Unit- 1

Study of human diseases caused by bacteria

i) Staphylococcus aureus

 # Introduction: -

Staphylococci are Gram-positive cocci that occur in grape-like clusters. Their ability to develop resistance to penicillin and other antibiotics enhances their importance as a human pathogen.

Staphylococci were first observed in human pyogenic lesions by von Recklinghausen in 1871. Pasteur (1880) obtained liquid cultures of cocci from pus and produced abscesses by inoculating them into rabbits. It was Sir Alexander Ogston, a Scottish surgeon, who established the causative role of the coccus in abscesses and also gave it the name Staphylococcus (*Staphyle*, in Greek, meaning **'bunch of grapes':** *kokkos* meaning a berry).

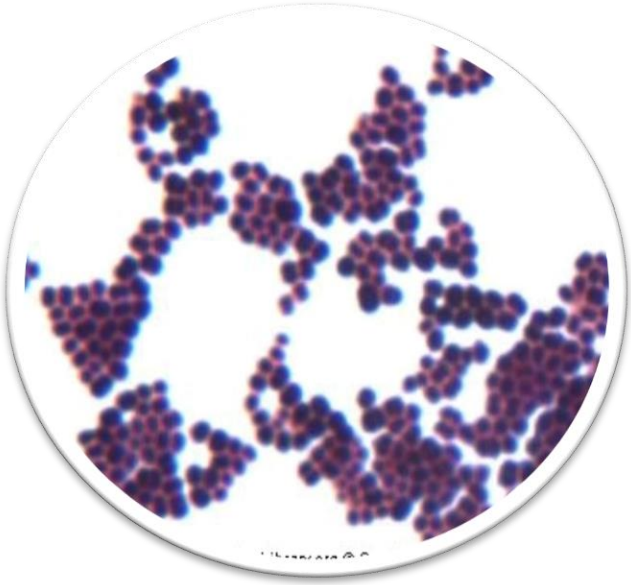
 # Morphology: -

⟶ They are spherical cocci, approximately 1µm in diameter, arranged characteristically in grape like clusters. Cluster formation is due to cell division occurring in three planes, with daughter cells tending to remain in close proximity. They may also be found singly, in pairs and in short chains of 3 or 4 cells.

⟶ They are non-motile and non-sporing, a few strains posses microscopically visible capsule. Many apparently are non-capsulated.

⟶ They stain readily with aniline dyes and are uniformly Gram-positive.

⟶ Under the influence of penicillin and certain chemicals, they may change to L forms.



 # Cultural characteristics: -

- They grow readily on ordinary media within a temperature range of 10 to 42 ^{0}C, the optimum temp. is 37 ^{0}C and pH 7.4 to 7.6.
- They are aerobes and facultative anaerobes.
- On nutrient agar, after incubation for 24 hours, the colonies are large (2 to 4 mm in diameter), circular, convex, smooth, shiny, opaque, and easily emulsifiable. Most strains produce golden **yellow** pigment, though some may be white, orange or yellow. Pigment production occurs optimally at 22 ^{0}C and only in aerobic cultures.
- The colonies on blood agar are similar to those on nutrient agar. Most strains are haemolytic, especially when incubated under 20 to 25% carbon dioxide. They produce a ß type of haemolysis.
- They grow on MacConkey's medium, producing smaller colonies that are pink due to lactose fermentation.
- In liquid media, uniform turbidity is produced.
- Several selective media have been devised for isolation of *Staphylococcus aureus.* These include media containing 8 –10 % NaCl (**Salt-milk agar /broth)**, Lithium chloride and tellurite (Ludlam's medium) and polymyxin.
- For primary isolation, sheep blood agar is recommended. Human blood should not be used as it may contain antibodies or other inhibitors.

 # Biochemical reactions: -

- They ferment a number of sugars, producing acid but no gas. Mannitol is fermented anaerobically by *Staphylococcus aureus* but not by other species.

