic but some are non-haemolytic. They are not inhibited by optochin and are not soluble in bile.

They are normal inhabitants of the oral cavity, GIT and female genital tract. They play an important role by inhibiting the colonization of many pathogenic streptococci through the production of bacteriocins. However, they can cause some diseases.

Diseases Caused by Viridans streptococci:

1- Subacute bacterial endocarditis (SBE) is the most serious infection of this group. It occurs in individuals with congenitally deformed, rheumatically affected or prosthetic heart valves. The infection is endogenous; the organism reaches the blood stream during tooth extraction or tonsillectomy. It settles on the deformed valve and leads to the inflammatory process. The infection can be prevented by giving such individuals amoxicillin preoperatively.

SBE is diagnosed by **blood culture.** At least 3 blood cultures withdrawn at the peak of the fever and from different sites are necessary to ensure isolation of the organisms (see blood culture technique page 177).

2- Most viridans streptococci are involved in **dental plaque** and **dental caries** e.g. *Str. mutans* synthesize large sticky polysaccharides such as dextrans or levans from sucrose which contribute to the occurrence of dental caries.

Streptococcus pneumoniae
PNEUMOCOCCI

According to thel6S rRNA sequencing, it is placed in the mitis group. It is exclusively a human pathogen that spreads by droplet. They cause pneumonia, meningitis, otitis media, sinusitis, conjunctivitis, endocarditis...etc

Morphology: Gram positive lancet-shaped cocci (i.e. oval with pointed ends) arranged in pairs (diplococci) or short chains. They are capsulated. Capsules may appear as unstained halos around the organism.

Cultural characters: They grow on blood agar producing a-haemolysis or greenish discolouration similar to viridans streptococci. Since both are found in sputum and their cultures and morphology are hard to differentiate and since pneumococci cause respiratory infections while viridans streptococci do not; the growth on blood agar should be differentiated by:

	Pneumococci	**Viridans Streptococci**
Solubility in bile	Soluble	Not soluble
Inulin fermentation	Fermented	Not fermented
Sensitivity to optochin	Sensitive	Not sensitive
Pathogenicity to mice	Pathogenic	Not pathogenic
Quellung test	Positive	Negative

Antigenic structure: There are at least 91 serotypes of pneumococci based on the specificity of the capsular polysaccharide. Types 1-8 are responsible for 75% of cases of pneumococcal pneumonia in adults and for more than half of the fatalities in pneumococcal bacteraemia. In children types 6,14,19,23 are frequent causes. Serotyping can be done by agglutination or capsule swelling 'quellung test'.

Pathogenesis and virulence factors: The capsule is the most important virulence factor. It interferes with phagocytosis and favours invasiveness. Anticapsular antibodies are protective and are opsonic. Other virulence factors include **IgAi protease** produced by the organism, enhances its ability to colonize the mucosa of the upper respiratory tract.

Autolysin, pneumolysin and **pilli** may contribute to pathogenesis. **Peptidoglycan** and **teichoic acid** in the cell wall, activate the complement, induce cytokine production and contribute to the inflammatory response and to the septic shock that occurs in some immunocompromised patients.

Predisposing factors: Virulent pneumococci are often carried in the normal respiratory tract of healthy people. Predisposition to disease occurs if the local or

general resistance is lowered by: 1- Viral or other respiratory infections, bronchial obstruction and respiratory tract injury. 2- Alcoholism or drug intoxication. 3- Abnormal circulatory dynamics e.g. heart failure and pulmonary congestion. 4- Certain chronic diseases e.g. sickle cell anaemia, nephrosis, diabetes, malnutrition. 5- Splenectomyzed and the elderly persons.

Diagnosis of pneumonia:

1- Direct microscopic examination of gram stained sputum smears will show the prevalent organism to be pneumococci among pus cells.

2- Fresh emulsified sputum or, CSF deposit in pneumococcal meningitis mixed with polyvalent anti-pneumococcal serum and methylene blue and examined under the microscope, reveals swollen capsules and clumping of the organism i.e. a positive **"quellung** reaction". This can be done for rapid identification.

3- Sputum is cultured on blood agar in 5-10% CO_2 (which enhances the growth). Alpha haemolytic colonies should be differentiated from viridans streptococci by applying an optochin disc on the inoculum, or by any of the tests mentioned above.

4- Intraperitoneal injection of sputum into mice. Animals die in 24-48 hrs. The organism can be seen in tissue smears and pure culture can be obtained from heart blood.The test is rarely used, as it requires maintaining a mouse colony.

5- Blood cultures are positive in 15-25% of pneumococcal infections.

In pneumococcal **meningitis,** prompt examination and culture as well as using tests that detect the capsular polysaccharide antigen (agglutination and ELISA) and DNA probes, directly applied to CSF deposits are very helpful.

Treatment: Most pneumococci are sensitive to penicillin. Resistance to penicillin as well as other antibiotics is reported. Third generation cephalosporins (e.g. ceftriaxone) and vancomycin are the drugs of choice for treatment of invasive infections with penicillin resistant pneumococci.

Prophylaxis:

1-Pneumococcal polysaccharide vaccine containing 23 types **(PPV-23)** is recommended for adults ages 19-64 years with chronic or immunosuppressing medical conditions. All persons 65 years of age or older should be routinely vaccinated with PPV-23

2-Pneumococcal conjugate vaccine **(PCV13)** containing the capsular polysaccharide of 13 pneumococcal serotypes coupled to a carrier protein is recently licenced in 2010 and replaced **PCV_7.** Four doses are recommended for all children at 2,4 and 6 months and at 12-15 months.

CHAPTER 3

ENTEROCOCCI

Most strains react with Lancefield group D antisera, however, they were placed in a genus separate from streptococci based on genetic differences. There are at least 37 species of enterococci, the commonest is *E. faecalis* causing 90% of enterococcal infections and *E.faecium* causing 5-10%.

They are gram positive cocci in short chains, produce pink colonies on MacConkey medium, usually non-haemolytic and occasionally a-haemolytic on blood agar. They grow in 6.5% NaCl and yield a positive pyrazine amidase (PYR) test. They can grow in presence of bile and hydrolyze the polysaccharide esculin (bile- esculin-positive). They grow well at between 10 and 45°C.

Enterococci are normal inhabitants of the intestine; however, they are a common cause of **nosocomial infections,** particularly in intensive care units. The commonest infections are **urinary tract infections,** where indwelling urinary catheters are important predisposing factors. The organism is transmitted from patient to patient by the hands of hospital personnel.

They cause 10% of cases of endocarditis in patients who have undergone GIT or urinary tract surgery or instrumentation. They also cause pelvic and mtra-abdominal infections, typically in combination with anaerobes e.g. peritonitis, cholecystitis, prostatitis and wound infections. They cause meningitis and bacteraemia in neonates.

A major problem with enterococci is that they can be very **resistant to several antibiotics.** They are intrinsically resistant to cephalosporins, penicillinase-resistant penicillins and monobactams. Combined penicillin and aminoglycosides are used for treatment. Vancomycin is used for treatment of penicillin resistant strains. However, enterococci resistant to penicillin, aminoglycosides and vancomycin have emerged. Vancomycin resistant enterococci **(VRE)** are now an important cause of nosocomial infections that are difficult to treat. Newer drugs such as **daptomycin, linezolid** (zyvox), **tigecycline** and a combination of two **streptogramins, quinupristin / dalfopristin (synercid)** are used for treatment of infections due to **VRE.**

(N.B. *E.faecium* is more likely to be vancomycin or multiply-resistant than *E. faecalis)*

CHAPTER 4
NEISSERIAE

Two members in the genus *Neisseriae; N. meningitidis* and *N. gonorrhoeae* are important human pathogens causing disease only in man. Other members occur as commensals in the oro- and naso-pharynx, and vagina. Morphology:

Gram negative cocci arranged in pairs with the adjacent sides flattened; "kidney shaped appearance". In pathological specimens, *N. meningitidis* and *N. gonorrhoeae* occur intracellularly in pus cells as well as extracellularly.

Cultural characters:

N. gonorrhoeae and *N. meningitidis* are aerobes and require for growth enriched media containing heated blood e.g. chocolate agar or the selective Modified Thayer-Martin (MTM) medium containing antibiotics that inhibit growth of other organisms present in the specimens. Cultures are incubated in a humid atmosphere containing 5-10% CO_2 at 35-37°C.

Biochemical activities:

Oxidase test: All neisseria species give a positive oxidase reaction as they possess the enzyme cytochrome C. The test is done by picking up a portion of the colony and smearing it on a strip of filter paper impregnated with the oxidase reagent; a deep purple colour develops.

Acid production from sugars, due to the action of neisseria species, can be used for differentiation of the pathogenic and commensal neisseria.

	Glucose	Maltose	Sucrose
N. meningitides	+ +		
N. gonorrhoeae	+	–	*m*
N.flavescens			
N. Sicca	+	+	+

Neisseria gonorrhoeae

GONOCOCCI

They cause gonorrhoea which is a sexually transmitted disease (STD).

Antigenic structure and virulence factors: **1**-Pili are important virulence factors as they mediate attachment to mucosal cells and are antiphagocytic. Non-piliated strains are avirulent. About 100 serotypes are known based on the antigenicity of pilus proteins, which undergo marked antigenic variation due to chromosomal rearrangement.

2- **Lipooligosaccharides (LOS)** in the cell wall are responsible for the endotoxic effects occurring in gonococcal infections.

3- **Opa proteins** are outer membrane proteins that plays a role in attachment
of gonococci to host cells. They undergo antigenic variation.

4- **Por protein** extends through the gonococcal cell membrane. It may prevent intracellular killing of gonococci within neutrophils by preventing phagosome-lysosome fusion. It undergoes antigenic variation.

5- **IgAi protease** released by gonococci hydro lyzes IgAi, a major mucosal immunoglobulin, and allows attachment of the organism to the mucosa (colonization).

Diseases caused by **gonococci:**

Gonococci attack mucous membranes of the genitourinary tract, eye, anorectal area and the throat producing acute suppuration that may lead to tissue invasion. This may be followed by chronic inflammation and fibrosis.

A- Gonorrhoea: It is a venereal disease transmitted sexually and affects both males and females.

a- Male gonorrhoea: It is usually in the form of acute anterior urethritis with purulent urethral discharge and dysuria. The infection may become chronic with scanty discharge (morning drop). It may be complicated by urethral stricture, prostatitis and epididymitis.

b- **Female gonorrhoea:** It is in the form of cervicitis and urethritis with mucopurulent discharge. It may extend to the uterine tubes causing **salpingitis** and pelvic inflammatory disease **(PID);** leading to fibrosis of the tubes, ectopic pregnancy and infertility in 20% of cases. Half of the infected females are asymptomatic. The infection does not involve the adult vagina, due to its acidity and the presence of normal microbiota.

c- Anorectal and throat infections occur mainly in heterosexual women and homosexual men.

d- In both sexes, blood invasion may occur, though rarely, leading to **disseminated gonococcal infections (DGI)** manifesting as septic arthritis, tenosynovitis, skin pustules and rarely endocarditis. It occurs usually in persons deficient in complement components mainly C6-C9.

Repeated gonococcal infections are common due to:

1-Antigenic variation in the pili and outer membrane proteins.

2-Superficial nature of infection so IgG has little protective action.

3-Secretory IgAi is destroyed by IgAi protease produced by the organism

B- Ophthalmia neonatorum: An infection of the newborn acquired from the birth canal of gonorrhoeal mothers. It may involve the cornea leading to blindness.

C- Vulvo-vaginitis may affect young girls due to sexual abuse.

Diagnosis:

In acute male urethritis, the urethral discharge is examined by direct smears stained by gram. The presence of gram negative diplococci intracelhilarly and extracellularly in pus cells is diagnostic.

In chronic male infections, acute and chronic female infections and when facing medicolegal problems as in sexual abuse:

Specimens include discharge from urethra, cervix, rectum, conjunctiva, throat, or synovial fluid. These are examined by:

1- Direct smears stained by gram are usually difficult to interpret due to presence of the organism in small numbers mixed with normal microbiota.

2- Nucleic acid amplification assays (PCR or probes) are available for direct detection of *N. gonorrhoeae* in genitourinary specimens. Advantages include better detection, more rapid results and the ability to use urine as a specimen source.

3- Cultures are done on chocolate agar or MTM medium and incubated at 35-37°C in a humid atmosphere in 5-10% **CO_2.** Suspected colonies are identified by their morphology, biochemical activities (oxidase positivity and acid production from glucose only), or serologically by fluorescent antibody staining or coagglutination tests, using specific antisera.

4- Blood cultures may be needed for diagnosis of DGI.

Other sexually transmitted diseases e.g. syphilis, non-gonoccocal urethritis caused by *Chlamydia trachomatis* and HIV can coexist with gonorrhoea and should be tested for.

Treatment: Gonococci are resistant to penicillin and other antibiotics. A single intramuscular dose of ceftriaxone is recommended for both partners in uncomplicated gonococcal infections. Resistance to spectinomycin or ciprofloxacin has been noticed.

Because mixed infection with C. *trachomatis* is common, tetracycline or azithromycin should also be prescribed.

Prevention: The prevention of gonorrhoea involves the use of condoms and the prompt treatment of symptomatic patients.

Neonatal ophthalmia is prevented by the use of erythromycin or tetracycline eye drops or eye ointment immediately after birth.

Neisseria meningitides

MENINGOCOCCI

Humans are the only natural hosts for whom meningococci are pathogenic. They cause meningococcaemia and meningitis which is a highly contagious disease. Three organisms cause more than 80% of cases of bacterial meningitis in persons over 2 month of age; *H. influenzae* type b, *Str. pneumoniae* and *N. meningitidis.* Of these organisms, meningococci are most likely to cause epidemics.

Antigenic composition and virulence factors:

1- Capsular polysaccharide antigens; according to which the meningococci are classified into 13 serogroups. The most important serogroups associated with disease in man are A, B, C, Y and W-135. Groups A and C cause epidemics of meningitis, while group B is associated with sporadic infections. Group A is more common in Africa.

Properties of the polysaccharide capsule: **a-** It enhances virulence by its antiphagocytic action, **b-** It is the antigen that defines the serologic groups, **c-** It is the antigen detected in the spinal fluid of patients with meningitis, **d-** It is the antigen in the vaccine that induces protective antibodies.

2- Pili mediate attachment to the nasopharyngeal mucosa.

3- **Outer membrane proteins** according to which the different groups are subdivided to serotypes.They play a role in attachment.

4- **Lipopolysaccharide** (endotoxin) is responsible for the septic shock due to septicaemia associated with meningococcal diseases.

5- IgAi protease hydrolyzes secretory IgAi, and helps the attachment of the organism to the mucous membrane (colonization).

MENINGOCOCCAEMIA and **MENINGITIS**

The organism occurs in the nasopharynx of healthy carriers. The carrier rate is 3-30%, this rate increases up to 80% during epidemics. It causes meningitis which may be preceded by meningococcaemia and usually occurs in epidemics among young adults.

The infection is transmitted by airborne droplets from cases or carriers. It starts in the nasopharynx where it may remain silent or gives rise to exudative pharyngitis.

From the nasopharynx, the organism may invade the blood stream **(meningococcaemia)** and spread to specific sites, such as the meninges or joints, or disseminate throughout the body. The most severe form of meningococcaemia is the life threatening Waterhouse-Friderichsen syndrome, which is characterized by high fever, shock, widespread purpura, DIC and adrenal insufficiency. Meningococcaemia occurs usually in persons deficient in complement components mainly **C6-C9.**

Meningitis is the most common complication of meningococcaemia. It usually begins suddenly, with severe headache, fever, vomiting and rigidity of the neck and back muscles. It may progress to coma within few hours.

Diagnosis: Specimens include CSF, blood and puncture material from petechiae or joint fluids.

1- The CSF is withdrawn by lumbar puncture under complete aseptic conditions. In meningitis, the CSF is under tension and turbid due to the large number of pus cells; 20000/cmm. On chemical examination, the proteins are elevated and glucose is reduced.

a-The CSF is centrifuged and the deposit is examined microscopically after staining with gram. The presence of gram negative diplococci intracellularly in pus cells is diagnostic.

b- Detection of meningococcal polysaccharide antigens in CSF by coagglutination test. Latex agglutination kits for the detection of antigens in the CSF, for the most important 3 causes of meningitis i.e. meningococci, pneumococci and *H. influenzae* type b, are very useful for rapid diagnosis.

Since meningitis is a serious condition: the detection of gram negative diplococci intracellularly in CSF smears, as well as. direct detection of meningococcal antigens should be immediately reported to the treating physician, who should start treatment immediately.

c- The deposit is cultured on chocolate agar and incubated at 35-37°C in a humid atmosphere containing 5-10% **CO_2.** Colonies appear in 2-3 days and are identified by:

- Morphology; gram negative diplococci.
- Biochemical reactions; oxidase production, acid production from glucose and maltose.
- Agglutination with anti-meningococcal serum.
- Fluorescent antibody staining may be used for identification.

2- Blood cultures commonly give positive results.

3- PCR test has been developed for the detection of meningococccal DNA in blood or CSF.

Treatment of bacterial meningitis will depend on the type of organism. Prompt treatment with combination of antibiotics I.V. should be used. Ampicillin + third generation cephalosporins e.g. cefotaxime or ceftriaxone are recommended as a first line of empirical treatment. Vancomycin with or without rifampicin may be added to cover resistant pneumococcal strains.

Chemoprophylaxis: Rifampicin, 600 mg orally twice daily for 2 days, oral ciprofloxacin or intramuscular ceftriaxone are used for contacts.

Vaccines: Two meningococcal vaccines are available. A meningococcal polysaccharide vaccine (MPSV4) available since 1970 and a meningococcal conjugate vaccine (MCV4) licensed in 2005, both are tetravalent and immunize against types A, C, Y and W-135.

MCV4 is recommended for adolescents and adults 11-55 years who are at increased risk for infection, before high school, college freshmen living in dorms, the military, travelers to crowded areas (e.g. Hajj), asplenic patients, persons with complement deficiencies and microbiologists.

MCV4 was licensed in 2007 for children 2-10 years and adults over 55 years who are at risk.

MPSV4 can be used as an alternative if MCV4 is not available.

Both vaccines are protective, reduce the carrier rate and are effective in preventing epidemics. However MCV4 is expected to give better, longer-lasting protection. Both vaccines do not include the group B polysaccharide which is poorly immunogenic in humans.

Commensal neisseria and the diagnosis **of *N. meningitidis*** carriers: Commensal neisseriae e.g. *N. sicca, N. flavescens, N. lactamica...*etc, are normal inhabitants of the throat and nasopharynx and very rarely cause disease. For diagnosis of meningococcal carriers, nasopharyngeal swabs are cultured on enriched selective media (MTM) as described above. Isolated gram negative cocci should be differentiated from commensal neisseria by the difference in their cultural characters, biochemical reactions and serologic identification with specific anti-meningococcal serum.

MORAXELLA

Moraxella is a genus in the family *Neisseriaceae.*

Moraxella catarrhalis (Branhamella catarrhalis) is a gram negative diplococcus. Although considered to be a member of the normal microbiota. in human oropharynx, it can cause bronchitis, pneumonia, sinusitis, otitis media and conjunctivitis, particularly in immuno-compromised patients. Most clinical isolates produce p-lactamase. It does not ferment carbohydrates, it is oxidase positive and is DNase and butyrate esterase positive. It is sensitive to amoxicillin/clavulanic acid, cephalosporins, macrolides and fluoroquinolones.

CHAPTER 5

CORYNEBACTERIUM

Corynebacterium diphtheriae is the most important member of the group. It produces a powerful exotoxin that causes diphtheria in humans. Other corynebacteria species (termed coryneforms or diphtheroids) resemble *C. diphtheriae* in morphology and occur as normal flora on the skin and mucous membranes, but some of them are implicated in opportunistic infections.

Corynebacterium diphtheriae

C. diphtheriae causes diphtheria which is an exclusively human disease.

Morphology:

Gram positive rods with a swollen end "club shaped". They lie in small groups at acute angles or parallel to each other, "chinese-letter arrangement". The bacilli are beaded due to the metachromatic granules that contain polyphosphate and are seen better when stained with methylene blue.

Cultural characters:

Facultative anaerobes; grow best on Loeffler's serum at 37°C, can grow on blood agar. On blood tellurite medium (selective differential medium), colonies appear grey to black. The four biotypes; gravis, mitis, intermedius, and belfanti are differentiated by colony morphology on blood tellurite and biochemical reactions. They are catalase positive.

Diphtheria toxin:

C. *diphtheriae* is a powerful exotoxin producer. The toxin is the virulence factor. Only diphtheria strains lysogenized by a prophage are toxigenic and virulent. Gravis strains of diphtheria produce large amounts of toxin and generally cause the severest cases of diphtheria. The amount of toxin produced depends on the extracellular iron concentration.

Diphtheria toxin inhibits protein synthesis. The toxin is composed of two fragments. Fragment B binds the toxin to cell surface receptors. Fragment A inactivates elongation factor 2 (by ADP-ribosylation of EF-2) leading to inhibition of polypeptide chain elongation (that requires EF-2) and abrupt arrest of protein synthesis, resulting in the necrotic and neurotoxic effects of the toxin.

The toxin is heat labile, highly toxic, highly antigenic and stimulates the production of antibodies (antitoxin) that neutralize the toxin's activity. Formalin treatment of the toxin produces a toxoid that retains the antigenicity but not the toxicity of the molecule. The toxoid is used for immunization.

Toxigenicity testing of *C. diphtheriae* isolates:

It is essential to demonstrate toxin production by diphtheria isolates obtained from throat swabs of cases or carriers, this is done by:

1- **Elek's test** (or its modification): A strip of filter paper saturated with antitoxic serum is embedded in a serum agar plate. The strain tested is streaked at right angle to the filter paper and the plate incubated at 37°C for 2 days. The antitoxin diffusing from the filter paper strip will form precipitation lines with the toxin diffusing from the toxigenic strain. Absence of precipitation lines indicates that the strain is non-toxigenic.

2- **PCR** based methods are used for detection of diphtheria toxin genes particularly the gene encoding for fragment A of the toxin.

3- **ELISA** can be used to detect diphtheria toxin from clinical isolates.

4- Immunochromographic strip assay allows detection of diphtheria toxin in few hours.

5- **Tissue culture cytotoxicity assay:** On incorporation of the test strain into an agar overlay of cell culture monolayer. The produced toxin diffuses into the cells below and kills them

DIPHTHERIA

Diphtheria has virtually disappeared in developed countries following mass immunization, but is still endemic in many regions of the world.

Tonsillar diphtheria is the commonest type and is transmitted by airborne droplets. Conjunctival or skin diphtheria is rare and spreads by contact.

In tonsillar diphtheria the organism **multiplies locally** releasing the toxin causing inflammation of the throat, local necrosis with fibrinous exudate resulting in formation of a spreading greyish white, tough, adherent **pseudo-membrane.** Any attempt to remove this membrane causes bleeding.

The released exotoxin diffuses to the blood stream causing **toxaemia** and affection of the heart, kidney, liver and nervous tissue.

Clinical findings:

The patient presents with mild fever, sore throat and general ill health. Cervical lymph nodes are enlarged (bull neck appearance), the tonsils are covered with a greyish membrane which may extend to the posterior pharyngeal wall or larynx. This may cause laryngeal obstruction and suffocation if not rapidly managed by tracheostomy. Generalized symptoms occur due to production and absorption of the toxin. Nerve involvement may lead to paralysis of muscles of the soft palate and pharynx causing difficulties in swallowing and speech, peripheral neuritis or paralysis of limbs.

Myocarditis accompanied by arrhythmias and circulatory failure may occur and may cause death.

Cutaneous diphtheria: A pucture wound or cut in the skin can result in introduction of C, diphtheria into the subcutaneous tissue, leading to chronic nonhealing ulcer with a gray membrane. Rarely, exotoxin production leads to tissue degeneration and death.This occurs chiefly in the tropics, among alcoholic, homeless and other impoverished groups **Diagnosis of a case:**

If the clinical picture is strongly suggestive of diphtheria, specific treatment with antitoxic serum should be started immediately to neutralize the toxin before irreversible damage occurs. The clinician should not wait for laboratory results which serve to confirm the clinical diagnosis.

Throat swabs from the membrane are examined as follows:

-**Direct smears** are stained with gram and methylene blue. Gram positive bacilli with the characteristic morphology of diphtheria may be seen.

-**Direct detection of diphtheria toxin genes** by **PCR** in clinical specimens is available, however, ft should be carefully interpreted.

-**Cultures** are made on Loeffler's serum and blood tellurite media. These are examined after 12-18 hrs. Colonies are picked and stained with gram and methylene blue. The presence of organisms morphologically similar to diphtheria is reported as "diphtheria-like" organisms. A blood agar plate is also cultured to exclude ***Str. pyogenes*** tonsillitis.

The physician should be notified of the high possibility of diphtheria which is being confirmed further by toxigenicity tests as previously described.

Carriers of diphtheria in the throat or nose are diagnosed as in a case.

Treatment:

1 -Diphtheria **antitoxic serum** should be given without delay, when there is a strong clinical suspicion of diphtheria. The antitoxin neutralizes the toxin before ft causes iiteversible damage. From 20,000 to 120,000 units are injected intramuscularly or intravenously after suitable precautions have been taken to rule out hypersensitivity to the animal serum

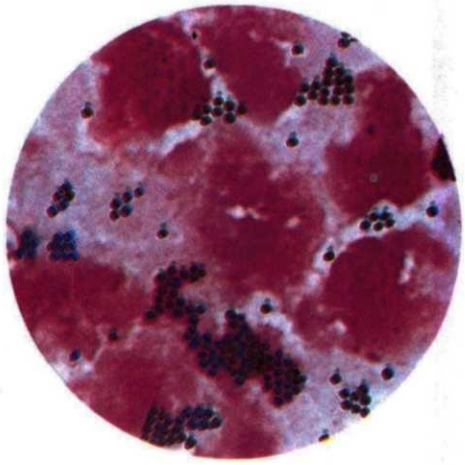
Complications of antitoxin and how to manage:

Since the antitoxic serum is produced in animals (e.g. horse) by the repeated injection of toxoid; it may result in hypersensitivity reactions e.g. anaphylactic shock or serum sickness.

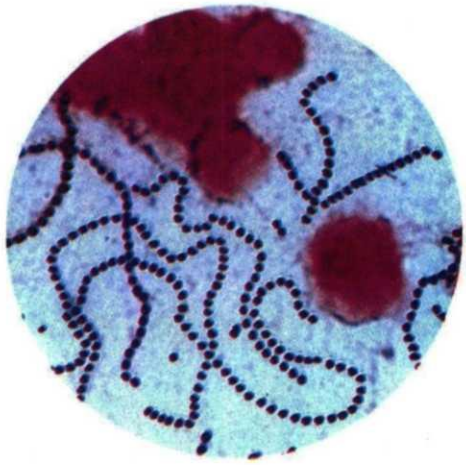
To avoid such complications, tests for sensitivity to horse serum must be performed before treatment With antitoxin as follows:

-Very minute amounts of diluted antitoxin (one drop or 0.02 ml) are dropped in the eye or injected intradermally.

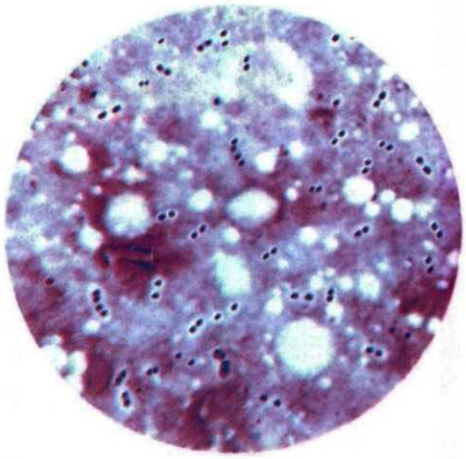
PLATE I



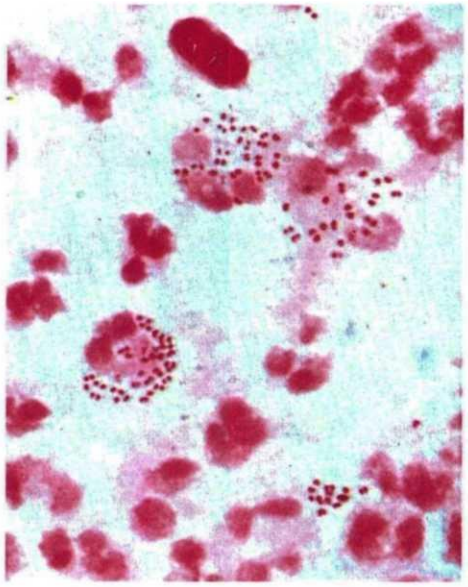
A) Gram stained smear from pus showing **staphylococci** in bunches among pus cells.



B) A gram stained smear from pus showing **streptococci** in chains among pus cells.



C) **Pneumococci** in pairs surrounded by a capsule which appears as an unstained halo, in tissues.



D) Gram stained smear from urethral discharge showing **gonococci** intra- and extracellular in pus cells.

If reaction occurs (conjunctivitis or erythema within 20 min) desensitization is done by injecting the antitoxin, using highly diluted slowly increasing doses at 20 minutes intervals. To guard against development of anaphylactic shock, pretreatment with cortisone and anti-histaminics is recommended. An epinephrine injection should be ready for use.

2-Antibiotics e.g. penicillin and erythromycin are given in adequate doses, however, they are not a substitute for antitoxins. They inhibit growth of the organism in the throat, thus reduce toxin production and decrease the incidence of carriers.

3- Respiratory support may be needed

Prophylaxis:

Active immunization: Diphtheria can be prevented by proper active immunization with diphtheria toxoid:

Diphtheria toxoid: A filtrate of broth culture of a toxigenic strain is treated with 0.3% formalin to remove toxicity but retain antigenicity. This fluid toxoid is purified, standardized and adsorbed onto aluminium hydroxide or aluminium phosphate, these act as adjuvants and delay absorption of the toxoid leading to a better immune response.

Such toxoids are commonly combined with tetanus toxoid and pertussis vaccine (DTP) and given intramuscularly to children at the age of 2,4, 6 and 18 months. A booster dose is given at school age. Adults should receive a booster every 10 years but it should contain diphtheria and tetanus toxoids Td only. Pertussis vaccine may cause encephalopathy if given after 6 years of age. DTaP (diphtheria and tetanus toxoids and acellular pertussis vaccine) is the vaccine used in USA and is recommended for use in Egypt by the WHO.

Close contacts of a case of diphtheria should receive a booster dose of diphtheria toxoid and a course of erythromycin or an injection of long acting penicillin. Antitoxic serum is no longer indicated for contacts.

Other Medically Important Corynebacteria (Diphtheroids)

They are normal inhabitants of the skin and mucous membranes of the respiratory tract, urinary tract and conjunctiva. Many species have been implicated in opportunistic infections in hospitalized patients.

C. ulcerans and C. *pseudotuberculosis* are closely related to *C. diphtheriae* and may carry the toxin gene. Toxin producing, C. *ulcerans* may cause disease clinically similar to diphtheria. *C. jeikeium* causes several infections associated with skin damage by wounds or invasive devices and it is very resistant to antibiotics.

Listeria monocytogenes

L. *monocytogenes* are small gram positive, non-spore forming bacilli similar in morphology to corynebacteria. The organism exhibits an unusual tumbling movement at 22-28°C but not at 37°C that distinguishes it from the corynebacteria that are non-motile. It is catalase positive. Colonies on blood agar produce a narrow zone of p-haemolysis.

Listeria is found in animals, soil and plants. From these reservoirs, it is transmitted to humans by contact with domestic farm animals or their faeces, by ingestion of contaminated unpasteurized milk or cheese and contaminated vegetables. The organism can grow at refrigerator temperature.

The pathogenesis of listeria is dependent upon its ability to invade mononuclear phagocytic cells. After internalization a **listeriolysin O** enzyme lyses the membrane of the phagolysosome and they escape destruction. It can move from cell to cell by means of **actin rockets,** a filament of actin that contracts and propels the bacteria through the membrane of one human cell and into another.

Since it grows intracellularly, cell mediated immunity (CMI) is a more important host defense than humoral immunity. Suppression of CMI predisposes to listeria infection. The disease is known as listeriosis and occurs in different forms:-

1-Infection of pregnant women passes to the foetus transplacentally causing granulomatosis infantiseptica characterized by pustular skin lesions and intrauterine sepsis leading to abortion, still-birth or premature labour.

2-Newborns infected during delivery from infected mothers, develop neonatal meningitis 1-4 weeks later.

3-Infection in irnmunosuppressed adults causes meningoencephalitis and bacteraemia, specially in renal transplant patients.

4-Gastroenteritis which is characterized by watery diarrhoea, fever, headache, myalgia and abdominal cramps but little vomiting. Outbreaks are usually caused by contaminated dairy products. Undercooked meats such as chicken and hot dogs have also been involved.

Diagnosis: Isolation from blood by blood culture and from CSF on blood agar and from stools on listeria selective media. Isolation is enhanced if specimens are refrigerated for few days before inoculation "cold enhancement". Identification is done by gram stain and detection of tumbling motility and positive catalase. It produces P− haemolysis, on sheep blood agar, which is enhanced in the vicinity of a streak of *Staph, aureus,* i.e. CAMP test positive (p. 14).

Treatment: Ampicillin and trimethoprim-sulfamethoxazole.

Erysipelothrix rhusiopathiae are gram positive rods widely distributed in land and sea animals in whom it causes disease. In man it causes erysipeloid, a skin infection that resembles erysipelas (caused by streptococci). Infection occurs by direct contact, and is usually on the hands of meat and fish handlers.

CHAPTER 6

CLOSTRIDIUM ANAEROBIC, GRAM POSITIVE, SPORE FORMING BACILLI

Some members of the genus *Clostridium* are saprophytes in soil, sewage and water. Others are commensals in the intestine of man and animals. The important members that cause disease in man are:

1- *CI. tetani* causing tetanus.
2- *CI. perfringens* causing gas gangrene and food poisoning.
3- *CI. botulinum* causing botulism
4- *CI. difficile* causing pseudomembranous colitis & antibiotic associated diarrhoea. The latter may be caused also by *CI. perfringens.*

Clostridium tetani

The organism is found in the intestine of man and animals and in manured soil. It causes tetanus in man and animals.

Morphology: Gram positive bacilli swollen at one end due to terminal spherical projecting spores; "drum-stick appearance". They are motile.

Cultural characters: Strict anaerobes, grow on nutrient agar, but better growth occurs on blood agar on which colonies are surrounded by a clear zone of haemolysis due to its tetanolysin toxin. The organism grows readily on Robertson cooked meat medium.

Animal pathogenicity: When mice are injected intramuscularly with supernatant of cooked meat culture of the organism, within few hours, the tail becomes stiff, the injected limb shows spastic paralysis which spreads to the rest of the body. The administration of tetanus antitoxin few hours before injecting the culture protects the animal.

Toxin production:

CI. tetani owes its pathogenicity to a potent neurotoxin "tetanospasmin". It is a polypeptide that exists as one antigenic type. The toxin is released from the site of the wound and diffuses *via* the blood stream to the peripheral nervous system and reaches the CNS by retrograde axonal transport. The toxin binds to ganglio sides receptors and blocks the release of inhibitory mediators (glycine and y-aminobutyric acid). Motor neurons are left under no inhibitory control and undergo sustained excitatory discharge, causing the characteristic motor spasms of tetanus. The toxin exerts its effect on the spinal cord, the brain stem, peripheral nerves, at neuromuscular junctions and directly on muscles. Thus leading to, generalized muscular spasm, hyper-reflexia, seizures and spastic paralysis.

Extremely small amounts of the toxin cause the disease and can be lethal for humans. Such amounts are too small to trigger immune mechanisms, and one attack of tetanus does not provide immunity to any future attacks.

TETANUS

Infection occurs by contamination of wounds with street dust (containing the spores). The injury may be a trivial nail prick, a contaminated surgical wound, a gun shot wound, or an infected umbilical stump leading to "tetanus neonatorum". The spores can also be introduced by "skin-popping," a technique used by drug addicts to inject drugs into the skin.

At the local site of infection the spores genninate releasing the toxin which exerts its action. Germination is enhanced by the presence of necrotic tissue or associated pyogenic infections. LP. varies from 4 days to several weeks.

The disease is characterized by convulsive tonic contractions of voluntary muscles including; spasm in jaw muscles leading to trismus **(lockjaw),** facial spasm (sardonic grin), pronounced arching of the back due to spasm of the muscles of the back (opisthotonos). Death rate is high and occurs due to respiratory or cardiac failure.

Neonatal tetanus follows contamination, of the umbilical stump or circumcision wound of the newborn infants, by tetanus spores. It is a major problem in developing countries. It occurs if the mother is not immune.

Diagnosis:

On clinical suspicion and history of contaminated wounds, treatment with antitoxin should be started without waiting for laboratory diagnosis, which is done for confirmation as follows:

Wound exudate - aspirated from deep sites of the wound - is examined microscopically for the presence of gram positive bacilli with drum-stick appearance. Direct immunofluorescent staining is used when available.

The exudate is cultured on blood agar incubated anaerobically, and on Robertson cooked meat medium. Proof of isolation of *CI. tetani* must rest on production of toxin and its neutralization by specific antitoxin in laboratory animals.

Prevention and control:

Tetanus is a totally preventable disease. The results of treatment of tetanus are unsatisfactory. Prophylactic immunization is the only way to control tetanus.

Active immunization: Alum precipitated tetanus toxoid is given in combination with diphtheria toxoid and pertussis vaccine "DTP" or DTaP in three intramuscular injections at the age of 2, 4 and 6 months.

-A booster dose is given a year later and another upon school entry.

-A booster dose of tetanus and diphtheria (DT) is recommended every 10 years, to maintain a good serum antitoxin level.

-Individuals wounded with a previous history of vaccination 5 or more years ago should receive a booster dose.

-Booster doses are given to military personnel before or during war.

-Boosters are recommended for pregnant women to guard against labour infection and to provide maternal immunity for the newborn.

Passive immunization:

Antitoxin is given to wounded persons without previous history of vaccination or those immunized more than 10 years ago. Intramuscular injection of 250-500 IU human tetanus immune globulin (HTIG) i.e. tetanus antitoxin made in humans to avoid hypersensitivity reactions that occur when antitoxin made in horses is used. That will give protection for 2-4 weeks.

Antitoxin prophylaxis should be accompanied by active immunization with tetanus toxoid at different sites i.e. **passive-active immunization.**

Treatment

1- Antitoxin should be given at once to **suspected cases** to neutralize any toxin that did not fix to the CNS. HTIG is given in a dose of3000-10,000 IU divided into three equal portions, given intramuscularly in three different sites at the same time. However, the efficacy of antitoxin treatment is doubtful except in neonatal tetanus, where it may be life saving.

2- Proper care of the wound and surgical debridement are very important.

3- Penicillin or metronidazole is given in big doses to eliminate the organism from tissues and to treat associated pyogenic infections.

4- An adequate airway must be maintained and respiratory support given.

5- Benzodiazepines e.g. valium, should be given to prevent spasm

Clostridium perfringens

This organism is the commonest of several members of the genus *Clostridium* associated with **gas gangrene** and myonecrosis (causing 90% of cases). They are found in the large intestines of man and animals. Some strains of *CI. perfringens* that produce a powerful enterotoxin cause **food poisoning.**

Morphology: Gram positive large bacilli. Spores are oval, subterminal and non-projecting. It is capsulated.

Cultural characters: Anaerobes, colonies on blood agar show zones of complete haemolysis.

Biochemical activities: They ferment glucose, lactose, maltose and sucrose producing acid and large amounts of gas. The organism causes rapid fermentation of lactose in litmus milk and the produced gas splits the clot "stormy clot reaction".

Nagler's reaction: All types of *CI. perfringens* produce opalescence in egg yolk media (5% egg yolk agar) due to the production of lecithinase which causes a visible precipitate around the colonies; such opalescence can be specifically inhibited by *CI. perfringens* antitoxin.

Toxins and other virulence factors:

CI. perfringens produces a variety of toxins and enzymes that result in spreading of infection:

1- Alpha toxin is a lecithinase which causes destruction of the cell membrane, cell death and necrosis.

2- Theta toxin has a haemolytic and necrotizing effect.

3- DNase, hyaluronidase, protease and collagenase.

4- Enterotoxin produced by some strains causes a type of food poisoning which follows ingestion of contaminated warmed meat dishes.

GAS GANGRENE (Myonecrosis)

Several species of **Clostridia** referred to as the histotoxic or tissue-destroying **Clostridia** cause gas gangrene, these include the saccahrolytic *CI. perfringens, CI. novyi* and *CI. septicum,* followed by the proteolytic *CI. histolyticum, CI. sporogenes* and others.

Pathogenesis:

The infection occurs when wounds are contaminated with soil containing the organism or its spores. The condition occurs in deep lacerated, devitalized wounds as in car accidents or war wounds. The presence of foreign bodies, mixed infection with aerobic pyogenic bacteria as well as the decreased blood supply, lowers the oxygen tension and favours germination of spores. Vegetative cells multiply and ferment sugars producing large amounts of gas which distends the tissues and interferes with their blood supply leading to their death. Necrotizing toxins, collagenase and hyaluronidase favour necrosis and spreading of infection.

Proteolytic **Clostridia** digest dead tissues leading to change in colour and foul odour of the wound. The wound is oedematous, foul smelling, dark in colour with crepitations in the adjacent tissues. The condition is accompanied by generalized toxaemia, shock, multiple organ failure and may be fatal.

Diagnosis is made clinically; laboratory diagnosis is used for confirmation.

- Exudate aspirated from deep sites of the wound is stained by gram, the presence of large gram positive rods is suggestive. Spores are rarely found.
- Cultures are made on chopped meat-glucose media, and thioglycolate broth and on 2 blood agar plates incubated aerobically and anaerobically. Colonies that grow only anaerobically causing haemolysis are further identified by:
-Morphology, sugar fermentation and stormy clot formation in litmus milk and Nagler's reaction (as previously mentioned).

Treatment:

1- Surgical debridement of devitalized tissues. Amputation may be needed.
2- Penicillin in big doses in combination with metronidazole and imipenem or an aminoglycoside are used.
3- Hyperbaric oxygen therapy. It detoxifies patients rapidly.
4- Polyvalent antitoxic sera are of doubtful value.

Prevention:

Prevention depends upon adequate cleaning of contaminated wounds, surgical debridement, removal of foreign bodies and administration of antibiotics (e.g. penicillin). Antitoxic serum for passive prophylaxis is unreliable. Toxoids are not available for active immunization.

CL perfringens* food poisoning** usually follows the ingestion of large numbers of **Clostridia** (10 or more) that have grown in contaminated reheated meat dishes. The enterotoxin forms when the organisms sporulate in the gut. The enterotoxin is a protein that may be a nonessential component of the spore coat. It causes marked hypersecretion of water and electrolytes in the lumen of the intestine causing diarrhoea and abdominal cramps, no vomiting or fever. The incubation period is 6-18 hours. Fluid replacement therapy is the only **treatment.** The **condition** is similar to the diarrhoeal type of food poisoning produced by ***B. *cereus.* It is self limited in 1-2 days.

Clostridium botulinum

CI. botulinum is essentially a saprophyte in soil. It is frequently present in the intestinal tract of domestic animals. It causes botulism, which is a type of food poisoning due to ingestion of inadequately sterilized canned meat, fish or alkaline vegetables e.g. green beans, green pepper or mushrooms. Canning provides proper anaerobic conditions for growth and production of exotoxin.

Morphology: Gram positive large straight rods, motile, non-capsulated. Spores are oval, central or subterminal.

Cultural characters: Strict anaerobes, grow on simple media.

Botulinum toxin: *CI. botulinum* produces a very potent **neurotoxin.** It is a polypeptide encoded by a lysogenic phage. The toxin is absorbed from the gut and is carried *via* the blood then binds irreversibly to the presynaptic nerve endings of the peripheral nervous system and cranial nerves, where it blocks the release of acetylcholine at the neuromuscular junction leading to **flaccid paralysis.** There are 7 antigenic types (A-G); types A,B,E and occasionally F cause botulism in man. The toxin is heat labile; can be destroyed by boiling for 20 minutes.

Botulinum toxin is considered to be a major agent for bioterrorism and biologic warfare.

Animal pathogenicity: On intraperitoneal injection of the toxin into mice, flaccid paralysis occurs all over the body in few hours.

BOTULISM

There are three clinical forms of botulism:

1- The classical type, which is an intoxication resulting from ingestion of canned food containing the preformed neurotoxin produced by *CI. botulinum.* The toxin has an affinity to the cranial motor nerves causing bulbar paralysis manifesting as diplopia, dysphagia and respiratory muscle failure. Symptoms begin 18-24 hrs after ingestion of the toxic food. Death results from respiratory or cardiac failure. There is no diarrhoea, vomiting or fever. Foods responsible include; canned green beans, mushrooms, tuna fish, liver paste, home preserved vegetables, meat or ham and others.

2- **Wound botulism,** in which spores contaminate a wound, germinate and produce toxin at the site. This type is associated with drug abuse, specially skin-popping with black tar heroin.

3- **Infant botulism** 'floppy baby' occurs due to ingestion of spores in the baby's food, where they germinate in the gut and produce the toxin, which is absorbed into the blood. Honey contaminated with the spores is the most frequent vehicle of infection. It affects infants below 6 months when the colonization resistance of the gut is poor. The infants develop poor feeding, weakness or paralysis and may need respiratory support but usually recover spontaneously. However, it may be one of the causes of sudden infant death syndrome. Infant botulism accounts for about half of the cases of botulism in USA.

Diagnosis is a clinical one but may need laboratory confirmation:

1- The toxin can be directly detected by ELISA or PCR in patient's serum, stool or food remnants. In infant botulism, *CI. botulinum* and toxin can be detected in bowel contents, but not in serum

2- The toxin can be demonstrated in patient's serum or food remnants by injection into mice, which will die with generalized flaccid paralysis, unless protected by antitoxin. Typing of the toxin can be done by neutralization with specific antitoxin in mice i.e. mouse protection tests.

3- Cultures of food remnants are rarely done.

Treatment: Trivalent antitoxin (types A, **B,** and E) is promptly administered I.V. within 12 hours after ingestion i.e. before the toxin binds to the tissues. Adequate ventilation by mechanical respirators should be used when needed.

Prevention is by careful sterilization of food before canning. Swollen cans should be discarded. Honey should not be given to infants.

Clostridium difficile The organism is carried in the GIT in about 3% of the general population and up to 30% of hospitalized patients. It causes pseudomembranous **colitis (PMC)** and **antibiotic associated diarrhoea** (AAD) and is the commonest cause of hospital acquired diarrhoea. It is transmitted by the faecal-oral route. The hands of hospital personnel are important intermediaries.

These conditions occur as a complication of antibiotic therapy. Clindamycin was the first antibiotic recognized to cause PMC, but many antibiotics are known to cause this disease. At present the second and third generation cephalosporins are the commonest causes as they are being more frequently used. The diarrhoea may occur 4-10 days after starting antibiotics. Cancer therapy also predisposes to these conditions.

The antibiotics suppress drug sensitive normal microbiota allowing the drug resistant *CI. difficile* to multiply and produce an exotoxin A which is an enterotoxin that causes outpouring of fluids i.e. watery diarrhoea and an exotoxin B, which is a cytotoxin that causes damage to the colonic mucosa, leading to the psuedomembrane formation.

AAD can be also caused by *CI. perfringens* and *CI. sordelli.*

Diagnosis can be done by:

1- Detection of the toxins in stool supernates by ELISA or latex agglutination, have largely replaced tissue culture cytotoxicity assays.

2- Isolation of the organism on special media.

Treatment: Withdraw the causative antibiotic. Treat with metronidazole or vancomycin and replacement fluids. Metronidazole is preferred because using vancomycin may select for vancomycin resistant enterococci.

CHAPTER7

BACILLUS

AEROBIC, GRAM POSITIVE, SPORE- FORMING BACILLI

The most important pathogenic member in this genus is ***Bacillus anthracis.*** Most of the other members are saprophytes in water, soil and air and are collectively termed anthracoids. Of these, ***B.*** *cereus* and ***B.*** *subtilis* may act as opportunistic pathogens in debilitated or immunocompromised persons in whom they cause bacteraemia, meningitis, endocarditis or endophthalmitis.

B. cereus *may* cause food poisoning after consumption of cooked rice in which it has grown profusely and produced an enterotoxin.

Bacillus anthracis

Morphology: Gram positive rectangular large organisms arranged in chains. They are capsulated *in vivo;* when stained with polychrome methylene blue, the capsule appears as pink rim around the blue bacillus (McFadyean reaction). They form spores *in vitro.* The spores are central, ovoid and are not stained with gram. They appear pink when stained with the acid fast spore stain. The spores can survive in dry soil for decades.

Cultural characters: Aerobes; grow on nutrient agar at 37°**C.** Gelatin is liquefied and growth in gelatin stabs resembles an inverted fir tree.

Pathogenesis and Virulence:

There is one antigenic type. The virulence of the organism is due to;

1- The capsular polypeptide (D-glutamate) which is anti-phagocytic.

2- Anthrax toxins: these are made up of three proteins, protective antigen (PA), lethal factor **(LF)** and oedema factor **(EF).** PA binds to specific cell receptors, and after proteolytic activation, it forms a membrane channel that mediates entry of EF and LF into the cell. EF is an adenylate cyclase; with PA, it forms a toxin known as oedema toxin. LF plus PA form lethal toxin, which is a major virulence factor and causes death of infected animals and humans. When injected into laboratory animals (e.g. rates) the lethal toxin can quickly kill the animals.

The name protective antigen refers to the fact that antibodies against PA protect against disease. The capsule and toxin are coded for by plasmids.

ANTHRAX

Anthrax is primarily a disease of farm animals e.g. cattle and sheep, man is infected by coming in contact with infected animals or their products or contaminated dust. Individuals more liable to get the infection are farmers, butchers, veterinarians or laboratory workers. The commonest infections are:

1- Cutaneous anthrax (malignant pustule). The organism enters through skin abrasions. A papule forms which rapidly changes to a vesicle then a pustule and a necrotic ulcer covered by a black eschar and surrounded by marked oedema. Infection may spread leading to septicaemia.

2- Pulmonary (inhalation) anthrax (wool sorter's disease) results from inhalation of spores and affects people handling wool from infected animals. It is characterized by haemorrhagic mediastinitis, bloody pleural effusion, septic shock and death.

3- Intestinal anthrax is rarely reported and results from eating infected meat. Pulmonary and intestinal anthrax are often fatal as they go unrecognized until it is beyond the time for effective therapy.

B. anthracis is a major agent of **bioterrorism** because it can be easily grown in large quantities, is resistant to destruction and can be formulated into an aerosol for wide dissemination. In 2001, an outbreak of 22 cases; 11 inhalation and 11 cutaneous anthrax, occurred in the USA. The outbreak was caused by sending spores of the organism through the mail. Five of the patients with inhalation anthrax died.

Diagnosis: Anthrax is suspected from clinical signs and history of exposure. Pathologic samples include; exudates from skin lesions, sputum, stools or blood from severely ill patients. These are examined as follows:

1- Smears are stained with gram and McFadyean stain. These will show the large gram positive rods in chains which may be capsulated. Dry smears may be stained by immunofluorescence for rapid diagnosis.

2- Cultures on nutrient or blood agar showing the characteristic colonies are identified by gram. Virulent anthrax cultures kill rates or guinea pigs upon intraperitoneal injection.

3- If the patient is severely ill, blood cultures should be done, although the isolation of the organism may not be possible after antibiotic therapy. Blood smears stained by McFadyean stain may reveal the capsulated rods.

4- ELISA to measure antibodies to EF, LF and PA may be of value retrospectively, but is seldom used diagnostically. Paired serum samples separated by 10 days may be useful in confirmation of the diagnosis.

5- In case of a bioterror attack, rapid diagnosis can be performed in special laboratories using PCR-based assays.

Treatment should be started early. Ciprofloxacin or doxycycline is used for cutaneous anthrax. Ciprofloxacin plus rifampin plus vancomycin are recommended for inhalation anthrax.

Prevention: Control measures include:

1- Postexposure prophylaxis with ciprofloxacin or doxycycline for 4 weeks while giving 3 doses of the vaccine or for 8 weeks without vaccination.

2- Anthrax vaccine adsorbed (AVA BioThrax) prepared from the culture filterate of a non-capsulated strain of *B. anthracis* containing PA adsorbed to aluminum hydroxide is available for human use in USA. The vaccine is weakly immunogenic and six doses of the vaccine over an 18-month period are given. Annual boosters are given to maintain protection. The vaccine is given to those with high occupational risk e.g. veterinarians, laboratory workers, woolen mill workers and livestock handlers.

A recombinant PA vaccine (rPA) adsorbed to aluminum hydroxide is under development.

3- Active immunization of domestic animals with live attenuated vaccines.

4- Disposal of animal carcasses by burning or by deep burial in lime pits.

5- Autoclaving of animal products.

6- Protective clothing and gloves for handling potentially infected material.

Bacillus cereus

B. cereus causes post-traumatic panophthalmitis, endocarditis, meningitis, osteomylitis and pneumonia mainly in immunosuppressed persons. These infections are resistant to antibiotics and should be treated with vancomycin with or without an aminoglycoside.

It causes two distinct forms of **food poisoning:-**

1- The emetic type, caused by a heat stable enterotoxin, characterized by nausea, vomiting and abdominal cramps 1^5 hr after eating (similar to staphylococcal food poisoning) and is associated mainly with rice dishes. It is caused by the preformed toxin which is formed when the spores survive steaming and rapid frying and then germinate when rice is kept warm for many hours (i.e. reheated rice). The condition is self limited in 24 hrs.

2- The diarrhoeal type, due to a heat labile enterotoxin, characterized by abdominal pain and cramps with profuse watery diarrhoea 8-24 hr after ingestion of the contaminated food (resembling *CI. perfringens* food poisoning) and is associated mainly with meat dishes and sauces. The enterotoxin may be preformed in the food or produced in the intestine.

Sterilization test bacilli: *Geobacillus stearothermophilus* spores are killed at 121°C for about 20 min. and are used for testing efficiency of autoclaves by including strips containing the organism within the material being autoclaved and are subsequently examined by culture for surviving spores.The same organism is used to test efficiency of plasma gas sterilizers. *B. atrophaeus* is used to test ethylene oxide sterilizers. *B. pumilus* is used to test efficacy of sterilization be ionizing radiation.

CHAPTER 8

ENTEROBACTERIACEAE

Members of the family *Enterobacteriaceae* are gram negative bacilli found primarily in the colon of humans and other animals, many as part of the normal flora. They cause a variety of diseases with different pathogenic mechanisms.

The family includes many genera e.g. *Escherichia, Shigella, Salmonella, Klebsiella, Enterobacter, Serratia, Citrobacter* and *Proteus.* They have certain features in common:

1- They are facultative anaerobes.
2- They all ferment glucose (fermentation of other sugars varies).
3- They are oxidase negative.
4- They reduce nitrates to nitrites as part of their energy-generating processes.

Commercially prepared biochemical test kits (API 20E) are used for the differentiation of the species of Enterobacteriaceae.

The pathogenic genera are the *Shigella, Salmonella,* and some strains of the *Escherichia.* The opportunistic pathogens are *Klebsiella, Enterobacter, Serratia, Citrobacter, Proteus* and *Escherichia.*

According to their effect on lactose, Enterobacteriaceae are divided to:

1- The lactose fermenters include the genera *Escherichia, Klebsiella, Citrobacter* and *Enterobacter.* They are collectively called conforms.

2- The lactose non-fermenters include the genera *Salmonella, Shigella,* and *Proteus.*

Escherichia coli

These are normal inhabitants of the intestine of man and animals, however, some can cause disease in man.

Morphology: Gram negative bacilli, motile, some strains are capsulated.

Cultural characters: Facultative anaerobes, grow on simple media. On MacConkey's medium, they produce rose-pink colonies due to lactose fermentation. *E. coli* strains causing urinary tract infections produce haemolysis on blood agar.

Biochemical **activity:** They ferment glucose, lactose, maltose, mannite, sucrose and salicin with production of acid and gas. They are indole positive, V.P. negative, M.R. positive and citrate, urease and FfcS negative.

Serological **characters:** In addition to O (somatic) and H (flagellar) antigens, many pathogenic *E. coli* possess K (capsular) antigen. The

enteropathogenic *E. coli* possess O antigens with numerical designations e.g. 26, 55, 111,119. Serotype 0157:H7 cause haemorrhagic colitis.

Virulence factors: *E. coli* has several clearly identified components that contribute to its ability to cause disease:

- Pili or colonization factors which enable the organism to adhere to mucosal cells. Genes for pili are carried on plasmids.
- K or capsular polysaccharide antigen which interferes with phagocytosis.

-Endotoxin which is the lipopolysaccharide (LPS) that causes endotoxic manifestations that may be associated with *E. coli* infections.

- Two **exotoxins** (enterotoxins) one is heat labile (LT) and the second is heat stable (ST) are produced by **enterotoxigenic** strains of *E. coli.* They are genetically determined by a plasmid.
- **Verotoxin** or **Shiga toxin** produced by enterohaemorrhagic *E. coli.*

Diseases caused by *E. coli:*

1- Urinary tract infections: *E. coli* causes 90% of <u>community acquired</u> urinary tract infections (UTI). Certain strains known as **uropathogenic *E. coli*** colonize the vagina and periurethral region from where they ascend to the bladder or kidney causing cystitis or pyelonephritis. These strains possess pili with adhesive proteins that bind to specific receptors on the urinary tract epithelium. They also possess **K** antigens and exotoxins (haemolysins).

2- 2?. *coli* is an important cause of **hospital acquired infections** including UTI, which is associated with urinary catheterization and drug resistant strains.

3- **Neonatal meningitis:** *E. coli* causes 40% of neonatal meningitis followed by group B streptococci. Such strains usually possess capsular antigen Kl.

4- Pneumonia, sepsis, bacteraemia and **endotoxic shock** may follow any *E. coli* infection specially in immunocompromised hosts or neonates.

5- Intestinal diseases: Diarrhoeagenic strains of *E. coli* cause diarrhoea through different mechanisms:

a- Enterotoxigenic *E. coli* (ETEC) cause "travellers' diarrhoea" and infantile diarrhoea. The diarrhoea is watery and ranges from mild to severe (cholera-like) and may be fatal.

The organism adheres to intestinal epithelium *via* the pili or colonization factors. Then they liberate LT and ST enterotoxins. LT causes the watery diarrhoea by stimulating adenylate cyclase activity in cells of the small intestine resulting in increase in the concentration of cAMP, which causes excretion of the chloride ion, inhibition of sodium ion absorption and

significant fluid and electrolyte loss into the lumen of the gut. ST activates guanylate cyclase in enteric epithelial cells and stimulates fluid secretion.

b- **Enteropathogenic *E. coli*** (EPEC) is an important cause of diarrhoea in infants. Certain serotypes e.g. **O55,** Om,026 previously caused outbreaks of neonatal diarrhoea in nurseries. They act mainly by adhering tightly to intestinal mucosa resulting in loss of microvilli and cupping of cells around the bacteria. Thus, preventing the normal functions of absorption and secretion. Resulting in sever watery diarrhoea, vomiting and fever. It is usually self limited.

c- Enteroinvasive *E. coli* (EIEC): These cause dysentery-like diarrhoea through invasion of intestinal epithelial cells. Like shigella, EIEC are non-lactose or late lactose fermenters and are non-motile.

d- Enterohaemorrhagic *E. coli* (EHEC): These belong mainly to serotype 0157:H7. They produce a toxin known as **"verotoxin"** (so called because it is toxic to Vero cells (monkey kidney cells in tissue culture). It is also called **shiga toxin** as it is similar to those produced by *Shigella* species. These strains are associated with outbreaks of haemorrhagic colitis, which is a severe form of bloody diarrhoea that mainly follows ingestion of undercooked hamburger at fast-food restaurants. Some patients may end up with a life-threatening complication called **haemolytic uraemic syndrome** (haemolytic anaemia, thrombocytopenia and acute renal failure). Treatment of the diarrhoea with antibiotics increases the risk of developing this syndrome by increasing the amount of verotoxin produced by the organism.

e- Enteroaggregative *E. coli* (EAEC): These exhibit a specific pattern of aggregative adherence to the mucosa in patches. They cause acute and persistent diarrhoea in children and in HIV patients. EAEC produce ST-like toxin and a haemolysin. The available methods used for their identification are DNA probes and HEp-2 cell cultures to determine the aggregative phenotype.

Diagnosis:

The specimen e.g. urine, pus, stools, CSF...etc are cultured on different media. Lactose fermenting pink colonies on MacConkey are further identified by their morphology and biochemical reactions.

In case of diarrhoea, isolated *E. coli* should be further tested serologically and virulence proved;

1- Serotyping by slide agglutination for EPEC and EHEC strains.

2- When EHEC infection is suspected; rapid diagnostic methods are used to detect the verotoxin by **ELISA,** or to detect the organism by immunofluorescence in stools.
3- Various *in vivo* assays, tissue cultures, immmu no assays, DNA probes and PCR may be used for detection of toxin production or its gene, mainly in reference laboratories.

Treatment of *E. coli* infections depend on the site and on the sensitivity pattern of the isolated organism Diarrhoeal diseases usually do not require treatment. However, the duration of the diarrhoea can be shortened by antibiotics. Rehydration is essential.

Indicators of faecal pollution of water: There are some organisms that normally occur in the stools and if isolated from a water sample, this means that the water is contaminated with stools. These organisms are; *E. coli, Enterococcus faecalis* and *CI. perfringens.*

KLEBSIELLA

Klebsiella pneumoniae are normal inhabitants of the intestine and respiratory tract. They are saprophytes in soil and water. Some may cause diseases in man, which are mainly nosocomial due to multi-drug resistant strains. There are 77 serotypes based on capsular polysaccharide, which is the most important virulence factor.

Morphology: Gram negative, non-motile, capsulated bacilli.

Cultural characters: They give pink colonies on MacConkey. Colonies are mucoid due to the production of abundant extracellular slime.

Biochemical activities: They ferment glucose, lactose, maltose, mannite, sucrose and salicin with production of acid and gas. They are indole negative, V.P. positive, M.R. negative and citrate positive.

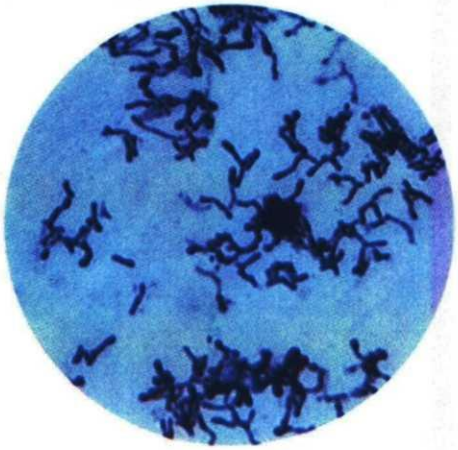
Diseases caused by Klebsiella:

K. pneumoniae causes lobar pneumonia, urinary tract infection, septicaemia and neonatal meningitis. It is a common cause of hospital acquired infections. It is highly pathogenic to mice and causes their death within 24-48 hrs when injected intra-peritoneally. Capsulated organisms can be seen in smears from tissues stained by gram.

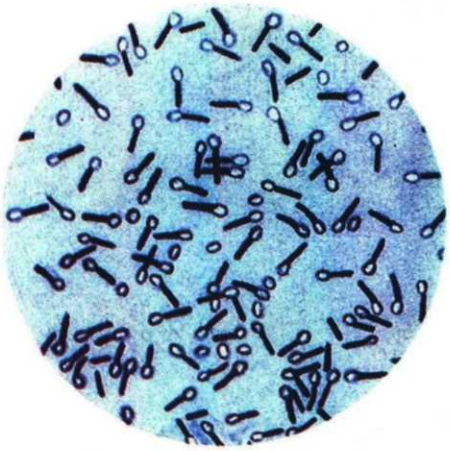
K. rhinoscleromatis causes rhinoscleroma which is a granulomatous lesion in the nose and throat. *K. ozaenae* is assosiated with atrophic rhinitis. *K. oxytoca* causes hospital acquired infections.

Enterobacter. Citrobacter and **Serratia** are found in soil, water and stools. They may cause urinary tract, wound and blood stream infections in hospitalized and immunocompromised patients, especially those under

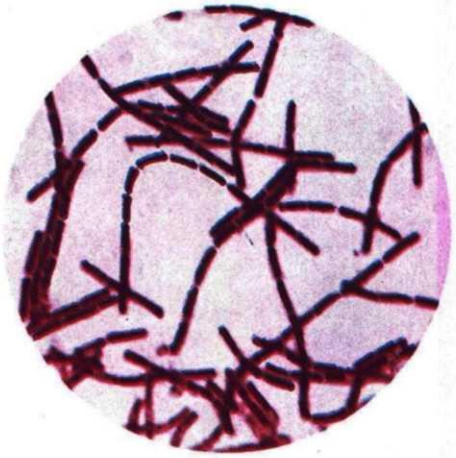
PLATE II



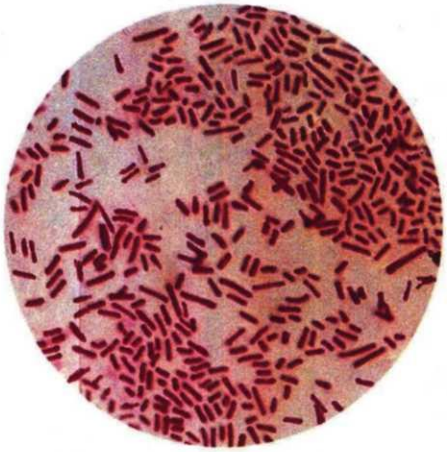
A) ***C. diphtheria*** stained by gram showing Chinese-letter arrangement and club-shaped ends.



B) ***Cl. tetani*** stained by gram showing drum-stick appearance.



C) **Anthrax** bacilli stained by gram.



D) Gram negative bacilli.

invasive procedures such as, respiratory intubations, intravenous and urinary catheters. They are gram negative bacilli, motile and are differentiated by their biochemical reactions.

SALMONELLA

Salmonella are recently classified according to DNA-DNA hybridization studies into 7 groups. Nearly all of the *Salmonella* serotypes that infect humans are in group I which includes more than 1400 serotypes. *Salmonella enterica* is the most important species. The most medically important serotypes are *Salmonella* Typhi, Paratyphi A B and C which cause enteric fever. *Salmonella* Typhimurium and *Salmonella* Enteritidis which cause salmonella food poisoning or enterocolitis. *Salmonella* Choleraesuis causes septicaemia with metastatic abscesses.

Morphology: Gram negative, motile, non-capsulated bacilli.

Cultural characters: Facultative anaerobes. They grow on simple media. On MacConkey and DC A they produce pale non-lactose fermenting colonies.

Biochemical activities: They ferment glucose, maltose and mannite with the production of acid only in case of *S.* Typhi, acid and gas in case of *S.* Paratyphi. Most of them produce **H_2S**. They are non-lactose, non-sucrose fermenters, indole negative and urease negative.

Serological characters: They possess O (somatic) and H (flagellar) antigens. Salmonella is divided into **serogroups** according to the **O** antigen, and into **serotypes** according to the specific **H** antigen. Some may possess a capsular **Vi** or virulence antigen.

Diseases caused by Salmonella:

1- Enteric fever.
2- Enterocolitis, gastroenteritis or food poisoning.
3- Septicaemia with metastatic abscesses.

ENTERIC FEVER (Typhoid and Paratyphoid)

Typhoid fever is endemic in Egypt and is caused by *S.* Typhi. *S.* Paratyphi A, B and very rarely C cause paratyphoid. The source of infection is the stools or urine of cases or carriers (man is the only reservoir). The organism enters by the oral route in contaminated food or drinks e.g. raw vegetables, fruits, raw shell fish and milk products. Infection begins in the small intestine where the organism multiplies in mesenteric lymph nodes then it passes through the lymphatics to the blood stream causing primary bacteraemia that persists for one week (during the incubation period). The organism spreads in phagocytic cells to the liver, gall bladder, spleen, kidney and bone marrow. It multiplies in these organs then passes into the blood causing a second heavier bacteraemia

that coincides with fever and signs of clinical illness. From the gall bladder, it reinvades the intestine where the organism multiplies in Peyer's patches and gut lymphoid tissues. It may rarely reach the periosteum, lungs or meninges. Manifestations appear after an incubation period of two weeks in the form of fever, malaise, headache, delirium, tender abdomen, constipation with enlargement of the spleen. Rose spots may appear on the abdomen.

The complications of typhoid fever which are intestinal haemorrhage and perforation are markedly reduced nowadays due to the use of antibiotics.

Carriers: After recovery, some individuals continue to harbour salmonella in their tissues as convalescent or chronic carriers. They may carry the organism in the gall bladder and intermittently excrete it in the stools. The organism may be carried in the urinary tract. The prevalence of urinary bilharziasis among Egyptians favours the occurrence of urinary carriers. Such individuals should not work as food handlers.

Diagnosis of Enteric Fever:

Diagnosis of enteric fever depends on the stage of the disease and can be accomplished by: Isolation of the organism from blood, urine, stools, bone marrow or serologically by the detection of antibodies in the serum of the patient.

I- During the first week:

Isolation from blood: The organism is found in the blood during the first week. A blood culture is done by adding 5-10 ml of blood to 50-100 ml of bile salt broth which is incubated at 37°C. Subcultures are made on MacConkey or DCA medium. Non-lactose fermenting colonies are further identified by morphology, biochemical activities and serologic typing by slide agglutination with anti-salmonella sera. The blood culture may have to be repeated to increase the chance of isolation of the organism.

It is to be noted that the organism disappears from the blood shortly after antibiotic therapy.

II. In the second week and onward:

1-Isolation from stools: The organism is found in the stools throughout the course of illness but is most readily isolated from stools during the second and third week of illness. Repeated stools examination should be attempted.

A stool sample is inoculated on MacConkey and DCA or salmonella -shigella (SS) agar. Samples are also inoculated into tubes of selenite and tetrathionate broth and incubated at 37°C overnight then, subcultures are made on MacConkey. Any pale non-lactose fermenting colonies are picked and identified morphologically, biochemically and serologically.

Accu-chek *S.* Typhi test for direct detection of *S.* Typhi antigens in stools is a rapid (15 min.) irnmunochromatographic kit. The test is sensitive and specific and is usually positive at the end of the first week.

2- Isolation from urine: The organism appears in urine from the second week onwards. Its appearance may be intermittent; hence isolation from urine needs repeated examination.

The urine sample is centrimged and the deposit is inoculated on MacConkey or DCA. Pale colonies are identified as described above.

3- Serological diagnosis:

I-Widal test: Antibodies to salmonella appear in the serum of the patient during the second week of illness and reach maximum titre during the 4^{th} week. They are detected by the Widal test which is a tube dilution agglutination test done as follows:

Serial dilutions of the patient's serum (1/20, 1/40, 1/80, 1/160... etc) are made in sets of test tubes; to each set, a suspension of O and H antigens of the different salmonella, is added. The tubes are incubated, then examined for presence of agglutination with the different suspensions. The highest dilution of the serum which gives agglutination is the end titre.

Interpretations of the Widal test:

For proper interpretations of the Widal test, two serum samples separated by 10 days interval should be tested. The detection of a **"rising titre"** in the second serum sample indicates active enteric fever. However, if only one serum sample is available, the following facts should be taken into consideration:

a- High titre of O and H (> 1/160) or rising titre indicates recent active infection. Note that O antibodies disappear faster than H antibodies.

b- High titres of H only (> 1/160) suggest past vaccination or past infection.

c- If the test is done during the first week, it gives false negative results as the antibodies start to appear during the second week and onward.

d- In Egypt, titres below 1/80 are of no significance due to endemicity of the disease in the area and previous subclinical infection.

e- Early antibiotic treatment lowers the antibody titre due to reduction of the antigenic mass.

f- Non-enteric infections by other salmonella or autoimmune diseases may cause a non-specific rise of antibody titre, due to the presence of cross-reacting antibodies.

g- High titres to Vi antigen (if included in the test) occur in some carriers.

II- ELISA and immunoblot tests are more sensitive and specific and have largely replaced the Widal test.

Diagnosis of carriers is done by isolation of the organism from stools after a cholagogue to empty the gall bladder (where the organism is present). This is followed by a saline purge.

-Urinary carriers are diagnosed as mentioned in a case.

-Repeated examination is necessary in both types of carriers.

-Carriers of ***S.*** Typhi have "Vi" agglutinins in their sera.

Prevention:

1- Sanitary measures must be taken to prevent contamination of food and water by the organism

2- Carriers must not be allowed to work as food handlers.

3- Vaccines: The following types of vaccines are available and are recommended for those who are travelling to high risk areas:

a-Oral living attenuated typhoid vaccine (Vivotif) containing avirulent mutant of ***S.*** Typhi, is given in four oral capsules taken one every other day. The last dose should be given at least one week before travel. It should not be given to children less than 6 years.

b- Inactivated single-dose vaccine containing Vi capsular polysaccharide of ***S.*** Typhi (ViCPS) given by intramuscular injection of 0.5 ml. It should be given 2 weeks before travel. The vaccine has fewer side effects than the old 2 dose vaccine. It should not be given to children less than 2 years.

c- The old inactivated whole cell vaccines containing, S. Typhi, ***S.*** Paratyphi A and B (TAB) given in two doses of 0.5 and 1 ml subcutaneously separated by one month interval may be still used in developing countries.

Treatment: Multiple drug resistance is a problem transmitted genetically by plasmids among enteric organisms. Antibiotic sensitivity of isolates is required. The drugs now used are ceftriaxone and ciprofloxacin. Ampicillin and ciprofloxacin are recommended for chronic carriers of salmonella.

Salmonella Enterocolitis
"Food poisoning"

It is the most common salmonella infection. It affects all ages. Many **non**-typhoidal salmonella are responsible for this condition. The most important are ***S.*** Typhimurium and **5. Enteritidis.** These organisms are common pathogens of animals and poultry. The organism is transmitted by eating improperly cooked meat of infected animals, eggs of infected birds or from food contaminated with rat excreta.

The organism invades and replicates in the epithelial cells of the small and large intestine leading to intestinal lesions and diarrhoea. The incubation period is 12-48 hours. The disease is characterized by nausea, vomiting, abdominal discomfort, severe diarrhoea and slight fever. The organism can be isolated from the stools. Blood cultures are negative.

Recovery follows within one week. It is self-limited and does not require medical treatment. Fluid and electrolyte replacement may be needed. Antibiotics are indicated only for neonates or persons with chronic diseases who are at risk of septicaemia and disseminated abscesses.

Septicaemia with Metastatic Abscesses

It is mainly caused by ***S.*** Choleraesuis. It occurs in patients with underlying chronic disease such as sickle cell anaemia or cancer or a child with enterocolitis. Following oral infection, bacteraemia occurs and results in the seeding of many organs, with osteomyelitis, pneumonia and meningitis as the most common sequelae. Metastatic abscesses frequently occur on top of damaged tissues e.g. infarcts and aortic aneurysms. Blood cultures are positive. Treatment as in typhoid fever.

SHIGELLA

Members of the genus *Shigella* cause **bacillary dysentery** in man. There is no animal reservoir. *Sh. dysenteriae* causes the severest form.

Morphology: Gram negative bacilli, non-motile and non-capsulated.

Cultural characters: They produce pale non-lactose fermenting colonies on MacConkey's medium and DCA.

Serological characters: They are divided into four groups according to O antigen: Group A: *Sh. dysenteriae* includes 13 serotypes. Group B: *Shflexneri* includes 8 serotypes. Group C: *Sh. boydii* includes 18 serotypes. Group D: *Sh. sonnei* includes one serotype.

Biochemical **activities:** *Sh. dysenteriae* ferments glucose only with production of acid only. *Sh. flexneri, Sh. boydii* and *Sh. sonnei* ferment glucose and mannite with acid production. *Sh. sonnei* is a late lactose fermenter. They do not produce **H_2S** and are urease negative.

Virulence **factors:**

1- Invasiveness of the mucosa of the distal ileum and colon is the critical factor in pathogenesis. The organism does not invade the blood stream.

2- Shiga **toxin** produced by *Sh. dysenteriae* type 1 is a heat labile exotoxin that affects both the gut and the CNS. It acts as an enterotoxin causing the diarrhoea similar to that caused by *E. coli* verotoxin. It acts as a neurotoxin causing meningism and coma in severe fatal infections with *Sh. dysenteriae.*

Pathogenesis **and** clinical picture:

Shigellosis is only a human disease. The organism is transmitted by the faecal-oral route. The four Fs -fingers, flies, food, and faeces- are the main factors in transmission. Food and water borne outbreaks occur in nurseries and mental hospitals where faecal-oral transmission is likely to occur.

The essential pathologic process is **invasion** of the mucosa and wall of the large intestine and terminal ileum leading to necrosis, superficial ulcers, pseudomembrane formation and bleeding. Infection is limited to the gastrointestinal tract without blood invasion.

The toxic activity is distinct from the invasive property of shigella in dysentery. The two may act in sequence, the toxin producing early non-bloody, voluminous diarrhoea and the invasion of the large intestine, resulting in late dysentery with blood and pus in stools.

The incubation period is 1-4 days. There is abdominal pain, diarrhoea, tenesmus and fever. Stools contain blood, mucous and pus. *Sh. dysenteriae* causes the severest local and systemic manifestations. *Sh. sonnei* causes mild disease, which is most common. Recovery occurs spontaneously in most cases. However, in children and elderly, dehydration, acidosis and even death may occur. Very few remain as chronic carriers.

Diagnosis:

-Macroscopic examination of stools to detect mucus and blood.

-Microscopic examination reveals pus and RBCs.

-Stools are inoculated on MacConkey and DCA and on selenite broth. Subcultures from broth are made on MacConkey and DCA after 24 hours. Non-lactose fermenting (pale) colonies are identified by; morphology, biochemical reactions and by agglutination with specific antisera.

Prevention: No specific prophylaxis is available.

-Prevention of shigellosis is dependent on interruption of faecal-oral transmission by proper sewage disposal, chlorinat ion of water and personal hygiene (hand washing by food handlers).

Treatment: The main treatment is restoration of the fluid and electrolyte balance. Fluoroquinolone (ciprofloxacin) is the drug of choice in severe cases. Trimethoprim-sulfamethoxazole is also used. Susceptibility testing should be done, as drug resistance is very high among shigella strains.

YERSINIA

The genus ***Yersinia*** includes three species of medical importance to man. ***Yersinia pestis*** which causes plague in man. ***Yersinia enterocolitica*** and ***Yersinia pseudotuberculosis,*** both are important causes of human diarrhoeal diseases.

Yersinia pestis

Plague is a zoonotic disease primarily affecting rats and rodents, which act as reservoirs for *Y. pestis.* The organism is transmitted to man by the rat-flea *Xenopsylla cheopis.*

Morphology: Gram negative short ovoid, non-motile bacilli. In tissue, they form a capsule-like outer envelope. When stained by Wayson stain, Geimsa or methylene blue, they show marked bipolar staining "safety pin appearance".

Cultural characters: Facultative anaerobes. They grow on blood agar and MacConkey producing non-lactose fermenting colonies at 27- 30°C.

The organism is highly pathogenic to laboratory animals. When injected subcutaneously into white rats, they die within few days. Films made from spleen, liver or blood show numerous organisms.

Virulence factors:

1-A lipopolysaccharide **endotoxin** that causes endotoxic shock.

2-F1 antigen is antiphagocytic and induces protective antibodies.

3-V-W antigens are present in virulent strains and are encoded by genes on plasmids. They are antiphagocytic.

4-Yops (Yersinia outer proteins) have a variety of activities, including inhibiting phagocytosis, inhibiting platelet aggregation and preventing an effective inflammatory response.

5- Plasminogen activator protease degrades fibrin and extracellular proteins and facilitates systemic spread from the inoculation site.

6- An **exotoxin** lethal to mice.

PLAGUE

Plague is a disease of wild rodents. It is transmitted from rat to rat and from rat to man by the bite of the infected flea. The inoculated organism multiplies in the draining lymph nodes, which become enlarged and tender, so the condition is called **bubonic plague.** While the invasion may stop there, the organism often reaches the blood stream and disseminates to form abscesses in many organs and may cause **pneumonic plague** or **meningitis.** Primary pneumonic plague may result from inhalation of infected droplets from a patient. Haemorrhagic consolidation, sepsis and death may occur.

Dissemination is associated with endotoxic shock, DIC and cutaneous haemorrhages i.e. septicaemic plague.

The organism may **be** used in bioterrorism and is delivered by; aerosol to cause pneumonic plague, or using infected fleas to cause bubonic plague.

Diagnosis:

A- Direct detection and isolation of the organism from; aspirate of lymph nodes, sputum or blood.

1- Direct detection of the organism in specimens after staining by Wayson stain, Giemsa or methylene blue to demonstrate bipolarity.

2- Direct detection of the capsular Fl antigen in specimens by specific fluorescent staining or detection of DNA by PCR offers a rapid and less hazardous means of diagnosis than cultures or animal inoculation.

3- Isolation by culture on blood agar at 27- 30°C. Colonies are identified by morphology and biochemical activities. Definite identification of culture by immunofluorescent stains or by inoculation into white rats or by PCR.

B-Serologic diagnosis by passive haemagglutination or ELISA. A rising antibody titre is diagnostic. It is useful mainly in convalescent stages.

Prophylaxis: The main preventive measures include:

- Patients with suspected plague should be isolated, particularly if pulmonary involvement has not been ruled out. Contacts of pneumonic plague cases should receive tetracyclines, as chemoprophylaxis.
- Anti-rat anti-flea measures.
- **Vaccination:** Numerous vaccines are currently under development.

Treatment: Streptomycin is the drug of choice. Gentamicin, doxycycline and the fluoroquinolones are alternative. They may be used in combination.

Y. enterocolitica and ***Y. pseudotuberculosis*** cause enterocolitis similar to that caused by salmonella and shigella, mesenteric adenitis clinically similar to appendicitis and rarely bacteraemia and abscesses in liver or spleen in persons with underlying disease. Immunologic sequelae e.g. reactive arthritis and erythema nodosum may occur. They are **transmitted** to humans by contamination of food with the excreta of domestic animals e.g. dogs, cats, cattle or pork

Isolation from stools can be improved by 'cold enrichment' by inoculation of buffered saline incubated at 4°C for 2 weeks (the organism multiplies in the cold). Subcultures are made at intervals on MacConkey incubated at 25°C. Most infections are self-limited. In case of bacteraemia or abscess, ciprofloxacin or trimethoprim-sulfamethoxazole is usually effective.

PROTEUS, PROVIDENCIA and MORGANELLA

Proteus species are found in soil and water, and are normal inhabitants of the intestine of man. They cause infections only when they leave the intestine. The genus includes two important species; *P. vulgaris* and *P. mirabilis.*

They cause urinary tract infections (UTI) -most commonly by *P. mirabilis*-wound infections, otitis media, pneumonia, meningitis and bacteraemia. Infections may be hospital or community acquired. Proteus infections are resistant to antibiotics and antibiotic sensitivity tests should be done before giving treatment.

Morphology: Gram negative bacilli, very pleomorphic and highly motile.

Cultural characters: Facultative anaerobes. Due to their high motility, they give colonies which **swarm** in successive waves over the surface of nutrient agar. On MacConkey, they produce pale **non-lactose fermenting** colonies.

Biochemical activities: They are phenylalanine deaminase positive and urease positive; which differentiate them from salmonella and shigella. They produce **H_2S** which blackens the butt of triple sugar iron (TSI) agar.

Diagnosis by colony morphology and biochemical reactions.

Treatment according to the results of antibiotic sensitivity tests. However, most strains are sensitive to aminoglycosides and trimethoprim-sulfamethoxazole. Cephalosporins are used for resistant strains.

Morganella morganii and ***Providencia rettgeri*** were found to be different from proteus by molecular DNA studies and were placed in separate genera.

They are similar to proteus in several characters. They are gram negative, non-lactose fermenters, phenylalanine deaminase and urease positive. However, they are differentiated by other biochemical properties.

They are found in soil and water, and are normal inhabitants of the intestine of man. They cause UTI and occasionally other infections. They are emerging as important agents of nosocomial infections. They are resistant to antibiotics.

ACINETOBACTER

Acinetobacter species are oxidase negative, non-fermentative, non-motile gram negative cocco-bacilli found in soil and water, but they can be part of the normal microbiota. They are opportunistic pathogens that readily colonize compromised patients. *A. baumannii* cause infections in hospitalized patients with respiratory therapy equipment and indwelling catheters including; pneumonia, urinary tract infections, meningitis, skin and wound infections that may progress to septicaemia. Antibiotic resistant strains prevail in hospitals and cause outbreaks in ICUs and CCUs. Colistin may be the only active agent.

CHAPTER 9

PSEUDOMONAS

P. aeruginosa is the commonest human pathogen of the Pseudomonas group. It is found in soil, sewage and water, some are commensals in the intestine. It causes urinary tract infection, wound infection, otitis externa and corneal ulcers in contact lens users, pneumonia and sepsis with ecthyma gangrenosum in the skin. It causes osteomylitis and endocarditis in intravenous drug users. Infections are severe and occur in hospitalized and compromised hosts e.g. neutropenic, burned and cystic fibrosis patients.

Morphology: Gram negative motile bacilli.

Cultural characters: Aerobe; grows on nutrient agar leading to greenish colouration of the medium due to its diffusible exopigment which consists of pyocyanin (blue) and pyoverdin (yellow-green fluorescent). Cultures have a sweet grape-like odour. Some strains haemolyze blood.

Biochemical activities: *P. aeruginosa* is oxidase positive and does not ferment any sugar. Acid is produced from glucose by oxidation only.

Pathogenesis: They are invasive and toxigenic due to several virulence factors: **Pili, endotoxin** and **enzymes** (elastase and protease), that facilitate invasion. **Exotoxin** A, very similar to diphtheria toxin in its action and causes tissue necrosis. Strains isolated from cystic fibrosis possess an **exopolysaccharide (glycocalyx)** which mediate adherence of the organism to mucous membranes and allows it to live in a biofilm away from antibodies and phagocytosis.

***P. aeruginosa* causes 10-20% of hospital acquired infections due to:-**

1- Their ability to grow in aqueous solutions that favours their persistence in hospital environment contaminating respiratory and anaesthesia equipment and I.V. fluids.
2- Their remarkable ability to withstand disinfectants. They were found growing in hexachlorophene-containing soap solutions, antiseptics and detergents.
3- The presence of the compromised patients in hospitals e.g. those with extensive burns, chronic respiratory diseases, cystic fibrosis and UTI in those with indwelling catheters.

Diagnosis of infections:

1- The pus from the lesions may be greenish blue.
2- Smears stained by gram show gram negative bacilli among pus cells.
3- Cultures on nutrient agar show the characteristic colonies with greenish colouration of the medium, sweet grape-like odour and are oxidase positive
4- Bacteriophage and pyocin typing are used for epidemiologic purposes.

Treatment: The organism is resistante to many antibiotics. Antibiotic sensitivity tests should be done to find the proper antibiotic. Penicillins -ticarcillin or piperacillin- plus an aminoglycoside e.g. gentamycin are commonly used, as well as, imepinem, ciprofloxacin and ceftazidime.

CHAPTER 10

VIBRIOS, CAMPYLOBACTER & HELICOBACTER

VIBRIOS

These are comma-shaped gram negative bacilli. Some are saprophytes in water and soil, other members cause disease in man or animal. The most clinically important species is *V. cholerae.*

Vibrio cholerae

V. cholerae serogroups 01 and 0139 cause classic epidemic cholera in humans, occasionally non-Ol/non-0139 *V. cholerae* cause sporadic choleralike disease and some of them are invasive and may cause septic infections.

Morphology: Gram negative curved or comma-shaped rods, **motile** with single terminal fiagellum **(darting motility)**

Cultural characters: Highly aerobic and grow on simple media. Growth is favoured by alkaline pH (8-9). On alkaline peptone, they grow forming a surface pellicle within 8 hrs. On thiosulfate-citrate-bile-sucrose (TCBS) agar, they produce yellow colonies.

They are string test positive: When a colony is emulsified in a drop of 0.5% Na deoxycholate in distilled H_20, within one minute, the cells lyse and DNA strings when a loopful is lifted from the slide. The test differentiates Vibrios from Aeromonas which is string test negative (see p.96).

Biochemical activities: They ferment glucose, maltose, mannite and sucrose with production of acid only. They are indole positive, oxidase positive and reduce nitrate. On triple sugar iron agar (TSI) it gives yellow slant and yellow butt and no gas.

Serological characters: The H flagellar antigen of *V. cholerae* is shared by all vibrios. *V. cholerae* are serogrouped according to O antigen into 206, O serogroups. The 01 serogroup includes 2 biotypes; the **classic** *V. cholerae* and **El Tor** and three serotypes; Inaba, Ogawa and Hikojima. The 2 biotypes could be differentiated by biochemical activities; El Tor strains are VP positive, lyse sheep RBC's, agglutinate chicken RBC's and are resistant to; polymyxin B and to lysis by cholera phage IV, while the classic biotypes have opposite reactions. *V. cholerae* **0139** is similar to El Tor, it differs in being capsulated. (Table p. 185)

Virulence factors:

***V. cholerae* enterotoxin** is a heat labile enterotoxin consisting of subunits A and B. The B subunit binds to ganglioside receptors on the surface of enterocytes, enabling the A subunit to enter the cell. Subunit A activates the enzyme adenylate cyclase which increases the level of intracellular cAMP

resulting in hypersecretion of water and electrolytes in the lumen of the intestine and severe diarrhoea occurs -^0 liters/day - leading to dehydration, which, if not promptly treated, leads to acidosis, shock and death.

Mucinase enzyme dissolves the protective glycoprotein coating over the intestinal cells helping adherence to the cells of the brush border of the gut.

CHOLERA

The disease is endemic in the Indian subcontinent and used to occur in worldwide epidemics. Cholera is an acute infectious disease characterized by severe vomiting and watery diarrhoea **(rice water stools)** resulting in dehydration and collapse.The incubation period is 1-4 days.

Pathogenesis: It is transmitted by faecal contamination of water and food, primarily from human cases or from carriers during the incubation period or convalescence. Marine shell-fish e.g. shrimp and oysters are the main animal reservoirs. Their ingestion without adequate cooking can transmit the disease. Infection is restricted to the intestine with no blood invasion. For infection to occur, a large number of bacteria must be ingested because the organism is sensitive to gastric acidity. The organisms attach to the microvilli of the brush border of epithelial cells in the intestine, where they multiply and liberate cholera enterotoxin, which exerts the above-mentioned effects. Convalescent carriers occur but chronic carriers are rare.

Diagnosis: There are two approaches for laboratory diagnosis;

A- First case in a non-endemic area:

Any comma-shaped motile organisms detected in a stool sample from the first case suspected to be cholera in a non-endemic area should be thoroughly identified before giving a report as positive for cholera.

1- Mucous flecks from stools are inoculated on alkaline peptone pH 8.5.

2- Subcultures are made from the surface pellicle after 6-8 hrs on TCBS or alkaline agar. The growing colonies are identified by:

- A wet mount which is examined for the characteristic darting motility.
- Smears stained with gram show gram negative comma-shaped bacilli.
- Biochemical activities; oxidase, sugar fermentation, indole, nitrate, string test, TSI inoculatioa
- Agglutination with specific anti-Ol and anti-0139 cholera sera.

B- Secondary case during an epidemic:

In an established epidemic, cases can be diagnosed by microscopic examination of stools for comma-shaped bacilli with characteristic motility, which can be immobilized by adding specific anti-0 cholera sera.

Direct methods for detection of *V. cholerae* 01 and 0139 in stools are available including fluorescent antibody staining, coagglutination and immunoassay. PCR assays have been developed for detection of cholera toxin genes, which are found on the chromosome.

Treatment: The most important part in therapy is to correct the fluid and electrolyte imbalance by giving intravenous fluids. Antibiotics have a secondary role in treatment. Tetracyclines are the most effective; however tetracycline resistance of *V. cholerae* has emerged in some endemic areas.

Prophylaxis: The main control measures are:
- Public health measures that ensure clean water and food supply and proper sewage disposal to prevent faeco-oral transmission.
- Chemoprophylaxis by using tetracyclines for exposed persons.

-Vaccines confer limited -50 %- protection and only for 6 months.
- An extract of killed bacteria given in 2 intramuscular doses with one week interval is available. -A recombinant live attenuated oral vaccine is available. -Oral vaccine that combines purified B subunit and killed whole cells is used in some countries and appears to be safe and protective.

V. parahaemolyticus is a halophilic (salt-loving) marine organism that causes gastroenteritis following ingestion of contaminated seafood e.g. raw fish or shellfish. Virulent strains produce a haemolysin and are urease positive. The disease is characterized by mild to severe watery diarrhoea, nausea, vomiting, abdominal cramps and fever. Incubation period is 6-30 hrs. The disease is self-limited; needs only correction of water and electrolyte imbalance.

CAMPYLOBACTER

Campylobacter have emerged as common human pathogens. ***C. jejuni*** causes 95% **of Campylobacter enterocolitis** especially in children. Other **Campylobacter** species e.g. *C. fetus* may cause systemic infections, bacteraemia, cholecystitis and meningitis in imunocompromised patients.

C. jejuni infections may be complicated two weeks later by **Guiltian-Barre** syndrome, which is an autoimmune disease, attributed to the formation **of** antibodies against ***C. jejuni*** that cross-react with antigens on neurons. Other autoimmune diseases may occur, e.g. reactive arthritis.

Morphology: Small gram negative rods with comma, S, or "gull-wing" shapes. **Motile** with a single flagellum at one or both poles. Motility is darting with cork screw-like movement.

Cultural characters: They are microaerophilic; grow best in presence of 5% oxygen and 10% CO_2. ***C. jejuni*** grows best at 42°C. Skirrow's medium, containing vancomycin, polymyxin and trimethoprim, is a selective medium used for their isolation from stools. C. *jejuni* is oxidase and catalase positive.