- Catalase Positive
- Coagulase Positive
- Urea hydrolysis Positive
- Nitrates to nitrites Positive
- Gelatin liquification Positive
- Methyl Red test Positive
- Voges Proaskeur test Positive
- Indole production Negative
- Lipolysis Positive
- Phosphatase test Positive
- Reduction of tellurite Positive **(Produce black colonies)**

 # Resistance: -

- *Staphylococci* are among the more resistant of nonsporing bacteria.
- Dried on threads, they retain their viability for 3-6 months and in pus 2-3 months.
- Withstand 60 ^{0}C for 30 minutes.
- Thermal death point is 62 ^{0}C for 30 minutes. Some require 80 ^{0}C for one hour to be killed.
- Most strains grow in presence of 10 to 15% NaCl.
- Resist 1% phenol for 15 minutes
- Mercury perchloride 1% solution kills them in 10 minutes.
- Killed in crystal violet (1:500,000) and brilliant green (1: 10,000000).
- Fatty acids inhibit the growth.
- Sensitive to *lysostaphin* – a mixture of enzymes produced by particular strains of *Staphylococcus epidermidis.*
- *Staphylococci* were uniformly sensitive to penicillin originally but are now resistant. Resistance is of 3 types--

1. **Production of beta lactamase (penicillinase): -** Which inactivates penicillin by splitting the beta lactam ring. *Staphylococci* produce 4 types of penicillinases, A to D. Penicillinase producing plasmids are transmitted by transduction or conjugation.

2. Change in bacterial surface receptors: -

Reduce binding of beta-lactam antibiotics to cells & show resistance to methicillins and cloxacillins. These strains have been called **'epidemic methicillin resistant *Staphylococcus aureus'* or EMR-SA.**

3. Development of tolerance to penicillin: -

By which the bacterium is only inhibited but not killed.

- *Staphylococci* also exhibit plasmid-borne resistance to erythromycins, tetracyclines, aminoglycosides and almost all clinically useful antibiotics except **vancomycin.**

 # Pathogenecity and virulence: -

Staphylococci produce 2 types of diseases—

1. **Infections: -** *Staphylococci* gain access to damaged skin, mucosal or tissue sites, colonise by adhering to cells or extra-cellular matrix, evade host defense mechanisms, multiply and cause tissue damage.
2. **Intoxications: -** The disease is caused by the bacterial toxins produced either in the infected host or preformed invitro.

The virulence factors described include the following: -

I) Cell associated polymers
II) Cell surface proteins
III) Extra-cellular enzymes
IV) Toxins
   - Cytolytic toxins
   - Enterotoxins
   - Toxic shock syndrome toxin (TSST)
   - Exfoliative (epidermolytic) toxin

I) Cell associated polymers: -

1. The cell wall polysaccharide peptidoglycan confers rigidity and structural integrity to the bacterial cell. It activates complement and induces release of inflammatory cytokines.
2. Teichoic acid, an antigenic component of cell wall facilitates adhesion of the cocci to the host cell surface and protects them from complement-mediated opsonisation.
3. Capsular polysaccharide surrounding the cell wall inhibits opsonisation.

II) Cell surface proteins: -

1. Protein A present on most *Staphylococcus aureus* strains has many biological properties, including chemotactic, antiphagocytic and anticomplementary effects.
2. Clumping factor, another surface protein is the 'bound coagulase'.

III) Extracellular enzymes: -

1. Coagulase is enzyme, which brings about clotting of human or rabbit plasma.
2. Lipases, which help them in infecting the skin and subcutaneous tissues.
3. Hyluronidase, break down the connective tissue. Staphylokinase (fibrinolysin). Fatty acid modifying enzymes and proteases help in initiation and spread of infection.
4. Nuclease.
5. Protein receptors facilitate adhesion to host cell and tissues.

IV) Toxins: -

1. Cytolytic toxins: -

Cytolytic toxins are membrane-active substances, consisting of 4 haemolysins and a leucocidin.

Alpha (α) haemolysin (Alpha toxin, a lysin): -

It is the most important among toxins. It is a protein inactivated at 70 ^{0}C, but reactivated paradoxically at 100 ^{0}C. This is because at 60-70 ^{0}C, the toxin combines with a heat labile inhibitor which is denatured at 100 ^{0}C, leaving the toxin free.

Alpha toxin lyses rabbit RBCs, but less active against sheep and human RBCs. It is also leucocidal, cytotoxic, dermonecrotic, neurotoxic and lethal. It is toxic to macrophages, lysosomes, muscle tissue, renal cortex and circulatory system.

Beta (β) haemolysin (Sphingomyelinase): -

It is haemolytic for sheep RBCs but not for human or rabbit RBCs. It exhibits a 'hot-cold phenomenon', the haemolysis being initiated at 37 ^{0}C, but becoming evident only after chilling.