Filtration **of** emulsified stools may be done using 0.45 um pore size filters that allow the small **Campylobacter** to pass and exclude other organisms present in stools. The filtrate is inoculated on non-selective media. This method is required for isolation of **Campylobacter** other than C. *jejuni* that are sensitive to the antibiotics in the selective media.

Pathogenesis and clinical picture of enteritis: Infection is acquired by the oral route. Untreated water and food, e.g. raw milk, meat, poultry, contaminated with domestic animal faeces, is the major source of human infection. Contact with infected animals e.g. puppies with diarrhoea are a common source for children. Human to human transmission occurs less frequently. Enteritis begins as watery foul-smelling diarrhoea followed by bloody stools accompanied by fever and severe abdominal pain. Localized tissue invasion and the toxic activity (enterotoxin and cytotoxin) appear to be responsible for the enteritis. Bacteraemia occurs occasionally mainly in neonates or debilitated adults. The illness is usually self limited in 7-10 days.

Laboratory diagnosis: Stools are examined.

1- Smears stained by gram or wet preparations to show the morphology and motility.
2- Stools are inoculated on Skirrow's medium, and incubated at 42°C at microaerophilic atmosphere. Colonies that arise are identified by their morphology, biochemical reactions and sensitivity to antibiotics.
3- Serologic detection of antibodies by ELISA is useful in patients presenting with arthritis or Guillain-Barre syndrome.

Treatment is mainly fluid and electrolyte replacement. C. *jejuni* is sensitive to erythromycin and ciprofloxacin.

Helicobacter pylori

Helicobacter pylori cause chronic gastritis, peptic and duodenal ulcers. Infection with *H. pylori* is a risk factor for gastric carcinoma and is linked to mucosa-associated lymphoid tissue (MALT) lymphoma and iron deficiency anaemia. It is categorized as group I carcinogen by the WHO.

H. pylori is similar to Campylobacters in morphology but different in having multiple flagellae at one pole while **Campylobacter** has a single flagellum at one or both poles. *H. pylori* is highly urease positive while **Campylobacter** is urease negative. *H. pylori* is oxidase and catalase positive.

Pathogenesis and virulence factors:

Man appears to be the only reservoir and source of *H. pylori.* Transmission is assumed to be oral-oral within families, or possibly, by the faecal-oral route. By virtue of its rapid motility (by flagellae) *H. Pylori* penetrate the mucus layer lining the epithelium and attach to the mucous-secreting cells deep in the gastric mucosa, near the epithelial surface (by adhesins), away from the acidity of the stomach. The production of ammonia from urea by the organism's urease, coupled with an inflammatory response, damage the mucosa. Loss of the protective mucous coating predisposes to gastritis and peptic ulcer. The ammonia produced neutralizes gastric acidity, allowing the organism to survive causing persistent colonization which may lead to chronic gastritis, gastric atrophy, peptic ulcers and gastric carcinoma.

Virulence factors that help in damaging the mucosa, produced by some strains, are the vacuolating cytotoxin (VacA) and a cytotoxin-associated protein (CagA) coded by a gene within the Cag pathogenicity island (a cluster of genes coding cytotoxins and associated proteins).These strains cause severe forms of gastritis, peptic ulcers and malignancy.

Diagnosis:

Gastric biopsy specimens obtained by endoscopy are minced in saline and cultured as in Campylobacter but incubated at 37°C in a humid atmosphere for 7 days. Smears stained with gram and histologic sections stained with special stains, show the curved or spiral organisms.

-Rapid urease test; in which gastric biopsy material is placed onto a medium containing urea with a colour indicator. If *H. pylori* is present, the urease splits the urea and results in shift of pH leading to colour change.

Non-invasive methods for diagnosis include:

a-"Urea breath" test: A capsule of ^{14}C-labelled urea is ingested by the patient. If the organism is present, the urease activity generates radiolabelled CO_2 that can be detected in patient's exhaled breath.

b- Direct detection of ***H. pylori*** antigen by ELISA in stools is a useful test for diagnosis and for follow up of the result of treatment, c- PCR applied on gastric juice, faeces or biopsy specimens.

d- Serologic detection of *H. pylori* antibodies by ELISA; high titres are found in chronically infected patients.

Treatment: Triple therapy, one week course that includes clarithromycin + amoxicillin or metronidazole + the acid-lowering proton pump inhibitors (PPI) omeprazole (prilosec) or lansoprazole (prevacid), results in eradication of *H. pylori* in 90% of patients and decreases recurrence rate of peptic ulcers.

CHAPTER11

BRUCELLA

Members of this genus are pathogenic to animals from which they are transmitted to man causing brucellosis (undulant or Malta fever). DNA relatedness studies have shown that there is only one species in the genus, *Br. melitensis*, with multiple biovars and include:

-*Br. melitensis* causing infection in goats and sheep.
-*Br. abortus* causing abortion of cattle.
-*Br. suis* causing infection in pigs.
-*Br. canis* causes infection in dogs.

Morphology: Gram negative short cocco-bacilli, non-motile, non-sporing and non-capsulated.

Cultural and biochemical characters: Aerobes, optimum temperature 35-37°C. They require enriched media for growth e.g. brucella agar, brain heart infusion, trypticase soy agar, or chocolate agar. 10% CO_2 is required for primary growth of *Br. abortus* but not for the others.

All are oxidase, catalase and urease positive. *Br. abortus* and *Br. suis* produce H_2S. They differ in their sensitivity to dyes.

Virulence and immunity: A lipopolysaccharide (LPS) is the major virulence factor as well as the major cell wall antigen. The LPS has endotoxic activity and elicits an antibody response (IgG, IgM, IgA) which may be protective. Brucella can survive and multiply within host cell phagocytes i.e. **facultative intracellular parasite,** so immunity is mainly cell mediated.

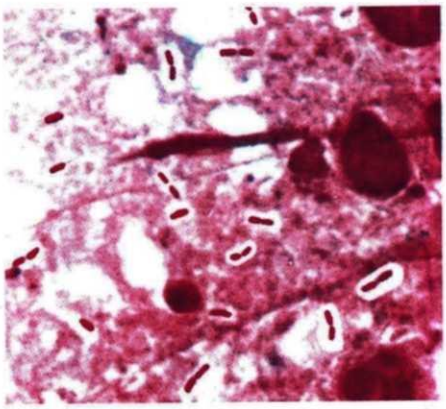
BRUCELLOSIS or UNDULANT FEVER

The organism is found in the uterine discharges and milk of infected animals. Man is infected by consumption of infected cow or goat milk or milk products. Farmers, butchers and hide porters are infected by coming in contact with sick or dead animals or their discharges. The organism can enter through abrasions in the skin, mucosa of the GIT, or by inhalation.

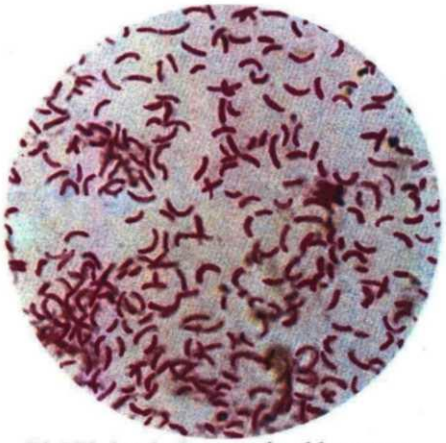
The incubation period is 1-6 weeks. The disease is characterized by an acute bacteraemic phase followed by a chronic stage that may extend over many years and may involve many tissues. The organism localizes in the reticulo-endothelial system; lymph nodes, liver, spleen and bone marrow. It can survive intracellularly in phagocytic cells. Cell mediated response of the host results in granuloma and abscess formation in different organs.

Systemic manifestations include fever, which may be intermittent, bouts of fever that remain for 3-4 weeks alternating with afebrile period of a similar

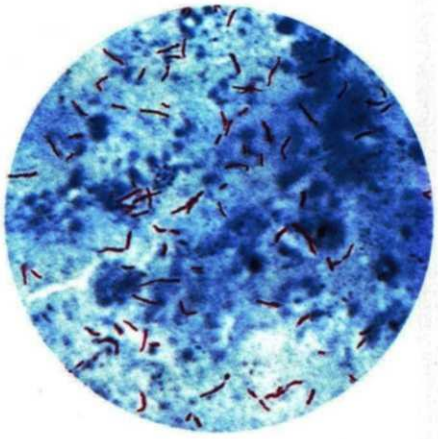
PLATE III



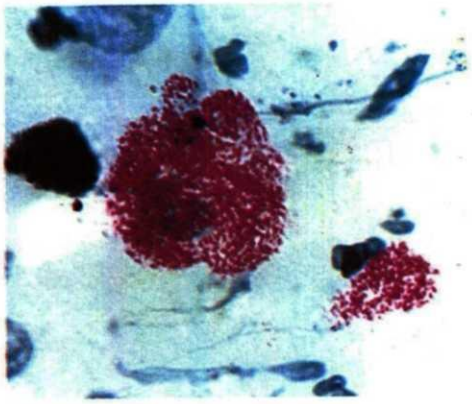
A) ***Klebsiella pneumoniae*** in animal tissues stained by gram, seen as gram negative diplobacilli surrounded by a capsule.



B) ***Vibrio cholerae*** stained by gram showing gram negative curved bacilli.



C) Sputum stained by Z.N. showing pink rods of **tubercle bacilli.**



D) **Lepra bacilli** in skin nodules stained by Z.N. appear as pink rods in bundles intracellularly.

duration. The disease runs a prolonged course accompanied with weakness, malaise, profuse sweating, headache, joint and muscle pain. Enlarged lymph nodes, liver and spleen are frequently found. It may be complicated by osteomyelitis, meningitis or cholecystitis. *Br. melitensis* infection is more acute and severe. *Br. suis* infection is more chronic.

Diagnosis: Specimens include blood, bone marrow and lymph node biopsy for culture and serum for serologic tests.

I- Blood culture: Isolation of the organism from the blood by repeated blood cultures on brain heart infusion broth, incubated at 35-37°C in 10% **CO_2.** Subcultures are done on any of the media mentioned above. Blood cultures should not be discarded as negative before 4 weeks incubation. Small, convex translucent, colonies are identified biochemically and serologically by specific antisera or by PCR to differentiate the various biovars.

II- Serologic diagnosis: Detection of antibodies in the serum of the patients;

a- Standard tube agglutination test (STAT) is done using a wide range of dilutions of the patient's serum (1/20-1/5120) to avoid the prozone phenomenon known to occur frequently with sera of brucellosis patients. In the absence of an acute-phase serum sample, a titre of 1/160 or more in convalescent serum sample is diagnostic. However, interpretations should be done in light of endemicity of the disease and the occupation of the patient.

c- Coomb's antiglobulin method may be used to detect the non-agglutinating IgA **"blocking antibodies"** that appear during the subacute stage of infection. These antibodies interfere with agglutination by IgG or IgM in the STAT causing the prozone phenomenon.

b- A rapid slide agglutination test with a buffered stained antigen is widely used for screening farm animals. The test gives good results in human brucellosis and is not affected by prozones or immunoglobulin switching.

d- ELISA for detection of IgG, IgM or IgA has replaced Coomb's test.

III- PCR is used for direct detection of organism in clinical material.

IV- Brucellin test; is a delayed type hypersensitivity test similar to tuberculin. It is rarely used.

Treatment: Due to chronicity and intracellular survival of the organism. Combined prolonged therapy 3-6 weeks with tetracyclines (doxycycline) and either streptomycin or rifampicin or gentamycin is recommended.

Prophylaxis: Live attenuated vaccine is used for cattle. No vaccine is available for humans. The most important measure is control of milk supply by pasteurization which kills the organism.

CHAPTER 12 HAEMOPHILUS and BORDETELLA HAEMOPHILUS

Members of this genus require certain growth factors present in blood (haemophilic) for their growth.

I- ***Haemophilus influenzae:***

Non-capsulated strains are often found in normal throats. Capsulated strains cause meningitis, epiglottitis and otitis media in children.

Morphology: Gram negative pleomorphic small cocco-bacilli. Long filamentous forms occur. Many strains are capsulated.

Cultural characters: Facultative anaerobes require heated blood containing media for their growth. They grow on chocolate agar which provides X **factor** (haematin) and V **factor** (NAD) that are essential for their growth. Around *Staph, aureus* colonies, the colonies of *H. influenzae* are much larger and denser, since staphylococci produce V factor. This is called **satellitism.**

Serological **characters:** Smooth capsulated strains can be classified into 6 types (a-f), depending on the capsular polysaccharide. *H. influenzae* type b **(Hib)** is the most pathogenic. The capsule is a major virulence factor.

Pathogenesis, virulence **factors and diseases:** The organism enters the upper respiratory tract by inhalation or droplets. The polysaccharide capsule is the major virulence factor that facilitates **invasion.** Other factors include fimbriae and IgA protease that degrades secretory IgA and facilitates attachment to respiratory mucosa, resulting in colonization. Outer membrane proteins and lipopolysaccharide contribute to invasion.

Capsulated strains mainly **Hib** cause **invasive** diseases in children below 5 years including meningitis, epiglottitis, septic arthritis, pneumonia, cellulitis and bacteraemia. **Non**-capsulated strains cause **non-invasive** diseases; otitis media and upper respiratory infections, initiated by viral infections. They may cause pneumonia and bacteraemia in adults in presence of predisposing factors e.g. viral infections, obstructive lung disease, malignancy or old age.

Diagnosis: Specimens are pus, sputum or CSF.

1- Smears are stained with gram When present in large numbers in specimens, the organism can be directly detected by Quellung reaction, immunofluorescence or by PCR.

2- Direct detection of *H. influenzae* polysaccharide in CSF by latex agglutination commercial kits.

3- Culture on chocolate agar at 37°C in 5% CO_2. Colonies are identified by; their morphology, inability to grow in absence of X-V growth factors, serologically with specific antisera or by DNA probes.

Treatment: Cefotaxime given intravenously or ceftriaxone are the drugs of choice for Hib meningitis.

Prevention Haemophilus b conjugated (Hib) vaccines containing the capsular polysaccharide conjugated to a carrier protein is recommended for children at 2, 4, 6 months and at 12-15 months. The vaccine has reduced the incidence of meningitis caused by this organism by 90% in immunized children.The vaccine may be given in combination with DTP i.e.DTPH.

Rifampin is giving prophylactically to nonimmune children in close contact with patients particularly those with meningitis.

U-Haemophilus aegyptius (Koch-weeks bacillus) causes acute purulent conjunctivitis (Pink eye). The condition is diagnosed by films and culture from the conjunctival discharge. *H.aegyptius* is closely related to *H. influenzae* biotype III causing Brazilian purpuric fever in children characterized by fever, purpura, shock and death.

Ill-Haemophilus ducreyi: This organism causes chancroid or soft sore, a sexually transmitted disease, which is an ulcer on the external genitalia. Draining lymph nodes are tender and enlarged.

Diagnosis: Smears made from the lesions or aspirate from lymph nodes, show gram negative cocco-bacilli that may be intracellular. The organism grows with difficulty on chocolate agar. It requires X but not V factor. PCR can be used to detect the organism directly in specimens. Detection of IgG or IgM antibodies by ELISA is very helpful in diagnosis.Treatment by azithromycin, cefatriaxone or ciprofloxacin.

BORDETELLA

Bordetella pertussis

Bordetella pertussis is the most important member of the genus *Bordetella.* It causes whooping cough which is mainly a disease of children. *B. parapertussis* can cause a similar disease.

Morphology: gram negative capsulated cocco-bacilli.

Cultural characters: It grows on complex enriched media e.g. Bordet-Gengou medium (potato-blood-glycerol agar) or Regan Lowe medium (charcoal-horse blood agar). Growth occurs after 3-7 days. Colonies are greyish-white with a shiny convex surface, "mercury drop" appearance.

Virulence factors and pathogenesis: Filamentous haemagglutinin (I HA) and **fimbriae** mediates adherence to the cilia of epithelial cells of the trachea and bronchi, where the organism multiplies and colonizes, interfering with ciliary action. **Pertussis toxin (PTx)** is partly cell bound and functions as

adhesins helping colonization and partly secreted acting as a toxin, which, with the **tracheal cytotoxin** and **endotoxin,** irritate and damage the ciliated cells of the respiratory tract causing the paroxysmal toxaemic stage. **Adenylate cyclase** inhibits bactericidal action of phagocytes, while **pertactin** (surface protein) help adhesion.

WHOOPING COUGH

Infection occurs by inhalation of droplets from early cases. After an incubation period of 2 weeks, the **catarrhal** stage develops with mild coughing and sneezing. During this stage (colonization), the patient is highly infectious. This is followed by severe **paroxysmal** cough (toxaemic stage) characterized by a series of hacking coughs that end with an inspiratory "whoop" that may be followed by vomiting, cyanosis and convulsions. It lasts for 1-4 weeks and is associated with marked leucocytosis and absolute lymphocytosis. The disease may be complicated by pneumonia, otitis media, sub-conjunctival haemorrhages and rarely encephalopathy or seizures.

B. pertussis is a common cause of prolonged cough in adults.

Diagnosis: Samples are nasopharyngeal swabs or saline nasal wash.

1- Immunofluorescence may be used to identify the organism in the samples directly. PCR may be used when available.

2- Isolation of the organism is positive in the catarrhal stage. Samples are inoculated on the above mentioned media and incubated in a humid atmosphere for 7 days. Colonies are identified by immunofluorescence or slide agglutination with specific antisera or PCR

3- Serologic detection of antibodies, which start to appear 3 weeks after onset of symptoms by agglutination or ELISA.

Treatment: Erythromycin is the drug of choice for treatment and prophylaxis.

Prophylaxis:

A heat killed vaccine prepared from capsulated strains of *B. pertussis* is given to children during the first year of life in combination with diphtheria and tetanus toxoid i.e. DTP vaccine. Adults should not receive this pertussis vaccine since it may cause encephalopathy if given after 6 years of age.

Acellular vaccines have at least two of the following antigens: Inactivated pertussis toxin which is the main immunogen in the vaccine, FHA, pertactin and 2 types of fimbriae. These vaccines have fewer side effects than the killed vaccine. The vaccine is given to children with diphtheria and tetanus toxoids (DTaP) at 2, 4, 6 months and boosters at 15-18 months and at 4-6 years. Recently in 2013, it was recommended by the Advisory Committee on Immunization Practices (ACIP) that all adolescents and adults as well as pregnant women should receive a booster dose of DTaP.

CHAPTER 13
MYCOBACTERIA

The genus *Mycobacterium* comprises the "acid-fast bacilli", which are difficult to stain, but once stained, they resist decolourization with acid or with acid and alcohol. The genus comprises over 50 species. Several members produce disease in man and animals while others are saprophytes. The pathogens include organisms responsible for human and bovine tuberculosis and leprosy. It is convenient to divide mycobacteria of clinical interest into:

1- *M. **tuberculosis*** **complex** (MTC) which includes *M. tuberculosis, M. bovis, M. qfricanum* and *M. microti.* These are associated with tuberculosis.

2- **Non-tuberculous mycobacteria** (NTM) may be associated with human diseases.

3- ***M. leprae*** causing leprosy.

Mycobacterium tuberculosis

M. tuberculosis and *M. bovis* are the cause of human tuberculosis.

Morphology:

They are thin straight or slightly curved rods. They cannot be stained by simple stains due to the high lipid (mycolic acid) content of the cell wall. They can be stained by Ziehl-Neelsen stain (Z.N.) and appear as thin pink rods arranged singly or in small groups in a contrasting blue background.They can be stained by fluorochrome stains (e.g. auramine, rhodamine) and appear as yellow orange fluorescent bacilli in a black background by fluorescent microscopy.

Constituents of tubercle bacilli:

1- Its cell wall contains several **complex lipids:**

a) Mycolic acids, which contribute to the organism's acid-fastness, resistance to phagocytosis and intracellular destruction, resistance to acids, alkalis and antibiotics.

b) Phosphatides, which play a role in caseation necrosis.

c) Wax D; the active component in Freund's adjuvant, used to enhance the immune response to many antigens in experimental animals.

d) Cord factor (trehalose-6, 6'-dimycolate) is correlated with virulence.

2- The organism contains several **proteins** which when combined with waxes elicit delayed hypersensitivity. These proteins are the antigens in the **PPD** (purified protein derivative) used in the tuberculin skin test.

3- The organism contains a variety of **polysaccharides.**

Cultural characters:

They are strictly aerobic; however, better growth occurs in presence of 5-10% CO_2. They grow on egg-enriched media such as Lowenstein-Jensen (L-J) medium They are **slow growers,** no growth appears before 2-4 weeks incubation at 35-37°C. The growth on L-J medium is irregular, dry and off-white. Other media used include Middlebrook's 7H10,7H11 agar and 7H9, 7H12 broth. Virulent strains grow in a characteristic serpentine cord-like pattern in fluid media due to cord factor.

Biochemical activities and resistance:

M. tuberculosis produces niacin and reduces nitrates while other mycobacteria do not. They are relatively resistant to acids, alkalis and dryness. They can survive in dried expectorated sputum, which is a factor in their transmission by aerosol. They are killed by sunlight and by pasteurization of milk.

TUBERCULOSIS

The problem of tuberculosis has increased recently due to the emergence of multidrug resistant strains and an immunosuppressed population e.g. AIDS patients. Human tuberculosis is caused by *M. tuberculosis* and *M. bovis.* The disease can affect any organ of the body. Infection with *M. tuberculosis* is airborne while infection with *M. bovis* occurs by ingestion of milk from infected cattle and causes intestinal tuberculosis which is rare.

Pathogenesis:

The organism produces disease exclusively by **invasion** of tissues. It is non-toxigenic. The organism preferentially infects macrophages and other reticuloendothelial cells. It resists the destructive properties of normal macrophages by inhibiting phagolysosome formation. It then multiplies within the cellular vacuole or phagosome. As it multiplies intracellularly, it stimulates cell mediated immunity (CMI) and hypersensitivity which leads to tissue damage. The disease is either primary or due to reinfection or reactivation of latent infection.

Primary tuberculosis

The site of initial infection is usually the lungs following inhalation of bacilli. They are engulfed by alveolar macrophages in which they replicate to form the initial exudative lesion or Ghon's focus. Some bacilli are carried in phagocytic cells to the draining hilar lymph nodes causing lymphadenitis. Ghon's focus together with the enlarged lymph nodes, form the **primary complex.** Activated macrophages form a granuloma around the site of primary infection which usually limits it and it heals in most cases by fibrosis

and may calcify, leaving the person immune and hypersensitive i.e. tuberculin positive.

Lymphatic and haematogenous spread to other parts of the lungs or to other organs may occur and depending on the immune status of the host, **infection** is stopped or it may progress to symptomatizing **disease.** In all cases, living bacilli remain in a **latent** state in any organ.

Latent tuberculosis infection (LTBI) is the condition that develops after exposure to a case of tuberculosis and acquiring TB infection. The person does not develop symptoms of disease but he has living inactive T.B. bacilli in his body and is tuberculin positive and may develop active disease in the future.

Re-infection or Activation of latent TB infection occurs months or years after the primary infection due to reactivation of LTBI -due to lowered host resistance- or exogenous re-infection. The lesions in the lungs are commonly apical (where the O_2 tension is high) and appear as coalescing tubercles with large areas of caseous necrosis and cavitations. Tubercles may erode into a bronchus leading to open tuberculosis with infective sputum The lesions may occur in any other organ e.g. the kidney, brain, bone.. .etc.

The Difference between Latent TB Infection and Active TB Disease

A person with latent TB infection (LTBI)	A person with active TB disease
Has a **positive** TST* or QFTG*.	Has a **positive** TST or QFTG*.
Has a normal chest x-ray and a negative sputum test.	May have an abnormal chest x-ray, or positive sputum smear or culture.
Has TB bacteria in his/her body that are alive, but inactive.	Has active TB bacteria in his /her body.
Does not feel sick.	Feels sick and may have symptoms such as coughing, fever and weight loss.
Cannot spread TB bacteria to others.	May spread TB bacteria to others.
Needs treatment for latent TB infection to prevent TB disease.	Needs treatment for active TB disease.

♦Tuberculin Skin Test or QuantiFERON-TB Gold (explained

Immunity and hypersensitivity:

On primary infection, cell mediated immunity (CMI) develops through the action of antigen specific sensitized T cells which secrete cytokines that activate macrophages to kill tubercle bacilli i.e. **immunity.** This leads to localization of tubercle bacilli, retards their multiplication, limits their spread and reduces lymphatic dissemination. In the course of primary infection, the host also acquires **hypersensitivity** to tubercle bacilli. This is made evident by the development of a positive tuberculin reaction. On re-infection, the severity and outcome of the disease depend on which of the two will take the upper

hand; immunity limits the disease while hypersensitivity will lead to inappropriate destruction of host tissues and progression of the disease.

Diagnosis of tuberculous infections:

Diagnosis depends on detection, identification and isolation of the tubercle bacilli (TB) from pathologic specimens.

Specimen: Tuberculosis can affect virtually every tissue in the body; thus the specimen can be; sputum or morning gastric aspirate, urine, CSF, stools and tissue biopsy. The following steps are made for detection or isolation of TB from **sputum.** Three morning sputum samples collected on three consecutive days should be examined as follows:

1- Direct smears are made from the specimen and stained with Ziehl-Neelsen stain. The detection of acid alcohol-fast bacilli in sputum gives a fairly strong indication of pulmonary tuberculosis. However, they are detected only if they are present in large numbers.

"The smear may be stained by fluorochrome stains auramine, rhooamine and examined by fluorescent microscopy for yellow orange fluorescing bacilli in a black background. This staining method is more sensitive than Z.N. staining.

2' Decontamination and concentration:

Contaminated or tenacious specimens e.g. pus, sputum, stools...etc. are subjected to decontamination and concentration before further processing. Specimens are mixed with an equal volume of N-acetyl-L-eystiene-2% NaOH solution. The mixture is held for 15 min. at room temperature, then diluted with buffer pH 6.8 or distilled water and centrifuged at 3000 rpm for 15 min. Supernatant is decanted and the sediment is processed. The technique leads to;

a- Liquefaction of the specimen, thus releasing the tubercle bacilli, b- Destruction of all bacteria other than tubercle bacilli by NaOH. c- By centrifugation, the tubercle bacilli are concentrated in a **small Volume.**

N.B. Specimens from sterile sites e.g. CSF do not need decontamination.

- Concentrated sediments are processed as follows:

3- Smears are made from the sediment and stained with Z.N. stain.

4* Sediments are cultured on L-J medium (which is the conventional method) and incubated at 35-37°C. Growth appears after 2-3 Weeks. Cultures should not be discarded as negative before 8 weeks. Isolated organisms could be identified by being, niacin positive and nitrate reduction positive and PCR.

5-The sediment may be cultured on fluid media using Middlebrook broth as the base for rapid diagnosis by the following test:

a- The BACTEC radiometric method in which the sediment is inoculated on liquid medium containing radioactive metabolites and incubated at 35-37°C. Growth is detected by production of radioactive carbon dioxide within two weeks.

b- Mycobacteria growth indicator tube (MGIT) in which the sediment is inoculated on liquid medium containing a fluorescence sensor that fluoresces when oxygen is depleted from the medium due to bacterial growth in 7-14 days. Fluorescence is detected by exposure to UV light.

6- Moleculdar techniques for rapid direct detection of *M. tuberculosis* in clinical specimens e.g. 1- The amplified *M. tuberculosis* direct test uses enzymes that rapidly make copies of *M. tuberculosis* 16s ribosomal RNA, which can be detected by genetic probes. 2- PCR for the detection of chromosomal DNA and for detection of drug resistance genes for rifampicin and others. Commercial PCR kits can confirm the diagnosis in 8hrs.

N.B: The conventional culture method on L-J should be done at the same time with any of the above methods since it is essential to isolate the organism on solid media to be identified and its antibiotic sensitivity tested.

The Mantoux tuberculin skin test (TST) is a delayed hypersensitivity skin test that determines whether a person is infected with *M. tuberculosis.The* test is performed by intradermal injection of 0.1 ml of the purified protein derivative (PPD), containing 5 tuberculin units, in the forearm. A positive test is read as an induration that appears after 48-72 hours.

The induration is due to accumulation of antigen specific CD4 T cells that are activated and release cytokines that attract macophages and polymorphs to the site of injection.

Groups that should be screened with TST include; HIV patients, close contacts of patients with active TB, low-income populations, alcoholics and IV drug users, prison inmates and individuals from countries with a high incidence of tuberculosis.

Interpretation of TST

A positive test cannot distinguish between latent infection and active disease. The diameter of the induration required to judge the test as positive varies depending upon the status of the individual being tested:-

Induration > **5** mm is considered positive in;

1- Recent contacts of a case of TB.
2- Patients with fibrosis on chest X-ray from prior TB.
3- Immunosuppressed persons e.g. HIV patients and solid organ transplant recipients.

Induration > 10 mm is considered positive in;

1- Unvaccinated children under 4 years and children exposed to high risk adults.
2- Persons with high risk factors, such as the homeless, IV drug users, nursing home residents, hospital and laboratory personnel.
3- Patients with high risk clinical conditions e.g. diabetes, silicosis, chronic kidney disease and cancer.

Induration > 15 mm is considered positive in any person, including persons with no risk factors for TB or those who received BCG vaccine.

False negative reaction may occur in presence of tuberculous infection when "anergy" develops due to overwhelming tuberculosis, measles, Hodgkin's disease, AIDS, sarcoidosis or immunosuppression.

False positive reaction may occur due to infection with NTM or previous BCG vaccination. However, BCG vaccination effect wanes in 5 years. The QFTG test mentioned below overcomes these false positive reactions.

QuantiFERON-TB Gold (QFTG) is a whole-blood test used for diagnosis of latent TB infections (LTBI) or TB disease. The test measures the amount of IFN-gamma released from the patient's white blood cells when mixed with two *M. tuberculosis* protein antigens (ES AT-6 and CFP-10, that are not present in BCG or in the NTM) and incubated for 16-24 hours. The amount of IFN-garnma released is measured by an ELISA test.

IFN gamma release assays **IGRAs** is another name for QFTG and related tests.

Advantages of QFTG: The tests require only a single blood specimen and results are read after 24 hrs only. The patient does not need to return for a reading. There is no reader bias. The result is not affected by prior BCG vaccination.

Disadvantages of QFTG: The tests are costly and the blood should be tested within 12 hrs. They should not be used in severely immunocompromised hosts or in children younger than 5 years. The tests are susceptible to biologic variation in the immune response.

Prevention and Control:

I-Treatment of active tuberculosis patients: Prompt adequate treatment of patients is the most important measure to prevent spread of infection. The policy in treatment of tuberculosis requires:

A-Prolonged course; treatment should **be** continued for 6-12 months as there is a slow response for treatment. This is due to: **a-** Most bacilli are intracellular.

b- The caseous material interferes with penetration of the drug, **c-** hi chronic lesions tubercle bacilli are not dividing i.e. "metabolically inactive" and need a long exposure to more than one drug.

B-Combination of drugs; 2-3 or more drugs are given at the same time and the **combination** is changed every few months; to reduce the drug toxicity and prevent emergence of drug **resistant** strains.

The first line of treatment drugs are; isoniazid (INH), rifampicin (RMP), ethambutol, streptomycin and pyrazinamide. In infections with multiple drug resistant strains, second line drugs should be used. These include para-arnino-salicylic acid, ethionamide, cycloserine, capreomycin, amikacin kanamycin and the fluorquinolones; ciprofloxacin and levofloxacin.

Multi-drug resistant TB **(MDR**-TB) is defined as those resistant to INH and rifampicin. MDR in tuberculosis is a serious problem that results from the spontaneous and random occurrence of chromosomal mutations. They are treated by second line drugs. The most important causes for development of resistance are; 1- Patients' non-compliance. 2- Inadequate treatment regimens.

Extreme drug resistant TB (XDR-TB) results from inadequate treatment of MDR-TB. It is defined as MDR + resistance to fluorquinolones and one of the injectable drugs (kanamycin, amikacin or capreomycin).

Because drug resistance is a problem, **antibiotic sensitivity testing** should be performed for all isolated organisms.

DOTS (directly observed therapy short-course) is recommended by the WHO to prevent patients' non-compliance in which the health care worker directly observes the patient taking the medication. It is a condensed course, consists of 2 months treatment with rifampicin, INH, pyrazinamide and ethambutol. This is followed by rifampicin and INH for 4-6 months. Ideally, the drugs are given daily but to ensure compliance by supervising the administration of the drug they may be given 3 times weekly.

II- Treatment of latent tuberculosis, to reduce the risk of progression to active tuberculosis. Treatment should not begin until active TB has been excluded by clinical, radiological and laboratory methods. Variable protocols are in use. Some use INH alone others use INH with RMP. Periods of treatment may vary from, 12 weeks to 9 months. The treatment is required for:-

(1) Individuals whose TST or QFTG test has recently converted to positive.
(2) Children who are close contacts of a case of infectious tuberculosis.
(3) Persons with a positive TST or QFTG test who are at high risk of reactivation including; a- Immunosuppressed persons e.g. HIV patients and organ transplant recipients, b- Patients with high risk clinical conditions e.g. diabetes, c- The homeless, IV drug users, hospital and laboratory personnel.

III- Specific prophylaxis = BCG **vaccine:**

The currently used vaccine for immunization against tuberculosis is a living attenuated *M. bovis* strain called BCG (bacillus, Calmette-Guerin). The vaccine creates a controlled focus of infection which stimulates cell mediated immunity. It is given by intradermal injection of 0.1 ml in the deltoid region.

In Egypt, the vaccine is given to newborns during the first month. In USA, it is given to children who are in contact with patients not complying to

treatment or those who have MDR tuberculosis. It should be given to health care workers who are TST negative and are working in areas where TB cannot be properly contained.

Advantages of BCG vaccination:

1- It protects against complications of TB e.g. milliary TB and tuberculous meningitis.
2- BCG is used in immunotherapy e.g. in treatment of bladder cancer. The vaccine is instilled into the bladder. It non-specifically stimulates cell mediated immunity, which can inhibit the growth of carcinoma cells.

Disadvantages of BCG vaccination:

1- It does not prevent infection. Its effectiveness varies from 0%-70%.
2- It gives a false sense of security.
3- It makes the tuberculin test useless in diagnosis especially in latent T.B.
4- The vaccine should not be given to immunocompromised children as it may cause disseminated infection, which requires treatment for 6 months.

P7-Eradication of TB in cattle and pasteurization of milk to reduce *M. bovis* infection.

V- Reducing overcrowding and improving socioeconomic conditions.

Non-Tubeculous Mycobacteria (NTM)

NTM differ from *M. tuberculosis* in a number of important aspects: (1) They are wide spread in the environment e.g. soil, water.. .etc. and are not transmitted from person to person. (2) They vary in their degree of pathogenicity. (3) They are commonly recovered from cultures from human secretions as contaminants or saprophytes. **(4)** Many are opportunistic pathogens and may cause human diseases including chronic pulmonary infection, lymphadenitis, skin and soft tissue infections and disseminated mycobacteraemia.

They are distinguished from tubercle bacilli by various cultural and biochemical characters e.g. their rate of growth and pigment production in relation to light. They are now identified by DNA probes. Some of the species that are significant causes of disease are:

M. avium intracellulare and ***M. kansasii:*** these cause pulmonary disease clinically indistinguishable from tuberculosis, primarily in immunocompromised patients e.g. AIDS patients and cervical adenitis in children. They are highly resistant to antituberculous drugs. However, M *kansasii* is more susceptible. ***M. ulcerans*** causes ulcerating infections of the skin (Buruli ulcers). ***M. marinum*** causes swimming pool granulomas.

Rapid growers that grow in 3-6 days, e.g. ***M.fortuitum*** and ***M. chelonae,*** are saprophytes in soil and water and rarely cause infections in immunocompromised patients and in those with prosthetic hip joint and indwelling catheters. Skin and soft tissue infections occur at the site of puncture wounds.

M. smegmatis is a rapid grower not associated with human disease. It is part of the normal microbiota of smegma, the material that collects under the foreskin of the penis and might be confused with acid-fast organisms in urine samples.

Mycobacterium leprae (Hansen's bacillus)

M. leprae is an **obligate intracellular, acid-fast** bacillus that causes leprosy in man, which affects mainly the mucous membrane of the nose, the skin and nerve fibers. The disease occurs worldwide with most cases in the tropical areas of Asia and Africa.

Morphology: They are similar in size and shape to the tubercle bacilli. They are acid-fast only to 5% H_2SO_4 or 1% HC1 in alcohol, which is used for decolourization in modified Ziehl-Neelsen stain, they are alcohol-fast. They appear in bundles or globular intracellular masses in smears prepared from nasal scrapings or tissue biopsy.

Cultural characters: *M. leprae* has not been grown in the laboratory, either on artificial media or on cell culture. However, ***in vivo*** injection of material containing lepra bacilli into the footpads of mice induces local granulomatous lesions with limited multiplication of bacilli. Inoculation of the armadillos results in extensive disease and the tissues containing large numbers of bacilli are used in the lepromin test and for vaccine production.The armadillo may be a natural host or a reservoir of the pathogen.

Pathogenesis:

The mode of transmission is not well known. However, infection may be acquired by prolonged contact with patients with lepromatous leprosy, who discharge *M. leprae* in large numbers in nasal secretions and from skin lesions. The lesions involve the cooler tissues of the body (optimal temperature for their growth is 30°C) i.e. skin, superficial nerves, nose, pharynx, eyes and testicles. The organism replicates intracellularly within skin histiocytes, endothelial cells and the cutaneous nerve cells (Schwann cells). There are two major clinical forms; the tuberculoid leprosy (TL) and the lepromatous leprosy (LL) with intermediate forms between the two i.e. border line.

The strength of the host's immune system influences the clinical form of the disease. A strong cell mediated immunity (Thl response with elevated levels of IL-2, IFN-Y and TNF-8) and a weak humoral response result in TL, the mild

form of disease, with few well defined nerves involved and low bacterial load. A strong humoral response (Th2 response with high levels of IL-4, IL-5 and IL-10), and absent cell mediated immunity, results in LL with widespread lesions, extensive skin and nerve involvement and high bacterial load.

In tuberculoid leprosy (TL), the course is benign and non-progressive with hypopigmented macular skin lesions and asymmetric nerve involvement with significant anaesthesia of the skin lesions. Few bacilli (paucibacillary) are found in the lesions. The lepromin test is positive i.e. the cell mediated immunity is intact causing the granulomatous response that causes nerve damage and the skin is infiltrated with Thl cells. The appearance of typical granulomas is sufficient for diagnosis. It may be self-limiting.

In lepromatous leprosy (LL), the course is progressive and malignant. Multiple nodular skin lesions occur and collapse of the nasal bone results in the typical leonine (lion-like) facies. There is symmetric nerve affection. Acid-fast bacilli are abundant (multibacillary) in skin lesions with bacteraemia. Lepromin test is negative indicating suppression of cell mediated immunity to the organism. Under treated LL patients often develop erythema nodosum leprosum, characterized by painful nodules, on the tibia and ulna, neuritis and uveitis (Table p. 184).

Diagnosis is mainly clinical and is confirmed by;

1- Microscopic examination of smears prepared from; nasal scrapings, slit skin smears from ear lobe, ulcerated skin nodules or biopsy from skin lesions; and stained by modified Ziehl-Neelsen. The presence of acid-fast bacilli in bunches intracellularly is diagnostic.

2- PCR assays are increasingly used to detect *M. leprae* in clinical specimens.

3- Lepromin test is a delayed hypersensitivity skin test similar to tuberculin. The test is done by intradermal injection of a heated bacillary suspension. It is positive in TL indicating intact cell mediated immunity and negative in LL

4- Non-treponemal serologic tests for syphilis e.g.VDRL and RPR, frequently yeild false positive results in leprosy patients.

Treatment: Due to development of resistance to dapsone, multidrug therapy (MDT) using dapsone, rifampicin and clofazimine are used for treatment of LL and dapsone and rifampicin for treatment of TL. Treatment is given for 2 years or until the lesions are free of organisms. Three more drugs that have shown bactericidal activity are ofloxacin, minocycline and clarithomycin.

Prevention: Treatment and isolation of lepromatous patients until they are noninfectious. BCG vaccination offers partial protection but a more effective vaccine based on killed *M. leprae* combined with BCG is being evaluated.

CHAPTER 14
SPIROCHAETES

Spirochaetes are a heterogeneous group of long, slender, delicate, spiral organisms, motile by periplasmic internal flagella (endoflagella). They include three genera that are pathogenic to man; ***Treponema, Borrelia*** and ***Leptospira.***

1- ***Treponema pallidum*** causes syphilis. Other treponema cause non-venereal infections (yaws, pinta and bejel). These are transmitted by direct contact.

2- Borrelia causes relapsing fever and Lyme disease.

3- Leptospira causes leptospirosis or Weil's disease.

TREPONEMA

The genus *Treponema* includes few pathogenic members. *Treponema pallidum* is the most important and is the causative organism of syphilis.

Treponema pallidum

Morphology:

They are delicate spiral filaments with small regular coils and characteristic corkscrew-like motility. They can be seen by dark-ground microscopy in unstained preparations. They are not stained by gram Silver impregnation and immunofluorescent methods are used for their detection.

Cultural characters:

Pathogenic treponema has not been cultivated on artificial media. Non-pathogenic treponema (e.g. Reiter strain) can be grown anaerobically *in vitro.* They are saprophytes antigenically related to *T. pallidum.* However human *T. pallidum* can be propagated by inoculation of positive specimens into the testicles of rabbits.

Serological characters:

Its antigenic make-up is unknown. However, in man the organism stimulates the formation of two types of antibodies; a treponemal antibody which reacts with treponema suspensions and a second antibody (reagin) which reacts with a non-specific antigen (cardiolipin). This is made use of in serologic diagnosis of syphilis.

Virulence factors and pathogenesis:

The outer membrane proteins are associated with adherence to the surface of host cells. The virulent spirochaetes produce hyaluronidase, which may facilitate perivascular infiltration.

SYPHLIS

It is a **sexually transmitted disease.** It can **be** acquired as a clinical hazard in hands of doctors and nurses by contact with patients. It can be transmitted from mother to foetus and by blood transfusion. The organism can survive in refrigerated blood for 24 hours which is of potential importance in blood transfusion.

The primary lesion is a hard painless ulcer called **chancre** that develops 2-10 weeks after exposure at the site of inoculation, mainly the genitalia. It appears as a papule which ulcerates. The regional lymph nodes are enlarged. The chancre will heal within 4-6 weeks, even without treatment.

Secondary stage lesions appear 6-12 weeks after the appearance of the chancre and are characterized by generalized manifestations e.g. skin rash, condylomata of anus and vulva and mucous patches in the mouth. ***T. pallidum*** is found in large numbers in the lesions of both stages. Systemic manifestations; fever, weight loss, joint pains and hair loss occur. Symptoms disappear spontaneously in 3-6 months.

A latent stage with no signs or symptoms -which may last for few months or for a lifetime- follows. However, the organism is still present as evidenced by positive serology.

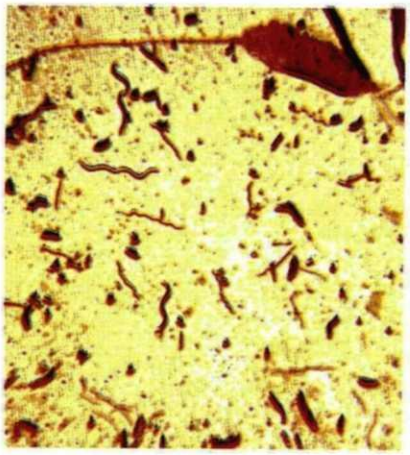
Tertiary syphilis may occur in 30% of untreated cases. It is characterized by appearance of granulomas (gumma) in the skin and bones; central nervous system involvement (e.g. paresis and tabes dorsalis); or cardiovascular lesions (e.g. aortitis, aneurysm of aorta). Treponemas are rarely seen in the lesions. Serology is positive.

Congenital **syphilis:** A pregnant syphilitic woman can transmit *T. pallidum* to the foetus through the placenta. This may lead to abortion or stillbirth at term, or a living baby who will develop congenital syphilis in childhood manifesting as; interstitial keratitis, Hutchinson's teeth, saddle nose, and several CNS anomalies. Adequate treatment of the mother during pregnancy prevents congenital syphilis. In congenital infection, the child makes IgM antitreponemal antibodies.

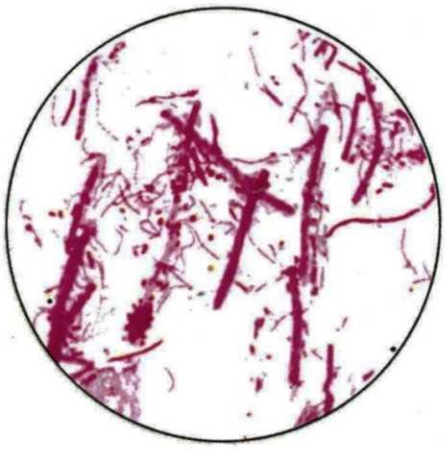
Diagnosis:

I- Detection of spirochaetes **in the lesion:** A wet mount is prepared from serous exudate collected from the chancre in primary stages or from the skin eruptions and mucous patches in secondary stages and examined by: **a-** Dark-ground microscope, which shows living motile spirochaetes with a characteristic slow movement and angulation. b-Direct immunofluorescence using fluorescein labelled antitreponemal antibodies.

PLATE IV

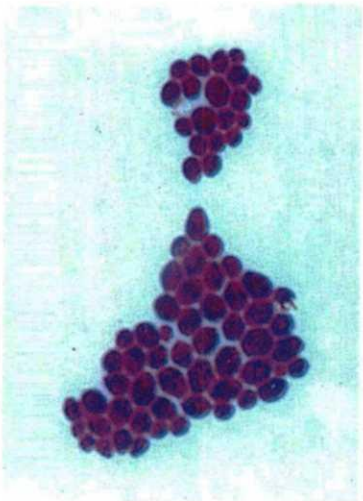


A) Commensal **spirochaetes** by Fontana stain.



B) Borrelia and fusiform bacilli in a smear from mouth ulcers. **Vincent angina**.

C) ***Pneumocystis jiroveci*** stained by silver stain in bronchoalveolar lavage from HIV patient.



D) **Candida albicans** stained by gram showing budding yeast cells.

c- PCR may be used to detect spirochaetes in clinical material.

Il-Serologic diagnosis:

Serological tests are the major diagnostic tool for syphilis. There are two kinds of these tests according to the type of antigen used:

A - **Non-treponemal antigen tests:**

These tests detect the "reagin" antibodies (mixture of IgM and IgG) which react with a non-specific antigen which is a mixture of cardio 1 ip in, cholesterol and lecithin. These tests are:

1- Venereal Disease Research Laboratory (VDRL) test is a flocculation test in which particles of cardiolipin form visible clumps when combined with reagin antibodies in serum, or in CSF in neurosyphilis. The suspension is shaken and the reaction occurs in few minutes. It is read by the microscope.

2- Rapid Plasma Reagin (RPR): It is another flocculation test performed on plasma and is read by the naked eye.

3- Toluidine red unhealed serum test (TRUST) is another flocculation test.

These tests are non-specific and may give false positive results in other diseases e.g. autoimmune diseases, measles, leprosy, malaria, hepatitis **B,** infectious mononucleosis. A positive test should be confirmed by one of the specific treponemal antigen tests. The non-treponemal antigen tests are mainly used for **screening** and for epidemiologic purposes because they are more rapid, simple and cheap. They may be used **to evaluate the effect of treatment** since they revert to negative in 6-18 months after effective treatment.

B- Treponemal **antigen tests:**

These tests use *T. pallidum* as the antigen. The tests are highly specific as they detect anti-treponemal antibodies but they are more complex and expensive. They are used primarily as confirmatory tests. They remain positive for life even after effective treatment.

1- Fluorescent treponemal **antibody absorption** (FTA- ABS) **test:**

It is an indirect immunofluorescence test in which the patient's serum is layered on killed treponema fixed to a slide. After incubation for some time, the excess is washed. Then, fluorescein labelled antihuman gamma globulin is added, incubated for some time, and then the excess is washed. Fluorescence is seen with positive sera. Sera are first absorbed with a suspension of saprophytic Reiter spirochetes.

The presence of IgM FTA in the blood of newborns is a good evidence of *in utero* infection.

2- ***Treponema pallidum*** **haemagglutination (TP-HA) test:**

It is a specific test easy to perform. It depends on the ability of antibodies in syphilitic patients' sera to bring about agglutination of sheep red blood cells coated with an extract of ***T. pallidum.*** The test is done in microtiter plates and is called **microhaemagglutination assay for *T. pallidum* antibodies (MH ATP)**.

3- ***Treponema pallidum****-particle* **agglutination (TP-PA)** test: Gelatin particles are coated with ***T. pallidum*** antigens. Serial dilutions of the patient's serum are added to the gelatin particles in microtiter plates. A mat of agglutinated particles indicates a positive test.

4-**Enzy me immunoassays (EIA)** are used, however they my give false positive results and should be confirmed by any of the above tests

Treatment: Penicillin is the drug of choice. Syphilitic pregnant women should be adequately treated to prevent congenital syphilis. Children born to syphilitic mothers should be treated with penicillin. For those allergic to penicillin, tetracycline, erythromycin or ceftriaxone may be used though they may be less effective.

BORRELIA

Many members of the genus are commensals. Pathogenic Borreliae cause relapsing fever and Lyme disease.

1

Morphology:

Large spirals with wide irregular coils. Some species are stained with ordinary stains and are gram negative, whereas other species are stained by Irishman, Wright or Giemsa stains.

Cultural characters:

Some members can be cultured in fluid media containing blood, serum or tissue e.g. Barbour-Stoenner-Kelly (BSK **n)** medium and its modifications. They should be incubated in a microaerophilic environment at 30-35°C. Cultures are monitored for growth by dark field microscopy for **4**-6 weeks.

Antigenic structure:

Borreliae are antigenically variable. *B. recurrentis* shows antigenic variation *in vivo.* These antigenic variants are the cause of the relapses and with each relapse a new antigenic variant arises.

RELAPSING FEVER

This is a disease characterized by repeated bouts of fever alternating with periods of apyrexia. **Epidemic relapsing fever** is caused by ***B.*** *recurrentis*

and is transmitted by lice, while **endemic relapsing fever** is transmitted by ticks and is caused by other borrelia species e.g. *B. duttoni* and *B. hermsii*. Man is the only host for *B. recurrentis* and *B. duttoni*. Rodents and small animals are the main reservoir for other borrelia.

Lice or ticks become infected by feeding on patients' or rodents' blood during the bacteraemic stage. Man is infected by rubbing crushed lice into bite wounds in case of epidemic relapsing fever and by the bite of the tick in case of endemic relapsing fever.

After an incubation period of 3-10 days, there is sudden onset of fever which lasts for about 4 days followed by afebrile period of 3 to 10 days. Then the patient develops another bout of fever, 3-10 relapses may occur. The organism is found in large numbers in the blood during the febrile stage. In fatal cases, the spirochetes invade many organs (heart, spleen, liver and kidney) with death generally due to myocarditis.

Antibodies against borreliae appear during the febrile stage; these agglutinate and destroy the organism and the attack is terminated. Antigenic variants of Borreliae, to which the patient has no antibodies, emerge and cause the relapse.

Diagnosis:

1- During the **febrile stage:** Blood films stained with Leishman, Wright or Giemsa stains reveal large numbers of spirochaetes.

2- Cultivation and mouse enrichment: During the **afebrile stage** the organism is scanty in the blood and blood films are negative. Diagnosis is done by injecting white mice intraperitoneally with the patient's blood. After 2-4 days, films from tail blood are stained and examined for presence of Borreliae. Mice blood is inoculated on BSKII medium.

3- DNA probes may be used for identification.

Treatment: Penicillin, tetracyclines and erythromycin have proved effective treatments.

LYME DISEASE

This illness is named after the town of Lyme, Connecticut, USA where a number of cases were first discovered in 1975. It is caused by *Borrelia burgdorferi* and is transmitted to man from the saliva of the tick which contaminates the site of the bite. Rodents and deer are the main animal reservoir.

The disease has early and late manifestations: The **early stages** are characterized by a distinctive skin lesion called **"erythema migrans"** associated with fever, chills, muscle pain and headache.

Late manifestations which appear weeks or months later are arthritis, myocarditis and neurologic manifestations e.g. meningitis.

Diagnosis:

1- PCR assay is used to detect *B. burgdorferi* DNA in skin biopsy, body fluids, CSF or joint fluid. The test is rapid, sensitive and specific.

2- *B. burgdorferi* in tissue sections can sometimes be identified using antibodies and immunohistochemical methods.

3- Culture is generally not performed because it takes 6-8 weeks to complete and lacks sensitivity.

4- Serologic detection of IgM or a rising titre of IgG by immuno-fluorescence or ELISA. However, the tests are not specific or sensitive. A positive test should be confirmed by Western blot assay.

Treatment: For early cases, doxycycline or amoxicillin are used. For severe or late cases, penicillin or ceftriaxone are more effective.

Prevention: Prevention is mainly by using tick control measures e.g. using tick repellents and wearing clothing that can protect those exposed to ticks.

FUSOSPIROCHAETAL DISEASE
"Vincent Angina"

Under certain conditions which cause injury or devitalization of the mucous membrane e.g. nutritional deficiency, stasis ulcers, human bites or herpes simplex lesions; the normal spirochaetes of the mouth together with anaerobic cigar-shaped fusiform bacilli (fusobacteria) multiply in the lesions causing **acute ulcerative gingivostomatitis.** Ulcers may become covered with a pseudomembrane containing pus cells and necrotic tissues, on the tonsils and gums, the condition is called **"Vincent angina"** and it should be differentiated from diphtheria and follicular tonsillitis.

Smears prepared from the pseudomembrane show gram negative spirochaetes in large numbers associated with fusiform gram negative bacilli (fusobacteria). Pus cells and other organisms are also found.

LEPTOSPIRAE

Leptospira interrogans causes leptospirosis in animals e.g. rats, dogs and field mice. Man is infected by coming in contact with water contaminated with their excreta. *L. interrogans* causes leptospirosis or Weil's disease also called infectious jaundice in man.

Morphology: Long thin finely coiled, one or both ends of the organism are hooked. It can be seen in fresh unstained preparation by dark-ground microscopy. They can be stained with silver stains. Members of the genus are motile with two subterminal periplasmic flagellae.

Cultural characters: They grow aerobicaly on serum containing semisolid media e.g. Stuart's or Fletcher's media. Optimum temperature is 30°C.

WEIL'S DISEASE, LEPTOSPIROSIS, (Infectious Jaundice)

Weil's disease is caused by *Leptospira interrogans* which is a natural pathogen of rats and other rodents and dogs. The organism is excreted in their urine and faeces where it contaminates stagnant water in which the organism can remain viable for several weeks.

Man is infected by coming in contact with water or other materials contaminated with the excreta of animal hosts. The organism can enter through mucous membranes or abrasions in the skin. Swimming in or ingestion of contaminated water or food can cause human infection. The disease occurs mainly in sewage workers, miners, rice farmers, sugar cane workers, military personnel and fishermen.

After an incubation period of 1-2 weeks, there is fever for few days followed by jaundice, haemorrhages and renal failure. Severe cases may be complicated by meningitis. The organism is found in the blood during the fever and is excreted in urine from the second week onwards.

Diagnosis:

I- Detection of the organism in:

a- Blood or CSF during the first week of fever, b- Urine from the 2nd week of fever.

1- Microscopic examination of films from blood or fresh urine deposit, with the dark-ground microscope, or after staining with silver stains or direct fluorescent antibody assay.

2- PCR based methods for rapid detection of leptospira DNA in specimens.

3- Culture on Fletcher's or Stuart's medium, incubated at 30°C. Growth apears after 1-2 weeks. Cultures are identified by agglutination or PCR.

II- **Serologic diagnosis;** by detection of specific antibodies in patients' sera which start to appear during the second week of fever:

1- ELISA for detection of IgM or a rising titre of IgG.

2- Agglutination tests using a killed suspension of the organism are used. The detection of a rising titre is the most convincing evidence.

Treatment: Penicillin and doxycycline are effective early in infection.

CHAPTER 15 MYCOPLASMA and UREAPLASMA

Mycoplasma are the smallest free living organisms. They **lack a cell wall** consequently they are pleomorphic, stain poorly with gram, and are resistant to cell wall inhibitors (penicillins and cephalosporins). They are the only bacteria that contain cholesterol in their cell membrane. They require special enriched media for growth. Important human pathogens are:

1- *M*. ***pneumoniae*** causes **atypical** pneumonia.

2- Ureaplasma urealyticum is urease positive. It causes 20% of non-gonococcal urethritis and neonatal sepsis in premature infants.

3- ***M. hominis*** is associated with postpartum fever and pelvic inflammatory disease e.g. salpingitis.

4- *M*. ***genitalium*** is associated with non-gonococcal urethritis.

-All may be the cause of septic arthritis in immunocompromised persons.

Morphology **and cultural characters of *M. pneumoniae'*.**

They are pleomorphic; varying in size from 50-300 nm. They can be stained with Geimsa. They grow on special media enriched with serum and other ingredients that provide sterols and nucleic acid precursors. They are facultative anaerobes, better growth occurs at 10% C02. They grow slowly and require at least one week to form visible colonies, which have a characteristic "fried egg appearance". Cultures can be identified by PCR.

Diagnosis of atypical pneumonia: Diagnosis is mainly a clinical one.

1- Direct detection in sputum or nasopharyngeal aspirates of:

a- Antigens by immunofluorescence using specific antisera. **b-** Specific nucleotide sequence using DNA probes or PCR.

2- Serology is most useful in diagnosis:

a- Detection of ***M.*** *pneumoniae* IgM or a rising titre of IgG by ELISA or complement fixation,

b- Detection of **cold agglutinin** at a titre of 1/128 or higher indicates recent infection. These are autoantibodies against type O red cells that agglutinate these cells at 4°C but not at 37°C. It is positive in 50% of cases. It is non-specific and may be positive in other diseases including viral infections, malaria and acquired haemolytic anaemia.

3- Isolation from sputum is difficult and time consuming.

Treatment by tetracyclines, erythromycin or azithromycin.

U. urealyticum, *M.* ***hominis,*** *M*. ***genitalium*** are collectively called genital mycoplasmas and can be directly detected in specimens as in ***M.*** *pneumoniae. U. urealyticum* can grow in culture in 2 days and is identified by its tiny colony morphology and urease production. All mycoplasmas and *U. urealyticum* are sensitive to quinolones, trovafloxacin and sparfloxacin.

CHAPTER 16

LEGIONELLA

There are about 40 species of *Legionella* found in soil, lakes and streams. They can multiply in free living amoebas in water and coexist with them in biofilms. *L. pneumophila* is the major cause of disease in man. It causes outbreaks of atypical pneumonia called "Legionnaire's disease" and a mild flulike condition without pneumonia called Pontiac fever which is self limited.

Mophology and cultural characters:

They are motile, weakly gram negative rods. They are fastidious and require for growth media containing L-cysteine, iron, and a- ketoglutarate as in buffered charcoal yeast extract (BCYE) agar. They are catalase and oxidase positive.

Pathogenesis of Legionnaire's disease

Infection occurs by inhalation of water aerosols contaminated by these organisms. It can be community-acquired or nosocomial. Outbreaks of pneumonia in hospitals are attributed to contaminated air conditions, showers or sinks. Risk groups include smokers, alcoholics, diabetics, AIDS, cancer, transplant, and chronic lung disease patients.

A virulence factor mportant for macrophage invasion is the Mip protein, which promotes adherence and phagocytosis. The organism multiplies in and can survive intracellularly in alveolar macrophages, as they inhibit phagosomal lysosomal granules fusion. Severe cases may be accompanied by damage to the vascular endothelium in the brain and kidney. CMI is the most important defence mechanism, because of the intracellular growth and survival of the organism.

Diagnosis:

1- Culture of bronchial aspirate, pleural fluid or lung biopsy on BCYE agar aerobically. Cultures are identified by immunofluorescent, Geimsa, or silver staining and by nucleic acid probes.

2- A rapid (within hours), specific, urinary antigen detection test by ELISA is available.

3- PCR can be used for direct detection, of *L. pneumophila* nucleic acids in sputum, urine and other specimens.

4- Serologic diagnosis, by detection of IgM or rising titre of IgG in convalescent sera by ELISA is useful in retrospective diagnosis of outbreaks

Treatment: Azithromycin or erythromycin (with or without rifampicin) is the treatment of choice. Levofloxacin and trovafloxacin are also used.

Prevention: Elimination of aerosols from water sources and reduction of the incidence of legionella in hospital water supplies, by super-heating and hyperchlorination could help controlling the spread of infection

CHAPTER 17 RICKETTSIA & ORIENTIA

Orientia is only one species *O. tsutsugamushi* and differs from **Rickettsia** in the 16S rRNA sequence and cell wall structure. Both are short bacilli that are difficult to stain by gram They are stained best by Giemsa stain. They are **obligate intracellular** parasites; therefore, they are grown in tissue culture, embryonated eggs or experimental animals. They are maintained in nature in certain arthropods such as ticks, lice, fleas and mites.They are transmitted to humans by the bite or faeces of the arthropod. Vectors, reservoirs and diseases caused by rickettsia and orientia are presented in the table.

Species	Disease	Reservoir	Vector	Natural Cycle
Typhus Group				
R. prowazeki	- Epidemic typhus	Man	Louse	Human-louse
R. typhi	- Endemic typhus	Rat	Flea	Rat-flea
O. tsutsugamushi	- Scrub typhus	Rodents	Mite	Transovarian in mites
Spotted Fever group				
R. rickettsii	- Rocky mountain spotted fever	Dogs and rodents	Tick	Transovarian in ticks
R. akari	- Rickettsial pox	Mice	Mite	Transovarian in mites
R. conorii	- Fiever boutonneuse	Rodents	Tick	Transovarian in ticks

EPIDEMIC TYPHUS

The disease is characterized by prolonged fever, severe prostration, skin rash and enlargement of spleen and liver. The disease is fatal specially in old age. It occurs in epidemics and is transmitted by the body louse. Infection occurs by contamination of the site of the bite by the faeces of infected lice.

The *Rickettsia prowazeki* circulate in the blood stream during the first week. They invade the capillary endothelium causing vasculitis in the brain, heart and other organs. Disseminated intravascular coagulation and vascular occlusion may occur. Survivors remain with long lasting immunity.

Brill- Zinsser disease:

In some individuals, recovery is not followed by eradication of infection but the rickettsia remains latent in lymph nodes for several years. If such individuals are exposed later to factors which lower their immunity, the rickettsia may be activated to produce disease again. This condition is known as Brill's disease in which the infection is endogenous. A person with Brill's

disease may be the point of start of an epidemic in a louse infested susceptible population.

ENDEMIC TYPHUS

It resembles epidemic typhus but is much milder and of low mortality. It occurs in sporadic endemic forms. It is caused by *R> typhi* and is transmitted from rat to man by fleas.

ROCKY MOUNTAIN SPOTTED FIVER

The name "Rocky mountain spotted fever" is derived from the region in which the disease was first found. It accounts for 95% of rickettsial diseases in USA The tick is an important reservoir and vector fof ft *rickettail* The organism is transmitted transovarian among ticks, Dogs and rodents are also reservoirs of infection. Humans are accidental hosts; no person to person transmission occurs.

Most cases occur in children. The disease is characterized by acute onset of non-specific symptoms e.g. fever, headache, malaise and prostration. The typical rash, which appears 2-6 days later, begins With macules that progress to petechiae that appear first on hands and feet then move to the trunk.

CNS manifestations e.g. delirium, coma, D1C and circulatory collapse may occur in severe cases. It can be fatal if untreated.

Diagnosis of Rickettsial diseases;

Early diagnosis and prompt treatment are lifesaving.

Direct defection in blood and skin biopsy specimens from the rash; by immuno-histochemical methods and PCR. These methods ate very usefu 1 for establishing a diagnosis in the acute stage.

Isolation procedures are only done in few laboratories, as they ate hazardous. Inoculation of guinea pigs, mice or yolk sac of embtyofiated eggs have been replaced by tissue culture methods, which give results in 2-3 days.

Serologic diagnosis is based on detection of it rising antibody titre by indirect immunofluorescence, ELISA, latex agglutination and Western blot, which detect specific antibodies. Serology is mainly used fo confirm the diagnosis for epidemiologic investigations.

Treatment of rickettsial diseases: The treatment of choice for all rickettsial diseases is tetracyclines, with chloramphenicol as the second choice;

Prevention of transmission by breaking the chain of Infection)

- For epidemic typhus: Anti-louse measures and personal hygiene.
- For endemic typhus: Rat proof buildings.

- For Spotted fever: Reduce exposure by wearing protective clothings andusing tick repellents,

CHAPTER 18

COX I EL LA and Q FEVER

Coxietta burnetii is the only species and causes Q fever. It is similar to rickettsia in most characters; however, it differs from rickettsia in being more resistant to drying, disinfectants and **UV.** It can survive for months in dried animal discharges (placental tissues or aminiotic fluid) faeces, urine or milk; due to **endospore formation** during an intracellular developmental cycle.

C. burnetii exist in two antigenic forms called phase I and phase II. Phase I is the virulent form that is found in humans with Q fever and in infected vertebrate animals, and it is the infectious form Phase II is the avirulent form

It is **not** transmitted to humans by the bite of an arthropod. However, the organism is found in ticks which transmit it to goats, sheep and cattle. These animals are the reservoir for human infection, passing the organism in their milk, discharges and excreta. Man gets infected by:

- Inhalation of dust contaminated with discharges or excreta of infected animals or inhalation of aerosols in slaughterhouses when handling infected animal tissues.
- Rarely by consumption of unpasteurized infected milk.

Q fever is usually an occupational hazard affecting mainly farmers, abattoir workers, veterinarians and laboratory personnel. Inhalation of **aerosols** containing the organism, which enter the lungs, results in infection of alveolar macrophages and a brief rickettsaemia. Many infections are subclinical. Incubation period is 2 weeks.

Acute disease begins with fever and influenza-like symptoms. Pneumonia occurs in about half of cases. Hepatitis is frequent enough that the combination of pneumonia and **hepatitis** should suggest Q fever.

Chronic Q fever characterized by life threatening endocarditis may occur in patients with abnormal valves. It is associated with a rise in antibody titre to phase I C. *burnetii* and negative blood cultures.

Diagnosis is mainly by serologic detection of a rising antibody titre to phase I or II C. *burnetii.* Indirect immunofluorescence is considered the best method PCR is useful in diagnosing culture negative endocarditis. Other methods as in rickettsia may be used.

Treatment: Doxycycline is used for treatment of acute infection. Combined, prolonged treatment of doxycycline with ciprofloxacin or rifampicin is essential in endocarditis.

Prevention by proper pasteurization of milk. A formalin-killed whole cell, phase I *C. burnetii* vaccine is available for those occupationally at risk.

CHAPTER 19

CHLAMYDIAE

Chlamydiae are a large group of **obligate intracellular parasites** closely related to gram negative bacteria. They were classified in the past as large viruses due to their small size (250-400 nm) and their obligate intracellular parasitism They differ from viruses and simulate bacteria in the following:

1- They possess both RNA and DNA like bacteria.

2- They multiply by a special developmental cycle that includes binary fission

3- They have a rigid cell wall similar to gram negative bacteria.

4- They possess ribosomes and synthesize their own proteins.

5- They have a variety of metabolically active enzymes.

6- They contain plasmids.

7- They are sensitive to antibiotics.

Chlamydiae can be considered as gram negative bacteria that lack mechanisms for the production of metabolic energy and can not synthesize ATP. Due to this defect, they need to multiply intracellularly where the host cell (e.g. tissue culture) provides energy and metabolites.

The chlamydiae that infect humans are divided into three species:-1

-Chlamydia trachomatis causes ocular, genital and respiratory infections.

2- ***Chlamydia (Chlamydophila) psittaci*** causes psittacosis in birds that may be transmitted to man.

3- ***Chlamydia (Chlamydophila) pneumoniae*** causes atypical pneumonia. Man is the only host.

Developmental cycle:

Two forms of the organism are seen; the **"elementary body"** which is the infectious form and measures 300 nm, this is taken into the cell by a phagocytosis-like process in which it passes to the larger form called "initial or **reticulate body".** The latter grows in size and divides by binary fission forming a large number of elementary bodies that are seen in the host cell as intracytoplasmic inclusion bodies. These are released to infect new cells.

Characters of the organism:

They are stained by Giemsa or Macchiavello stain. They grow in tissue culture and in the yolk sac of chick embryo. C. *psittaci* and *C. pneumoniae* have one serotype, while C. *trachomatis* has at least 15 serotypes.

Diseases caused by *C. trachomatis:*

C. trachomatis infects only humans and is transmitted by close personal contact, e.g. finger to eye, sexually or from the birth canal during delivery.

I- Ocular infections:

1- Trachoma which is a chronic keratoconjunctivitis that starts with acute infection of the conjunctiva and cornea and progresses to scarring and blindness. The disease is endemic in Egypt, many African, Asian and South American countries. Infection is transmitted from eye to eye by fingers, fomites or flies. It is caused by serotypes A B and C.

2- Inclusion conjunctivitis is a milder form in which the cornea is not involved. Healing occurs without scar formation. It affects adults as well as neonates who acquire the infection from the birth canal of an infected mother. It is caused by serotypes D-K.

II- Genital infections:

C. *trachomatis* serotypes D-K is a prominent cause of **non-gonococcal urethritis** and rarely epididymitis in males. In females it causes cervicitis, salpingitis and pelvic inflammatory disease. It can lead to sterility and predispose to ectopic pregnancy. It is transmitted to sex partners.

Patients with genital *C. trachomatis* infections have a high incidence of Reiter's syndrome, which is characterized by urethritis, arthritis and uveitis. It is an autoimmune disease caused by antibodies formed against C. *trachomatis* cross-reacting with antigens on the cells of the urethra, joints and uveal tract.

HI- Lymphogranuloma venereum (LGV):

LGV is a sexually transmitted disease caused by *C. trachomatis* serotypes L-l, L-2 and L-3. The initial lesion is a painless papule on the external genital organs which ulcerates. The inguinal lymph nodes suppurate and the pus is drained through the overlying skin. Healing by scar formation leads to stricture and lymphatic obstruction. Frie test is a hypersensitivity skin test similar to tuberculin test that may be used for diagnosis.

IV- Respiratory infection:

Adults with inclusion conjunctivitis may show upper respiratory infection e.g. otitis, pharyngitis and nasal obstruction. This is due to drainage of infectious chlamydia through the nasolacrimal duct. Pneumonitis due to *C. trachomatis* may occur in immunocompromised patients.

Neonatal pneumonia occurs due to *C. trachomatis* acquired from the birth canal of an infected mother. Detection of an IgM antibody titre 1/32 or more is diagnostic. Oral erythromycin for 14 days is recommended

Chlamydia (Chlamydophila) psittaci

It causes psittacosis in birds that may be transmitted to man. Infected birds pass the organism in their respiratory secretions and in the faeces. Man gets infected by inhalation of dust containing the dried faeces, by infected aerosols or by handling the infected tissues. The disease in man is in the form of bronchitis or pneumonia similar to that produced by viruses (atypical pneumonia). It occurs mainly in people handling birds.

Chlamydia (Chlamydophila) pneumoniae

C. pneumoniae causes both upper and lower respiratory tract infection in humans only and is transmitted by aerosol. Most infections are asymptomatic or mild. It causes atypical pneumonia similar to *Mycoplasma pneumoniae.*

An association with atherosclerotic coronary artery and cerebrovascular disease has been suggested based on seroepidemiologic studies and detection of *C. pneumoniae* in atherosclerotic tissues by PCR. However, additional work is needed for confirmation.

Diagnosis of chlamydial infections:

Specimens include; conjunctival and urethral discharge, cervical scrapings, sputum, urine, pus .. .etc. They are examined as follows:

1- Direct detection in the specimens of;

a- Intracytoplasmic inclusions by Giemsa or immunofluorescent staining.

b- Chlamydial antigens in exudates or urine by commercially available kits that use ELISA or fluorescent antibody staining.

c- Nucleic acids in specimens by DNA probes or **PCR,** which can be used on urine to diagnose chlamydial sexually transmitted diseases.

2- Isolation on McCoy cells for C. *trachomatis* and C. *psittaci* and on HEP**-2** cells for C. *pneumoniae* and detection of inclusion bodies in cell cultures.

3- Serologic diagnosis by detection of specific IgM or a rising titres of IgG using CF test or ELISA. Serologic tests are useful in the diagnosis of *C. pneumoniae* and *C. psittaci* infections. They are generally not useful for diagnosis of genital tract chlamydial infections because the frequency of infection is so high that many people already have antibodies.

Treatment of chlamydial infections:

Chlamydia are sensitive to tetracyclines e.g. doxycycline and the macrolides, e.g. erythromycin and azithromycin. The drug of choice for sexually transmitted C. *trachomatis* infections is azithromycin. Both partners and offsprings should be also treated, to prevent reinfection. Patients with a diagnosis of gonorrhoea should also be treated for C. *trachomatis.*

CHAPTER 20

ACTINOMYCETES

They are a diverse group of filamentous branching gram positive bacilli, but some are also acid-fast. Most are saprophytes in soil The genera of medical importance are *Actinomyces, Nocardia, Actinomadura* and *Streptomyces.* They cause three diseases in humans i.e. actinomycosis, nocardiosis and actinomycetoma (see p. 91).

Actinomyces israelii causes actinomycosis

It is an **anaerobic** bacterium and is one of the normal microbiota of the oral cavity and GIT. However, after local trauma, it invades the tissues causing actinomycosis, which is a chronic inflammatory, granulomatous lesion that drains pus -through sinus tracts-, which contains yellow "sulphur granules". These are composed of a central mycelial mass with a peripheral zone of swollen clubs.

Most lesions are cervico-facial (associated with poor dental hygiene or tooth extraction), abdominal or thoracic. Pelvic actinomycosis can occur in women keeping an intrauterine device for a long period.

Diagnosis: Pus from lesions is crushed between two slides. On direct examination, the sulphur granules are seen with radially arranged clubs.

Gram staining will reveal filamentous branching gram positive bacilli with bacillary and coccoid forms.

Culture anaerobically on thioglycolate broth and brain-heart infusion blood agar. Colonies are identified by morphology and immunofluorescent staining.

Treatment by prolonged administration of penicillin G and surgical drainage. Clindamycin and erythromycin are used for penicillin allergic individuals.

Nocardia asteroides causes Nocardiosis

Nocardia species are **aerobes** found in soil. They are gram positive branching filaments. Some are **weakly acid-fast.**

N. asteroides is the common cause of nocardiosis. Inhalation of the organism by an immunocompromised person causes, chronic pulmonary infections that mimic tuberculosis with consolidation and may progress to form abscesses and sinus tracts. It may spread to the brain, skin, or kidneys.

Diagnosis by detection of gram positive bacilli, coccobacillary cells and branching filaments that are weakly acid-fast in specimens. It grows aerobically on brain-heart infusion agar in few days.

Treatment with trimethoprim-sulfamethoxazole for long periods (about 1 year) and surgical drainage. Ceftriaxone and minocyclin may be effective.

Actinomycetoma (see p. 91)

CHAPTER 21

MYCOLOGY

There are thousands species of fungi. Most of them are saprophytes. Few species cause disease in man. Fungi are eukaryotic organisms. Their cell wall consists primarily of chitin and their cell membrane contains ergosterol, in contrast to human cell membrane that contains cholesterol. They can be classified morphologically or clinically:

I- Morphological classification:

1- Yeasts: These are oval or round cells that reproduce by asexual budding and may form pseudohyphae e.g. *Candida* and *Cryptococcus neoformans.*

2- **Filamentous fungi:** These are branching filaments (hyphae) which may be septate or non-septate. They reproduce by asexual spores (conidia), which may be unicellular and called microconidia or multicellular and called macroconidia e.g. the dermatophytes *(Microsporum, Trichophyton* and *Epidermophyton)* and *Aspergillus.*

3- **Dimorphic fungi:** These occur in 2 forms; a yeast form in tissues or when grown at 37°**C;** and a filamentous form (hyphea) when grown at 22°**C,** e.g. *Histoplasma, Blastomyces, Coccidioides.*

II- **Clinical classification:**

1- Superficial mycoses: These are fungal infections that are confined to the stratum corneum without tissue invasion e.g. pityriasis versicolor or tinea versicolor caused by *Malassezia furfur.*

2- **Cutaneous mycoses:** These are fungal infections that involve the skin, nail or hair with tissue destruction and immunological reaction e.g. dermatophytes and cutaneous candidiasis.

3- **Subcutaneous mycoses:** These are infections confined to the subcutaneous tissue without dissemination to distant sites e.g. mycetoma, chromomycosis and sporotrichosis.

4- Systemic (endemic) mycoses: These are primary pulmonary lesions that may disseminate to any organ mainly in immunocompromised patients. They are caused by the dimorphic fungi.

5- Opportunistic mycoses e.g. systemic candidiasis, cryptococcosis, aspergillosis, mucor mycosis and *Pneumocystis* infections.

In addition to the above mentioned mycotic infections there are two other kinds of fungal diseases;

a- Allergies to fungal spores, particularly those of Aspergillus, Alternaria and others. They cause mainly type I hypersensitivity reactions or atopy manifesting as bronchial asthma, hay fever, urticaria .. ..etc.

b- Vlycotoxicosis: These are diseases due to the consumption of food containing fungal toxins e.g.

- **Amanita mushrooms** produce fungal toxins, when ingested, they cause sever fatal damage to the liver and kidney. A disease called **mycetismus.**
- Another mycotoxicosis, **ergotism,** is caused by the mould *Claviceps purpura,* which infects grains and produces alkaloids (e.g. ergotamine and lysergic acid diethylamide LSD) that cause neurologic effects.
- Other toxins ingested with spoiled grains and peanuts are the **anatoxins** which are metabolized in the liver to epoxide, a potent carcinogen. Afla-toxins are coumarin derivatives produced by *Aspergillus flaws;* they are hepatotoxic, cause tumours in animals and are suspected of causing hepatic carcinoma in man. Aflatoxin Bl induces a mutation in the pS3 tumour suppressor gene and loss of growth control in the hepatocytes.

SUPERFICIAL MYCOSES Tinea

Versicolor (Pityriasis versicolor)

It is a superficial chronic skin infection of the stratum corneum, characterized by superficial brownish scaly areas on light-skinned persons and lighter (depigmented) areas on dark-skinned persons. It has a world wide distribution and is caused by *Malassezia furfur* and is of cosmetic importance.

KOH preparations of skin scales show short thick septate hyphae and clusters of budding yeast cells.

It is treated by topical miconazole. Lesions tend to recur and permanent cure is difficult to achieve.

CUTANEOUS MYCOSES

Dermatophytes

The dermatophytes include 3 genera *Epidermophyton*, *Microsporum* and *Trichophyton.* These organisms affect the keratinized tissues; skin, hair and nails.They spread peripherally from foci to produce ring-like lesions. Hence, the name **ringworm** or **tinea.** Infection does not spread to deeper tissues.

The source of infection is man to man by direct contact, from the animals e.g. cats and dogs or from the soil. The intact skin is an important barrier against infection. Heat and humidity enhance the infection. The clinical forms of the disease are named according to the site affected e.g. tinea capitis in the head, tinea cruris in the groin area, tinea unguinum in the nail.. ..etc.

Diagnosis of ringworm:

1- Skin scales, nail and hair clippings are examined microscopically after digestion using 10% KOH. Branching hyphae are detected among epithelial cells in skin or nails. In the hair, hyphae or spores are detected. The latter may be outside the hair (ectothrix) or inside the hair (endothrix).

2- Cultures are done on Sabouraud's dextrose agar (SDA) containing actidione
(cycloheximide) to inhibit saprophytes and chloramphenicol to inhibit bacteria and incubated at room temperature for up to 4 weeks. Colonies are identified by morphology and colour on surface and reverse, and by microscopic examination using lactophenol cotton blue or other stains.

Treatment by local antifungal creams e.g. miconazole or oral griseofulvin.

SUBCUTANEOUS MYCOSES

These are caused by fungi that grow in soil and on vegetation and are introduced into the subcutaneous tissue through **trauma.**

Mycetoma (Madura foot)

Mycetoma is a chronic granulomatous infection that usually involves the lower limbs. The condition is characterized by swelling, purplish discolouration and multiple sinuses that drain pus containing yellow, white, red or black granules. It is common in tropical areas in bare footed persons. According to aetiology, it is of two types:

1- Actinomycotic (bacterial) mycetoma which is caused by species of the **aerobic** actinomycetes including; *Nocardia brasiliensis, Actinomadura madurae* and *Streptomyces somaliensis.*

2- **Eumycotic (fungal)** mycetoma caused by a heterogeneous group of species having true septate hyphae e.g. *Madurella mycetomatis* and others.

Laboratory diagnosis is important to identify the specific aetio logic agent of mycetoma since the **actinomycotic mycetoma** is curable by **antibiotics** e.g. streptomycin, trimethoprim-sulfamethoxazole, oxasole or dapsone, while **fungal mycetoma** is resistant to antibiotics and needs **surgical** interference or amputation. However, prolonged fungal chemotherapy may be tried, e.g. ketoconazole, flucytosine and even amphotericin B.

Diagnosis: Macroscopic examination of granules. Black and white granules are common with fungi, yellow granules are common with actinomycetoma

- Microscopic examination of crushed granules will reveal hyphae in fungal infection and fragmented filaments in bacterial infection.
- Cultivation on SDA incubated at room temperature and on blood agar incubated aerobically and anaerobically. (Table p. 185)

SYSTEMIC (ENDEMIC) MYCOSES

These infections result from inhalation of the spores of dimorphic fungi that have their saprophytic filamentous forms in the soil. Within the lungs, the spores differentiate into yeasts or other specialized forms. Most lung infections are asymptomatic and self-limited. However, in some persons, mainly irrmiunocompromised patients, infection may disseminate to other organs and may be fatal. Infected persons do not transmit the disease to others.These diseases are endemic in the Americas, rarely in Africa and the Middle East and include; histoplasmosis, coccidioidomycosis and blastomycosis.

Histoplasmosis caused by *Histoplasma capsulation*

The infection occurs in the lungs as acute pneumonia or as chronic cavitary lesions. The organism may spread to the spleen and liver inside macrophages causing lymphadenopathy or hepato-splenomegaly.

Coccidioidomycosis caused by *Coccidioides immitis*

Lesions occur in the lungs and can spread by direct extention or *via* the blood causing granulomatous lesions in any organ mainly the bone and CNS. Skin test is positive.

Blastomycosis caused by *Blastomyces dermatitidis*

Respiratory infections are mild or asymptomatic. Dissemination may result in ulcerated granulomas of the skin, bone or other sites.

Diagnosis of systemic mycoses:

1- Microscopic examination of biopsy specimens, bone marrow aspirates or blood films, after staining by periodic acid- Schiff or calcofluor white or Giemsa stains.
2- Culture of sputum, urine, bone marrow aspirate and tissue biopsy, on SDA at 37°C and at 25°C. Cultures are identified by detection of specific antigens or by DNA probes.
3- Serologic tests show a rise in antibody titres when dissemination occurs.
4- Skin tests using fungal antigens e.g. coccidioidin or histoplasmin.
5- Detection of fungal antigens in specimens by RIA and fungal nucleic acids by DNA probes or PCR.

OPPORTUNISTIC MYCOSES

Opportunistic fungi fail to induce disease in most normal persons. But can do so in those with impaired host defenses.

Candida

Candida albicans, is the most important species of Candida. Other species include C. *tropicalis, C. parapsilosis, C. krusei* and others.

C. *albicans* are gram positive oval budding yeasts which produce pseudohyphae. It is part of the normal flora of mucous membranes of the upper respiratory, gastrointestinal and female genital tract. In these sites, it may predominate and cause superinfection.

Pathogenesis and clinical findings:

Predisposing factors to **Candida** infections are diabetes mellitus, general debility, immunodeficiency, indwelling urinary catheters, intravenous drug

abuse, prolonged treatment with broad-spectrum antibiotics, and corticosteroids. Clinical affections include:

1- In the mouth, overgrowth of C. *albicans* produces white patches i.e. oral thrush or moniliasis.
2- Vulvovaginitis with itching and discharge which is favoured by prolonged use of antibiotics and diabetes.
3- Skin invasion occurs in warm moist areas, which become red and weeping such as the axilla, intergluteal folds, or inframammary folds most common in obese and diabetics.
4- Nails become involved when repeatedly immersed in water; as in persons involved in dish washing. Painful redness and swelling of nail folds, thickening and loss of nail i.e. paronychia.
5- Systemic candidaisis and chronic mucocutaneous candidiasis may occur in debilitated children, diabetics, immunosuppressed patients or drug addicts. They are usually associated with deficient CMI. Many patients are unable to mount an effective Thl7 response to *Candida*

Laboratory diagnosis:

1- Direct microscopic examination of smear or exudates from the lesions shows large oval gram positive budding yeast cells with pseudohyphae.
2- Cultures are done on nutrient agar, corn meal agar and SDA. Colonies are soft, cream-coloured with a yeasty odour. They are also identified by;
a- Morphology; oval budding gram positive yeast cells.
b- Germ tube formation in serum incubated at 37°C for 30min -2hrs.
c- Chlamydospore formation on corn meal agar.
d- Biochemical reactions: C. *albicans* ferments glucose and maltose with acid and gas production. The last three properties (b, c, d) differentiate *C. albicans* from other **Candida.**

Treatment: Fluconazole is the drug of choice for oropharyngeal or oesophageal thrush. Nystatin and clotrimazole ointments are used for skin lesions. Ketoconazole is used for mucocutaneous candidiasis and with amphotericin B for disseminated candidiasis.

Cryptococcus neoformans

C. neoformans are yeast cells with a gelatinous capsule. It is found in soil contaminated with the excreta of birds specially pigeons' faeces. It is an opportunistic pathogen affecting mainly immunosuppressed individuals specially AIDS patients. Infection occurs by inhalation where it causes subclinical lung affection or pneumonia. It may spread systemically to the CNS causing meningoencephalitis or to, the skin, adrenals, bone or eyes.

Laboratory diagnosis:

1- Direct microscopic examination of sputum and CSF after staining with India ink, reveals large gelatinous capsule around budding yeast cells.

2- Cultures done on SDA without actidione, at 20-37°C show mucoid colonies which are identified by India ink staining, biochemical reactions (urease positive), pathogenicity to mice and DNA probes.

3- Capsular antigens are detected in CSF, serum or urine by using anticapsular antibodies in a latex agglutination test or enzyme immunoassays.

4- Antibodies are detected in patients' sera.

Aspergillosis

Aspergillus species are wide spread saprophytic moulds. *Aspergillus fumigatus* is the most common human pathogen. *A.flavus* and *A. niger* may be involved. Infection occurs by inhalation of the spores (conidia). It causes the following clinical conditions:

1- Allergy in atopic individuals e.g. asthma.

2- Aspergilloma "fungus ball" which is due to growth of the fungus inside lung cavities specially those caused by tuberculosis.

3-Invasive aspergillosis affects mainly immunocompromised persons causing acute pneumonic process with or without dissemination.

4-Aflatoxin production in spoiled grains. It is hepatotoxic and carcinogenic.

Diagnosis: Aspergillus grows on most media at room temperature.

Pneumocystis jiroveci

Pneumocystis is an opportunistic **fungus.** It is present in the lungs of many animals but rarely causes disease unless the host is immunosuppressed. *P. jiroveci* is the human species and the more familiar *P. carinii* is found only in rats. Serologic evidence suggests that most individuals are infected in early childhood and the organism has worldwide distribution. It causes **interstitial pneumonia** in malnourished infants and immunosuppressed patients (AIDS, corticosteroid therapy, transplant recipients...etc). It was a major cause of pneumonia and death in AIDS patients until the use of chemoprophylactic regimens. Cell mediated immunity plays a major role in resistance to disease.

P. jiroveci has two morphologicaly distinct forms; thin-walled trophozoites and cysts, which are thick-walled, spherical to elliptical and contain 4-8 nuclei. In clinical specimens, the trophozoites and cysts are present in a tight mass.

It is transmitted by inhalation; growth in the lungs is limited to the surfactant layer above the alveolar epithelium causing interstitial pneumonitis and frothy exudates that blocks oxygen exchange. Extrapulmonary infections

occur in the late stages of AIDS and affect the liver, spleen, lymph nodes and bone marrow. Mortality is 100% in untreated cases.

Diagnosis: Bronchoalveolar lavage, lung biopsy or induced sputum are stained by Giemsa, toluidine blue, methenamine silver or calcofluor white and examined for the presence of trophozoites or cysts. Direct immunofluorescent staining using monoclonal antibodies and PCR are used for diagnosis.

Treatment: Trimethoprim-sulfamethoxazole (TMP-SMZ) is the drug of choice. Pentamidine and atovaquone are alternative drugs.

Prophylaxis: TMP-SMZ or aerosolized pentamidine can be used for chemoprophylaxis in AIDS patients whose CD4 counts are below 200.

ANTIFUNGAL DRUGS

Selective toxicity is very limited in antifungal drugs due to the fact that fungi, like human cells are eukaryotes. The available drugs are those which bind to ergosterol in the cell membrane or inhibit its synthesis. Others act by inhibiting chitin synthesis in the cell wall e.g.:

Amphotericin B acts by binding to ergosterol in the cell membrane causing its destruction. It is used in severe systemic and opportunistic mycoses. The drug is nephrotoxic, however, the new lipoidal emulsions are less toxic.

Flucytosine inhibits fungal DNA synthesis. It is an oral antifungal drug used in conjunction with amphotericin B to treat candidiasis and cryptococcosis. Side effects include bone marrow suppression, liver affection and hair loss.

Azoles including ketoconazole, triazoles (fluconazole, itraconazole, voriconazole) are oral drugs used to treat a wide range of systemic and localized fungal infections. They act by inhibiting ergosterol synthesis. Ketoconazole is the most toxic, and may inhibit the synthesis of testosterone and Cortisol leading to gynaecomastia, impotence, menstrual irregularity.

Griseofulvin is an oral drug for treatment of dermatophytes. It is concentrated in the stratum corneum and keratinized tissues, where it inhibits hyphal growth. Prolonged treatment for months is required.

Terbinafine inhibits ergosterol synthesis. It is used to treat dermatophyte infections.

Caspofungin is a new antifungal drug that blocks cell wall synthesis. It is used to treat invasive aspirgillosis, oropharyngeal, oesophageal and invasive candidiasis. It is given by a slow I. V. infusion and has minimal side effects

Topical antifungal agents include; Nystatin used to treat candidal infections of the skin, mouth and vagina. Clotrimazole, miconazole and other azoles have a broad-spectrum of activity against; ringworm, tinea versicolor and cutaneous candidiasis. Tolnaftate and naftifine are more effective.

CHAPTER **22**
MINOR BACTERIAL PATHOGENS

Gardnerella vaginalis **is a normal inhabitant of the vagina. However, in association with anaerobic bacteria *(Mobilluncus)*, they cause "bacterial vaginosis" which is characterized by: 1-A foul-smelling discharge. 2- Detection of "clue cells" which are epithelial cells coated with gram variable cocco-bacilli of** *G. vaginalis* **in vaginal or cervical swabs. 3-The "whiff" test, which consists of treating the vaginal discharge with 10% KOH and smelling a pungent, "fishy" odour, is often positive. 4- Vaginal pH is more than 4.5. These 4 criteria used for the diagnosis of bacterial vaginosis are known as Amsel's Criteria. Women with bacterial vaginosis have a higher incidence of morbidity or mortality in their newborn. Metronidazole is the drug of choice for treatment.**

Lactobacilli are obligate anaerobic gram positive bacilli. They are normal microbiota in the mouth, colon and vagina. In the mouth, they may play a role in the production of dental caries. In the vagina, they have beneficial protective effect due to production of lactic acid, which keeps the pH low and inhibits colonization of the vagina with pathogenic organisms. Suppression of lactobacilli by antibiotics can lead to superinfection with *C.albicans* **resulting in** *Candida* **vaginitis. Lactobacilli are rare causes of opportunistic infections.**

Francisella tularensis **causes tularaemia in wild animals. Rabbits, deers and rodents are the most important reservoirs. Man is accidentally infected through the bite of the vector (ticks, mites, lice and flies), by contact with infected animals, by ingestion of infected meat or by inhalation. It presents by an ulcer at the site of the bite, fever and lymphadenopathy ulceroglandular type. Other clinical forms of the disease include; glandular, oculoglandular. tvphoidal, oropharyngeal and pneumonic tularaemia. Laboratory workers are at high risk of infection while handling infected specimens. A vaccine is required for these personnel and for those whose occupation brings them to close contact with wild animals. Due to its highly infectious pathogenic nature, it is a dangerous potential agent of bioterrorism and is classified as category A agent. It is diagnosed serologically and treated with streptomycin or gentamycin. *Aeromonas*** Species **are gram negative bacilli found in water, soil, food, animals and human faeces. *A. hydrophila* causes wound infection and diarrhoea which may be cholera or desentry-like. In immunocompromised patients it causes bacteraemia and septicaemia. It is oxidase positive, catalase positive and is differentiated from vibrios by being string test negative. It is motile in distilled water while vibrios are not. It is resistant to the compound 0/129 to which vibrios are susceptible. *Bartonella* species are pleomorphic gram negative bacilli. *Bartonella henselae* is the cause of cat-scratch fever characterized by cutaneous papule or pastule and localized lymphadenopathy and bacillary angiomatosis in immunocompromised individuals, especially AIDS patients. It is a proliferative, vascular lesion resembling Kaposi sarcoma. The organism is a member of the oral flora of cats. It is transmitted from cat to cat by fleas. Cat scratches or bites, are the main mode of transmission to humans. *B. quintana* causes trench fever transmitted by body lice. Humans and body lice are the reservoir for the organism. It may cause bacillary angiomatosis.**

PART II MEDICAL

VIROLOGY

CHAPTER 23

GENERAL PROPERTIES OF VIRUSES

Viruses are parasites at the genetic level. They are the smallest infectious agents known. They can infect man, animals, insects, plants and bacteria. The following properties distinguish viruses from other microorganisms:

1-They are very small in size.