Gamma (γ) haemolysin: -

It is composed of two separate proteins, both of which are necessary for haemolytic activity.

Delta (δ) haemolysin: -

It has a detergent like effect on cell membranes of RBCs, WBCs, macrophages, and platelets.

Leucocidin: -

It is also a two-component toxin (S and F). It destroys WBCs.

2. Enterotoxin: - This toxin is responsible for the manifestations of staphylococcal food poisoning – nausea, vomiting and diarrhoea 2 to 6 hours after consuming contaminated food containing preformed toxin. The toxin is relatively heat stable, resisting 100 ^{0}C for 10 to 40 minutes.

8 antigenic types of enterotoxin are currently known, named A, B, C_{1-3} **,** D, E, and H. The toxin is believed to act directly on the autonomous nervous system to cause the illness. The toxin is antigenic and neutralised by antitoxin. The toxin also exhibits pyogenic, mitogenic, hypotensive, thrombocytopenic and cytotoxic effects.

3. Toxic shock syndrome toxin (TSST): -

TSS is a potentially fatal multisystem disease presenting with fever, hypotension, myalgia, vomiting, diarrhoea, mucosal hypermia and an erythromatous rash.

TSS became widely known in 1880 following outbreaks in the USA in menstruating women using highly absorbent vaginal tampons.

4. Exfoliative (epidermolytic) toxin: -

This toxin also known as ET is responsible for the **'staphylococcal scalded skin syndrome' (SSSS),** exfoliative skin diseases in which the outer layer of epidermis gets separated from the underlying tissues. The severe form of SSSS is known as Ritter's disease in the newborn and toxic epidermal necrolysis in older patients.

 # Staphylococcal diseases: -

Staphylococcal infections are among the most common of bacterial infections and range from the trivial to the fatal. Common Staphylococcal infections are as follows—

1. Skin and soft tissue: -

- ➔ Foliiculitis
- ➔ Furuncle (boil)
- ➔ Abscess (particularly breast abscess)
- ➔ Wound infection
- ➔ Carbuncle
- ➔ Impetigo
- ➔ Paronychia

2. Musculoskeletal: -

- ➔ Osteomyelitis
- ➔ Arthritis
- ➔ Bursitis
- ➔ Pyomyositis

3. Respiratory: -

- ➔ Tonsillitis
- ➔ Pharyngitis
- ➔ Sinusitis
- ➔ Otitis
- ➔ Bronchopneumonia
- ➔ Lung abscess
- ➔ Pneumonia

4. Central nervous system: -

- ➔ Abscess
- ➔ Meningitis

5. Endovascular: -

- ➔ Bacteraemia
- ➔ Septicaemia
- ➔ Pyemia
- ➔ Endocarditis

6. Urinary: -

- ➔ Staphylococci are uncommon in routine urinary tract infections, though they cause infection in association with local instrumentation, implants or diabetes.

 # Bacteriophage typing: -

Staphylococci may be typed, based on their susceptibility to bacteriophages. The reference centre for staphylococcal phage typing in India is located in the Department of Microbiology, Maulana Azad Medical College, New Delhi.

 # Epidemiology: -

Staphylococci are primary parasites of human beings and animals, colonising the skin, skin glands and mucous membranes. The most common sources of infection are human patients and carriers; animals and inanimate objects being less important. Patients with superficial infections and respiratory infections disseminate large numbers of staphylococci into the environment. About 10 to 30 % of healthy persons carry staphylococci in the nose and about 10 % in the perineum and also on the hair. Vaginal carriage is about 5 to 10 %, which rises greatly during menses.

Staphylococcal carriage starts early in life, colonisation of the umbilical stump being very common in babies borne in hospital. Some carriers, called 'shedders', disseminate very large numbers of cocci for prolonged periods. The cocci shed by patients and carriers contaminate fomites such as handkerchief, bed linen, and blankets and may persist on them for days or weeks. Staphylococci may also come from infected domestic animals such as cows.

The modes of transmission may be contact, direct or through fomites, by dust or by airborne droplets.

Hospital infections by staphylococci deserve special attention because of their frequency and because they are caused by strains resistant to various antibiotics. Staphylococci are a common cause of post-operative wound infections and other hospital cross infections.

Measures for the control of Staphylococcal infection in hospitals include:

1. Isolation of patients with open Staphylococcal lesions.
2. Detection of Staphylococcal lesions among surgeons, nurses and other hospital staff and keeping them away from work till the lesions are healed.
3. Strict aseptic techniques in operation theatres.