2-They contain one kind of nucleic acid (RNA or DNA) as their genome.

3-They are metabolically inert, as they do not possess ribosomes or protein synthesizing apparatus.

4-They are **obligate intracellular parasites** i.e. can only replicate inside living cells. They require the biological machinery of a host cell for reproduction and survival.

5-Hence, they cannot be grown on artificial culture media and are grown in tissue culture, embryonated eggs or living animals.

6-They are not susceptible to antibacterial antibiotics.

Size of viruses:

They vary in size from 20-300 nm Due to their small size: **a-**

They can pass through bacterial filters.

b-They require high speeds (ultracentrifugation) for their sedimentation 10,000-30,000 rpm (bacteria require 1,000-3,000 rpm).

c- They are only seen by electron microscopy (EM), except poxviruses. EM is the best method for measuring the size of viruses.

STRUCTURE OF VIRUSES:

Each virus particle or virion is composed of a protein coat or **capsid** and a **nucleic acid core.** The capsid with its enclosed nucleic acid is called the **nucleocapsid.** Many viruses are naked but some viruses are enveloped (Fig. 1). Other internal proteins found in some viruses are **enzymes,** and **matrix proteins** which are present in enveloped viruses and mediate the interactions between the capsid and envelope.

Viral Capsid:

It is composed of small protein subunits called capsomers.The arrangement of the capsomers determines virus symmetry. Functions of the capsid are:

1- It protects the viral genome (DNA or RNA) against inactivation by nucleases.

2- It is responsible for the structural symmetry of virions i.e. icosahedral or helical.

3- It participates in attachment of virions to susceptible cells.

4- Capsid proteins are important antigens that induce antibodies that neutralize virus infectivity and, activate cytotoxic T cells to kill virus-infected cells.

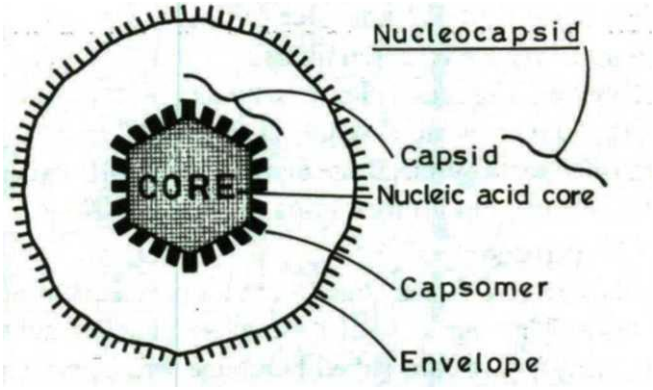
5- Variation in capsid proteins is responsible for the different viral serotypes in non-enveloped viruses.

Viral Nucleic Acid (genome):

Viruses contain either DNA or RNA but not both. Most DNA viruses are double stranded, while most RNA viruses are single stranded. The nucleic acid may be linear or circular. Some RNA viruses have segmented genome e.g. rotavirus and influenza virus. The molecular weight and type of nucleic acid are specific for each virus group. All viruses have one copy of their genome (haploid) except retroviruses which have two copies (diploid).Viral genomes are used as vectors in gene therapy and in recombinant avirulent virus vector vaccines. Functions of the nucleic acid are:

1- It is the infectious part of the virus; coreless particles are non-infectious.

2- It carries the genetic information for (a) Virus replication, (b) Virulence or ability to parasitize cells, (c) Antigenic specificity of the protein coat.



(Fig. 1): Diagram showing the structure of a complete enveloped virus particle.

Viral Envelope:

Many viruses are surrounded by a lipid or lipoprotein envelope, which may be covered by glycoprotein spike-like projections, which attach to host cell receptors during the entry of the virus into the cell, e.g. haemagglutinin (HA) and neuraminidase (NA) spikes in influenza virus. Due to their lipid content, such viruses are sensitive to ether. Loss of lipids results in disruption of virus and loss of infectivity. The envelope may be partially or completely derived from host membranes during release from the cell by budding.

The surface proteins; whether the virus capsid proteins or the envelope glycoproteins are the principal antigens against which the host mounts its immune response. They are also the determinants of type specificity.

Viral Enzymes; some viruses carry enzymes e.g. RNA polymerase, which is present in negative sense RNA viruses to copy their mRNA e.g. orthomyxoviruses and the reverse transcriptase enzyme (RT) present in retroviruses to make a cDNA copy of the viral RNA.

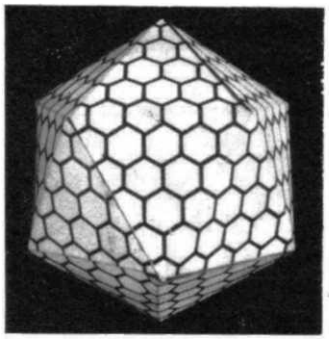
Virus Symmetry: The arrangement of the capsomers, in the capsid, gives the virus its geometric symmetry. Viruses have 3 types of symmetry:

1- Cubical symmetry: These viruses resemble a crystal and are called icosahedral viruses e.g. herpesviruses and adenoviruses (Fig. 2).

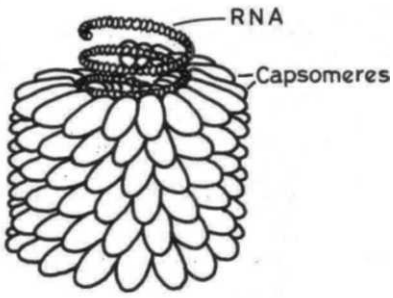
2- Helical symmetry in which the particle is elongated. The capsomers are arranged in a ribbon which is wound in the form of a helix or spiral around the spiral nucleic acid (Fig. 3). All human viruses that have helical symmetry are enveloped e.g. influenza virus.

3- Complex symmetry in which the viruses are complicated in structure e.g. poxviruses which are brick shaped with ridges on the external surface. The bacteriophage is another example of complex symmetry.

Atypical virus-like agents:



(Fig. 2): Icosahedral viral symmetry.



(Fig. 3): Helical viral symmetry.

1- Defective viruses are composed of viral nucleic acid and proteins but cannot replicate without a helper virus, which provides the missing function. These usually have a mutation or a deletion of part of their genetic material. During the growth of most human viruses, many more defective than infectious virus particles are produced.

2- Pseudovirions contain host cell DNA instead of viral DNA within the capsid. They are formed during infection with certain viruses when the host cell DNA is fragmented and pieces are incorporated within the capsid. Pseudovirions can infect cells, but they do not replicate.

3- Prions are infectious particles that are composed solely of protein. They contain no detectable nucleic acid. They cause slow diseases (Chapter 37).

***An icosahedron is a geometric partem with 20 triangular facets and 12 corners e.g. adenoviruses.**

METHODS OF CULTIVATION OF VIRUSES

Since viruses are obligate intracellular parasites, they have to be grown in living cells. There are three systems for their cultivation:

1- Cell cultures. **2-** Embryonated eggs. **3-** Laboratory animals.

Although embryonated eggs and laboratory animals are useful for the isolation of certain viruses, cell cultures in monolayer are the main isolation system used in most clinical virology laboratories. **I- Cell cultures:**

Pieces of animal or human tissues are trypsinized to get separate cells. These are grown in presence of growth medium containing serum, on glass or plastic tubes, bottles or plates with a flat side. A monolayer or sheet of cells is formed on the flat side of the container within few days. Viruses are inoculated on the monolayer. There are three types of tissue cultures:

1- **Primary cell lines:** These are prepared from organ fragments e.g. monkey kidney. Such cells can only divide for several passages (4-6) and then degenerate.

2- **Human diploid cell lines:** These are usually fibroblasts derived from human embryo tissues. They have diploid number of chromosomes. They grow rapidly and can be subcultured up to about 50 passages in culture e.g. human embryo lung tissues.

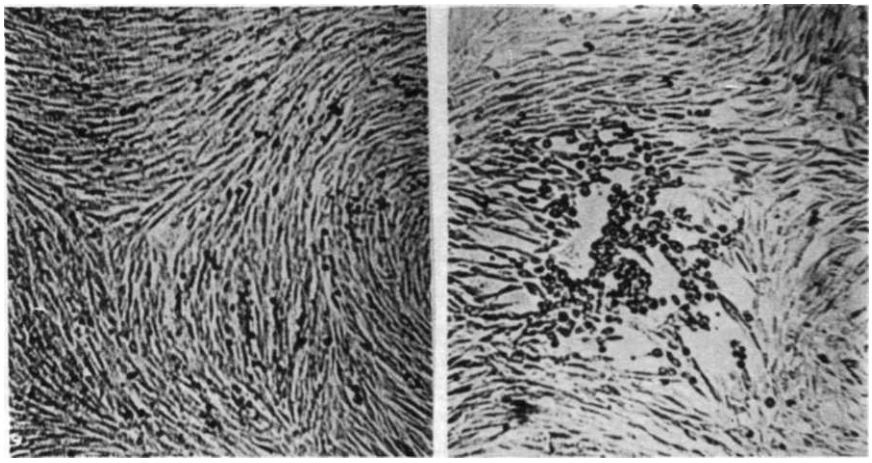
3- **Continuous (heteroploid) cell lines:** These are derived from tumour cells and they can divide indefinitely e.g. HeLa cell line, derived from carcinoma of the cervix, or HEp-2 cell line, derived from human epidermoid carcinoma of the larynx.

Detection of virus replication in cell culture:

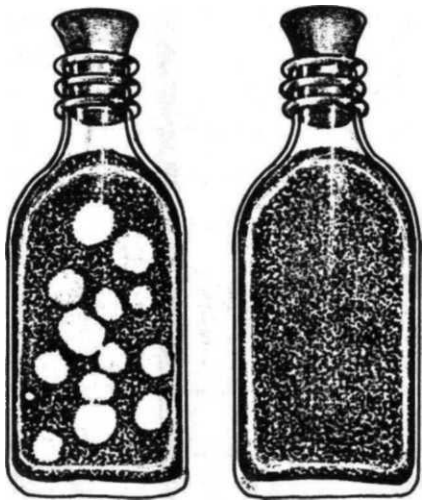
1- Cytopathogenic effects (CPE): These are changes in cells that can be observed microscopically: **a-** Cell death and detachment from the glass surface is produced by many viruses e.g. pohovirus (Fig. 4). **b-** Rounding and grape-like cluster formation is produced by adenovirus, **c-** Syncytium or multinucleated giant cell formation are characteristic of measles or mumps, **d-** Cell transformation: The cells lose the property of contact inhibition* present in normal cells and pile up to form foci of malignantly transformed cells e.g. when infected with tumour viruses.

'Contact inhibition: In monolayer cell cultures, normal cells stop division when they come in contact with each other.

2-Plaque formation: **Plaques are virally infected areas in cell culture monolayer covered with an agar overlay. They can be seen by the naked eye as unstained areas when using vital stains, e.g. neutral red, and can be used for quantitation of virus**



(Fig. 4): To the left, uninfected tissue culture monolayer. To the right, infected tissue culture showing CPE; cells are lysed and fall off the glass.



(Fig. 5): To the right, a bottle of uninfected tissue culture monolayer. To the left, a bottle of infected tissue culture showing plaques.

3- Inclusion bodies: **These are intranuclear (e.g. herpesviruses) or intracytoplasmic (e.g. poxvirus, or rabies virus) structures which may appear in virus infected cells and can be seen by light microscopy. They are often the site of virus replication. Their presence is of diagnostic value e.g. the "Negri bodies" in the cytoplasm of nerve cells of rabid animals.**

particles as each plaque is produced by a single virus particle (Fig. 5).

4- A decrease in acid production by infected dying cells, which is detected visually by a colour change in the phenol red (a pH indicator) in the culture medium.

5- Haemadsorption (Fig. 6): When RBCs are added to infected cells they will appear as rosettes or clumps on the areas where the virus is growing. This is useful in haemagglutinating viruses e.g. mumps, influenza and parainfluenza viruses.

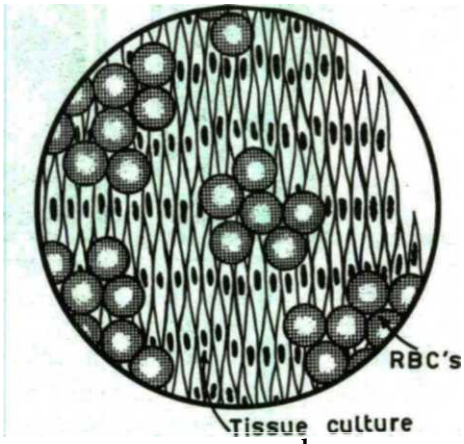
6- Fluorescent-antibody staining: Infected cell sheets on cover slips or microtitre plates may be treated with fluorescein labelled specific antibody and examined for positive fluorescence.

7- **Interference:** In some viruses, which do not produce CPE, their growth can be proved by their ability to interfere with the growth of a second CPE producing virus. For example, rubella virus, which does not cause a CPE, can be detected by interference with the formation of a CPE by certain enteroviruses e.g. echovirus.

8- Detection of viral antigens by serology: Soluble antigens which diffuse in the nutrient medium or those released after freezing and thawing of tissue culture cells, can be detected by any serologic method including complement fixation, haemagglutination inhibition, ELISA ....etc.

9- Neutralization tests: Neutralization of the effects of virus on tissue culture. This is done by mixing the virus with specific antiserum then the mixture is added to an appropriate cell line. Absence of CPE indicates virus neutralization. This can be used to identify and type the virus isolated.

10- Detection of virus specific nucleic acid by PCR provides rapid, sensitive,



(Fig. 6): Diagram of haemadsorption: Clumps of RBCs are adhering to infected cells in the tissue culture monolayer.

and specific methods for detection.

II- Embryonated Eggs:

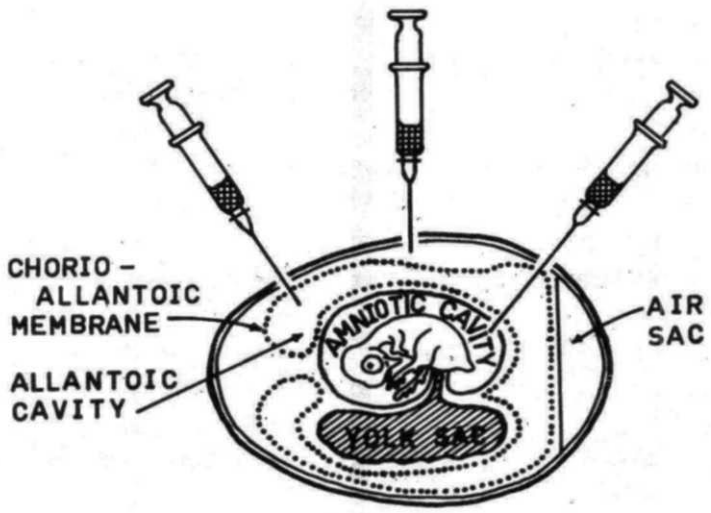
Different viruses can grow in various cavities of embryonated eggs or in the developing embryo itself. The age of the embryo used and the site of inoculation vary according to the virus inoculated (Fig. **7).**

Chorioallantoic membrane inoculation is used in pox and herpesviruses. The influenza virus can readily grow in the amniotic sac and in the respiratory cells of the embryo.

Ill- Laboratory Animals:

Animal inoculation was mainly used in the past when tissue culture methods were not known. However, animal inoculation is still used for studying viral oncogenesis, pathogenesis of viral diseases, immune response to viruses and for primary isolation of some viruses.

The white suckling mice are the most widely used; they are susceptible to the togaviruses that cause encephalitis, when they are injected intracerebrally. However, several cell lines and cell cultures derived from insects are now available for many of these viruses.



(Fig. 7): Diagram of chick embryo showing routes of inoculation for viral isolation.

DIAGNOSIS OF VIRUS INFECTIONS

The laboratory procedures used in diagnosis of viral diseases include: **A-** Direct detection of viruses, their antigens, or their nucleic acids in clinical specimens. B-
Isolation of viruses.

C- Serologic detection of antiviral antibodies.

D- Skin tests to detect cell mediated immunity in some viruses.

A- Direct detection of viruses, their antigens, or their nucleic acid in clinical specimens can be achieved by different techniques:

1- **Light microscopy:** This can be used to visualize some large viruses e.g. poxviruses in which elementary bodies can be seen in skin lesions (papules and vesicles). Inclusion bodies can also be seen under the light microscope in several viral infections. In rabies, intracytoplasmic inclusions called "Negri bodies" can be detected in nerve cells.

2- **Electron microscopy** is used to demonstrate virus particles in vesicular fluid or tissue extracts treated with special stains. It is only successful if large numbers of particles (10^9 /ml) are present.

3- **Immunoelectron microscopy** (HEM): Addition of specific antisera to the clinical material leads to aggregation of virus, so it can be detected more readily than separate virus particles e.g. diagnosis of hepatitis A virus and rotavirus in stools (Fig. 8).

4- **Immunofluorescence microscopy:** Detection of virus in smears from lesions using fluorescein labelled specific antisera e.g. diagnosis of rabies in brain smears.

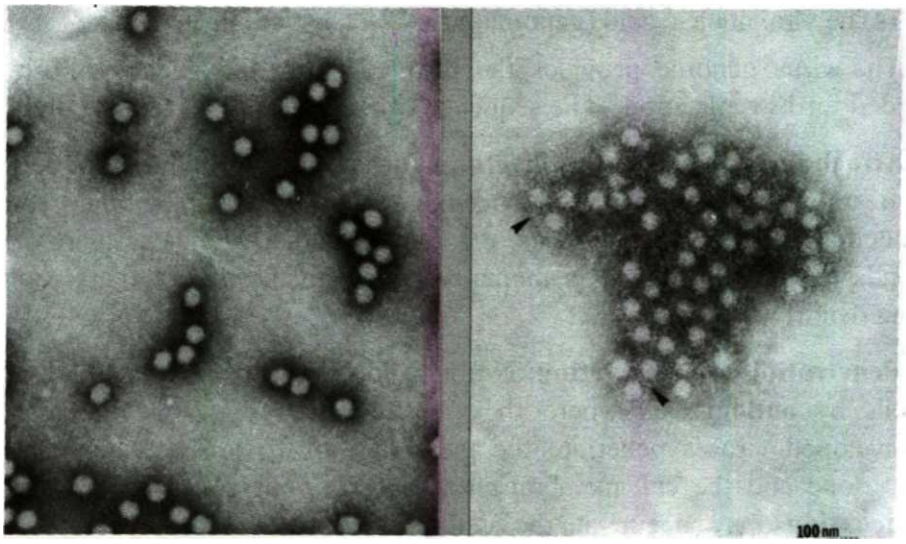
5- **Solid-phase immunoassays:** Both radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) can be used for detection of **viral antigens** in different clinical specimens e.g. detection of hepatitis A and rotavirus in stools and the detection of hepatitis B surface antigen or p24 antigen of the human immunodeficiency virus (HIV) in blood.

6- **Nucleic acid hybridization:** Using DNA probes, it is possible to detect virus nucleic acid in pathologic specimens or in tissue samples. The probe which is a single strand of the nucleic acid of the virus in question will hybridize with its complementary strand in the specimen. Probes are labelled and can be easily detected.

7- **Polymerase chain reaction (PCR):** This technique involves amplification of a short sequence of a target DNA or RNA (which may be in low concentration e.g. one copy) leading to accumulation of large amounts of that sequence, so it can be easily detected.

N.B. In the near future nucleic acid based technology using high density microar-ray and deep sequencing will likely change approaches to vial

PCR is used to determine the quantity of viruses in patients' blood i.e. **virus load** e.g. in HIV patients, which helps in monitoring the course of the disease and in evaluation of treatment and prognosis.



(Fig. 8): E.M. micrograph showing; to the left dispersed virus particles. To the right the same virus aggregated by specific antibodies.

B- Isolation of viruses:

Isolation of virus from clinical specimens by inoculation on cell culture, chick embryo or laboratory animals according to the virus in question.

C- Serologic detection of antiviral antibodies:

Serologic diagnosis of virus infections can be established by detecting a rising antibody titre to the virus. The first sample should be collected early after onset (acute phase), the second sample 10-14 days later. A 4-fold rise in antibody titre in the second sample indicates infection.

If paired sera are not available or rapid diagnosis is needed, as in diagnosis of rubella in early pregnancy; detection of IgM antibodies to the virus is resorted to. The detection of IgM in a single serum sample, indicates recent infection. The presence of IgM antibodies to any virus in the newborn serum indicates infection *in utero* (IgM does not cross the placenta) e.g. CMV, rubella, herpes viruses or varicella.

The tests used include virus neutralization, complement fixation, ELISA haemagglutination inhibition, immunofluorescence, radioimmunoassay, or Western blot.

D- Skin tests are used to diagnose cell mediated immune response against some viral infections e.g. mumps.

VIRUS REPLICATION

Viruses have no metabolic activity of their own. Therefore, they depend on living host cells for providing energy and the synthetic machinery and the low molecular-weigh precursors for the synthesis of:

1-The viral nucleic acid (genome) and **2-** The viral proteins.

The virus genome provides the host cell with the genetic information needed for its replication. The sequence of events for virus replication is:

I- Attachment: Virus and cell are brought into contact by random collision, but attachment is specific and occurs only if the cell membrane contains specific receptors for the virus, e.g. human immunodeficiency virus (HTV) binds to CD4 receptors on immune cells i.e. helper T cells, macrophages and dendritic cells.

II-Penetration and uncoating: Non-enveloped virions are taken into animal cells by **endocytosis** where they are uncoated by lysosomal enzymes. Enveloped viruses penetrate the membrane by **fusion** between the virus envelope and the cell membrane releasing the nucleocapsid into the cell; **uncoating** may occur at the cell surface e.g. bacteriophages, in the cytoplasm e.g. pohovirus, or in the nucleus e.g. herpesvirus. Uncoating renders viral nucleic acid accessible for transcription and replication.

III-Eclipse: It is the period after penetration during which no infectious virus can be detected inside the host cell. During this phase, the cell is redirected, by the viral nucleic acid (genome), toward synthesizing viral components. The eclipse phase ends with the appearance of virus particles.

IV-Intracellular viral synthesis: It includes synthesis of both viral nucleic acid and proteins. The viral nucleic acid (genome) replicates by using a strand of the parental nucleic acid as a template for the production of progeny DNA or RNA molecules.

The essential step in protein synthesis is transcription of mRNA from viral nucleic acid. The mRNA is translated by the host cell ribosomes into viral proteins, some of which are early proteins i.e. enzymes required for replication of the viral genome, and others are late proteins, i.e. structural proteins of the progeny viruses (capsids and enzymes).

Some viral mRNAs are translated into precursor polypeptides that must be cleaved by proteases to produce the functional structural proteins as in HIV, whereas other viral mRNAs are translated directly into structural proteins.

The mRNA transcription varies depending on the nucleic acid type:

a- In double stranded (ds) DNA viruses, mRNA is transcribed from the negative strand of DNA by DNA dependent RNA polymerase (transcriptase) e.g. herpesviruses.

ds DNA ____ - +ss DNA - ss DNA —RNA polymerase→ mRNA

b- In single stranded (ss) RNA viruses of positive polarity (+ sense), the ssRNA itself acts as mRNA for translation into proteins e.g. picornaviruses, togaviruses and flaviviruses.

+ ssRNA = mRNA

c-The ssRNA viruses of negative polarity (- sense) must be transcribed by RNA dependent RNA polymerase - which is present in the virus - into complementary (+ sense) mRNA e.g. rabies or influenza viruses.

- ssRNA —polymerase→ + ssRNA = mRNA

d-The ssRNA of retroviruses (+ sense) is transcribed by a unique virion associated reverse transcriptase enzyme into complementary ssDNA which is converted into dsDNA, which becomes integrated into the cellular genome causing malignant transformation of cells *in vivo* and *in vitro.* It may be transcribed into mRNA as in HIV.

+ ssRNA —Reverse transcriptase→ ssDNA -4 dsDNA totegrated ^S, Transcribed to mRN/

V- **Assembly** of viral nucleic acid and protein coats to form mature virus particles occurs in the cytoplasm e.g. pohovirus or in the nucleus e.g. herpes viruses.

VI- **Release:** Mature virus particles accumulate in the cell in enormous numbers and are released by either of two processes. One is by rapturing the cell i.e. **cytolysis,** which usually occurs with non-enveloped viruses. The other is by a slow process of leaking or **budding** through the cell membrane, which occurs with enveloped viruses, where they acquire lipoprotein envelope during budding from the outer cell membrane (this is mediated by matrix proteins). Herpes viruses acquire their envelope from the nuclear membrane.

CHAPTER 24

PATHOGENESIS AND TREATMENT OF VIRUS INFECTIONS

PATHOGENESIS OF VIRUS INFECTIONS

Viruses should reach a susceptible cell before they can produce disease. Therefore, they should have:

1- Portal of entry; namely the respiratory tract, gastrointestinal tract (GIT), skin, urogenital tract or conjunctiva.

2- A pathway through the body; namely the blood, lymphatics or nerves.

3- A target organ which may be CNS, skin, glands, liver ... etc.

Many viral infections are subclinical or **inapparent.** The same virus may produce a variety of diseases. The same disease may be produced by a variety of viruses. The outcome of virus infections is determined by the interactions of the virus and the host and is influenced by the genetics of each. Viruses may produce **local** or **systemic** infections, which may reach the full-blown picture, or more commonly end in a subclinical form. Some viruses **persist.**

I- Local infections which occur **at the portal of entry** with **no viraemia** e.g. viral influenza and common cold at the mucous membrane of the respiratory tract and rotavirus infection at the GIT causing diarrhoea.

Local infections are characterized by **short incubation period, short lasting immunity** that is mediated by **IgA** and **interferon.**

II- Systemic infections: After primary replication at the site of entry, the virus travels through the blood or lymphatics causing **viraemia,** or through the nerves to reach a **distant target organ** that has specific receptors for the virus e.g. pohovirus, mumps, measles, rabies and hepatitis.

Systemic infections are characterized by: **Long incubation period, long lasting immunity** that is mediated by **IgM** and **IgG** . Infection can be **stopped** at the viraemic stage by immune mechanisms i.e. **neutralizing antibodies.** This leads to subclinical or abortive infections (Vol. I, p. 96). **Gamma globulins** given to contacts of a case may **abort infection,** if given during the incubation period before the viraemic stage.

III- Persistent viral infections: Sometimes viruses persist for a long time in the host in one of the following forms:

a-Chronic infections in which the virus can be continuously detected with no or mild symptoms e.g. hepatitis B and C chronic carriers.

b- Latent infections in which the virus persists hidden most of the time, with periodic reactivation and development of clinical lesions containing the virus e.g. herpes viruses and HIV infections.

c-"Slow virus" infections: These have a very long incubation period of months or years with no clinical symptoms e.g. subacute sclerosing panencephalitis with incubation period of 2-20 years and is caused by a variant of measles virus and AIDS. Other slow infections caused by non-conventional agents called prions e.g. Creutzfeldt-Jakob disease and kuru in man, scrapie in sheep and mad cow disease in cattle (Chapter 37).

Mechanisms that help persistence of viruses include:

1- Integration of a DNA provirus into host cell DNA e.g. retroviruses.
2- Rapid antigenic variation.
3- Virus-spread from cell to cell without an extracellular phase.
4- Location within an immunologically sheltered sanctuary e.g. the brain.
5- Decreased expression of MHC-I by the action of viral genes which leads to decreased recognition of virus infected cells by CTLs.
6- Formation of virus-antibody complexes which remain infectious.
7- Immunosuppression as in AIDS.

TREATMENT OF VIRUS INFECTIONS

Since viruses are obligate intracellular parasites, it is difficult to find an antiviral drug that selectively inhibits the virus without affecting the cell (selective toxicity). Few antiviral drugs are in clinical use. They act by stopping virus attachment, penetration, uncoating or intracellular synthesis.

Human immunodeficiency virus (HIV) chemotherapy: The drugs used act by stopping virus-cell fusion and entry or intracellular synthesis by inhibiting the reverse transcriptase, protease or integrase enzymes. These drugs inhibit production of infectious viral particles but do not affect the proviral DNA.

I- Reverse transcriptase (RT) inhibitors

a-Nucleoside/nucleotide RT inhibitors

Zidovudine (AZT): Is a thymidine analogue that inhibits the replication of HIV by inhibiting viral RT. AZT reduces morbidity and mortality. It causes bone marrow depression and is very expensive. Dideoxyinosine (ddl) and dideoxycytidine (ddC): They also inhibit HIV as described for AZT; however, they are less toxic. Stavudine (d4T) and lamivudine (3TC) are used in HIV patients intolerant or resistant to AZT or ddl. b-Non-nucleoside RT inhibitors e.g. Nevirapine, efavirenz and delaviridine.

II-Protease inhibitors

Saquinavir, ritonavir, indinavir, lopinavir and nelfinavir (Viracept) are inhibitors of the protease encoded by HIV thus inhibiting cleavage of precursor polypeptides required for viral replication. The main side effect of this group is the deposition of fat in the back of the neck "buffalo hump" and in other parts of the body.

III- Fusion inhibitors; **enfuvirtide (fuzeon). It is a synthetic peptide that binds to gp41 on the viral envelope, blocks fusion with the cell membrane and inhibits entry of HIV into the cell. It is administered by injection and is expensive. It is approved as "salvage therapy" for those whose previous therapy is no longer working.**

IV- Integrase inhbitors; Raltegravir (RA1, isentress) inhibits integration of viral genetic material in host chromosome.

V- Chemokine receptor antagonists; Maraviroc and Vicriviroc, these prevent the binding of HIV to the CCR5 receptor.

A combination of 2 nucleoside analogues (AZT and ddl or 3TC or d4T) in addition to a protease inhibitor is the treatment of choice for acute HIV infections. This regimen lowers the virus load to undetectable levels, increases the CD4 count and increases CD8 activity. It prolongs and improves the quality of life, but does not cure the latent infection.

The combined antiretroviral therapies for treatment of HIV are called HAART or "highly active antiretroviral therapy". These combinations help in delaying the emergence of resistant variants. However, drug resistant mutants have emerged that affect the ability of both reverse transcriptase inhibitors and protease inhibitors to sustain their clinical efficacy. Other combinations are used.

Drugs used for treatment of herpes viruses, or influenza viruses:

Acyclovir is an analogue of guanosine that inhibits herpes simplex virus with little effects on host cells. It inhibits virus specific DNA polymerase. It is used topically for treatment of herpetic corneal ulcers and for herpetic skin lesions. Parenteral aa^ninistration is used in treatment of serious systemic infections, e.g. herpesvirus encephalitis. It is effective in prevention of systemic infection by HSV-1 or varicella-zoster virus in immuno-comprornised patients. However, acyclovir resistant mutants have emerged.

Ganciclovir: It is a nucleoside analogue of guanosine, structurally similar to acyclovir but is more active against CMV than acyclovir. It is effective in treatment of retinitis caused by CMV in AIDS patients.

Foscarnet inhibits the DNA polymerases of all herpesviruses. It is used for treatment of acyclovir resistant herpesvirus infections and CMV retinitis.

Ribavirin is a synthetic nucleotide that is effective against many DNA and RNA viruses *in vitro.* **It acts by interfering with the synthesis of mRNA. It is used for treatment of HCV and as aerosol preparations for treatment of respiratory syncytial virus infections in children.**

Amantadine and Rimantadine are used for treatment or prophylaxis of seasonal influenza A but not B. They inhibit virus uncoating.

Interferon is used for treatment of several viral diseases (see vol.1, p.97).

Viral vaccines for prevention of viral diseases are discussed in vol I chapter 19.

CHAPTER 25

CLASSIFICATION of VIRUSES

Most clinically important viruses can be classified into groups according to their structural characters into:

RNA VIRUSES

RNA Non-Enveloped Viruses

= Picornaviruses
-Enteroviruses
Pohovirus, Coxackievirus, Echovirus, Enteroviruses 68-78
- Hepatovirus HAV -Rhino viruses = **Reoviruses;**
Rotavirus = **Caliciviruses;**
Norwalk virus = **Astroviruses**

RNA Enveloped Viruses

= Orthomyxoviruses;
Influenza virus =
Paramyxoviruses; RSV, Measles, Mumps, Parainfluenza =
Rhabdoviruses; Rabies virus =
Retroviruses; HIV, HTLV =
Togaviruses; Encephalitis viruses. =
Flaviviruses; Encephalitis,Yellow fever
Dengue, West Nile viruses, HCV. =
Bunyaviruses; Sandfly, Rift Valley fever viruses and Hantavirus =
Filoviruses; Ebola virus =
Arenaviruses; Lassa fever virus =
Coronaviruses; SARS = **Deltavirus;** HDV

DNA VIRUSES

DNA Enveloped Viruses

= Herpes viruses;
HSV1& 2, Varicella-zoster, CMV, EB, HHV 6, 7 & 8. =
Hepadnavirus; HBV = **Poxviruses;**
Smallpox, Molluscum contagiosum, Monkeypox, Cowpox

DNA Non-Enveloped Viruses

= Adenoviruses
= Papillomavirus
= Parvoviruses; B19 virus
= Polyomaviruses; JC & BK viruses

N.B.

-**Hepatitis viruses** are a heterogeneous group belonging to different families in which HAV is a Hepatovirus in the Picornavirus family, HBV is a Hepadnavirus, HCV is a Flaviviruses but is not transmitted by arthropods, HDV is a defective Deltavirus, HEV *(Hepevirus)* resembles caliciviruses and is now classified as a genus in the family Hepeviridea.

-**Arboviruses** are transmitted by arthropods and belong to several virus families, including togavirus, flavivirus, bunyavirus, rhabdovirus, arenavirus, and reovirus.

-**Rubella** is a Togavirus but is not transmitted by arthropods.

-**Hantaviruses** are Bunyaviruses but not transmitted by insects. The disease is acquired by inhalation of rodents excreta.

PICORNAVIRUSES
(Pico = small, rna = RNA)

They are the smallest RNA viruses; 25-30 nm **Picornaviruses** that cause human diseases include the following genera:

1 -**Human Enteroviruses** (HEV) are stable at pH 3 and they can resist the acidity of the stomach, so they can infect by the oral route. They include 5 species and several serotypes. **The following is the new classification:-**
   a-Polioviruses types 1-3
   b- HEV-A includes coxsackieviruses A-12 types.
   c- HEV-B includes coxsackieviruses B-7 types and echoA^uses 33 types.
   d- HEV-C includes coxsackieviruses A-12 types.
   e- Enteroviruses type 68-116

2- Hepatovirus is hepatitis A virus which was classified as enterovirus type 72. It is now placed in a separate genus. It is described in chapter 35.

3- **Rhinoviruses;** which include more than 150 antigenic types. These infect by the respiratory route and are acid-labile at pH 3.

4- Parechoviruses 14 serotypes; they cause common cold, gastroenteritis, neonatal sepsis, aseptic meningitis, encephalitis and myocarditis.

5- A**phtho**viruses cause foot and mouth disease in cattle, sheep and goats, which may be transmitted to man by contact or ingestion of infected meat.

POLIOVIRUSES

They cause poliomyelitis, which in its full blown picture affects the CNS, destroys the motor neurons in the spinal cord, causing flaccid paralysis. Fortunately, most pohovirus infections are subclinical. Man is the only natural host or reservoir of infection. **Properties of the virus:**

1- It is an icosahedral non-enveloped virus, the genome is a positive sense single stranded RNA, 25-30 nm in diameter.

2- There are 3 antigenic types.

3- The virus infects only primates, e.g. man and monkeys as they possess specific receptors for viral attachment.

4- They are grown in primary or continuous cell lines derived from man or monkey tissues causing characteristic cytopathogenic effects (CPE).

Replication begins by attachment to specific receptors on the cell membrane and entry into the cell, this is followed by uncoating. The genome RNA functions as mRNA and is translated into one large polypeptide, which is cleaved by virus encoded protease into structural proteins and enzymes. Replication of the genome occurs by synthesis of a complementary negative strand, which then serves as the template for the positive strands. Some of the positive strands function as mRNA to make more viral proteins and the remainder become progeny viral genome. Assembly then occurs and the virus accumulates in the cytoplasm and is released by cell lysis.

Pathogenesis:

Infection occurs by the ingestion of food or drink contaminated by stools of cases or carriers. Incubation period is 7-14 days. The organism multiplies in the oropharynx (tonsils) and the peyer's patches in the intestine and is excreted in stools. Infection may stop at this stage i.e. **inapparent infection.**

Infection may continue and the virus passes to the deep cervical and deep mesenteric lymph nodes. Then it invades the blood stream Viraemia is associated with mild symptoms of fever, malaise, headache, nausea, and vomiting. The disease may be stopped at this stage i.e. **abortive infection.**

Aseptic meningitis (non-paralytic poliomyelitis) may occur and manifests by **stiffness** and pain in the back and neck. It usually recovers but it may progress to paralysis.

Paralytic poliomyelitis occurs only in 0.1-1% of cases. The virus affects the anterior horn cells of the spinal cord leading to flaccid paralysis. In severe cases, it may affect the posterior horn cells, the vestibular nuclei and motor cortex. Death may occur due to respiratory paralysis.

No permanent carrier state occurs, but virus excretion in stools can occur for several months. Immunity is permanent to the type of pohovirus causing the infection.

Diagnosis:

1- Isolation of the virus from stools or throat washings is done on tissue culture. CPEs are identified by neutralization, immunofluorescence or by PCR.

2- Paired serum samples are tested to demonstrate a rising antibody titre by neutralization or complement fixation tests.

3- PCR is used for rapid detection of viral RNA in blood.

Prophylaxis:

Active immunization: There are two vaccines that contain the three types of virus and produce neutralizing antibodies and prevent CNS infection.

I- Salk inactivated polio-vaccine **(IPV):**

It is a formalin inactivated vaccine prepared from the three types of the virus grown in monkey kidney cell cultures. It is given in 4 subcutaneous doses at 2, 4 and 6 months and a booster injection is given at 4-6 years.

The vaccine produces neutralizing antibodies **(IgG** and **IgM)** and prevents infection of the CNS. However, it does not prevent virus replication in the intestine. So, the vaccine protects against paralytic poliomyelitis but not against non-paralytic forms of infection. The vaccine can be safely given to

immunosuppressed children and pregnant mothers, in whom Sabin vaccine is contraindicated.

II- Sabin living attenuated oral polio-vaccine **(OPV):**

It is a living attenuated vaccine prepared from non-paralytogenic mutants of the three types of pohovirus grown on human diploid cell cultures and stabilized by MgCfe. Four doses are given **orally** at the age of 2, **4** and 6 months and a booster dose is given at **4**-6 years.

The vaccine has the following advantages:

1- It is easily administered i.e. orally.

2- The live vaccine virus multiplies locally in the intestine, thus leads to production of serum neutralizing antibodies **(IgG** and **IgM)** and local immunity in the intestine by **IgA** and **interferon.** Hence, it prevents intestinal infection with the wild virus, thus preventing both paralytic and non-paralytic pohovirus infections.

3- The vaccine strains pass with the stools and are disseminated in the environment and can be transmitted to non-immunized children by faeco-oral route. This leads to spread of immunity in the community called **"herd immunity"** which may eventually lead to eradication of the wild pohovirus.

Disadvantages of Sabin vaccine:

1- Failure of vaccination which may be due to:

a- Loss of the potency of the vaccine due to improper refrigeration during transport or storage, **b-** Interference with replication of the virus in the intestine if the child is already infected with another enterovirus.

2- The vaccine may cause paralytic disease in immunodeficient children. Salk vaccine is recommended for these children.

3- Rarely (1/milhon), vaccine-associated paralytic poliomyelitis (VAPP) may occur due to reversion of the attenuated virus to the virulent type during its rephcation in vaccinated children (particularly type 2 and 3).

In USA they use IPV and not OPV for the 4 doses of vaccination of children to avoid occurrence of VAPP. The current version of the inactivated vaccine used in USA in **2007** is the enhanced polio-vaccine **elPV** which proved to be as immunogenic as OPV and is not associated with the development of VAPP.

A major campaign is under way by the WHO using OPV mass vaccination to eradicate pohovirus from the world as was done with smallpox.

Passive immunization:

Gamma globulins given early to susceptible unimmunized contacts may be effective in preventing paralytic poliomyelitis.

Summarv of differences between	Salk tIPV)	Sabin fOPV)
Prevents disease	Yes	Yes
Interrupts transmission	No	Yes
Induces humoral IgG	Yes	Yes
Induces intestinal IgA	No	Yes
Affords herd immunity	No	Yes
Prevents rephcation of wild strain in the gut	No	Yes
May revert to virulent	No	Yes
Coinfection with other enteroviruses may impair immunization	No	Yes
Requires refrigeration	No	Yes
Can cause disease in the immunocompromised	No	Yes
Route of administration	Injection	Oral
Duration of immunity	Shorter	Longer
Used in Egypt	No	Yes
Used in USA	Yes	No

COXSACKIEVIRUSES

Coxsackieviruses are classified into A and B based on their pathogenicity in **newborn suckling mice:-**

- Coxsackieviruses A 12 types in HEV-A species and 12 types in HEV- C species. They produce wide spread myositis and flaccid paralysis which is rapidly fatal without other observable lesions.
- Coxsackieviruses B **(7** types) in HEV-B species. They produce focal myositis and other generalized mild lesions of the CNS, heart and pancreas.

They cause several disease syndromes **in man.** They are transmitted by the faeco-oral or respiratory route. They multiply in the GIT or oropharynx and disseminate *via* the blood stream. The syndromes include:

1- Herpangina caused by group **A** affects children mainly and is characterized by fever, sore throat, anorexia, dysphagia, vomiting and abdominal pain. Vesicles appear on the throat and tongue. It is a self limited disease.

2- Acute haemorrhagic conjunctivitis caused by group **A 24.**

3- **Hand foot and mouth** disease caused mainly by group **A.** The disease is characterized by a vesicular rash on the hands and feet and ulceration in the mouth mainly in children.
4- **Pleurodynia** caused by group **B,** and is characterized by fever and chest pain. Abdominal pain may occur in some cases.
5- **Myocarditis** and **pericarditis** caused by group **B,** infection may be fatal in neonates or may cause permanent heart damage and cardiomyopathy.
6- **Diabetes mellitus:** Coxsackievirus **B** 3**.4** is suspected to have a role in type I diabetes mellitus.
7- **Aseptic meningitis;** caused by groups **A and B.** It is characterized by fever, malaise, headache, nausea and vomiting, stiff neck or back.
8- **Minor febrile illness** and **upper respiratory infections** with or without rash is caused by both groups **A and B.**
9- **Diarrhea** and **hepatitis** caused by group **A and B.**

Diagnosis by isolation in cell culture or in suckling mice or by detection of rising antibody titres

ECHOVIRUSES

There are 33 serotypes in HEV-B species. They are transmitted by the faeco-oral route. Diseases caused by echoviruses are; aseptic meningitis, encephalitis, febrile illness with or without rash, common cold, diarrhea, hepatitis, pleurodynia, myocarditis and pericarditis

HUMAN ENTEROVIRUS TYPES 68-116

-Enterovirus **68** causes pneumonia in children.
-Enterovirus **70** causes acute haemorrhagic conjunctivitis, and encephalitis.
-Enterovirus **71** causes meningitis, encephalitis and paralysis resembling poliomyelitis. They cause also hand-foot-and-mouth disease, and herpangina.

RHTNOVIRUSES

More than 150 types are known. These cause upper respiratory tract infections specially, **common cold.** They are responsible for about half of asthma exacerbations. They grow better at a temperature of 33°C which is the temperature of the nasopharynx. Infection is transmitted by contact or by airborne particles.

The virus enters ***via*** the respiratory tract and multiplies locally in the mucous membrane of the nasopharynx. Incubation period is short 2-4 days followed by headache, nasal discharge, mild cough and malaise.

There is no blood invasion and the disease is self-limited. Immunity is due to local IgA and interferon. That is why immunity is short lived and several attacks may be acquired during one season. This is also due to multiplicity of antigenic types. Secondary bacterial infections may cause otitis media, sinusitis, bronchitis or pneumonitis, especially in children.

CHAPTER 26
ARTHROPOD - BORNE and RODENT-BORNE VIRUSES

Many viral diseases are transmitted by the bite of an arthropod vector. These viruses are called arthropod-borne viruses or **arboviruses.**

Rodent-borne viruses or **roboviruses,** are transmitted directly from rodents to humans without an arthropod vector by contact with rodent's body fluids or inhalation of their dry excreta. Major rodent-borne viral diseases are; hantavirus infections and lassa fever, these cause haemorrhagic fevers.

Other causes of **haemorrhagic fevers** include yellow fever, dengue fever and Rift Valley Fever (arboviruses) mentioned in this chapter. African haemorrhagic fevers caused by Marburg and Ebola viruses (filoviruses) are mentioned in chapter 38.

ARTHROPOD - BORNE VIRUSES Arboviruses

are extremely numerous and include many unrelated viruses belonging to different genera, some of which are mentioned below:

1-Togaviruses - Genus alphavirus: Some important members are:	
- Eastern equine encephalitis virus.	Icosahedral, enveloped
- Western equine encephalitis virus.	(+) ssRNA viruses
- Venezuelan equine encephalitis virus.	
- Sindbis virus.	
2-Flaviviruses: Some important members are:	Icosahedral, enveloped
- Brazilian encephalitis virus.	(+) ssRNA viruses.
- Japanese B encephalitis virus	
- Yellow fever virus.	
- West Nile fever virus.	
- Dengue virus.	
3-Bunyaviruses - Genus phlebovirus:	Helical, enveloped
- Sandfly fever virus.	(-) ssRNA viruses
- Rift Valley fever virus.	

Arboviruses are infectious agents **transmitted** by blood-sucking arthropods e.g. mosquitoes, ticks, or sandflies. Arthropods become infected by feeding on blood of animals or man during the viraemic stage. The virus multiplies in the body of the insect without causing disease and is excreted in the saliva whereby the insect becomes infective. Various animals, rodents and birds act as reservoirs of infection.

Diseases caused by arboviruses range in severity from mild to rapidly fatal. The **clinical picture** varies, but usually present in one or more of the following pictures: **1-** Encephalitis which is fatal. **2-** Haemorrhagic fevers, frequently severe and fatal. **3-** Fever with myalgias, arthralgias, and non-haemorrhagic rash. Lymphadenopathy may occur in some.

The pathogenesis of these diseases involves not only the cytocidal effect of the virus but also, in some a prominent immunopathologic component. Most infections are subclinical. Infants and the elderly are the most susceptible. After recovery from the disease, immunity is hfelong.

Diagnosis of diseases caused by Arboviruses:

1- Antigen detection and PCR assays are available for direct detection of viral proteins or RNA in clinical specimens for some arboviruses.

2- Serologic diagnosis by ELISA for detection of specific IgM or a rising titre of IgG has largely replaced the classic methods, i.e. haemagglutination inhibition, complement fixation and neutralization of virus infectivity.

3- Isolation of the virus from the blood during the acute phase or from CSF, skin, and tissue biopsy depending on the agent. Several cell lines are used including mosquito cell lines and intracerebral inoculation of suckling mice

ARBOVIRUS ENCEPHALITIS

Different encephalitis arboviruses in the togavirus and flavivirus groups are endemic in many parts of the world. In Egypt, two such viruses exist; the **West Nile virus** and the **Sindbis virus** which are transmitted by mosquitoes. The reservoir of infection is various animals, rodents and birds.

These viruses are enveloped, icosahedral, containing single stranded positive sense RNA. They are haemagglutinating viruses. They can be grown in the laboratory in tissue culture, on chick embryo as well as in mouse brain by intracerebral inoculation.

Pathogenesis:

Infection occurs through the bite of the infective arthropod. The virus multiphes in the local lymphoid tissue then reaches the blood stream. Viraemia coincides with onset of fever, which is associated with generalized lymphadenopathy and maculopapular rash. Most infections are subclinical without affection of CNS.

Invasion of CNS leads to disseminated encephalitis, meningitis and myelitis. West Nile and Sindbis viruses cause mild meningitic disease.

YELLOW FEVER

Yellow fever is an acute febrile life threatening, mosquito-borne disease characterized by fever, jaundice, haemorrhages and albuminuria. The virus is a flavivirus. It is an icosahedral, enveloped, (+) ssRNA virus.

The disease is endemic in equatorial countries of Africa and South America. The main reservoir is monkey, man is an accidental host. Monkey to monkey transmission occurs by the mosquito *Aedes africanus* in Africa

and *Haemagogus* in South America. Man becomes infected if he visits the jungle for cutting trees, picking nuts, road construction or hunting. This transmission cycle is called "jungle yellow fever" as it occurs in the forests.

When infected persons return to urban areas, man to man transmission occurs by the mosquito *Aedes aegypti* which breeds in stagnant water. This is called "urban yellow fever" and it may have an epidemic form

Pathogenesis and clinical picture

The virus is introduced by the bite of an infective mosquito. It multiphes in the local lymph nodes and spreads to the blood this coincides with sudden onset of fever, headache, myalgias and photophobia; some patients recover at this point. The virus may reach the liver, spleen, kidney and bone marrow leading to their destruction. This results in jaundice, haemorrhages and proteinuria. It may affect the heart or GIT leading to shock, prostration haematemesis (black vomit). Death may result from kidney or liver failure.

Prophylaxis:

1- Mosquito control on airplanes going to or corning from endemic areas.

2- Specific prophylaxis: A live attenuated vaccine called 17-D vaccine is available. It is prepared in eggs and is supphed as dried powder. It must be refrigerated and is rehydrated before use. It is given in one subcutaneous injection, which gives immunity for 10 years.

The vaccine is contraindicated for infants less than 9 months of age, during pregnancy and in persons with altered immune systems e.g. HIV or organ transplant patients.

DENGUE "Breakbone fever"

Dengue occurs in the tropical and subtropical areas where the mosquito vector prevails. The virus is a flavivirus. Four antigenic types are known. Dengue viruses are transmitted principally between humans by the mosquito *Aedes aegypti.*

Classic dengue begins suddenly with an influenza-like syndrome consisting of fever, malaise, headache, severe muscle, joint, bone and back pain. Nausea, vomiting and eye pain. Enlarged lymph nodes and maculopapular rash may occur. This form of disease is rarely fatal and recovery occurs after few weeks.

Dengue haemorrhagic fever is a severe form of dengue that is fatal. The initial picture is the same as classic dengue, but then shock and haemorrhage, especially into the GIT and skin, develop.

Pathogenesis: The patient recovers from classic dengue caused by one of the 4 types, and antibody is produced. When the patient is infected with another serotype an anamnestic response occurs and large amounts of cross-reacting

antibodies to the first type are produced. It is postulated that virus-antibody complexes are formed within few days of the second infection; these activate the complement causing increased vascular permeability and thrombocytopenia. It is also postulated that the antibodies increase the entry of virus into monocytes and macrophages with the consequent release of large amounts of cytokines vasoactive mediators causing DIC, haemorrhages and shock.

SANDFLY FEVER

It is caused by a bunyavirus of the phlebovirus genus. It is a mild non-fatal disease characterized by fever, headache and malaise. It occurs in the Mediterranean area where the sandfly *Phlebotomus papatasii* exists.

RIFT VALLEY FEVER

It is caused by a bunyavirus of the phlebovirus genus and is transmitted by mosquitoes *{Aedes* species). It is primarily a pathogen of sheep and domestic animals. Man is secondarily infected during an epizootic in domestic animals by coming in contact with infected animal body fluids or mosquito bites.

Disease in humans is usually a mild febrile illness, and recovery is complete. Complications include retinitis, encephalitis and haemorrhagic fever. Permanent loss of vision may occur.

An epizootic has been reported in Egypt in 1977 which caused enormous losses of sheep and cattle. Thousands of human cases occurred with 600 deaths. A similar outbreak occurred in Eastern Africa in 1998. A recent outbreak of 404 cases and 118 deaths was reported in Kenya November 2006-January 2007. A living attenuated vaccine is used for animal immunization.

WEST NILE FEVER

It is caused by the West Nile virus (WNV) which is a flavivirus and is transmitted by the *Culex* mosquito. Wild birds are the main reservoir.

The disease is characterized by mild fever, lymphadenopathy and rash. Transitory meningeal involvement may occur during the acute stage. It may cause fatal encephalitis in older people. Less than 1% of those infected have symptomatic disease. There is only one antigenic type and immunity is lifelong.

It occurs mainly in the Middle East, Africa and Southwest Asia. An outbreak appeared unexpectedly in the USA in 1999 causing encephalitis which led to death of several humans as well as many birds. The infection may have entered the USA by an infected bird, infected traveler or mosquito coming in airplanes. In 2002 WNV epidemic included the first documented cases of person-to-person transmission through blood transfusion or organ transplantation. In 2003, there were 7700 cases, 166 died.

Blood banks use PCR for screening donated blood for WNV. Mosquito control is the only preventive measure, there are no vaccines.

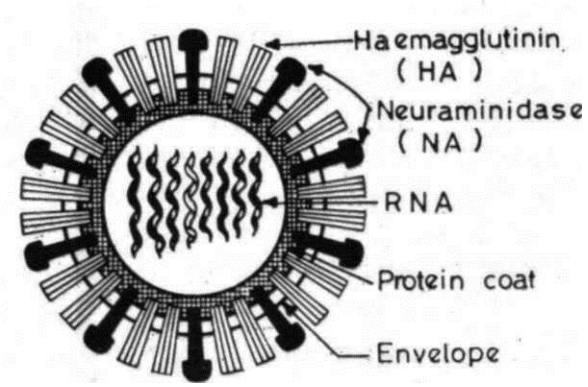
CHAPTER 27
ORTHOMYXOVIRUSES
INFLUENZA VIRUSES

Influenza viruses are members of the family Orthomyxoviridae. Three types of influenza virus are known; A, B and C Influenza. A causes worldwide influenza epidemics every 10-20 years (pandemics) and outbreaks every year. Influenza B causes outbreaks, but less often than influenza A. Influenza C causes mild respiratory disease.

Influenza A virus is a single stranded segmented RNA virus (8 gene segments). The nucleocapsid is helical and is surrounded by a lipoprotein envelope. The envelope is covered with two glycoprotein spikes, a haemagglutinin (HA) and a neuraminidase (NA). HA binds to the cell surface receptor (sialic acid) to initiate infection. NA facilitates virus release from infected cells. Changes in HA and NA determine the antigenicity of the virus and according to which influenza A virus includes 16 HA and 9 NA subtypes that are circulating in birds, humans, swine and horses. Currently the most famous subtypes are:

•A (HINT) circulating in humans caused the 2009 influenza pandemic (swine flu).

•A (H5NI) circulating in birds causing avian flu and infecting humans who closely handle infected birds. The virus jumped directly from birds to humans. It contains gene segments from avian viruses only. Cases are still appearing and if **reassortment** with human strains occurs, it will spread from person to person. The virus represents a potential source of a pandemic.



(Fig. 9): Structure of influenza virus.

Antigenic variation is a common phenomenon in influenza viruses due to changes in **HA** and **NA.** It continually occurs in type **A,** less so in type B while type C is antigenically stable. There are two types;

1- Antigenic drifts; these are minor changes due to mutation and occur in both **A** and B viruses, resulting in the strains that cause yearly outbreaks.

2- Antigenic shifts; these are major changes due to **reassortment** of gene segments. This occurs when one cell is infected simultaneously with two different influenza A viruses (e.g. an avian and a human influenza A virus), mixtures of parental gene segments may be assembled into progeny virions, resulting in a new variant of human influenza A virus, bearing the avian virus HA. Pig cells have receptors for both avian and human influenza strains and can be co-infected by more than one strain acting as a mixing pot in which **reassortment** between two or more viruses (e.g. avian, human and pig influenza A viruses) may occur. The 2009 pandemic due to A/H1N1 represents a quadruple **reassortment** of two swine strains, one human strain and one avian strain of influenza (Fig. 10).

Influenza B virus is only a human virus, there is no animal source of new RNA segments, and it does not undergo antigenic shifts.

Antigenic shifts appear less frequently, about every 10 years causing epidemics, whereas drift variants appear virtually every year and are the cause for changing the strains used for vaccine production on yearly basis.

Pathogenesis: Infection occurs by inhalation of airborne droplets. The neuraminidase of the inhaled virus degrades the protective mucous layer, allowing the virus to reach the mucous membrane of the respiratory tract where it multiplies locally causing rhinitis, pharyngitis and bronchitis. The incubation period is short 1-4 days. Manifestations are fever, myalgia, headache, dry cough, malaise and anorexia. The systemic manifestations are due to circulating cytokines. The disease is self-limited; however, it may be complicated by viral or bacterial pneumonia mainly in the elderly and/or debilitated individuals. **Avian flu** caused by A/H5N1 causes severe disease with pneumonia and multi-organ failure. Mortality rate may reach 50% mainly due to progressive pneumonia.

Diagnosis: Laboratory confirmation is essential for the new influenza A subtypes, specially at the beginning of a new community outbreak. Nasal aspirates, gargles, throat swabs or sputum are examined by:

1- **Direct,** rapid detection of viral antigens in specimens using commercially available kits that depend on ELISA and other immunoassay methods.

2- Detection of viral RNA in clinical specimens by probes or RT-PCR.

3- Isolation of virus in cell culture or embryonated eggs.

Prevention by **vaccination;** two types of vaccines are available. **1- Inactivated Influenza Vaccines (IIV)** are either inactivate whole virus or subvirion containing purified virus disrupted with detergents or purified surface antigen glycoproteins (HA and NA). All preparations contain the annually recommended strains that represent the seasonal influenza viruses that are predicted to be circulating during the influenza season. It is given by

intramuscular injections. It is licensed for all persons above 6 months including those that are healthy or those with chronic conditions and pregnant women.

2- Living Attenuated Influenza Vaccines (LAIV) is a cold adapted vaccine containing temperature-sensitive mutants of influenza A and B (FluMist). These can replicate in the cooler (33°C) nasal mucosa where they induce IgA, but not in the warmer (37°C) lower respiratory tract. It is administered by nasal spray. It is licensed for non-pregnant healthy persons 2-49 years old.

Both IIV and LAIV contain strains of influenza virus that are antigenically equivalent to the annually recommended strains of influenza. The viruses used for vaccine preparation up to 2012 are, influenza A (H3N2), influenza A (H1N1) and influenza B virus. The vaccine is reformulated every year to contain the current antigenic strains that are the result of the antigenic drift or shift. Yearly boosters are recommended before the flu season in October. A quadrivalent vaccine will be available in 2013 -2014.

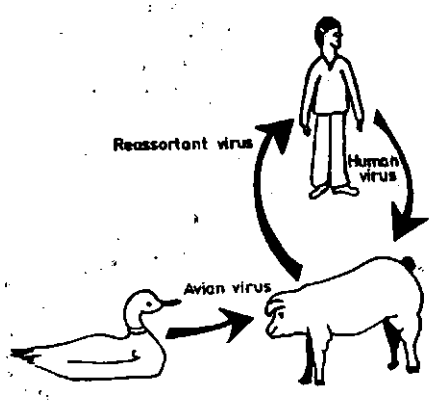
All children 6 months to 8 years old, who have not been previously vaccinated, should receive 2 doses of the age appropriate vaccine in the same season with a single dose during subsequent seasons.

Since **2007** when the FDA approved **the first vaccine for humans** against H5Nl,the vaccine is prepared yearly from the circulating stains of the virus. It is an inactivated whole virus vaccine given I.M. in two doses separated by one month. The vaccine could be used if the current H5N1 avian virus starts to spread from human to human.

Prevention and treatment by drugs:

The neuraminidase inhibitors; zanamivir (Relenza) delivered by inhalation and oseltamivir (Tamiflu) given orally are used for treatment and prevention of influenza A and B. They reduce the spread of the virus from cell to another and reduce the duration of symptoms by 1-2 days.

Currently amantadine and rimantadine are not recommended for treatment or prophylaxis of influenza in USA until susceptibility to the drugs has been established among circulating influenza A



(Fig.10): The new A/HlNlpandemic strain of 2009 resulted from quadruple reassortement -in the pig- of two swine, one human and one avian influenza strains

CHAPTER 28 PARAMYXOVIRUSES AND RUBELLA VIRUS

PARAMYXOVIRUSES

These include important agents of respiratory infections of infants and young children i.e. **parainfluenza viruses, respiratory syncytial virus (RSV), mumps and measles.**

Paramyxoviruses are composed of a non-segmented single stranded RNA, a helical nucleocapsid, and an outer lipoprotein envelope, which is covered with spikes, that contain haemagglutinin (HA), neuraminidase (NA), and fusion proteins. They are antigenically stable.

PARAINFLUENZA VIRUS

There are four types. It is a major cause of respiratory disease in infants and young adults. Infection is transmitted by respiratory droplets. The virus multiplies locally without viraemia. Incubation period is 2-6 days. Type 3 causes common cold, rhinitis, pharyngitis and bronchitis often with fever and pneumonia. Types 1 and 2 are major causes of **croup** which is a harsh cough and hoarseness due to **laryngotracheobronchitis. Diagnosis** as in influenza. **Prophylaxis:** No prophylaxis is available.

RESPIRATORY SYNCYTIAL VIRUS (RSV)

RSV is the most important cause of **bronchiolitis** and **pneumonia in infants.** In older children and adults, it causes mild upper respiratory infection and otitis media. It causes outbreaks of respiratory infections, yearly in winter, and in hospitalized infants.

The virus surface spikes are fusion proteins, which cause syncytia in cell cultures. Antibodies to the fusion proteins neutralize virus infectivity.

Humans and chimpanzees are the natural host. It is transmitted by respiratory droplets or direct contact with contaminated hands. The virus multiplies and spreads locally in the respiratory epithelium i.e. no viraemia.

Rapid diagnosis can be made by direct detection of viral antigens in respiratory secretions by commercial kits using immunoflourscence or ELISA. Detection of viral RNA by PCR. Virus isolation and identification of the characteristic syncytia and giant cell formation in cell culture.

Treatment: Aerosolized ribavirin is used for treatment of infants with serious RSV. It may be combined with specific hyperimmune globulins.

Prevention: Monoclonal antibodies against fusion proteins or hyperimmune globulins can be used for prophylaxis of premature or immunocompromised infants.

MUMPS

It is an acute contagious disease characterized by non-suppurative enlargement of one or both parotid glands. There is only one antigenic type. The internal nucleocapsid protein is the S (soluble) antigen detected early in infection. Antibodies against the haemagglutinin (HA) are neutralizing. Humans are the natural host. Pathogenesis, clinical findings and immunity:

Infection occurs by respiratory droplets. Primary multiplication occurs in the respiratory mucosa followed by viraemia and localization of virus in the parotids and other salivary glands. It may affect the testes, ovaries, pancreas and the CNS. One third of cases are subclinical.

The incubation period is 14-18 days followed by fever, malaise, anorexia and swelling of parotid glands. The disease is benign and resolves spontaneously. Orchitis occurs in adults and may rarely lead to sterility. Meningitis may comphcate some cases and it usually resolves without sequelae. Pancreatitis is a rare complication. Immunity is permanent after a single infection. Infants are immune for the first 6 months due to maternal antibodies.

Diagnosis is usually made clinically, but can be confirmed by;

1- Isolation of the virus from sahva, CSF, or urine.
2- Detection of IgM or a rising titre of IgG by haemagglutination inhibition or ELISA.
3- Rt-PCR detects mumps genome sequence in clinical samples

Prophylaxis:

A living attenuated vaccine is given to children combined with measles and rubella (MMR) vaccines. Two doses are recommended the first at 15 months and the second at 4-6 years. Due to a recent outbreak in 2006, a second dose of the vaccine is recommended for those who received only one dose specially health care workers, and students at college entry.

MEASLES

Measles is a highly infectious disease that affects children. It is characterized by fever and maculopapular skin rash. Measles occurs worldwide, usually in outbreaks every 2-3 years. There is only one type of measles virus, and HA is the antigen against which neutralizing antibody is directed. Humans are the only host.

Pathogenesis, clinical findings and immunity:

Infection occurs by droplets. Primary multiplication occurs in the respiratory mucosa from where it passes to the regional lymph nodes and

the reticulo-endothehal system to pour in the blood causing viraemia then it localizes in the skin and mucosa of the conjunctive and respiratory tract.

Incubation period is 8-15 days followed by fever which lasts for 4 days associated with conjunctivitis, cough and "koplik's spots" in the mouth. These are bluish-white ulcerations of the buccal mucosa opposite the lower molars. As the fever subsides the maculopapular rash appears all over the body. The rash is caused primarily by cytotoxic T cells attacking the virus-infected vascular endothehal cells in the skin.

Complications may occur in debilitated children e.g. bronchopneumonia otitis media, and rarely encephalitis. Subacute sclerosing panencephalitis is a rare, fatal disease of the CNS that occurs several years after measles. Measles in a pregnant woman causes stillbirth.

One attack of measles is followed by long lasting immunity. Although antibodies may play a role in neutralization of the virus during the viraemia, cell mediated immunity is more important. Maternal antibodies protect the infant during the first 6 months. Infection with measles causes transient depression of cell mediated immunity against other intracellular organisms e.g. *M. tuberculosis* leading to loss of tuberculin skin test reactivity.
Diagnosis: Measles is easy to diagnose clinically. Laboratory diagnosis is rarely needed.

Prophylaxis:

A living attenuated vaccine is given by injection to children at the age of 15 months. Another dose is recommended before school entry. It may be given in combination with mumps and rubella (MMR) vaccines.

RUBELLA (GERMAN MEASLES)

Rubella is an acute infectious disease characterized by fever, rash and enlargement of occipital and cervical lymph nodes.

Rubella virus belongs to the Togavirus family, but it is not transmitted by arthropods. It is a single stranded RNA virus, having an icosahedral nucleocapsid and a lipoprotein envelope. Its surface spikes contain haemagglutinin. It is one antigenic type. Man is the only host. It causes:

1- Postnatal rubella:

Infection of neonates, children and adults occurs by respiratory droplets. Incubation period is 2-3 weeks. Initial replication of the virus occurs in the nasopharynx and the local lymph nodes; it then spreads via the blood to the internal organs and skin. Maculopapular rash appears associated with fever and enlargement of the cervical or occipital lymph nodes. The rash may be due to antigen-antibody mediated vasculitis. Polyarthritis due to immune complexes may occur in adult women. Infection is asymptomatic in 20- 50%

of cases. The disease is mild and self-limited and one attack is followed by solid immunity.

2- Congenital rubella syndrome:

Rubella infection of pregnant women results in transplacental transmission of the virus and infection of the foetus. If this happens during the first trimester of pregnancy, during which, the very sensitive organs develop, it may lead to congenital anomalies of the foetus in the form of congenital heart defects, cataract, deafness, blindness, meningoencephahtis, mental retardation and hepatosplenomegaly. Infection may lead to intrauterine foetal death and abortion.

Children infected *in utero* can continue to excrete the virus for up to 18 months after birth. The virus is found in pharyngeal secretions and other body fluids. They can be a source of infection to pregnant women. The problem is more serious when such chronic shedders are asymptomatic. They can be diagnosed only by isolation of the virus or by the detection of IgM or a rising IgG titre.

Diagnosis:

1- Detection by ELISA of a rising titre of rubella IgG in paired serum samples separated by 10 days, or detection of rubella IgM antibodies in a single serum sample in pregnant women is diagnostic of recent rubella infection. This is an indication for therapeutic abortion especially during the first trimester of pregnancy. Various diagnostic kits are available.

   If recent infection of a pregnant woman has occurred, isolation of virus from amniotic fluid indicates definite foetal infection.

2- Detection of rubella IgM antibodies in the newborn is diagnostic of infection *in utero.* Since IgM does not cross the placenta so its presence indicates that it must have been synthesized by the foetus *in utero.*

3- Isolation of the virus from newborn specimens or PCR may be used.

Prophylaxis:

A living attenuated rubella vaccine is effective and induces life long immunity in 95% of recipients. It is available alone or in combination with measles and mumps (MMR) vaccines. It is given subcutaneously to children at the age of 15 months and a booster dose at school entery. The single vaccine is recommended for unimmunized young adult women if they are not pregnant and they should use contraception for the next 3 months.

A proof of immunity (positive serologic tests or documented rubella vaccination) is required for women entering college and for female hospital personnel who might come in contact with rubella patients or pregnant women.

CHAPTER 29

REOVIRUSE

S ROTAVIRUS

Rotaviruses are the most important human pathogen in the Reoviridae family. They are the single most important worldwide cause of viral gastroenteritis in young children. By the age of three, 90% of children have antibodies to at least one serotype. Up to 50% of cases of acute gastroenteritis of hospitalized children are caused by rotaviruses.

The virus is a non-enveloped, icosahedral 60-80 nm particle having a double layered capsid. The genome is double stranded **segmented** RNA. There are at least 6 serotypes. It is difficult to grow in culture. **Pathogenesis:**

Incubation period is **1-4** days. It is common in children between the age of 6 months and 2 years. The virus infects by the faeco-oral route, multiplies in the enterocytes in the villi of the small intestine, and damages their transport mechanisms. Sodium and glucose absorption is impaired. This causes watery non-bloody diarrhoea and vomiting associated with fever and abdominal pain ending with dehydration, acidosis and shock. It may be fatal if untreated

Diagnosis:

Stools are collected during the first few days of illness.

1- Rapid diagnosis using commercially available kits that detect the virus in stools by ELISA, latex agglutination or RIA.

2- Demonstration of the virus in stools by immunoelectron microscopy, a technique which is not feasible for routine diagnosis.

3- PCR or dot hybridization using rotavirus specific nucleic acid probe to detect the virus in stools or typing it if needed.

4- A rising antibody titre can be detected by ELISA or complement fixation, in paired serum samples.

Treatment and vaccines:

- Treatment by replacement of fluids and restoration of electrolyte balance.

-RotaTeq is the vaccine licensed in 2006. It is a live oral pentavalent hurnan/bovine reassortant vaccine containing 5 reassortant rotaviruses developed from human and bovine parent rotavirus strains. It is given to infants in USA in 3 oral doses; 2 ml each at 2, **4** and 6 months. The vaccine should not be given before 6 weeks or after 8 months.

-Rotarix is a live attenuated monovalent oral vaccine based on an attenuated single human rotavirus strain that gives protection against other prevalent strains. It is given in 2 doses; 1ml each in the first six months of life.

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RABIES VIRUS

Rabies is an acute infection of the CNS that is almost always fatal. The virus is transmitted to man from the bite of a rabid animal.

The virus is a rhabdovirus 75x180 nm It is a bullet shaped enveloped, single stranded (negative sense) RNA virus and contains an RNA polymerase. The envelope is covered with glycoprotein spikes, which stimulate neutralizing antibodies.

The virus infects all warm-blooded animals e.g. dogs, cats, wolves, foxes cattle, skunks, raccoons; in all of which it is fatal. The virus is present in the saliva and CNS of these animals. Only in certain bats, the virus may adapt to the salivary glands without symptoms, acting as reservoirs for infection. Rodents and rabbits do not transmit rabies.

There is only one serotype of the virus but there are two biologic forms:

1- Street virus: This is the virus propagated in nature. It infects by peripheral inoculation leading to encephalitis. All laboratory animals are susceptible by intramuscular or intracerebral inoculation. The incubation period is variable (3-8 weeks). It produces intracytoplasmic inclusion bodies "Negri bodies" in the brain.

2- Fixed virus: This results from repeated passage of the virus in brains of laboratory animals. It is attenuated and causes encephalitis only when inoculated intracerebrally, after a fixed short incubation period (4-6 days). Inclusion bodies are absent or found with difficulty.

Pathogenesis:

The virus is introduced through the bite of a rabid animal in its saliva. It multiplies locally then travels *via* the peripheral nerves to the CNS where it multiplies causing fulminating fatal encephalitis. Eosinophilic intra-cytoplasmic inclusion bodies appear in the affected cells called "Negri bodies". There is no viraemia. From the CNS it travels down in the peripheral nerves to the salivary glands passing in the saliva.

It may pass to other organs e.g. the cornea which may be a rare, non-bite source of infection, in persons receiving corneal transplants taken from patients who died of undiagnosed rabies.

The incubation period (LP.) in humans varies from 2 to 16 weeks or more depending on the distance between the site of the bite and the brain. It also depends on the severity of laceration and amount of virus introduced. The LP. is shorter in persons bitten on the face or head. The long LP. gives time for effective vaccination and prevention of the virus from reaching the CNS.

Rare modes of **non-bite transmission** are by exposure to the virus in open cuts in the skin or onto mucous membranes, also by exposure to aerosols of bat secretions in bat caves.

Manifestations start by headache, malaise, fever, anorexia, and abnormal sensations around the site of the bite followed by, irritabihty, personahty changes, hydrophobia, sahvation, and painful swallowing due to spasm in throat muscles, finally convulsions, paralysis and coma ending in death.

Antemortum diagnosis in humans:

It is recommended as it allows early identification and post-exposure prophylaxis of family members and health care staff exposed to patients' saliva through, mucous membrane contact, scratches or abrasions.

Specimens include full-thickness skin biopsy from the nape of the neck including hair follicles, corneal impression smears (taken by an ophthalmologist), saliva, CSF and serum. These are examined as follows:

1- Antigen detection by immunofluorescence in skin biopsy and corneal smears.
2- Virus isolation from sahva and CSF using continuous cell culture, or by intracerebral inoculation of mice.
3- RT-PCR applied to the above specimens except the serum
4- Antibodies appear 2 weeks after illness in serum and CSF and are tested for by ELISA, neutralization or immunofluorescence.

Diagnosis in animals:

Immediately after the bite, the ariimal should be captured and observed for 10 days. If no symptoms appear during this period the diagnosis of rabies is excluded. If the animal dies or symptoms appear the animal is sacrificed, and diagnosis is done as follows:

1- Antigen detection by direct immunofluorescence in brain smears.
2- Detection of "Negri bodies" in stained brain smears.
3- Isolation of the virus from brain or sahva as above mentioned.
4- RT-PCR apphed to brain tissues.

Management of rabies:

If the animal is available, diagnosis is done as mentioned above. If the biting animal is not available, the case is managed as impending rabies.

1- The wound is immediately cleaned with soap and water and irrigated by a virucidal agent such as povidone-iodine solution. Avoid suturing.
2- Administer tetanus vaccine and antibiotics to control bacterial infections.
3- Post-exposure prophylaxis by vaccination and passive immunization with rabies immune globulins is required in the following situations:

a- Non-provoked bites by domestic or wild animals.
b- If the diagnosis was established in the captured animal.
c- In severe bites on the head and neck, even if the animal is available due to short incubation period, which may be as short as 2 weeks. Vaccination should start immediately to be stopped if the animal proved free of rabies.
d- Bite or non-bite exposure to bats.
e- Non-bite exposure of family members and health care staff to patients' saliva through mucous membrane contact, scratches or abrasions.

Types of Vaccines: All vaccines for human use contain inactivated virus.

1- Human diploid cell vaccine (HDCV): The virus is grown in human normal fibroblast cell line. It is inactivated by P-propiolactone.

2- Purified chick embryo cell vaccines (PCEC); prepared from fixed rabies virus strain (Fluty LEP) grown in chicken fibroblasts and inactivated with P–propiolactone, then purified by zonal centrifugation.

3- Rabies vaccine adsorbed (RVA); prepared in diploid cell line derived from rhesus monkey foetal lung, inactivated by P-propiolactone, and adsorbed on aluminium phosphate. This vaccine is no longer available in USA since 2009

The vaccines induce immunity in 7 days, which lasts for 2 years. Five doses given intramuscularly 1 ml each (4 doses are recommended in 2012) in the deltoid area in adults or in the anterolateral aspect of the thigh in children, on days 0, 3, 7, 14 and 28 post-exposure. It should not be given in the gluteal area.

N.B: The old nerve tissue and duck embryo vaccines are not recommended as they are less immunogenic, require 25 injections and cause complications.

Passive immunization with human rabies immune globulin (HRIG). The recommended dose is 20 IU per Kg of body weight. As much as possible of the immune globulin is given into the site of the bite and the remainder is given intramuscularly at a site distant from the site of vaccination (in the gluteal region). HRIG neutralizes the virus at the site, and gives time for the vaccine to stimulate active immunity before the virus reaches the CNS. An immune serum of equine origin is used in developing countries.

Control measures include:

1- Pre-exposure vaccination is recommended for persons at risk including; veterinarians, animal control and wildlife workers, rabies laboratory workers. HDCV is given in three doses on days 0, 7, and 21, or 28. Booster doses are needed every 2-3 years, to maintain a neutralizing antibody titre of 1:5.

2- Destruction of stray dogs and quarantine of imported dogs.

3- Vaccination of dogs, cats and other pets.

Animal vaccines; a recombinant viral vaccine consisting of vaccinia virus carrying the rabies glycoprotein gene has successfully immunized animals when orally administered. Vaccines added to baits are used to reduce rabies in wildlife reservoir species.

CHAPTER 31
RETROVIRUS ES

Retroviruses are RNA viruses that contain the reverse transcriptase enzyme and replicate in a unique manner. They cause tumours in several species of animals. There are two important human retroviruses in the *Retroviridae* family.

1- Human immunodeficiency virus (HIV) in the *Lentivirus* genus. It causes chronic **slow** infections. It is **cytolytic** and **non-transforming.**

2- Human T cell lymphotropic virus (HTLV) in the *Deltaretrovirus* genus, (chapter 36).

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HIV viruses are non-oncogenic cytolytic slow retroviruses that cause the acquired immunodeficiency syndrome (AIDS). HIV-1 was isolated in 1983 and HIV-2 in 1986. HIV in humans originated from cross-species infections by simian viruses in rural Africa. Infected individuals may remain asymptomatic, but they are infected for life. A high percentage of carriers develop fatal illness after several years.

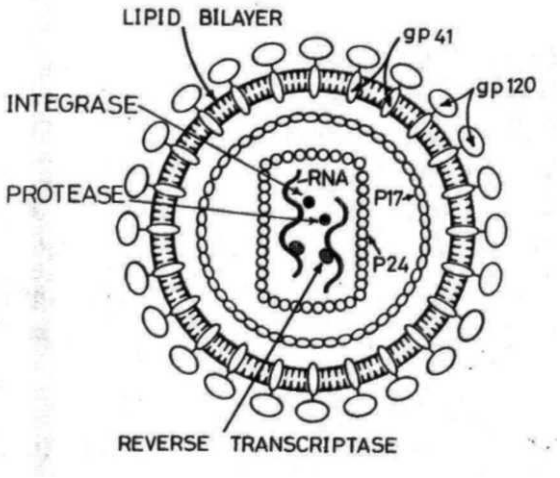
HIV-1 and HIV-2 differ in genome organization and phylogeny (evolution). The proteins of HIV-2 are only 40% identical to those of HIV-1. HIV-2 is prevalent in West Africa and is much less transmissible than HIV-1. Many groups and subtypes are described for both viruses.

The virion is a 100 nm spherical particle composed of a cylinderical internal **core** of proteins. The most important core protein is **p24** (the capsid), it appears in the serum early after infection and is a serologic marker for virus replication. It is surrounded by a matrix protein pi7. The core contains the viral **genome** which is a diploid single stranded positive sense RNA and the **enzymes;** reverse transcriptase (RT), integrase and a protease.

The core is surrounded by an **envelope** composed of a lipid bilayer which contains the precursor glycoprotein (gpl60) which is cleaved to form the two envelope type specific glycoproteins **gpl20,** and **gp41.** The latter is a transmembrane protein stem embedded in the envelope, while gpl20 protrudes from the surface and interacts with the CD4 molecules on T cells, monocytes, macrophages and dendritic cells (Fig.l 1).

The **genome** contains 9 genes. Three of the genes; ***gag*** gene encodes the core proteins, ***pol*** gene encodes the enzymes, and ***env*** gene encodes the envelope proteins. The *env* gene mutates rapidly resulting in many antigenic variants. The *tat* and *rev* genes are regulatory genes required for its replication. The other four are accessory genes.

(Fig. 11):
Structure of HIV particle.



The replicative cycle follows the retroviral cycle. The initial step is **binding** of the **gpl20** to the **CD4** protein and the coreceptors on the cell surface, then the **gp41 mediates fusion** of the viral envelope with the ceil membrane, and the virion enters the cell. After uncoating, **RT** transcribes the genome RNA into double stranded DNA **(provirus),** which **integrates** into the host cell DNA by the action of the **integrase** enzyme. Viral **mRNA** is **transcribed** from the proviral DNA and transported to the cytoplasm, where it is **translated** into large **polyproteins.** These are **cleaved** by the **protease** enzyme - to produce the different viral structures and enzymes- during viral assembly and budding from the cell. Cleavage by the protease enzyme is essential for maturation and production of infectious virus.

Pathogenesis and immunity:

HTV attacks **CD4** helper T cells leading to their depletion. The **CD4** molecules on these cells are the major receptors for attachment of the virus glycoprotein, **gpl20.** A protein coreceptor **CXCR4** (chemokine receptor) on T cells is required for infection of these cells.

Macrophages, monocytes and dendritic cells express **CD4** surface molecules and are infected by the virus. A coreceptor **CCR5** on these cells is required for their infection. The virus survives in these cells which act as a reservoir and can transport it to other organs e.g. brain and lungs.

The initial infection of the genital tract occurs in dendritic cells that line the mucosa, these migrate to local lymph nodes where helper T cells become infected.

During the acute stage cytotoxic **CD8** lymphocytes (CTLs) and antibodies dramatically reduce HIV levels, then, the **virus escapes** from the immune system and establishes chronic latent infection (clinical latency) through:-

I-Viral mechanisms: **a-** Latent infection of host cells as a **provirus.** b- Rapid genetic **mutation, c-** Trapping of infectious virus in lymphoid tissues in and on the surface of follicular dendritic cells, which act as **reservoirs.**

II-Suppression of immune mechanisms which are more evident during late stages of infection: a-Deletion of CD4 T cells, b-Deletion of HIV-specific CTL clones, c- Dysfunction of CTLs due to; decreased production of IL-2, and decreased expression of MHC-I by the action of viral genes, leading to decreased recognition of virus infected cells by CTLs. d- Impaired functions of APC. e- Interference with humoral response which are T cell dependent.

The massive destruction of T cells during the late stages is explained as follows; a- It is found that infected CD4 T cells express high levels of HIV envelope glycoproteins on their surface, this leads to fusion with CD4 molecules on neighbouring uninfected cells. This is followed by lysis of large numbers of fused cells and rapid depletion of CD4 helper T cells leading to marked suppression of the immune response, b- Strains of HIV found in the late stages are more virulent and cytopathic than the strains of virus found early in infection and may act as superantigens.

Mode of transmission:

1-Sexual contact: Both homosexual and heterosexuals are at high risk. The virus is present in semen and vaginal secretions.

2-Parenteral transmission by transfusion of blood or its products has been greatly reduced by screening donated blood for HIV antibodies. However, there is a window period early in infection when the blood of an infected person can contain HIV but no antibodies. Pricking by contaminated needles or syringes can transmit infection to those at risk e.g. drug abusers and health care workers.

3-Mother to foetus, transplacental, or to the newborn during delivery by exposure to blood in the birth canal or from the milk on breast feeding.

Clinical findings: The clinical picture of HIV infection can be divided into three stages: an early, acute stage; a middle, latent stage; and a late, immunodeficiency stage.

The acute stage begins 2-4 weeks after infection with a mononucleosis-like picture of generalized lymphadenopathy, fever, sore throat and a maculopapular rash. This stage occurs in 87% of those infected, however, it resolves spontaneously in 2 weeks. Antibodies to HIV appear 3-4 weeks after infection. There is rapid replication of the virus, which disseminates to various organs specially lymph nodes. An immune response, by cytotoxic T cells and humoral immunity, controls the infection.

A latent stage (clinical latency) for up to 10 years follows. The patient is asymptomatic, viraemia is low or absent, a large amount of HIV is being

produced and is trapped by the follicular dendritic cells in lymph nodes where they remain sequestrated. A syndrome called AIDS related complex (ARC) may occur late in the latent stage, characterized by fatigue, wasting, fever, chronic diarrhoea, and persistent lymphadenopathy. ARC often progresses to AIDS.

The late stage of HIV infections is **AIDS.** There is decline in the number of CD4 cells to below 400/uL and an increase in the frequency of opportunistic infections. The most characteristic are Kaposi's sarcoma and *Pneumocystis jiroveci* pneumonia. Others are viral infections e.g. CMV, disseminated herpes simplex and herpes zoster infections; fungal infections such as *C. albicans,* cryptococcal meningitis; bacterial infections such as, M *avium intracellular, M. tuberculosis;* protozoal infections such as toxoplasmosis and cancer e.g.. non-hodgkin's lymphoma and cervical cancer. Many patients have neurologic problems e.g. dementia and neuropathy.

Pediatric AIDS; Newborns that acquire HIV infection from the mothers -if untreated- have a poor prognosis. They usually present with clinical symptoms by 2 years of age; death follows in another 2 years from opportunistic infections. High levels of plasma HIV load indicate that the infant is at risk of rapid progression of the disease.

Laboratory Diagnosis:

1- Decreased CD4 cells count and inversion of the CD4/CD8 ratio.

2- **Detection of HIV antibodies** which appear 6-12 weeks after infection. Antibodies are detected by ELISA. A positive test must be repeated and confirmed by the Western blot technique which is more specific and it detects antibodies against viral core protein p24 or envelope glycoproteins gp 41, gp 120 or gpl60 and others. Simple rapid tests are available.

3- Detection of viral nucleic acid in clinical samples by PCR based assays. Quantitative PCR is used to determine plasma HIV RNA i.e. **viral load,** which helps in monitoring the effectiveness of antiviral therapies.

4-**Virus isolation** from lymphocytes, bone marrow or plasma in special laboratories.

5- **Detection of viral antigens** i.e. p24 by ELISA, which may be the only marker in the window period early in infection when antibodies are not detected. Donated blood should be tested for this antigen to assure its safety. However, the antigen often becomes undetectable after antibodies appear (because the p24 protein is complexed with p24 antibodies) but may reappear late in the course of infection, indicating a poor prognosis.

During the acute stage of HIV infection, antibody testing is usually negative. Diagnosis is made by viral isolation or by detection of viral **RNA** by PCR assay or by detection of p24 antigen. Diagnosis during this stage is important as it allows early treatment and helps in preventing infection of sexual partners. Patients with more than 10,000 copies of viral RNA/ml plasma are more likely to progress to AIDS than those with less numbers.

Diagnosis of pediatric AIDS is best established by detecting viral nucleic acids by PCR, viral isolation or detection of p24 antigen. Assays for IgM antibodies are remarkably insensitive because of its low concentration. Commercial kits are not available.

Treatment: A combination of 2 nucleoside analogues (reverse transcriptase inhibitors) in addition to a protease inhibitor is the treatment of choice for acute HIV infections. Other regimens are used (Chapter 24).

Therapy with combination of antiretroviral drugs is referred to as HAART or "highly active antiretroviral therapy". The first once-daily pill containing 3 HIV drugs was approved in USA in 2006. These regimens lower virus load to undetectable levels, delay emergence of resistant mutants, allow the recovery of the immune responses to opportunistic pathogens and prolong patient survival. However, the virus persists and reappears when HAART is discontinued. So combination therapy has turned HIV infection into a chronic treatable disease. New drugs are, integrase inhibitors and chemokine receptor (CCR5) antagonists that inhibit viral entry into cells (Chapter24).

Prevention and control:

No vaccine is available and several are under trial. However, vaccine production for HIV is difficult due to several factors; e.g. rapid mutation of the virus, absence of an appropriate animal model as well as the unclear understanding of which host immune responses are protective.

Prevention consists of taking measures to avoid exposure to the virus, e.g. sex education, not sharing needles, screening of donated blood for HIV. Contaminated wounds should be washed with soap and water. Blood spills on surfaces are disinfected by chlorine 1% for 10 min.

Post-exposure chemoprophylaxis after a needle-stick injury by AZT, lamivudine and a protease inhibitor for 2-4 weeks prevents infection.

Measures to reduce transmission of HIV infection from infected mothers to foetus or newborn include: Screening of pregnant mothers for HIV, and those infected should receive AZT or nevirapine during pregnancy and intravenous AZT during delivery. Neonates should receive the same drugs. Delivery by cesarean section is recommended. Infected mothers should not breast feed their infants.

HERPESVIRUSES

The herpesvirus family contains several important human pathogens. An outstanding characteristic of this family is the ability to establish **latent** infections which persist indefinitely in infected hosts with periodic reactivation specially in immunosuppressed hosts. Some members cause cancer. This group is susceptible to antiviral chemotherapy. Herpesviruses that commonly infect man include:

- **Herpes simplex virus** (HSV) type 1 and 2.
- **Varicella-Zoster virus.**
- **Cytomegalovirus** (CMV).
- **Epstein-Barr virus** (EBV).
- **Human herpesvirus *6*** (HHV 6) infects T lymphocytes. It is acquired in early infancy and causes the childhood rash; roseola infantum (sixth disease) associated with lymph-adenopathy, sore throat and fever.
- **HHV** 7 is another T-lymphotropic virus that was isolated in 1990 from CD4 lymphocytes. Its disease association is not clear.
- **HHV 8** or the Kaposi's sarcoma-associated herpesvirus (KSHV) was first detected inl994 in Kaposi's sarcoma (KS) specimens from AIDS patients. It causes KS, which is a vasocutaneous, multifocal tumour. The viral DNA is found in tumour cells and can be detected in biopsy specimens by PCR It causes malignant transformation by inactivation of the Rb (retinoblastoma) tumour suppressor gene. It is transmitted sexually and in transplanted organs.

The virus: Different members of the herpesvirus family are morphologically similar. They are double stranded DNA icosahedral viruses 120-200 nm The nucleocapsid is surrounded by an envelope, which is derived from the nuclear membrane of the infected cell and contains glycoprotein spikes.

HERPES SIMPLEX VIRUSES

There are two distinct herpes simplex viruses, type 1 and type 2. The two viruses cross-react serologically but some unique proteins exist for each type. They differ in their mode of transmission; HSV-1 spreads by contact with, or droplets of infected sahva, whereas HSV-2 is transmitted sexually or to newborns during birth. This results in different clinical forms of infection. Most HSV-1 lesions are above the waist, while most HSV-2 lesions are below the waist.

Primary infection commonly occurs in children 2-4 years of age or newborn infants can contract the infection from the birth canal or *in utero.*

HSV multiplies locally in the mucous membrane or abraded skin causing cytolysis, necrosis, ballooning, multinucleated giant cell formation and intranuclear inclusion bodies in infected cells. Cell fusion provides cell-to-cell spread of HSV, even in the presence of neutralizing antibodies.

Clinical manifestations are vesicular lesions, which may change to shallow ulcers. Scabs form and lesions heal without scarring. Most primary infections are asymptomatic. Those due to HSV-1 occur during childhood, while HSV-2 infections occur mainly at the age of sexual activity.

Latent infections: From the primary lesions, the virus migrates up the neurons and remains latent in the trigeminal ganglia (HSV-1) or the sacral ganglia (HSV-2) where the virus persists for the life time of the host.

Reactivation of the virus is provoked by several stimuli including; fever, physical or emotional stress, immunosuppression, menstruation and exposure to sun light. The virus migrates down the neurons and replicates in the skin, causing the lesions. Reactivation may be asymptomatic resulting in virus shedding only.

Clinical syndromes:

1- **Gingivostomatitis** due to HSV-1. Primary infection occurs in children and is characterized by vesicular lesions in the mouth accompanied by fever, malaise and myalgia. In adults it causes **pharyngotonsillitis.**
2- **Herpes labialis** (fever blisters) caused by HSV-1, characterized by crops of vesicles at the mucocutaneous junction of the lips or nose. Recurrences occur at the same site.
3- **Keratoconjunctivitis:** HSV-1 infection of the eye causing corneal ulcers which may leave opacities and lead to blindness.
4- **Encephalitis** due to HSV-1 with a high mortality rate.
5- **Meningitis** due to HSV-1 or HSV-2.
6- **Disseminated infections,** such as pneumonia in immunosuppressed patients e.g. AIDS or transplant patient.
7- **Herpetic whitlow** is a pustular lesion on the fingers of medical staff acquired from patients due to HSV-1 or HSV-2. Another skin affection is **eczema herpeticum** that occurs in persons with chronic eczema due to HSV-1.
8- **Genital herpes** due to HSV-2 is characterized by vesiculo-ulcerative lesions on the external genitalia as well as the cervix. It is associated with fever and inguinal lymphadenopathy.
9- **Neonatal herpes** acquired from vesicular lesions in the birth canal. The most serious infection is disseminated disease of the newborn including meningitis or encephalitis. Cesarean section may be used to avoid infection. Both HSV-1 and HSV-2 acquired postaatally from family members or hospital personnel who are shedding virus can cause neonatal herpes.

Diagnosis is needed when the clinical diagnosis is not clear, or where rapid confirmation is needed to support the choice of therapy.

1- Tzanck smear; in which scrapings from the base of skin lesions are stained with Giemsa. The presence of multinucleated giant cells suggests HSV infection

2- Detection of viral antigens or viral DNA in vesicular fluid by immunofluorescence or by DNA probes or PCR.

3- A rapid diagnosis of encephalitis can be made by detecting HSV DNA in CSF by PCR.

4- Isolation of the virus from herpetic lesions. Cytopathic effect occurs in 1-3 days. Virus is identified by immunofluorescence or ELISA.

5- Serologic diagnosis by detection of a rise in antibody titre may be useful in primary infection. Detection of IgM antibodies in the newborn sera indicates intra-uterine infection.

Treatment:

Acyclovir is used for all herpetic infections and is useful if given early and intravenously in treatment of encephalitis, systemic disease and neonatal infections. Vidarabine, foscarnet and valacyclovir (orally), are also used. Penciclovir and acyclovir are used as ointments for treatment of herpetic keratitis or orolabial infections. The drugs have no effect on the latent state.

Prophylaxis:

Vaccines of various types are being developed. A recombinant HSV-2 glycoprotein vaccine recently tested, failed to prevent herpesvirus infections in a large clinical trial in 2010. Cesarean section is recommended for women who are at term and have genital lesions or positive cultures.

VARICELLA - ZOSTER VIRUS (VZV) There are two distinct diseases caused by the same virus. Varicella is the primary disease characterized by generalized rash, while zoster is the recurrent form and manifests by localized eruptions. There is only one type of the virus and man is the natural host.