 # Laboratory diagnosis: -

Collection of specimens: - The specimens to be collected depend on type of lesion (for example, pus from suppurative lesions, sputum from respiratory infections). In case of food poisoning, faeces and the remains of suspected food should be collected. For the detection of carriers, the usual specimen is the nasal

swab. Swabs from the perineum, pieces of hair and umbilical stump may be necessary in special situations.

Direct microscopic observation: - Direct microscopy with Gram stained smears is useful in the case of pus, where cocci in clusters may be seen. In case of sputum mixed bacterial flora is normally present hence it is of no value.

Diagnosis may readily be made by culture. The specimens are plated n blood agar. Colonies appear after overnight incubation. Also specimens are inoculated on selective media like Ludlam's or Salt milk agar or Robertson's cooked meat medium containing 10 % NaCl. Smears are examined from the cultures and the coagulase test done when staphylococci are isolated.

Tube coagulase test: - 0.1 ml of young broth culture or agar culture suspension of the isolate is added to about 0.5 ml of human or rabbit plasma in a narrow tube. Positive & negative controls are also set up. The tubes are incubated in a water bath at 37 ^{0}C for 3-6 hours.. If positive plasma clots and does not flow when the tube is tilted.

Slide coagulase test: - The isolate is emulsified in a drop of saline on a slide. A drop of human or rabbit plasma is added to the emulsion and mixed. Prompt clumping of cocci indicates a positive test. Positive and negative controls also set up.

Antibiotic sensitivity test: - It should be performed as a guide to treatment. This is important as staphylococci readily develop resistance to drugs.

Bacteriophage typing: - It may be done if information is desired for epidemiological purposes.

Serological tests: - These may sometimes be help in the diagnosis of hidden deep infections.

Antistaphylolysin (Antialphalysin) titre: - These titres of more than 2 units per ml may be of value in the diagnosis of deep-seated infections such as bone abscesses.

Other tests: - Antibiogram pattern, Plasmid profile, DNA fingerprinting, Ribotyping and PCR based analysis for genetic pleomorphism.

 # Treatment: -

As drug resistance is so common among staphylococci, the appropriate antibiotic should be chosen based on antibiotic sensitivity tests.

Benzyl penicillin is the most effective antibiotic, if the strain is sensitive. Methicillin, cloxacillin are used against penicillinase producing strains. But methicillin resistant strains of *Staphylococcus aureus (MRSA)* became common.

For life threatening staphylococcal infections, Vancomycin is the drug of choice. Also strains resistant to vancomycin appeared in the hospital.

For mild superficial lesions, topical applications of antiseptics such as povidone iodine, chlorhexidine and antibiotics such as bacitracin or mupirocin may be sufficient.

Cephalexins, Cefadroxil, Cefuroxime, Sparfloxacin, Gaietyfloxacin are the new antibiotics nowadays used.

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ii) Streptococcus pneumoniae (*Pneumococcus*)

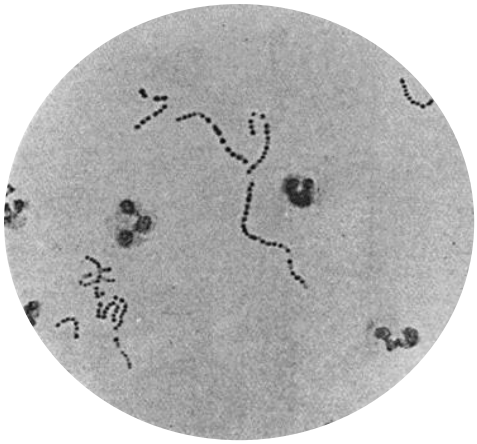
 # Introduction: -

Pneumococcus, a Gram-positive lanceolate diplococcus, formerly classified as *Diplococcus pneumoniae,* has been reclassified as *Streptococcus pneumoniae* because of its genetic relatedness to streptococcus. They are the single most prevalent bacterial agent in pneumonia and otitis media in children. They can also cause sinusitis, bronchitis, bacteraemia, meningitis and other infectious processes.

Pneumococci were first noticed in 1881 by Pasteur and Sternberg independently. But the relationship between pneumococci and pneumonia was established only later by Fraenkel and Weichselbaum independently in 1886.