Varicella (chickenpox) is a highly contagious disease of children that occurs in epidemics. Infection is transmitted by respiratory droplets or by contact with the lesions. Incubation period is 10-21 days. VZV multiplies in the mucosa of the respiratory tract then it spreads *via* the blood to the skin causing the typical rash, which evolves from papules to vesicles, pustules, and finally crusts. It starts on the trunk and spreads to the limbs and face. Multinucleated giant cells with intranuclear inclusions can be detected in the base of the lesions. Recovery is the rule and lesions heal

without scar formation. The disease is mild in children but more severe in adults and in immunosuppressed children. After recovery the virus becomes latent in the dorsal root ganglia.

Congenital varicella syndrome following maternal cases of chickenpox during pregnancy may occur and is fatal. Neonates may acquire the infection from the mother before or just after birth with a high fatality rate.

Immunity following varicella is life-long, but zoster can occur despite this immunity to varicella.

Zoster (Shingles) is a sporadic incapacitating disease that occurs in immunosuppressed persons as a result of therapy, disease or aging and occasionally in healthy adults. It results from **reactivation** of latent VZV later in life at times of reduced cell mediated immunity or local trauma. The virus affects the sensory nerves and ganglia leading to severe pain in the area of skin supplied by these nerves, and then crops of vesicles appear over the skin supplied by the affected nerves. The eruptions are unilateral affecting the trunk and neck. Disseminated zoster may occur in immunosuppressed patients e.g. pneumonia. Susceptible children develop varicella when exposed to an adult zoster patient.

Diagnosis is mainly clinical. However, laboratory diagnosis can be done on the same lines used for HSV.

Electron microscopy of vesicular fluid may be needed, although rarely, for morphologic differentiation between varicella and poxviruses.

Prophylaxis:

- Varivax is a living attenuated vaccine that prevents varicella but zoster can still occur in those previously infected, as it does not eliminate the latent state, however, recurrences are very mild. One dose is recommended for children 12-15 months and a second dose at 4-6 years, by subcutaneous injection. It should not be given to immunosuppressed persons or pregnant women.
-Zostavax is a living attenuated vaccine licensed in 2006, given as a single dose, subcutaneous injection, to individuals 60 years of age or older, to reduce the risk of getting zoster (shingles). The vaccine should not be used for children and is not a substitute for Varivax.
- Acyclovir and varicella-zoster immune globulin which contains high titre of antibody to VZV are useful in preventing varicella and disseminated zoster in immunosuppressed persons exposed to infection.

Treatment: Acyclovir, valacyclovir or foscarnet are used to treat adults with chickenpox, zoster or disseminated disease, and immunosuppressed children with varicella. They reduce the duration and severity of symptoms.

CYTOMEGALOVIRUS (CMV)

CMV is a common cause of human diseases. Cytomegalovirus-infected cells are massively enlarged and hence the name. The virus is excreted in the urine, saliva, semen, breast milk and cervical secretions.

It is **transmitted** early in life transplacentally, within the birth canal, and commonly in breast milk. Later in life it is transmitted *via* saliva, sexually, by blood transfusion and organ transplants. Clinical forms are:

1- In the normal host it causes:

- **a-** Asymptomatic **latent** infection with the virus persisting in leucocytes and kidneys. Intermittent virus shedding in saliva and urine may occur.
- **b- Infectious mononucleosis-like** syndrome clinically similar to EBV infection. However, they are heterophile antibodies-negative.
- **c-** An association was observed between the presence of CMV and restenosis in coronary angioplasty. It is speculated that the virus may cause proliferation of cells of the smooth muscles, leading to restenosis.

2- In the immunocompromised host e.g. AIDS patients, those receiving organ transplants and those receiving chemotherapy; CMV causes pneumonia, retinitis, graft rejection or disseminated disease. In these patients, infection may be due to reactivation of their own latent virus.

3- Congenital infection *in utero* -from primary or reactivated infection of the mother- causes abortion, still-birth or **'cytomegalic inclusion disease'** characterized by congenital anomalies e.g. blindness, deafness, mental retardation or microcephaly. Hepatosplenomegaly, jaundice and purpura are common. The anomalies are more common when the foetus is infected during the first trimester. Perinatal infection from the birth canal or from breast milk usually results in subclinical infection.

Diagnosis:

1- Detection of intranuclear cytomegalic inclusions, which are oval "owl's-eye" shape in tissues or in desquamated cells in the urine.

2- Detection of CMV nucleic acids in tissues or body fluids e.g. blood, CSF and amniotic fluid by PCR, which is also used to determine the viral load.

3- Isolation of virus from throat washings and urine.

4- Detection of IgM or rising titre of IgG in congenitally infected infants is diagnostic.

Treatment and Prevention:

- Ganciclovir is used in treatment of AIDS patients. Valganciclovir (orally), cidofovir and foscarnet are used for treatment of CMV retinitis.
- Infants with cytomegalic inclusion disease should be isolated. Blood transfused to newborns should be CMV negative. If possible, only organs from CMV negative donors should be transplanted in CMV negative recipients.
- A genetically engineered subunit glycoprotein B vaccine is under trial.

EPSTEIN-BARR VIRUS (EBV)

EB virus is the causative agent of infectious mononucleosis. It is associated with Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin and non-Hodgkin lymphomas, gastric cancer, oral hairy leukoplakia in AIDS patients and lymphoprohferative disorders in immunodeficient persons.

Properties: EBV is morphologically similar to herpesviruses but differ antigenically. Important antigens are; viral capsid antigen (VCA), early antigens (EA), nuclear antigens (EBNA) and viral membrane antigens (MA) against which neutralizing protective antibodies are formed. It infects primarily **B** cells in some of which it remains **latent.** Viral DNA integrates into the cell genome, but many copies are found in the cytoplasm. EBV can transform B cells; they become immortal and proliferate ^definitely.

Pathogenesis: EBV is transmitted by infected sahva, very rarely by blood transfusion. Infection starts in the oropharynx then spreads to the blood where the virus infects **B** lymphocytes. Cytotoxic T lymphocytes react against infected B cells, change in morphology and appear as atypical T lymphocytes in the peripheral blood.

Primary infection during early childhood is asymptomatic resulting in life-long immunity; however, when it occurs during adolescence 50% manifest as **infectious mononucleosis (IM)** in which there is polyclonal activation of B cells, which secrete autoimmune, heterophil antibodies. The disease is characterized by fever, sore throat, enlarged lymph nodes and spleen, rash may appear and hepatitis commonly occurs. The disease is self-limited. However, IM in children with an inherited immunodeficiency results in a serious condition called X-linked lymphoprohferative syndrome.

Diagnosis of IM:

1- Blood picture shows increased leucocytic count (25,000/cmm) with absolute lymphocytosis, monocytosis and atypical lymphocytes.

2- Detection of EB virus in patients' materials i.e. peripheral lymphocytes, saliva, throat washings or lymphoid tissues by DNA probes or PCR.

3- Detection of **heterophil antibodies** that agglutinate sheep RBCs; by the Mono-spot or Paul Bunnel commercially available test kits.

4- Detection of antibodies to EBV specific antigens by ELISA or immunofluorescence. IgM to VCA indicates recent infection. IgG to VCA or EBNA indicate past infection.

5- Isolation of EBV from patients' materials though possible, yet it is difficult.

CHAPTER 33

POXVIRUSES

Poxviruses are the largest and most complex viruses. The group includes **variola virus;** the aetiologic agent of smallpox, vaccinia virus and cowpox virus, the three are antigenically related. The group includes also molluscum contagiosum and monkeypox viruses.

Vaccinia virus is the agent used for preparation of smallpox vaccine. It is also being studied to be used as a delivery vector to carry immunizing genes for several viruses, to be used as a polyvalent virus vaccine. It is also used as a vector in certain gene therapy experiments.

SMALLPOX

Smallpox is the first disease to be controlled by immunization and the first to be eradicated. A brief description should be included for several reasons:

1- Differentiation from similar clinical conditions e.g. chickenpox, monkeypox, pustular acne, meningococcaemia and drug rash.
2- The vaccine is still used on a small scale for military personnel and laboratory workers in contact with virus, and may cause complications.
3- There is concern that the virus could be reintroduced as a **biologic weapon.**

The virus is a large enveloped double stranded DNA, brick shaped, 230 nm diameter x 400 nm length. It can be seen by light microscopy. It grows on the chorioallantoic membrane of chick embryo producing characteristic lesions called "pocks".

Pathogenesis and clinical picture:

The virus was transmitted by respiratory aerosol or by direct contact with skin lesions or contaminated fomites. It is a systemic disease with a viraemic stage and final localization in the skin causing the rash. Incubation period was 12 days, followed by fever for 1-5 days. The rash started on the face and extremities then appeared on the trunk. The rash evolved through stages from macules to papules, vesicles, pustules and finally crusts in 2-3 weeks. Crusts fell off leaving scarred area. All stages of the rash as well as respiratory discharges and saliva were infectious. Immunity following disease is life-long.

Diagnosis:

1- Material collected from skin lesions was used for:
- Detection of the virus by electron microscopy.
- Detection of viral antigens by immunofluorescence.
- Isolation of the virus using chick embryo or tissue culture.
- PCR is available and can be used for diagnosis.

2- Detection of serum antibodies by neutralization tests, ELISA or RIA.

Vaccination by the live vaccinia virus:

The virus is naturally attenuated for man, giving rise only to local lesions that are followed by humoral and cell mediated immunity to smallpox. Immunity following vaccination lasted for about 10 years.

The vaccine is prepared from vesicular lesions (lymph) produced on shaved skin of calves or from virus grown on chick embryo; the final vaccine contains 40% glycerol to stabilize the virus and 0.4% phenol to destroy bacteria.

A new cell culture-produced vaccine is being evaluated.

The success of smallpox eradication officially declared in 1980 meant that routine vaccination is no longer recommended. **Recent** concern about a possible terrorist attack involving smallpox have resulted in recommendations for using smallpox vaccine on a limited scale e.g. starting with health care workers since they will be the first to be exposed to smallpox cases (first responders).

Complications of vaccination, which are treated by methisazone or cidofovir and vaccinia immunoglobulins, include;

1- Bacterial infection of the vaccination site.
2- Generalized vaccinia; spread of the virus through the blood causing generalized skin lesions. It occurs in immunosuppressed children.
3- Post-vaccination encephalitis which is rare.

Causes of successful eradication:

1- Man is the only host, and there is no animal reservoir of infection.
2- There is only one stable serotype of the virus.
3- There is no carrier state or subclinical infection.
4- Effective vaccine that is highly immunogenic and was used world wide.
5- A surveillance-containment program was used by the WHO. Smallpox cases are easily recognized clinically. Cases were traced and all susceptible contacts were identified and vaccinated. The vaccine is protective if given within 4 days after exposure.

Monkeypox can infect both monkey and humans and may resemble smallpox clinically. Human infections were discovered in 1970 in Africa after eradication of smallpox from the region. It is acquired by contact with wild animals killed for food or for their skins. It affects all ages but mainly children below IS years. Symptoms are similar to smallpox, but differ in occurrence of lymphadenopathy, lower mortality and transmissibility. Complications are common and serious, mainly pulmonary distress and secondary bacterial infections.

Although human infection is rare; recently monkeypox has reemerged on a greater scale than previously seen. An outbreak occurred in Zaire 1997. It is important to ensure that new cases of smallpox-like disease are due to monkeypox. The latter is antigenically distinct and produces different lesions on the chorioallantoic membrane of chick embryo. PCR is used for diagnosis.

Molluscum contagiosum causes human warts (benign skin nodules). It is transmitted by close contact and sexually. It is common in children and immunocompromised persons. The virus resembles smallpox in morphology but is not antigenically related. Diagnosis is clinical; however, PCR or electron microscopy may be used for the detection of virus in material expressed from lesions. Treatment is surgical removal.

CHAPTER 34
ADENOVIRUS
ES

In humans, adenoviruses can cause disease in several organs. The virus causes **latent** infection in the adenoids, tonsils and lymphoid tissues.

Types 12, 18 and 31 induce tumours in newborn hamsters. Some adenoviruses can cause transformation of cells in culture. However, adenovirus **oncogenesis** has never been observed in humans.

The virus is 70-90 nm. It is a non-enveloped double stranded DNA virus with an icosahedral nucleocapsid. They are the only viruses with a **fiber** protruding from each of the 12 vertices of the capsid. The fiber is the organ of attachment and is a haemagglutinin. There are about 51 human antigenic types. The fiber protein is the type specific protein.

Diseases caused by adenoviruses occur by respiratory droplets, faeco-oral and by direct contact. Most infections resolve spontaneously, 50% are asymptomatic or mild and induce life-long type specific immunity against reinfection. Infections include:

1- Acute febrile pharyngitis and pneumonia which is common in children and military recruits by types 3,4, 7 and 21.

2- Pharyngoconjunctival fever may occur in outbreaks in summer camps (swimming pool conjunctivitis) by types 3 and 7.

3- Conjunctivitis and epidemic keratoconjunctivitis by types 8,19 and 37.

4- Gastroenteritis and intussusception in infants by types 40 and 41.

5- Acute haemorrhagic cystitis in children, especially boys, by types 11 and 21.

6- Immunocompromised e.g. AIDS patients may suffer severe GIT infections or fatal pneumonia. Transplant patients may suffer hepatitis or myocarditis in the transplanted organ mostly due to endogenous reactivation of a latent virus.

Diagnosis: Body secretions are examined by: a) E.M. b) Immunofluorescence to detect clustering of cells and inclusion bodies, c) Antigen detection by ELISA. d) PCR. e) Isolation of the virus in cell culture, f) A 4-fold rise of antibody titre is a good evidence of infection.

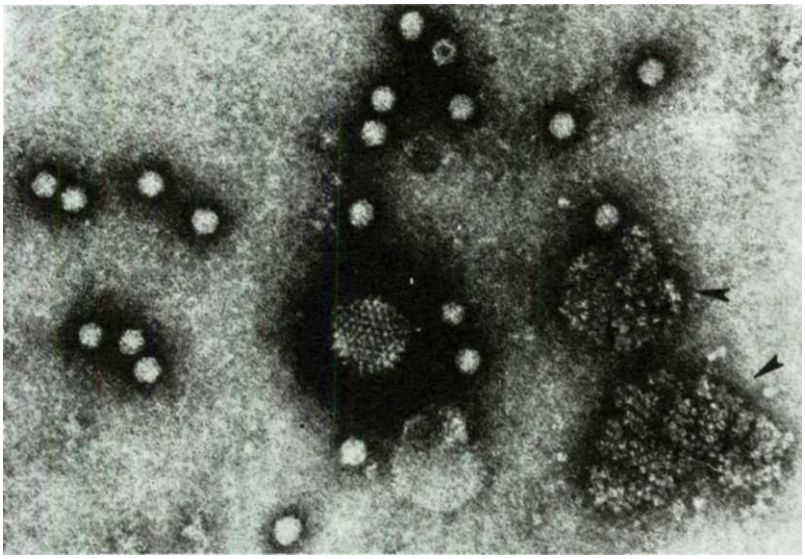
Prevention: Live vaccines containing types 4 and 7, available in gelatin-coated capsules, and given orally to military recruits was introduced in 1971. The vaccine proved effective but since 1999 its manufacture had been discontinued,

Gene therapy: Adenoviruses are being used as gene delivery vehicles for cancer therapy, gene therapy and genetic inununization studies. A novel anticancer therapy uses an attenuated replication-competent adenovirus

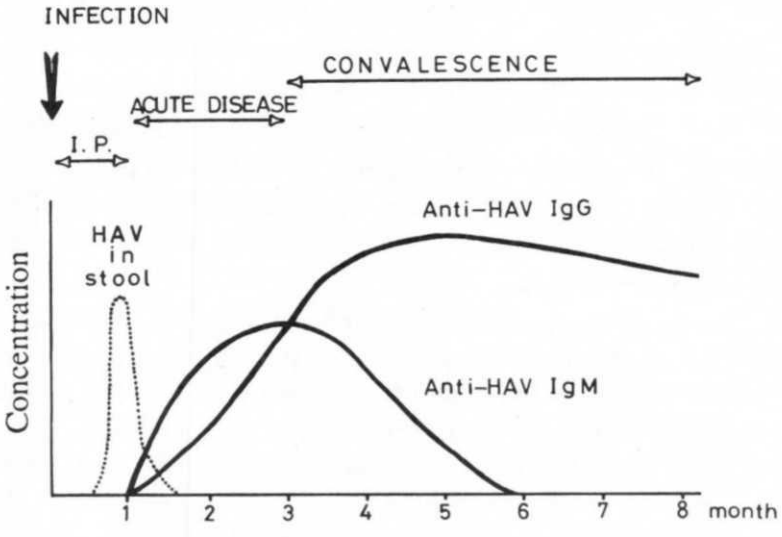
engineered to replicate only in targeted cancer cells. This "oncolytic therapy" is aimed at directly killing tumour cells due to viral lytic replication.

Adenoassociated Viruses or Defective Parvoviruses

These are small 20 nm particles that have been found in adenovirus preparations (Fig. 12). They are defective viruses that can not replicate unless adenovirus is present as a helper. Their role in disease production is not known.



(Fig. 12): Electron micrograph of an adenovirus particle surrounded by the smaller, adenoassociated virus particles. Arrows point to disrupted capsomcrcs.



(Fig. 13): Diagnosis of hepatitis A virus infection.

HEPATITIS VIRUSES

Hepatitis can occur in the course of several viral infections e.g. cytomegalovirus, yellow fever, Epstein-Barr, herpes simplex, rubella and entero-virus infections. However, some viruses primarily infect the liver and are called hepatitis viruses, these include: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV).

HEPATITIS A VIRUS (HAV)

HAV is a *Hepatovirus* genus in the Picornaviridea family. It is a non-enveloped ss-RNA icosahedral virus 27-30 nm in diameter. Only one serotype is known. The virus is relatively heat resistant; it withstands 60°C for 1 hour. It is destroyed by boiling for 5 minutes and by autoclaving. It is inactivated by formalin and chlorine.

It is pathogenic to primates e.g. man and chimpanzees. It grows on primary cell lines of primate hver and human diploid cells.

Epidemiology and clinical picture:

It is transmitted by the faeco-oral route usually through ingestion of contaminated food and water, incubation period is 2-6 weeks i.e. shorter than that of HBV. The manifestations are the same in all hepatitis viruses. The virus replicates in cytoplasm of hepatocytes and then is shed in bile, resulting in high titre of infectious HAV in stools. Manifestations are jaundice, fever, abdominal pain, anorexia and hepatomegaly. HAV infection is usually milder than HBV. Many cases are anicteric or asymptomatic. Recovery occurs without complications; no chronic hepatitis or chronic carrier state occurs, and there is no predisposition to hepatocellular carcinoma (HCC). Infection is followed by long lasting immunity.

The virus is found in the stools 2 weeks before and 2 weeks after the jaundice appears (Fig. 13). It affects mainly children and young adults (5-15 years). It may occur in sporadic or epidemic forms in summer camps or schools. It occurs mainly in autumn.

Laboratory diagnosis: (Fig. 13)

1- Increased level of liver enzymes and billirubin.
2- Detection of HAV IgM by ELISA or RIA indicates acute infection.
3- Detection of a rising titre of IgG. It persists for decades and indicates immunity.
4- Detection of the virus in stools by immunoassays, nucleic acid probes, PCR or immunoelectron microscopy.

Prophylaxis:

Hepatitis A vaccine **(Havrix)** a formalin inactivated vaccine is recommended for all children in 2 doses. The first dose is given at 12-23 months, the second 6 months later. It is given intramuscularly in the deltoid region. The vaccine may be given to those traveling to developing countries.

Twinrix is a combination vaccine that immunizes against both HAV and HBV. It contains the same immunogens as the individual vaccines.

Immune (y) globulin if given early after exposure renders the infection milder or subclinical mainly in the immunodeficient.

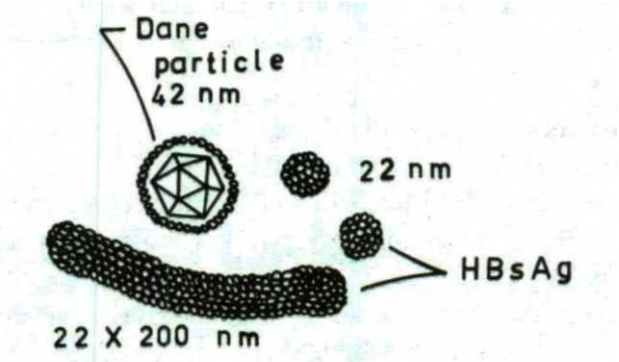
HEPATITIS B VIRUS (HBV)

HBV causes serum hepatitis. It is an enveloped partially double stranded DNA virus classified as a "Hepadnavirus". Three different types of particles are found in patient's serum (Fig. 14):

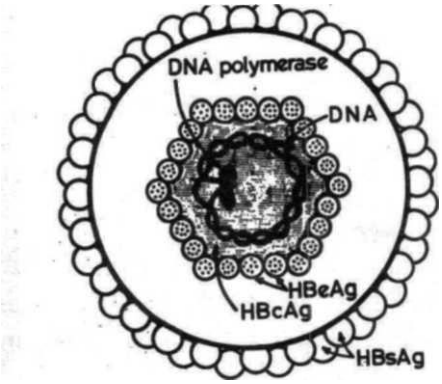
"Dane particles" are 42 nm spherical particles. These are the complete infectious virions of HBV and consist of an outer envelope which contains a protein called the surface antigen (HBsAg); which is important for laboratory diagnosis and immunization. The envelope surrounds the nucleocapsid core (HBcAg) which surrounds the viral DNA (genome) and the DNA polymerase. HBeAg is also a part of the core (Fig. 15).

Many **22 nm spherical particles** and **22 nm tubular particles** that are over 200 nm long are also found in serum. Both are composed of hepatitis B surface antigen (HBsAg) and are non-infectious.

For vaccine purposes, HBV has one serotype based on HBsAg. However, for epidemiologic purposes, there are four serologic subtypes of HBsAg based on a group specific antigen, "a", and two sets of epitopes d or y and w or r. These serotypes are -adw, adr, ayw, ayr- which are useful in epidemiologic studies because they are concentrated in certain geographic areas.



(Fig. 14): Three morphologic forms of HBV.



(Fig. 15): A diagram of a complete HBV particle showing relation of structure to antigens.

Mode of transmission:

It should be assumed that all body fluids from HBV-infected patients may be infectious especially those who are HBeAg positive.

1- Parenteral transmission, by blood and plasma transfusion. However, screening of donated blood has reduced the risk of transfusion-associated hepatitis. Transmission through the prick of contaminated needles or scalpels and even tattooing or ear piercing occurs. Risk groups include; drug abusers, medical personnel, renal dialysis patients and those receiving repeated blood transfusion e.g. haemophiliacs or transplant patients.

2- Transmission from carriers to close contacts by the sexual route or by other intimate exposures. Saliva can be a vehicle of transmission through bites. The virus is found in saliva, semen and vaginal secretions.

3- Perinatal transmission from mothers with hepatitis B to the newborn during delivery, and from an infected household contact to an infant.

Pathogenesis:

Humans are the only natural hosts of HBV. There is no animal reservoir. After entering the blood, the virus infects hepatocytes (as they have the virus-specific receptors) and viral antigens are displayed on the surface of the cells. Cytotoxic T cells mediate an immune attack against the viral antigens, causing necrosis and inflammation. The pathogenesis of hepatitis B is probably the result of this cell mediated immune injury, because HBV itself does not cause a cytopathic effect (during replication, the virus is released from the cell by budding). Antigen-antibody complexes cause some of the early symptoms, e.g., arthritis and urticaria, and some of the complications in chronic hepatitis, e.g., glomerulonephritis, vasculitis and cryoglobulinaemia.

Clinical findings and outcome of infection: Many HBV infections are asymptomatic and are detected only by the presence of antibody to HBsAg. After exposure, there is an incubation period of 2-6 months, which is much longer than that of hepatitis A. The symptoms are anorexia, nausea,

vomiting, abdominal pain and mild fever. Jaundice may appear after few days; however, anicteric hepatitis is more common. The symptoms are more severe in hepatitis B than in hepatitis A and can lead to life threatening **complications.**

Unlike hepatitis A, about 10% of infected adults and 90% of infected neonates become chronic carriers with persistent HBV antigenaemia, which is due to persistent infection of hepatocytes. The virus is present in the blood and other body fluids e.g. semen, sahva and vaginal secretions.

Most carriers are asymptomatic, but some are complicated by chronic active hepatitis, which can lead to cirrhosis, fulminant hepatitis and death.

A high rate of hepatocellular carcinoma (HCC) occurs in chronic carriers. During virus replication, it is found that some copies of viral DNA are integrated into the host cell genome and this seems likely to be the DNA that maintains the carrier state and predisposes to malignancy, through the activation of a cellular oncogene, leading to loss of growth controL

Recovery is associated with life-long immunity mediated by anti- HBsAg, which neutralizes viral infectivity. Anti- HBc is not protective.

Laboratory diagnosis:

1- Increased levels of liver enzymes and billirubin.

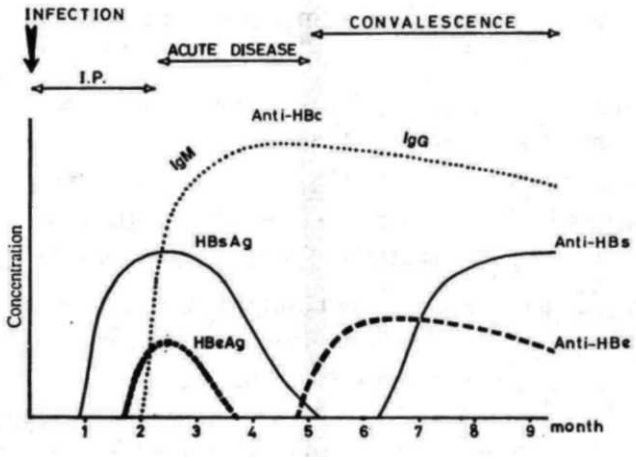
2- Detection of hepatitis markers i.e. antigens (Ag) and antibodies (Ab) in blood by ELISA (Fig. 16):

- **HBsAg** is detected during the incubation period and during active disease. It typically disappears 6 months after exposure, however, it may remain for more than 6 months or years in chronic carriers.
- **HBcAg** is not detected in the blood. It is detected only in the nuclei of liver cells.
- **HBeAg** appears during the LP., shortly after appearance of HBsAg. It is detected in the acute stage of the disease and in some chronic carriers. Its presence indicates that the serum contains high concentrations of infective HBV and is highly contagious.
- **Anti-HBs** appears after disappearance of HBsAg and its presence indicates immunity. It is the only antibody present after vaccination.
- **Anti-HBe** appears as HBeAg disappears and it indicates recovery. It is not detectable after 6 months.
- **Anti-HBc** IgM appears with the onset of clinical disease and declines within 6 months to be replaced by anti-HBc IgG which remains ^definitely indicating past infection.

There is a period of several weeks when HBsAg has disappeared and anti-HBs is not yet detectable. This is the window phase. At this time, anti-HBc IgM is always positive and is used to make the diagnosis.

3- Detection of viral DNA by PCR is a strong evidence of viraemia. It is useful in monitoring the effect of antiviral therapy, by measuring the viral load and for screening donated blood in the window phase.

4- Detection of viral DNA polymerase activity during the LP. and early in the disease i.e. during the viraemia.



(Fig. 16) Serologic markers used in the diagnosis of HBV infections.

In the **acute stage;** HBsAg is positive, HBsAb is negative, HBcAb* is positive. In the **window phase;** HBsAg is negative, HBsAb is negative, HBcAb is positive. In **complete recovery;** HBsAg is negative, HBsAb is positive, HBcAb is positive. In **chronic carriers;** HBsAg is positive, HBsAb is negative, HBcAb is positive.

* HBc IgM is found in the acute and window stages. IgG is found in other stages.

Ocult HBV infections are those in which patients lack detectable HBsAg but HBV- DNA can be identified in liver or serum samples. It occurs frequently (33%) in patients with chronic HCV liver disease. **Prophylaxis**

Pre-exposure prophylaxis by active vaccination of those at high risk. It is recommended for all newborns as part of their immunization schedule.

1- Plasma derived vaccine (Heptavax B): Prepared by purifying HBsAg from pooled plasma from healthy HBsAg positive carriers and inactivated. It is safe and effective and is still used in some countries.

2- Recombinant hepatitis B vaccine (Recombivax) has replaced Heptavax B in several parts of the world. It contains HBsAg produced in yeast cells

by the recombinant DNA technique. The vaccine is given in three intramuscular injections at 0, 1-2 months and the third dose at 6 months in USA. In Egypt it is given at 2,4 and 6 months.

The site of injection is the deltoid region in adults and children and in the anterolateral thigh muscles in infants and neonates.

The vaccine is recommended for adults at high risk who were not vaccinated e.g. health care workers, drug addicts, repeated transfusions or dialysis patients and those with frequent sexually transmitted diseases.

People immunized with HBV vaccine have anti-HBs but not anti-HBc because the immunogen in the vaccine is purified HBsAg.

Post-exposure prophylaxis: Persons exposed to a needle-stick injury from a patient with HBsAg positive blood and newborns whose mothers are HBsAg positive, should immediately receive both hepatitis B specific immunoglobulin **(HBIG)** and hepatitis B vaccine, given simultaneously at different sites. This is a good example of passive-active prophylaxis.

Control measures include implementation of infection control measures e.g. proper screening of blood donors, use of plastic disposable syringes, hand hygiene, avoid injury with sharp instrument... etc.

Treatment: Interferon-a and pegylated interferon-a are used in the treatment of chronic HBV. Lamivudine and adefovir are nucleoside analogues that inhibit viral DNA polymerase and are used for treatment.

HEPATITIS C VIRUS (HCV)

It is a member of the Flaviviridae family, genus *Hepacivirus.* It is a 60 nm spherical enveloped single stranded RNA virus. There are at least six genotypes and many subtypes. Different genotypes prevail in different parts of the world. Genotype 4a constitutes the majority of infections in Egypt. The virus undergoes variation during chronic infection resulting in a complex virus population in one host. This is called quasi-species. this difference complicates response to antiviral therapy which is genotype dependant. HCV is the most prevalent blood-borne pathogen.

Pathogenesis **and clinical findings** are similar to HBV. Incubation period is 8 weeks. Many acute and chronic infections are asymptomatic. Only 5% of individuals with acute HCV infection have symptomatic disease. However, the acute illness is milder than in HBV. The sequelae are similar to HBV; however, they are more common and severe. Chronic carriage rate is higher than in HBV and 85% of cases progress to chronic disease i.e. chronic active hepatitis, cirrhosis and HCC.

Transmission is mainly parenteral as in HBV. Sexual and perinatal transmission have been very difficult to document and are considerably less efficient than for HBV. However, it was reported possible with severely immunosuppressed HTV patients. There is no evidence of insect vector. In 10-50% of cases, the source of HCV infection is not known. **Laboratory diagnosis:**

1- Detection of anti-HCV antibodies in blood by ELISA and confirmation by a more specific test RIB A (recombinant immunoblot assay).

2- PCR for the detection of viral RNA in blood i.e. active infection. It is useful in early diagnosis before seroconversion occurs, for genotyping and for monitoring the effect of antiviral therapy, by measuring the viral load.

Prevention: No vaccine. Control measures are like those used for HBV.

Treatment; Telaprevir or boceprevir are protease inhibitor approved in 2011 used in triple therapy with long acting pegylated IFN-a and ribavirin. Sofosbuvir (sovaldi) is a nucleotide analogue inhibitor approved in 2013. It is more effective with shorter treatment time and less side effects. It is used with ribavirin for treatment of types 2 and 3 and + pegylated IFN-a for treatment of types 1 and 4.

HEPATITIS D VIRUS (HDV)

HDV is a **defective RNA virus** that replicates only in cells also infected by HBV, because HDV uses the surface antigen of HBV (HBsAg) as its envelope. HBV provides a rescue function for HDV. Its mode of transmission is similar to HBV. The incubation period is 2-12 weeks.

Hepatitis in chronic HBV carriers who become super-infected with HDV is more severe and the incidence of complications e.g. chronic active or fulminant hepatitis is much higher. Super-infection or co-infection with the two viruses occurs mainly in those receiving repeated blood transfusions or pooled plasma as well as drug addicts.

Diagnosis is done by the detection of HDV antigen or HDV antibodies. PCR is used for the detection of viral RNA.

Prevention of HBV infection by vaccination will consequently prevent HDV infection. However, vaccination does not prevent HBV carriers from super-infection by HDV.

HEPATITIS E VIRUS (HEV)

HEV is classified in the family, Hepeviridea, in the genus *Hepevirus.* It is a non-enveloped single stranded RNA virus. It infects by the faeco-oral route. Water- borne epidemics are reported. It resembles hepatitis A clinically with the exception that, it causes high mortality rate (20%) in pregnant women if fulminant hepatitis develops. Diagnosis: PCR is used to detect virus in stools. Detection of anti-HEV IgM or a rising titre of IgG by RIA or ELISA

CHAPTER 36

TUMOUR VIRUSES AND ONCOGENESIS

It is well proved that viruses cause cancer in animals. However, proving absolute association between viruses and human cancer is difficult since: **a-** The virus cannot be always isolated from the tumour, **b-** Many of the viruses isolated from human tumours do not produce tumours in experimental animals.

Cell Transformation:

Tumour viruses when grown on cell cultures lead to change of characters of these cells called **transformation.** It is a stable heritable change in growth control of these cells. Transformed cells have several characters of which:

1-Alteration in morphological, metabohc and genetic properties of the cell.
2-Increased growth rate.
3-Loss of the property of contact inhibition and piling up (p. 103).
4-They can divide indefinitely in serial culture (immortalization).
5-Reduced serum requirement for growth.
6-Appearance of new antigens, which are mainly virus specific antigens.
7-They produce tumours when injected in appropriate animals.

Mechanism of cell transformation by tumour viruses:

The change of a normal cell to a neoplastic cell is attributed to the integration of certain viral genes into the host cell genome. With DNA tumour viruses, a portion of the DNA of the viral genome becomes integrated into the host chromosome. With RNA tumour viruses (retroviruses), viral RNA acts as a template for the synthesis of viral DNA through the action of the reverse transcriptase enzyme (which is contained in all retroviruses). The DNA copy of the viral RNA is integrated into the host cell chromosome. The integrated DNA "provirus" causes cell transformation and carcinogenesis through:

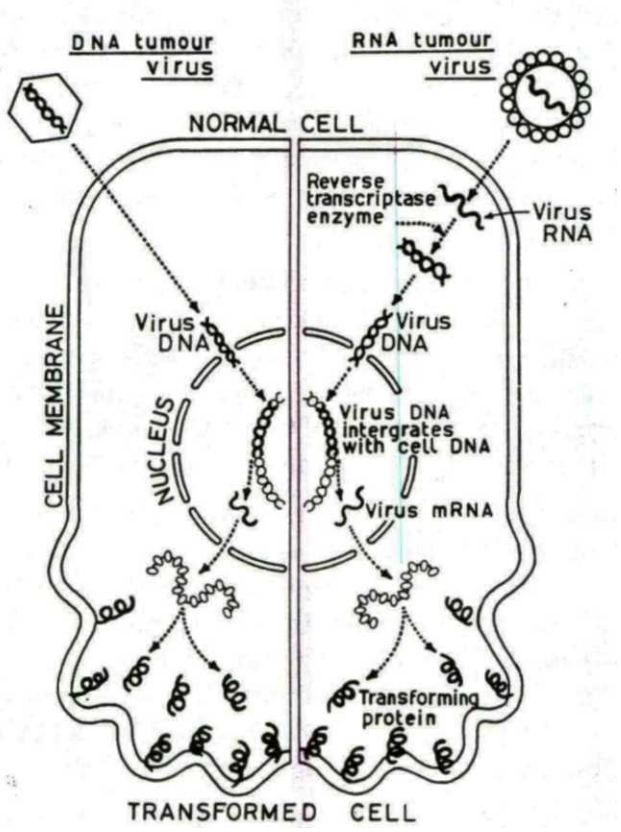
1- Introduction of new transforming genes i.e. **"viral oncogenes".**

2- Activation or over expression of pre-existing cellular genes **"proto-oncogenes"** which are, normally suppressed (or expressed at low regulated levels). This can occur due to mutation, translocation or amphfication of these genes induced by the tumour virus.

Insertion of a retroviral promoter element adjacent to a cellular oncogene may result in enhanced expression of that gene i.e. "promoter-insertion oncogenesis". Expression of a cellular gene also may be increased through the action of nearby viral "enhancer" sequences.

3- Inactivation of a tumour suppressor gene e.g. the Rb and the p53 gene. The p53 encodes a protein that activates the synthesis of a second protein, which blocks the cyclin-dependent kinases required for cell division to occur. The p53 protein also promotes apoptosis of cells that have sustained DNA damage or contain activated cellular oncogenes. Loss of Rb gene function results in development of retinoblastoma. Inactivation of tumour suppressor genes appears likely to be an important general mechanism of viral oncogenesis.

Tumour suppressor genes are involved in the formation of many cancers e.g. in many colon carcinomas, 2 genes are inactivated, the p53 gene and the DCC (deleted in colon carcinoma) gene. More than 50% of human cancers have a mutated p53 gene in the DNA of the malignant cells.



(Fig. 17): Mechanism of cell transformation by DNA or RNA tumour viruses.

Human tumour viruses:

One or more of the following criteria are used as evidence for the association of some viruses and human tumours:

a- Detection of virus nucleic acid integrated in the tumour cell chromosome or free in the cytoplasm as in EB virus.

b- Detection of viral antigens in or on tumour cells.
c- The ability of viruses isolated from human tumours to cause transformation of
cells *in vitro* e.g. EBV, or to induce tumours in experimental animals, **d-** The most definitive proof of a causal relationship is decreased tumour incidence by prevention of viral infection e.g. HBV and HPV vaccination.

Only two viruses HTLV and human papillomavirus are considered to be definite human tumour viruses. Several others are considered candidates.

1- **Human papillomavirus** (HPV) is a dsDNA non-enveloped virus (100 types). It is transmitted by direct and sexual contact. It causes infections at cutaneous and mucosal sites that vary from benign warts to cervical carcinoma. Types 6 and 11 cause anogenital warts; types 16 and 18, are implicated as the cause of carcinoma of the cervix. About 90% of anogenital cancers caused by HPV contain the DNA of these types. In most of these tumour cells, viral DNA integrated into host cell DNA leads to over-expressed E6 and E7 genes with overproduction of E6 and E7 proteins which are HPV transforming proteins. These inactivate tumour suppressor proteins encoded by p53 and Rb genes and induce abnormal mitosis.

HPV quadrivalent recombinant vaccine is composed of HPV LI proteins of types 6, 11, 16 and 18. It protects against cancer cervix and anogenital warts. It is recommended for all adolescents aged 9-12 years (before sexual activity). A catch-up vaccination is recommended at 13-26 years. It is given by I.M. injections in three doses at a schedule of 0,1-2 and 6 months. A bivalent vaccine containing types 16 and 18 is used for females only.

2- **Human** T **cell lymphotropic retrovirus** (HTLV) has been established as the causative agent of certain cutaneous T cell lymphomas and leukemias as well as tropical spastic paraparesis; an autoimmune disease. It infects preferentially CD4 T cells and causes their transformation *in vitro.* The proviral DNA is found integrated in the DNA of the malignant lymphoma cells only. It is transmitted by blood transfusion, I**.V.** drug users, sexually in semen and from mother to infant in breast milk. It is diagnosed by PCR and by the detection of antibodies by ELISA and Western blot technique.

3- **Epstein-Barr** virus is associated with nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkins, non Hodgkins lymphomas and gastric cancer.

4- **Human Herpesvirus 8;** causes Kaposi's sarcoma. The DNA of the virus has been detected in the sarcoma cells, but its role in oncogenesis remains to be determined.

5- **Hepatitis B and** C **virus** infections and their sequelae predispose to hepatocellular carcinoma (HCC). The integration of HBV DNA may cause insertional mutagenesis, resulting in the activation of a cellular oncogene.

SLOW VIRUS INFECTIONS and PRIONS DISEASES

Slow infectious diseases are caused by a heterogeneous group of agents including conventional viruses and unconventional agents i.e. prions. These agents cause CNS disease characterized by long incubation period, a gradual onset, and a progressive, invariably fatal course.

Prions are infectious particles composed entirely of proteins (PrP) with no detectable nucleic acids, which differentiates them from viruses. E.M. reveals filaments rather than virus particles. With scrapie prion as the model, it was found that this protein is encoded by a single cellular gene. When these proteins are in the normal, alpha-helix configuration PrP^{c}, they are non-pathogenic, but when their configuration changes to a beta-pleated sheet PrP^{80}, they aggregate into filaments that disrupt neuronal functions and result in the symptoms of the disease. This conversion is enhanced by specific cellular RNAs. Prion protein in normal cells is protease-sensitive whereas prion protein in infected cells is **protease-resistant** probably because of the change in conformation.

They are highly resistant to inactivation by heat, U.V. light and formaldehyde, but are inactivated by phenol, ether, hypochlorite, NaOH and autoclaving. They are resistant to temperatures used for cooking, a fact that may be important in their suspected ability to be transmitted by food.

Characters of prion-mediated diseases:

1- They are confined to CNS causing neurodegeneration (amyloid) and spongiform changes due to neuronal vacuolation and neuronal loss.

2- They have a long incubation period and a chronic progressive course with dementia and end fatally.

3- There is no inflammatory or immune response to these diseases as they are normal human proteins.

4- They are transmissible by ingestion of infected tissues mainly the brain, transplanted tissues and contaminated surgical instruments.

Slow diseases caused by conventional <u>viruses</u>:

1- Progressive multifocal leukoencephalopathy is caused by **JC** virus, (see p. 162), a member of the Polyomaviridae family. It is a fatal demyelinating disease which occurs when latent JC virus is activated in immunosuppressed AIDS, organ transplanted, or cancer patients on chemotherapy.

2- Subacute sclerosing panencephalitis is a rare persistent infection caused by a measles virus variant that cannot complete its replication. It causes slowly progressive demyelination in the CNS leading to personality changes and ending in dementia and death. Incubation period varies from 2-20 years and affects children and young adults. Patients have high antibody titre to measles virus

3- HIV has a long incubation period, progressive course leading to dementia.

Slow diseases caused by unconventional agents or prions:

The human prion-mediated diseases are; kuru, Creutzfeldt-Jakob disease (CJD), variant CJD (vCJD), Gerstmann-Straussler-Scheinker syndrome and fatal familial insomnia. They are called "transmissible spongiform encephalopathies" because they cause spongy, Swiss cheese-like holes in the brain parenchyma. The agents of these diseases have been transmitted serially in primates.

The animal prion diseases include scrapie of sheep and bovine spongiform encephalopathy **(BSE)** of cattle and others.

Kuru occurred only among certain tribes in New Guinea who had the tradition of eating the brains of the dead. However, the disease disappeared since that practice stopped.

CJD is found sporadically worldwide. It is transmitted in corneal transplants, in hormones extracted from human pituitaries and in grafts of cadaveric dura mater and in neurosurgical instrument. The main clinical findings are dementia and **early** neurologic signs i.e. jerky movements (myoclonus), ataxia, aphasia, visual loss and hemiparesis. It progresses to coma and death in less than one year (median 4 months). Most cases occur at **50-70** years of age and 10% of cases are hereditary.

Diagnosis of CJD is supported by the following investigations:

1- Detection of spongiform and amyloid changes in brain biopsy.
2- Immunohistochemical analysis of brain tissues show accumulation of protease-resistant prion protein, which is marked in vCJD.
3- A normal brain protein 14-3-3 is found in the CSF.
4- Monoclonal antibody-based assays are used for the detection of prion proteins in tonsillar or other lymphoid tissues. These are detected in variant CJD patients.
5- Electroencephalogram shows periodic (triphasic spikes) sharp waves. These are not detected in vCJD.
6- MRI of the brain shows characteristic changes. Some of these changes are specific for vCJD and differ from those in CJD (Table p. 185).

Scrapie is a disease of sheep. **BSE** also called "**mad cow disease**" affects cattle, both are caused by prions and are experimentally transmissible to mice.

Variant CJD (vCJD): In 1996, several cases of CJD occurred in Great Britain and were attributed to the ingestion of beef. These cases proved to be new variant of CJD that occurred in persons **under 30** years of age and differed clinically and pathologically from classic CJD which affected older persons (68 years old).The prions isolated from "**vCJD**" cases in humans, chemically resemble the prions isolated from BSE indicating that BSE agent infected humans. It is now accepted that vCJD and BSE are caused by the same agent. The clinical findings are long LP. (years), psychiatric/behavioral symptoms, painful dyesthesiasis and **delayed** neurologic signs. Through 2004, most of the 150 people who had been diagnosed with vCJD in England had died. A case of vCJD was reported in USA in 2006.

CHAPTER 38 MINOR VIRAL PATHOGENS

Parvovirus B19 is the only parvovirus that causes human disease. Parvoviruses are very small (22 nm) naked icosahedral viruses with single stranded DNA. Infection is transmitted by the respiratory route, transplacental or by blood transfusion. The diseases caused by B19 virus are:

1-The commonest is erythema infectiosum; a self limited disease of children that is characterized by a "slapped-cheek" rash (fifth disease).

2-In adults it causes arthritis resembling rheumatoid arthritis.

Deposition of immune complexes contributes to the pathogenesis of the rash in children and arthritis in adults.

3- Transient aplastic crisis in patients with sickle cell anaemia.

4- Chronic anaemia in immune suppressed (including AIDS) patients.

B19 virus preferentially infects and kills the immature red blood cell precursors in the bone marrow.

5- In pregnant women, it may cause hydrops foetalis or foetal death due to heart failure from severe anaemia.

Diagnosis is made by the detection of viral DNA by PCR in blood or amniotic fluid, to determine foetal infection or by serology in children and adults.

Coronaviruses are enveloped, RNA viruses with club-shaped surface spikes that resemble a "corona". They cause about 20% of upper respiratory tract infections (colds) in adults. Laboratory diagnosis is rarely needed.

Late in 2002, a new disease caused by a coronavirus, an atypical pneumonia called SARS (severe acute respiratory syndrome) emerged in China and spread rapidly to other countries. It is transmitted by droplets and close contact. Incubation period is 2-10 days. There is fever, malaise, headache, cough, dyspnea and hypoxia. Chest x-ray reveals interstitial "ground glass" infiltrates. Acute respiratory failure and death occurred in 10% of cases. Antibody-based and PCR-based tests can be used for diagnosis.

Hantaviruses are members of the Bunyaviridae family. The prototype is Hantaan virus that causes Korean haemorrhagic fever, which is characterized by headache, petechial haemorrhages, shock and renal failure. Hantaviruses are a heterogeneous group of viruses called roboviruses i.e., rodent-borne viruses.

In 1993, an outbreak of a new disease, characterized by influenza-like symptoms followed rapidly by acute respiratory failure, occurred in the western United States, centered in New Mexico and Arisona. This disease, now called "Hantavirus Pulmonary Syndrome", is caused by a hantavirus endemic in deer mice and is acquired by inhalation of aerosols of the rodent's urine and faeces. There is no person to person transmission. Diagnosis is

made by detecting viral RNA in lung tissue by PCR or by detecting IgM antibodies in patient's serum.

Lassa fever virus was first seen in1969 in a Nigerian town of that name. It causes a severe, often fatal **haemorrhagic fever** characterized by multiple organ involvement. The virus is a member of the Arenaviridae family that are RNA viruses, enveloped with surface spikes and a helical nucleocapsid. The natural hosts are small rodents which undergo a chronic infection. The virus is transmitted to man by contaminated food or water with animal urine. Asymptomatic infection is widespread.

Lymphocytic choriomeningitis virus which is a rare human pathogen causing **aseptic meningitis,** is a member of the Arenaviridae family. It is similar to lassa fever virus in host range and mode of transmission.

Ebola virus is named after the river in Zaire that was the site of an outbreak of **haemorrhagic fever** in 1976. The disease begins with fever, headache, vomiting and diarrhoea. Later, bleeding into the skin, from the nose and the gastrointestinal tract occurs due to thrombocytopenia, followed by shock and disseminated intravascular coagulation. The mortality rate approaches 100%. Most cases arise by secondary transmission from contact with the patient's blood or secretions, e.g. in hospital staff.

Ebola virus is a member of the Filoviridae family. It is an enveloped helical RNA virus that appears as filaments of varying length. The natural cycle of this virus is uncertain, but monkeys may be the natural reservoir, as they are for **Marburg virus,** another filovirus that causes **haemorrhagic fever** but is antigenically distinct. Diagnosis is made by isolating the virus or by detecting a rise in antibody titre. Prevention centers on limiting secondary spread by proper handling of patient's secretions and blood.

Astroviruses are nonenveloped RNA viruses similar to pohoviruses in size. They have a characteristic 5 or 6 pointed star-shaped morphology. These viruses cause watery **diarrhoea,** especially in children. Most adults have antibodies against astroviruses; suggesting that infection is common.

Norwalk virus is one cause of outbreaks of **gastroenteritis,** usually in schools, camps, cruise ships and similar confined populations. An outbreak was reported in 1969 in a school in Norwalk. It is an RNA virus with an icosahedral protein capsid. It is a member of the **Caliciviridae** family.

JC and **BK** are **polyomaviruses** that cause inapparent persistant infections transmitted by the respiratory route or contaminated food and water. Their reactivation in immunosuppressed patients may cause serious diseases. **JCV** causes progressive multifocal leukoencephalopathy. **BKV** causes haemorrhagic urinary tract infections in transplant and AIDS patients.

PART **in**

APPLIED MICROBIOLOGY

CHAPTER 39

NORMAL HUMAN MICROBIOTA

Microbiota are a heterogeneous population of microorganisms that inhabit the skin and mucous membranes of healthy persons. They consist of:

1- Resident microbiota which are relatively fixed types of microorganisms regularly found in a given area at a given age; if disturbed e.g. by hand washing,
they promptly reestablishe themselves.

2- transient microbiota are non-pathogenic or potentially pathogenic organisms derived from the environment contaminating the site. They may remain for hours, days or weeks but do not become permanently established. They can be readily removed e.g. by hand washing. They do not produce disease, except when the resident microbiota are disturbed.

Role of the resident microbiota in maintenance of health:

1- They perform important metabolic functions e.g.:
a- Synthesis of vitamin K and several B vitamins,
b- Conversion of bile pigments and bile acids.
c- Help in absorption of nutrients from the intestine.

2- They inhibit colonization and infection by pathogenic bacteria (bacterial antagonism or interference) as they: a- Interfere with bacterial adherence, by occupying receptor sites on host cells,
thereby preventing pathogens from binding to those receptors, b- Compete for essential nutrients, c- Produce inhibitory substances e.g. lactic acids and bacteriocins. d- Maintain inhibitory pH in vagina and skin.

3- Bacterial colonization of a newborn infant acts as a powerful stimulus for development of the immune system.

Role of the resident microbiota in disease:

Under certain conditions, microbiota or commensal bacteria may cause disease and are considered potential or opportunistic pathogens,

1-Lowered host defense mechanisms due to immunosuppression, diabetes.. .etc
2-Antimicrobial agents disturb the microbiota and cause super-infection.
3-Alteration of the host tissues due to obstruction, trauma or hormonal changes.
4-Change of the normal habitat of the organism e.g. viridans streptococci, a normal inhabitant of the mouth and throat may reach the blood stream and cause endocarditis after tooth extraction in rheumatic heart patients.(Vol.I p.40)

Common microbiota in the skin and nasopharynx: *Staph, epidermidis,* viridans streptococci, diphtheroids, commensal neisseria, **Candida** and anaerobes.
Common microbiota in the intestine: *E. coli,* proteus, pseudomonas, enterococci, Candida and anaerobes mainly bacteroides and *CI. perfringens.*
Common microbiota in the vagina: lactobacilli, which keep vaginal pH low, group B streptococci, **Candida,** *Gardnerella vaginalis* and *Staph, epidermidis.*

CHAPTER 40 ANAEROBIC INFECTIONS

The obligate anaerobes form the major part of the indigenous microbiota in the intestine, mouth and female genital tract of humans. They are the cause of a wide range of infections and most of them are of endogenous origin.

Classification of anaerobes:

I- Spore-forming gram positive bacilli = Clostridia.

II- Non-srx>re-forrning:

Gram positive bacilli	Gram negative bacilli
- Actinomyces	- Bacteroides
- Propiombacteriv jm	- Fusobacterium
- Lactobacilli	- Prevotella
- Eubacterium	- Porphyromonas
	- Leptotrichia
Gram positive cocci	Gram negative cocci - Veillonella
- Peptostreptococcu s	
- Peptococci	

III- Spirochaetes = Treponema.

Bacteroides species are the most important anaerobic pathogens. They are normal inhabitants of the intestinal tract and female genital tract. ***B. fragilis*** is the commonest pathogen. Abscess formation is a characteristic of lesions caused by bacteroides and most lesions are below the diaphragm and are associated with bacteraemia. They are resistant to penicillins as they produce fl-lactamase.

Factors that predispose to anaerobic infections:

A fall in oxidation-reduction potential (Eh) is the major factor that favours proliferation and invasion of tissues by anaerobes. Reduced Eh in tissues may occur due to:

1- Trauma, which leads to, deep lacerated wounds, loss of blood supply and tissue necrosis.

2- Foreign bodies e.g. clothing or soil inserted into a wound following an accident or war injury.

3- Decreased blood supply to a limb due to, pressure by a cast or ischaemic arterial disease as in diabetic patients.

4- Mixed infection with facultative anaerobes which consume sufficient oxygen to allow the anaerobes to flourish.

Sites and types of anaerobic infections:

- Brain abscesses.

- Upper respiratory tract infections e.g. periodontitis, chronic sinusitis, otitis media, mastoiditis and Vincent angina.
- Lower respiratory tract infections e.g. aspiration pneumonia, empyema and lung abscesses.
- Intra-abdominal infections e.g. appendicitis, peritonitis and abscesses.
- Gynaecological and obstetrical infections e.g. endometritis, salpingitis, tubo-ovarian abscesses and septic abortion.
- Soft tissue infections e.g. gas gangrene, necrotizing fasciitis and cellulitis. This affects mainly compromised hosts e.g. diabetics.
- Bacteraemia leading to thrombophlebitis, endocarditis and septic shock.

Diagnosis of anaerobic infections: Anaerobic infections are suspected clinically when there is a foul-smelling discharge, gas in tissues, the wound is deep lacerated, tissues are necrotic and aerobic cultures are negative.

Unless specific anaerobic techniques for sampling, transport, processing and culture are used, these sensitive anaerobes will fail to grow.

Large samples should be aspirated from deep sites away from atmospheric oxygen and rapidly transported to the laboratory in closed syringes or on reduced transport media.

Naked eye examination of pus may show sulphur granules in case of actinomycosis or red fluorescence under UV light in case of prevotella infections.

1- Specimens are directly examined after staining with gram The presence of organisms in the absence of growth in aerobic culture, points to the presence of an anaerobe. Vincent angina caused by fusobacteria and spirochaetes, can be diagnosed by gram stained smears.

2- Specimens are cultured on two plates of blood agar; one incubated aerobicalry and the second anaerobically to differentiate between anaerobes and facultative anaerobes. The strict anaerobes will grow on the anaerobic plate **only.** Selective media for different anaerobes may be used. They are incubated at 37°C in anaerobic jar (Gaspak system) with 10% $C0_2$ for 2-5 days as anaerobes are usually slow growers.

3- Colonies are identified by their morphology, biochemical reactions (API-A)
gas liquid chromatography to detect short chain fatty acids, serology and nucleic acid probes.

Treatment: Surgical drainage of pus, debridement and removal of necrotic tissues. Clindamycin, metronidazole, cefoxitin, and linomycin are effective. Penicillin is effective against most anaerobes except *B. fragilis.* Clindamycin use may be complicated with pseudo-membranous colitis.

CHAPTER 41

HOSPITAL ACQUIRED (HAI) or HEALTH CARE ASSOCIATED INFECTIONS (HCAI) or NOSOCOMIAL INFECTIONS

HCAJ is either an infection which is acquired during hospitalization (usually after 48 hrs and is related to receiving healthcare) and was not present or incubating in patients at the time of admission, or an infection which is acquired in hospital and becomes evident after discharge.

Factors that favour HCAI:

The hospital is an environment in which several factors are present increasing the risk of infections, these include:

I- Susceptible host: Most hospital patients are in a poor state of health e.g.

1- Extremes of age i.e. neonates and the old are vulnerable to infection.

2- Lowered host resistance due to diseases e.g. diabetes and burns, or those under immunosuppressive therapy e.g. transplant or cancer patients.

3- Instrumentation e.g. urinary, venous or arterial catheters, endoscopy, mechanical ventilation, or dental procedures.

II- Microorganisms in hospital environment have special characters:

1- They are highly pathogenic e.g. *Staph, aureus,* pseudomonas, *E. coli,* klebsiella, proteus, *M. tuberculosis* , avian influenza (A/H5N1) or pandemic influenza (A/H1N1). However non-pathogenic or opportunistic organisms may cause infections in immunosuppressed patients.

2- They are drug resistant being selected for by the wide use of antibiotics in hospitals e.g. resistant *Staph, aureus* (MRSA, VIRSA or VRSA) and vancomycin resistant enterococci (VRE).

III- Sources of infections:

A- Endogenous with normal microbiota of the patient himself.

B- Exogenous sources:

1- Infected patients transmit virulent organisms to other patients.

2- Carriers e.g. hospital personnel and medical staff.

3- Environmental sources e.g. water, surgical instruments, urinals, anaesthesia apparatus, ventilators, bed pans, blankets and air conditioning.

4- Blood and its products can be the source of HIV, HBV, HCV and others.

5- Intravenous manipulations; cannulae, shunts for haemodialysis may cause bacteraemia and septicaemia.

IV- Modes of transmission of infection:

1- Contact; direct by **hands** of health care workers (HCWs) or indirect by contaminated objects or vehicles used in common for patients.

2- **Air-borne** e.g. aerosols. 3- **Droplets** or dust. **4-** Blood or needle prick

5- Vector-borne e.g. mosquitoes...etc.

Most common types of HCAI:

1-Urinary tract infections by *E. coli,* **Klebsiella,** pseudomonas and proteus.

2-Surgical wound infection by staphylococci, anaerobes, gram negative bacilli
3-Lower respiratory tract infections especially pneumonia commonly caused by *Staph, aureus* and gram negative bacilli.
4-Bacteraemia or septicaemia by bacteroides, *Staph, aureus,* serratia ..etc.
5- Gastrointestinal infections e.g. pseudomembranous colitis by *CI. difficile*

INFECTION CONTROL of HCAI can be achieved by:

Implementation of a comprehensive infection control (IC) program and surveillance of infections in the hospital by an infection control committee, which includes representative staff from the different departments and service areas, headed by the hospital director and helped by the infection control team (ICT), which is the core of the committee, and includes the microbiologist and the infection control nurses. Their role includes;

1- Preparing evidence-based policies and procedures guidelines based on globally accepted references in an IC manual.

2- Use of surveillance data to monitor performance of IC practices and policies.

3- Use of surveillance cultures and investigation of infection outbreaks.

4- Education of HCWs how to implement standard precautions which should reduce the risk of transmission of infections among HCWs and patients. These IC practices and pohcies include:-

a- Frequent hand washing is the single most important measure to reduce transmission. It is indicated in the following moments: 1- Before and after touching a patient or touching a wound. 2-Before aseptic technique e.g. withdrawing blood samples or handling a medication. 3- After touching patient surroundings or contaminated environmental sources. 4-After touching blood, body fluids or items contaminated with them. 5- After removing gloves

Hand washing is done by: 1-Alcohol hand rub by alcohol gel is effective in killing microbes, but it does not act well in presence of dirt. 2- Washing by soap and water will remove 90% of transient microbiota. Antimicrobial soap e.g. chlorhexidene is also used. 3- Surgical hand scrub is done before surgery,

b- Personal protection by gloves, masks and gowns.

c-Avoid needle pricks or injuries by other sharps. Recapping of syringe needle should be prohibited. If unavoidable, recap using one hand only, d- Hygiene in theater, proper surgical technique ...etc. e-Prompt cleanup of blood spills by household bleach.

f-Free vaccination of HCW (p. 181). Post-exposure prophylaxis for those exposed to HBsAg positive source by HBIG and vaccine (pi54) and those exposed to HIV positive source by drugs (p. 138).

5- Standard precautions must be applied for all patients. In addition, transmission-

based precautions should be followed. With airborne- transmitted infections;

measles, pulmonary TB, and chickenpox, patients are isolated in negative pressure rooms. With droplet-transmitted infections; meningitis, influenza, mumps, rubella and contact-transmitted infections; MRSA, HAV, diarrhoea; patients are placed in ordinary single rooms. Before entering rooms HCWs should wear masks (surgical or N95(TB) for air-borne transmitted infections) . Gowns and gloves are worn with contact transmitted infections to be discarded in infectious waste bags. Upon entry, the patient is asked to wear a mask if tolerable. Before leaving the room hand wash or alcohol scrub is performed

6- Protective isolation of patients at high risk of contracting infections e.g. severe burns, immunodeficient, neutropenic and transplant patients.

7- Monitoring of disinfection and sterilization of patient care equipment.

8- Conservative antibiotic use and implementation of antibiotic policies.

9- Proper disposal of infectious hospital waste.

High risk areas that need supervision by ICT include; Intensive care units (ICUs), operation rooms, haemodialysis or renal dialysis units, central supply and sterilization department, emergency rooms, out-patient and dental clinics, areas for preparation of nutritional formulas.

Services that need close supervision by ICT include; kitchens, blood bank, clinical laboratory, endoscopy and physical therapy. **Investigations of outbreaks of HCAI by the ICT:**

An outbreak is the occurrence of a larger than usual number of cases of a specific infection acquired in an institution or certain population. Examples include outbreaks of surgical wound infection, MRSA outbreak in ICUs and outbreaks of food poisoning. Identifying the cause of the outbreak by:

1- Isolation of organisms from patients and suspected sources of infection.

2- Identification of organisms to species level

3- Typing of similar species to recognize identical strains in patients and source. **Typing methods include: Non-molecular typing methods:**

a- Biochemical typing according to biochemical reactions, **b-** Antibiotic resistance patterns, **c-** Serologic typing, **d-** Phage typing. **Molecular typing methods:**

a- Plasmid profile analysis.[1] **b-** Restriction endonuclease analysis and pulsed-field gel electrophoresis.[2] **c-** PCR based ribotyping to identify species and strains.[3] **d-**Nucleotide sequence analysis.[4]

1- It is a technique that allows isolation of plasmids from bacteria and determining their size. Bacterial isolates carrying identical plasmids are considered similar.

2-Chromosomal DNA from bacterial strains of one species is extracted and digested with particular restriction endonuclease enzymes and the resulting DNA fragments separated by pulsed-field gel electrophoresis (PFGE) are compared

3- In which primer sets complementary to various conserved regions of the 16S and 23S parts of rRNA gene are often used. Following amplification, the various size products of nucleotides are clectrophorescd on agarose gel, stained with ethidium bromide, exposed to UV light and photographed to compare the nucleotide patterns of different strains.

4-11 is used to compare multiple isolates after amplification of a certain locus in all isolates. The amplified product is sequenced usually by an automated sequencer.

IMPORTANT CLINICAL CONDITIONS

URINARY TRACT INFECTIONS

Urine is normally sterile. Urinary tract infection (UTI) is defined as **bacteriuria** i.e. the multiplication of bacteria in urine within the renal tract. A count of 100,000 organisms per ml is regarded as **significant bacteriuria.**

Pyuria is the presence of pus in urine (more man 10 cells/HPF). It usually accompanies bacteriuria.

Pathogenesis of UTI: Faecal flora my reach the UT by **ascending** from the perineum and peri-urethral sites. Rarely **descending** infections occur from the kidney, which is infected haematogenously during bacteraemia by some organisms.

Predisposing factors include: Shortness of the female urethra, sexual intercourse, senile prostatic hypertrophy, structural abnormalities in the UT that lead to incomplete emptying of the bladder, immunosuppression and diabetes. Urinary catheterization is the main predisposing factor for hospital acquired UTI.

Causative organisms:

- *E. coli* causes 60-90% of urinary infections.
- Klebsiella, proteus and pseudomonas.
- *Staph aureus, Staph, epidermidis* and *Staph, saprophyticus.*
- *Enterococcus faecalis, Bacteroides Jragilis, N. gonorrhoeae.*
- *M. tuberculosa.*
- *Candida albicans.*
- Adenoviruses.

Diagnosis:

Specimen: A **"mid stream"** urine sample must be obtained under aseptic precautions, which include cleaning the external genitalia with tap water and drying, and then the middle sample of urine is collected in a sterile container.

In babies the urine is collected in sterile self-adhesive plastic bags. However, suprapubic needle aspiration of the full bladder may be necessary.

Catheter samples should be avoided as they carry the risk of causing ascending infection, unless the patient is already catheterized.

Transport: Samples should reach the laboratory within one hour after voiding or kept refrigerated at 4°C for 24 hours maximally, to avoid multiplication of bacteria in urine resulting in false high bacterial counts.

-**Microscopic examination** of a wet film of urine deposit for the presence of pus cells, RBCs, bilharzial ova, casts or crystals.

- **Bacterial count** is done within one hour by using a calibrated loop that carries a known volume of the uncentrifuged urine. It is spread on solid media and incubated at 37°C. The number of bacteria is estimated and interpreted as follows:
  - 100,000 bacteria per ml or more indicates urinary tract infection.
  - Less than 10,000 bacteria per ml is regarded as contamination.
  - A count of 10,000 is considered significant if the organism is gram positive and only one type.
- **Cultures** are made on nutrient agar, blood agar and MacConkey's medium or CLED agar and incubated overnight at 37°C. The colonies are identified by morphology and biochemical reactions.
- **Antibiotic sensitivity** test is done for the isolated organism.
- When **TB** is suspected, five successive morning urine samples are examined by Z.N. stain and cultured on L.J. medium (see chapter 13).

Sterile pyuria means the presence of pus in urine, in the absence of bacterial growth on ordinary media. This can be due to:

1- Renal tuberculosis.

2- Taking antibiotics that suppress growth of organisms.

3- Anaerobes, mycoplasma, ureaplasma, chlamydia or viruses.

N.B: Prostatitis, vaginitis, cervicitis or renal calculi may be associated with pyuria with insignificant bacterial count i.e. 10^3 or less.

MENINGITIS

The diagnosis of meningitis is an emergency that requires prompt diagnosis and treatment. Meningitis is described as **septic** or pyogenic when the CSF contains mainly polymorphonuclear leucocytes as in bacterial meningitis. It is described as **aseptic** or lymphocytic when the CSF contains mainly lymphocytes as in viral, tuberculous or fungal meningitis.

Causative agents:

1- Bacterial meningitis is caused by:

- *N. meningitidis, H. influenzae* and *Str. pneumoniae* are the commonest cause of pyogenic meningitis.
- *M. tuberculosis* meningitis.
- Staphylococci, streptococci, pneumococci, *E. coli,* Klebsiella, proteus, pseudomonas or anaerobes may be the cause, after trauma or surgery.
- Spirochetes; syphilis and leptospirosis are rare causes.
- **Neonatal meningitis** is caused by group **B** haemolytic streptococci *{Str. agalactiae), E. coli* and other intestinal flora e.g. klebsiella and proteus. *Listeria monocytogenes* is a less common cause. *H. influenzae* is the commonest cause in **infants** 2-5 years.

2- Viral meningitis (aseptic meningitis)

Enteroviruses i.e. coxsackieviruses, echoviruses, pohovirus and mumps virus are the main viral causes. Arboviruses, herpesviruses, rubella, CMV, rabies and many other viral agents can cause meningitis and encephalitis.

3- Fungal meningitis: *Cryptococcus neoformans, coccidiodes immitis.*

Diagnosis:

CSF is obtained by lumbar puncture under complete aseptic conditions in 2-3 sterile tubes. It should be immediately examined as follows:

Physical examination: In septic pyogenic meningitis the CSF is turbid and under tension due to the large number of pus cells. In aseptic viral meningitis, it is clear or slightly turbid due to small numbers of lymphocytes. In tuberculous meningitis, a coagulum forms on standing.

Chemical examination for the level of proteins and glucose. In pyogenic and tuberculous meningitis, proteins are markedly increased while the glucose is reduced. In viral meningitis, proteins are slightly increased while the glucose is normal.

Cytological examination: Normal cell count in CSF is 0-5 / cmm. In pyogenic meningitis, the number of cells may be 20,000 / cmm or higher and are mainly polymorphs. In tuberculous or viral meningitis, the number of cells does not exceed 1000/cmm and are mainly lymphocytes.

Bacteriologic examination: CSF is centrifuged and the deposit is examined by:

1- Direct smears stained by gram and by Z.N., if indicated or by India ink, if cryptococcosis is suspected.

2- Detection of bacterial antigens in CSF by coagglutination and latex agglutination using antisera to the three common organisms i.e. *N. meningitides, H. influenzae* and *Str. pneumoniae.* Cryptoccocal antigens can be detected by latex agglutination.

Due to the need for prompt treatment, results of step 1 and 2 should be immediately reported to the treating physician.

3- Cultures are made on blood and chocolate agar and incubated aerobically and at 5-10% C02 atmosphere. Culture for tuberculosis is done if indicated. Cultures for fungi or leptospira may be needed.

4- Blood cultures should be done at the same time, since bacteraemia frequently occurs in pyogenic meningitis.

5- PCR may be used for detection of viral or bacterial nucleic acids in CSF or blood for rapid diagnosis.

6- Serologic diagnosis of viral causes by detecting IgM or IgG rising titre.

BACTERIAL FOOD POISONING

It is an acute condition that affects a group of people sharing the same food. It usually manifests by diarrhoea and vomiting except botulism which affects the nervous system. The common causes of food poisoning are:

1- *Staphylococcus aureus.* 2- *Salmonella* Typhimurium and Enterhidis. 3- *Clostridium botulinum.* 4- *Clostridium perfringens.* 5- *Bacillus cereus.* 6- *Vibrio parahaemolyticus.* 7- *Listeria monocytogenes.*

The condition is due to preformed enterotoxins in *Staph, aureus* and *B. cereus* (see p.38) and is due to a preformed neurotoxin in botulism. Salmonella food poisoning is due to multiplication of organisms in the gut; hence, the incubation period is long. In *CI. perfringens,* the organism multiplies in the gut and releases the enterotoxin during sporulation.

Diagnosis depends on the manifestations and type of food, as well as detection of the organism or toxin in food remnants, vomitus or stools.

For tracing the source of food poisoning, isolated organisms from food, patients and food handlers are identified by typing methods (chapter 41).

Comparison of Types of Food Poisoning

Organism	Incubation period	Signs & Symptoms	Pathogenesis	Type of Food	Outcome
Staph, aureus	1-8 hrs	Vomiting & diarrhoea	Enterotoxin in food	Carbo-hydrates milk & milk products	Recovery in 1-2 days
Salmonella	12-48 hrs	Vomiting, diarrhoea & fever	Multiplication in gut. No toxin	Infected meat or eggs	Treatment may be needed
CI. Botulinum	18-24 hrs	Neurologic	Neurotoxin in food	Canned Food	High mortality rate
CI. perfringens	6-18 hrs	Diarrhoea	Multiplication & enterotoxin	Reheated meat dishes	Recovery in 1-2 days
B. census: Emetic type Diarrhoea! type	 1-5 hrs 8-24 hrs	 Vomiting Diarrhoea	 Heat stable enterotoxin Heat labile enterotoxin	 Reheated cooked rice Meat & sauces	 Recovery in few days Recovery in few days
V. parahaemoly-ticus	6-30 hrs	Vomiting diarrhoea & fever	Multiplication in gut & haemolysin	Contaminat-ed sea food	Recovery in few days
L monocytogenes	8-48 hrs	Diarrhoea & fever	Multiplication & invasion	Cheese & undercooked meat	Recovery in few days

GASTROENTERITIS

Organisms infecting the small and/or large intestine cause diarrhoea or dysentery. Diarrhoeal diseases are a major cause of morbidity and mortality world wide. The main sources of infection are ingestion of contaminated food and drinks, or improperly cooked meat or fish of infected animals. The most common causative organisms are:

Bacterial causes other than those mentioned in food poisoning, include:

1- Shigella i.e. bacillary dysentery.
2- *E. coli* (ETEC, EPEC, EIEC, EHEC, EAggEC).
3- *Campylobacter jejuni.*
4- *V. cholerae,* serogroups 01 and 0139.
5- *CI. difficile* (antibiotic-associated diarrhoea & pseudomembranous colitis).
6- Yersinia *(Y. enterocolitica* and *Y. pseudotuberculosis).*
7- Aeromonas hydrophila (p. 96).

Viral causes:

Many cases of acute infectious diarrhoeas are due to viruses. Rotaviruses are the major cause of infantile gastroenteritis. Enteroviruses. adenoviruses, caliciviruses (e.g. Norwalk virus") and astroviruses are among the common causes of viral diarrhoea. However, many of these viruses can multiply in the gut without causing GIT symptoms.

Fungal causes: *Candida albicans* causes diarrhoea in predisposed individuals.

Protozoal causes: *Giardia lamblia,* Cryptosporidium and *Entamoeba histolytica.*

Diagnosis of gastroenteritis:

1- Stools are examined macroscopically for blood, mucus or worms.

2- Wet and stained smears are examined for RBCs, leucocytes, parasitic ova or cysts or abnormal organisms e.g. **Candida.**

3- Stools are directly cultured on ordinary and selective differential media. They are also subcultured on the same media after enrichment on fluid media (e.g. selenite broth, tctrathionate broth or alkaline peptone) and incubated aerobically or micro-aerophilically, according to the organism in question. Growing colonies are identified by; morphology, biochemical reactions and serologically by slide agglutination using specific antisera. Anaerobic incubation is used if *CI. difficile* is suspected.

4- Toxin production by certain organisms (e.g. enterotoxigenic *E. coli* and *CI. difficile)* can be detected by ELISA and gene probes.

5- Rotavirus can be detected in stools by ELISA or probes. Viral isolation is rarely needed.

6- Serologic diagnosis by detection of a rising antibody titre to suspected organisms supports the diagnosis of viral infections.

PYREXIA OF UNKNOWN ORIGIN (PUO)

It is defined as fever (38°C or more) that persists for long periods; 1-3 weeks or more without localizing signs or symptoms. This may be due to:-

1- Infections

a- Specific infections; including:

Bacterial causes	**Viral causes**
- Tuberculosis (TB).	- HIV infection (AIDS).
- Enteric fever.	- CMV infections.
- Brucellosis.	- Infectious mononucleosis (EBV).
- Relapsing fever.	- Lassa fever
- Epidemic or endemic typhus.	
- Q fever, Leptosirosis, Nocardiosis	

b- Non- specific infections:
- Urinary tract infection.
- Deep seated abscesses.
- Infective endocarditis.
- Dental, ear or sinus infections.

2- Non-infectious causes include: Malignancies, autoimmune diseases e.g. acute rheumatic fever and drug induced fever.

Laboratory diagnosis of infective causes of PUO

1- Blood culture.

2- Urine analysis and culture. 3-Stools analysis and culture.

4- Serologic tests:
- Widal test.....for enteric fever.
- Monospot test .....for infectious mononucleosis.
- Latex agglutination or ELISA.. .for typhus.
- Agglutination.....for brucellosis & Q fever.
- ASO,CRP .....for acute rheumatic fever.
- Specific IgM by ELISA.....for CMV.
- ELISA & Western blot .....for HIV.

5- Diagnosis of TB is done as previously mentioned (p. 66).

6- Other tests include sedimentation rate and blood picture.

Rash With or Without Fever occurs in the following infections: **Viral infections;** measles, rubella, chickenpox, smallpox, herpes simplex 1, and herpesvirus 6, herpes zoster, coxsakie viruses and echoviruses, HIV, parvovirus B19, arboviruses e.g. West Nile fever and encephalitis viruses, HPV.

Bacterial infections; Staphylococcal scalded skin syndrome and toxic shock syndrome, streptococcal diseases i.e. scarlet fever and pyoderma, early meningococcaemia, syphilis secondary stages, Lyme's disease, rickettsial infections e.g. epidemic typhus and Rocky mountain spotted fever.

Fever with Jaundice or Hepatitis can be due to the following infections:

- Hepatitis viruses infections: HAV, HBV, HCV, HDV or HEV.
- Yellow fever. - CMV infections. - Congenital rubella syndrome.
- EBV infectious mononucleosis. - Q fever *{Coxiella burnetii).*
- Leptospirosis *(L. interrogans).*

Other causes include hver abscesses, hydatid cysts, tumours, autoimmune hepatitis, systemic lupus erythematosis, ergot and aflatoxin poisoning and drugs.

Infectious Diseases with Bacteraemia

In these diseases bacteraemia is one of its main features and the disease can be diagnosed by **blood culture.** These diseases include: -

- Endocarditis - Enteric fever. -Puerperal sepsis.
- Brucellosis. - Meningitis.
-Toxic shock syndrome and necrotizing fasciitis caused by *Str. pyogenes.*

In most cases of bacteraemia, the organisms are scanty in blood and direct plating of a drop of blood on solid media will give a negative result. Blood culture when properly done will give positive results in 90% of cases.

Blood Culture Technique

Blood culture is made by withdrawing 5-10 ml of blood under complete aseptic conditions; these are added to 50-100 ml broth and incubated aerobically and anaerobically at 37°C. Subcultures are made every 48 hrs on suitable solid media and continued for 10-14 days before the sample is discarded as negative. Growing organisms are identified systematically.

For proper results 2-3 blood samples should be withdrawn from different anatomic sites at the peak of the fever and before starting antibiotic treatment. The samples may be separated by 10 min in some diseases or they can be withdrawn over 24 hrs in others.

The large volume of broth has the following advantages:

- It dilutes out antibacterial substances naturally occurring in serum.
- It allows for multiplication of organisms present in few numbers in blood.
- Antagonists to antibiotics (taken by the patient) can be added to the broth.

Organisms Causing Toxaemia (toxins in blood)

C. diphtheria, CI. tetani, CI. botulinum, CI. perfringens, CI. difficile, Staph, aureus TSST and *Str. pyogenes* pyrogenic toxin.

LOWER RESPIRATORY TRACT INFECTIONS

PNEUMONIA

Causative organisms include:-

Bacterial causes: *Str. pneumoniae, Kl. pneumoniae, Staph, aureus, Str. pyogenes, H. influenzae, P. aeruginosa, Proteus* species, *Y. pestis, M. tuberculosis* and anaerobic organisms.
Mycoplasma pneumoniae, Chlamydia pneumoniae and *C. psittaci, Legionella pneumophila* and *Coxiella burnetii.*
C. trachomatis is the commonest cause of neonatal pneumonia.

Viral causes: Influenza and parainfluenza viruses, respiratory syncytial virus (RSV), measles, adenoviruses, varicella-zoster and SARS.

Fungal causes: *Pneumocystis jiroveci, Cr. neoformans, Histoplasma,* and *Coccidioides.*

Diagnosis: Specimens include: Morning sputum sample which is rapidly processed -and liquefied by sputasol, bronchoalveolar lavage and blood.
1- Smears are stained by Gram or Z.N. and other stains when needed.
2- Cultures are made on different media.
3- Direct detection of organisms in smears by immunofluorescence.
4- Detection of viral or bacterial nucleic acids by molecular methods.
5- Serologic diagnosis by detecting a rising antibody titre.
6- Blood cultures are done; they are positive in 30% of pneumonias.

ATYPICAL PNEUMONIA

It is diagnosed when the causative agent cannot be isolated on ordinary laboratory media or when its clinical picture does not resemble that of typical pneumococcal pneumonia. It presents with unproductive cough, scanty sputum and low grade fever Le. influenza-like illness. Causative agents include; *Mycoplasma pneumoniae, Legionella pneumophila, Coxiella burnetii, C. psittaci, C. pneumoniae,* viral and fungal pneumonias.

UPPER RESPIRATORY TRACT INFECTIONS

Sore throat, Tonsillitis and **Pharyngitis** are caused by:

Bacterial causes

- *Streptococcus pyogenes.*
- *Corynebacterium diphtheriae.*
- *H. influenzae* type

b. -Vincent angina.

- Candidiasis (oral thrush).

Viral causes
-Adenovirus, influenza and parainfluenza viruses, coxsackievirus, echovirus, rhinoviruses, herpes simplex, EBV, CMV and coronaviruses.

Acute Otitis Media: 50% of infections are due to viruses, however, it is difficult clinically to differentiate viral from bacterial causes.
Bacterial causes are:

Str. pneumoniae, H. influenzae, Pseudomonas aeruginosa, Staph.aureus, Proteus species, *Str. pyogenes, E. coli, Klebsiella* species, *Moraxella catarrhalis.* Anaerobic organisms and Fungi.

Viruses: adenoviruses, influenza and parainfluenza viruses e.g. RSV and measles.

Conjunctivitis: is caused by;

Bacteria: *Str. pneumococci, H. influenzae, H. aegyptius, Moraxella lacunata,* gonococci, *E. coli,* staphylococci, streptococci, and *Chlamydia trachomatis.* Pseudomonas causes conjunctivitis after trauma, presence of foreign body or operation, **Neonatal conjunctivitis** is caused by; gonococci, C. *trachomatis* and *Staph, aureus.*

Viruses: Herpes simplex, adenoviruses, coxsackievirus A and enterovirus

70. **Fungi:** *Candida* and *Aspergillus* are rare but serious causes.

Sexually Transmitted Diseases (STD)

- The causative organisms are delicate and do not remain viable for long periods outside the body; hence close contact between mucosal surfaces -as in sexual contact- is needed for their transmission.
- They tend to produce genital lesions, but several organisms give rise to systemic disease.
- More than one infection can be acquired at the same time. The main sexually transmitted diseases are

1- Gonorrhoea ..... (TV*, *gonorrhoeae).*
2- Syphilis .....(*T. pallidum).*
3- Chancroid or soft sore ..... *(H. ducreyi).*
4- Lymphogranuloma venereum....(C. *trachomatis* types L_1-L_3).
5- Non-specific urethritis ..... (C. *trachomatis* types D-K).
6- Non-specific urethritis .....(Ureaplasma urealyticum)
7-Genital herpes .....*(Herpes simplex* type 2).
8-Genital wart and cervical carcinoma. (Human papilloma virus HPV).
9-Genital warts ..... (Molluscum contagiosum)
10-Hepatitis B and C less commonly.
11-AIDS .....HIV.
12-T cell lymphoma .....HTLV

Urethritis can be sexually transmitted or post-traumatic

1-Infectious causes of sexually transmitted urethritis are;

Bacterial causes

= Gonococcal urethritis.

= Non- gonococcal urethritis due to:

- *Chlamydia trachomatis* (types D-K) is the main cause.
- *Mycoplasma hominis, - Ureaplasma urealyticum.*
- *Candida albicans.* - *Trichomonas vaginalis* (parasite).

Viral causes: Adenoviruses, herpes simplex virus and human papilloma virus.

2-Post-traumatic infections after catheterization or instrumentation are mainly due

to: Staphylococci, *E. coli* and streptococci.

Diagnosis:

1- Urethral discharge is examined directly by:

- Wet smear for trichomonas.
- Gram stained smear will show pus cells and the organisms.
- Immunofluorescence or ELISA are used to test for chlamydia.

2- Discharge is cultured for the different organisms.

Organisms Transmitted by Blood Transfusion

Blood or plasma products may contain viable organisms from a blood donor and are passed to the patient at the time of infusion:

- HIV 1 and 2.
- Hepatitis viruses B, C and D.
- CMV, EBV, HTLV; are transmitted in blood cells but not in plasma.
- Parvovirus B19 rarely.
- Brucella
- *Treponema pallidum* and malaria are transmitted in fresh blood as they die when refrigerated after few days.

Donated blood should be tested for: HBsAg, HBc IgM, HCV, HIV 1 and 2, HTLV-I and II, syphilis (VDRL) and serum transaminases assay, which if elevated indicate hver affection.

Acute Bacterial Endocarditis

The most important and common causes are:

Staph, aureus, coagulase negative staphylococci, Viridans streptococci *Strept. bovis* and other streptococci, enterococci, *Heamophilus* sp., Q fever *{Coxiella), Chlamydia psittacae* and *Acinetobacter.*

Other less common causes are: *Pseudomonas, Brucella, Kingella, Eikenella corrodans, Cardiobacterium hominis, Acinobacillus, Candida* and *Aspergillus.*

Emerging pathogens e.g. *Bartonella* sp. and *Tropheryma whipplei.*

Diseases Transmitted from Mother to Foetus or Newborn

The organisms can be transmitted transplacentally (pre-natal) or they are contracted from the birth canal during labour (peri-natal) due to contamination of the baby by maternal blood, vaginal discharges and stools or other forms of contact e.g. breast milk or sahva shortly after birth.

A- **Pre-natal transmission** (Transplacental):

1- *Treponema pallidum* = Congenital syphilis.
2- *Listeria monocytogenes* = Abortion or still-birth.
3- Rubella = Congenital rubella syndrome.
4- Cytomegalovirus = Cytomegalic inclusion disease.
5- Varicella = Congenital varicella syndrome.
6- HIV = AIDS.
7- Parvovirus B19 = Hydrops foetalis.
8- Toxoplasma (protozoa) = Toxoplasmosis.

B- Peri-natal transmission:

1- Herpes simplex = Herpes neonatorum
2- HIV = AIDS.
3- HTLV = T cell lymphoma.
4- Hepatitis B = Hepatitis B carrier state.
5- Cytomegalovirus = subclinical infection.
6- Coxsackie B = neonatal myocarditis.
7- Gonococci = neonatal ophthalmia.
8- Chlamydia trachomatis = inclusion conjunctivitis, pneumonia
9- *Str. agalactiae* = neonatal sepsis and meningitis.
10- *Listeria monocytogenes* rarely = neonatal septicaemia and meningitis.

Diagnosis by the detection of IgM antibodies to the different viruses and serologic tests for syphilis. The **TORCH** test used for diagnosis of neonatal infections includes detection of IgM antibodies for, Toxoplasma, Rubella, CMV, Herpes simplex virus, in sera of the newborns. **O** stands for others which include varicella and syphilis.

Recommended Vaccinations for Health Care Workers (HCW):

HCW are at high risk of contracting diseases from patients. They should be immunized for their safety and the patient's safety by the following vaccines; **hepatitis** B, **measles, mumps** and **rubella** only for females, **varicella** and **influenza** virus current yearly vaccine, **tetanus** and **diphtheria. Zoster** and **HPV** vaccines are given if the HCW is in the recommended age group. Pneumococcal and meningococcal vaccines are

given if the HCW has another risk factor. Microbiologists routinely exposed to *N. meningitides* should receive **meningococcal** vaccine. Recently, it has been recommended to start vaccinating HCW against **smallpox** as they will be the first to be exposed to any emerging cases due to bioterrorism or biologic warfare.

Organisms Transmitted by Milk and Milk Products

1- From the infected animals and excreted in milk:

- Bovine tuberculosis.
- Brucellosis.
- Q fever.
- *Listeria monocytogenes* rarely.

2- From milk contaminated by milk handlers or other sources:

- Salmonella infections.
- Staphylococcal food poisoning.
- Shigellosis
- Pohomylitis
- Cholera.
- Hepatitis A and E.
- Campylobacter.
- Rotavirus.
- *Strep, pyogenes*
- Enteroviruses
- *E. coli* verotoxin producer

Zoonotic Diseases or Diseases Acquired from Animals

Zoonotic diseases are primarily diseases of animals. Man acquires the infection from the animal reservoir by direct contact, by ingestion of infectious tissue or milk, by inhalation of infected aerosols or by vectors e.g. fleas, ticks or mosquitoes.

Bacterial causes: *Mycobacterium bovis, Brucella, B. anthracis, L. monocytogenes, Erysipelothrix rhusiopathiae, Francisella tularensis, Y. pestis, Y. enterocolitica, Salmonella* Enteritidis and Typhimurium, *E. coli* 0157:H7, and *Campylobacter jejuni. Pasteurella multocida, Borrelia burgdorferi, Leptospira interrogans, Chlamydophila psittaci, Rickettsia rickettsii* and *Coxiella burnetii. Bartonella henselae* cause disease in cats, man is infected by the bite or skin scratch, leading to cellulitis or cat scratch disease.

Viral Causes include; rabies virus, yellow fever virus, Rift valley fever virus dengue virus, encephalitis viruses, lassa fever virus and hanta virus.

Biologic Warfare and Bioterrorism

Bioterrorism agents are microorganisms (or toxins) that could be used to produce death and disease in human populations, animal livestock's, or plant crops, for terrorist purposes. Such microorganisms could be genetically modified to increase their virulence, make them resistant to drugs or vaccines, or enhance their ability to be disseminated in the environment.

Potential bioterrorism agents are classified into risk categories based on the ease of dissemination or transmission from person to person, mortality rate, ability to cause public panic and requirement for public health preparedness.

Agents in the highest risk category are **small pox, anthrax, botulism toxin, plague,** haemorrhagic fever viruses and *Francisella tularensis.*

The first four agents are at the top of the list. However plague needs a vector (the flea) and botulism may be difficult to deliver. Anthrax and smallpox stand out as potential major problems. The most serious agent, if used, is smallpox virus as the smallpox vaccine was not given since 1972 in USA leaving a nonimmune population, 42 years old and younger.

In response to the possibility of a bioterrorism attack using smallpox virus, the US government has instituted a program to vaccinate "first responders" i.e. HCW, so that they can give emergency medical care without fear of contracting the disease. To protect the unimmumzed general population, the concept of "ring vaccination" will be used. This is based on the knowledge that an exposed individual can be immunized as long as 4 days after exposure and be protected. Therefore, if an attack occurs, people known to be exposed will be immunized as well as the direct contacts of those people and then the contacts of the contacts, in an expanding ring.

The Value of Direct Smear in Diagnosis

Proper examination of direct smears stained by different stains is very useful in several situations:

1- Detection of organisms in direct smear may be sufficient for diagnosis in: **a-** Leprosy (nasal scrapings by modified Z.N. stain).
b- Vincent angina (smears from lesions show pus cells, spirochaetes and fusiform bacilli).
c- Syphilis (smears from chancre show spirochaetes).
d- Relapsing fever during the fever (borrelia in blood films).
e- Acute male gonorrhoea (gram negative diplococci in pus cells).
f- A case of cholera during an epidemic (motile curved rods in stools).

2- The information given by direct smears may be urgently needed to start treatment before culture results appear as in:
a- Meningitis (CSF deposit may show the causative organism).
b- Tuberculosis (acid-fast bacilli in sputum by Z.N.).
c- Diphtheria-like organisms are reported to help clinicians start treatment

3- The presence and concentration of organisms in smears may give an indication of the organism involved:
a- Presence of pneumococci in large numbers in sputum smears are suggestive of
pneumococcal pneumonia, **b-** Appearance of organisms in smears and no growth in aerobic cultures may
point to anaerobes. **Recommended Schedule for Active Immunization of Children and Approaches to Develop New Vaccines (see** Vol. **I chapter 19).**

Difference between Acute Rheumatic fever (ARF) and Acute Glomerulonephritis (AGN)

	ARF	AGN
Occurrence	1-4 weeks after 5. *pyogenes* throat infection	3 weeks after *S. pyogenes* skin infection mainly
Strains of *S. pyogenes* involved	Rheumatogenic strains 1,3,5,6,18 and others	Nephritogenic strains M types 2,4,12,25,42,49,56,57 and 60
Pathogenesis	Autoimmune due to cross-reactivity between heart tissues and antibodies to M-protein	Antigen-antibody complex deposition on basement membrane of glomeruli
Frequency	More common complication	Less common complication
Recurrence	Common	Not common
Prophylaxis	Required (long acting penicillin)	Not needed
Diagnosis	ASO, CRP, sedimentation rate	Anti-DNase B
Sequelae	Damage to heart muscle and valves	Majority recover, rarely chronic glomerulonephritis

Difference between Tuberculoid (TL) and Lepromatous leprosy (LL)

	TL	LL
Course	Benign non-progressive	Malignant progressive
Lesions	Hypopigmented macular skin lesions	Nodular skin lesions
Nerve affection	Asymmetric	Symmetric
Bacteraemia	Absent	Present
AFB* in lesions	Few (paucibacillary)	Abundant (multibacillary)
Immune response CMI	Strong Weak	Weak Strong
Antibody response T cells	Thl with elevated IL-2, IFN-y, TNF-fi	Th2 with elevated IL-4, IL-5, IL-10
Lepromin test	Positive	Negative
Prognosis	Good	Bad
Treatment	2 drugs dapsone + Rifamicin	3 drugs dapsone + Rifamicin + Clofazimine

*AFB - acid-fast bacilli.

	El Tor Vibrio	Classic *V. cholerae*

Difference between Actinomy

	Actino
Causative agent	Bacteria e.g. *Actinomadura Nocardia bra Streptomysis*
Granules	Mainly yellov
Microscopic exam.	Gram positive bacillary chai
Culture	On blood aga anaerobic
Treatment	Respond well

Clinical and Pathogenic C
Classic CJD f

Characteristic	Cla
Median age at death	68 yea
Median duration of illness	4-5 mo
Clinical signs and symptoms	Demer neurol
Periodic sharp waves on electroencephalogram	Often
Presence of "florid plaques" on neuropathology.	Rare o
"Pulvinar sign" on MRI*	Not re
Immunohistochemical analysis of brain tissue	Variab accum
Presence of theagent in tonsillar and other lymphoid tissue	Not re
Increased glycoform ratio on immunoblot analysis of protease-resistant prion protein	Not re

***An abnormal signal in the posterior thalami on T2-and di sequences on brain magnetic resonance imaging (MRI); in vCJD. (Source: CDC)**

VP	Positive	Negative
Lysis of sheep RBCs	Positive	Negative
Haemagglutination of chicken RBCs	Positive	Negative
Resistance to polymyxin B	Yes	No
Resistance to lysis by cholera phage IV	Yes	No

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