 # Morphology: -

- ➔ Pneumococci are typically small (1 µm), slightly elongated cocci, with one end broad or rounded and the other pointed, presenting a flame shaped or lanceolate appearance.
- ➔ They occur in pairs (diplococci), with the broad ends in opposition, the long axis of the coccus parallel to the line joining the two cocci in a pair.
- ➔ They are capsulated, the capsule enclosing a pair.
- ➔ They are nonmotile and nonsporing.
- ➔ They are readily stained with aniline dyes and are Gram-positive.
- ➔ The capsule may be demonstrated as a clear halo in India ink preparations or may be stained directly by special techniques.



 # Cultural characteristics: -

- ➔ Pneumococci have complex growth requirements and grow only in enriched media.
- ➔ They are aerobes and facultative anaerobes
- ➔ The optimum temperature being 37 ^{0}C (range 25-42 ^{0}C) and optimum pH 7.8 (range 6.5 – 8.3)
- ➔ Growth is improved by 5-10 % CO_2
- ➔ On blood agar, after incubation for 18 hours, the colonies are small (0.5-1mm), dome shaped and glistening, with an area of green discoloration (alpha haemolysis) around them. On further incubation the colonies become flat with raised edges and central umbonation. Some strains that develop abundant capsular material form large mucoid colonies.
- ➔ Under anaerobic conditions, colonies on blood agar are surrounded by a zone of beta haemolysis due to oxygen labile haemolysin O.
- ➔ In liquid media such as glucose broth, growth occurs as uniform turbidity. The cocci readily undergo autolysis due to the activity of intracellular

enzymes. Autolysis is enhanced by bile salts, sodium lauryl sulphate and other surface-active agents.

 # Biochemical reactions: -

- ➔ Pneumococci ferment several sugars, forming acids only.
- ➔ Fermentation of inulin is a useful test for differentiating them from streptococci, as the latter do not ferment it.
- ➔ These are bile soluble. If a few drops of 10 % sodium deoxycholate solution are added to 1 ml of an overnight broth culture, the culture clears due to the lysis of the cocci.
- ➔ These are catalase and oxidase negative.

Difference between *Pneumococcus* and *Stre. viridans*

Sr. No.	**Particulars**	**Streptococcus pneumoniae**	**Streptococcus viridans**
1	Morphology	Capsulated, lanceolate	Noncapsulated, oval or round in chains
2	Quellung test	Positive	Negative
3	Colonies	Initially dome shaped, later ‘draughtsman’ colonies	Dome shaped
4	Growth in liquid media	Uniform turbidity	Granular turbidity, powdery deposit
5	Bile solubility	Invariably positive	Invariably negative
6	Inulin fermentation	Positive	Negative
7	Optochin sensitivity	Positive	Negative
8	Intraperitoneal inoculation in mice	Fatal infection	Non-pathogenic

 # Resistance: -

- ➔ Pneumococci are delicate organisms and are readily destroyed by heat (thermal death point 52 ^{0}C for 15 minutes) and antiseptics.
- ➔ In cultures they die on prolonged incubation, perhaps due to accumulation of toxic peroxides.
- ➔ They are sensitive to most antibiotics, beta lactam antibiotics being the drugs of choice. Almost all strains were sensitive to 0.05-µg penicillin till 1967, and then resistant strains began to appear. The mode of resistance is not production of beta lactamase, but alteration in the penicillin binding proteins on the bacterial surface. Such strains are also resistant to multiple drugs.
- ➔ The sensitivity of pneumococci to optochin (ethyl hydrocuprein) 1: 500,000 is useful in differentiating them from streptococci.

 # Antigenic properties: -

Capsular polysaccharide: - It is also called the 'specific soluble substance (SSS). Pneumococci are classified into types based on the antigenic nature of the capsular polysaccharide as Type I, II, III and IV. Now more than 90 different serotypes are recognised, named, 1, 2, 3, and so on.

Typing may be carried out by—

- ➔ Agglutination of the cocci with the type specific antiserum
- ➔ Precipitation of the SSS with the specific serum or
- ➔ By the "**Quellung" or Capsule swelling reaction: -** a suspension of pneumococci is mixed on the slide with a drop of the type specific antiserum and a loopful of methylene blue solution. The capsule becomes apparently swollen, sharply delineated and refractile.

Nucleoprotein

C carbohydrate antigen

C reactive protein: - An abnormal protein beta globulin that precipitate with the somatic C antigen of pneumococci appears in the acute phase sera of cases of pneumonia known as 'C-reactive protein' (CRP).

 # Variation: -

On repeated subculture, pneumococci undergo a smooth-to-rough (S-R) variation. In the R form, the colonies are rough and the cocci are noncapsulated, autoagglutinable and avirulent. R form arise as spontaneous mutants and outgrow the parental S forms in artificial culture, in tissues, such R mutants are eliminated by phagocytosis.

 # Toxins and other virulence factors: -

Pneumococci produce following toxins and virulence factors—

Haemolysin: - It is oxygen labile

Leucocidin

Pneumolysin: - It is a membrane-damaging toxin produced by pneumococci has cytotoxic and complement activating properties and so may be a virulence factor. It is immunogenic.

Capsular polysaccharide: - The virulence of pneumococci depends on its capsular polysaccharide. Its acidic and hydrophilic properties protect the cocci from phagocytosis. Capsulated pneumococci are not phagocytosed efficiently. The enhanced virulence of type 3 pneumococcus is due to the abundance of its capsular material. Noncapsulated strains are avirulent. The antibody to the capsular polysaccharide affords protection against infection.

 # Pathogenecity: -

1. Pneumococci colonise the human nasopharynx and may cause infection of the middle ear, paranasal sinuses and respiratory tract by direct spread. Infection of the meninges can also occur, by contiguity or through blood. Pneumococcal bacteraemia may also lead to infections in heart, peritoneum or joints.
2. **Otitis media and sinusitis: -** These are commonest pneumococcal infections. Prior respiratory infection or allergy causing congestion and blockage predispose to these conditions. Serotypes 6, 14, 19F and 23F are commonly encountered in these conditions. Pneumococci are one of the most common bacteria causing **pneumonia,** both lobar and bronchopneumonia. They also cause acute tracheobronchitis and empyema.

Bacteraemia is common during the early stage of lobar pneumonia. Toxaemia is due to the diffusion of the capsular polysaccharide into the blood and tissues.

In adults, types 1-8 are responsible for about 75 % of cases of pneumococcal pneumonia and for more than 50 % of all fatalities due to pneumococcal bacteraemia. In children, types 6, 14, 19 and 23 are frequent causes.

3. **Bronchopneumonia: -** It is almost always a secondary infection. The damage to the respiratory epithelium and excessive bronchial secretions caused by the primary infection facilitate the invasion of pneumococci along the bronchial tree. Bronchopneumonia is frequently a terminal event in aged and debilitated patients.

4. Acute exacerbations: - Pneumococci are commonly associated with this. The copious respiratory secretions in chronic bronchitis aid pneumococcal invasion.

5. Meningitis: - It is the most serious of pneumococcal infections. It is usually secondary to other pneumococcal infections such as pneumonia, otitis media or sinusitis. It occurs at all ages. Untreated cases are almost invariably fatal. Even with antibiotic therapy, the case fatality rate is about 25 %.

6. Suppurative lesions: - Pneumococci may also produce suppurative lesions in other parts of the body – empyemia, pericarditis, otitis media, sinusitis, conjunctivitis, suppurative arthritis and peritonitis usually are complications of pneumonia.

 # Epidemiology: -

The source of human infection is the respiratory tract of carriers and less often of patients. Pneumococci occur in the throat of approximately half the population sampled at any time. They are transmitted from one another by fingers or by inhalation of contaminated droplets or droplet nuclei. Dissemination is facilitated by crowding.

Disease results only when the host resistance is lowered by contributory factors such as respiratory viral infections, pulmonary congestion, stress, malnutrition, immunodeficiency or alcoholism.

Pneumococcal serotypes vary greatly in virulence. Type 3 is the most virulent and also its fatality rate is more. Epidemics may occur among closed communities as in army camps. The incidence of bronchopneumonia increases when an epidemic of influenza or other viral infection of the respiratory tract occurs. Cases are more common in winter and affect the younger children and older persons.

 # Laboratory diagnosis: -

Microscopic examination: - In the acute phase of lobar pneumonia, the rusty sputum contains pneumococci in large numbers. They may be demonstrated by Gram-staining. In acute otitis media, fluid aspirated from the middle ear is taken and stained. In case of meningitis, CSF (Cerebrospinal Fluid) is stained.

Quellung test: - "**Quellung" or Capsule swelling reaction: -** a suspension of pneumococci is mixed on the slide with a drop of the type specific antiserum and a loopful of methylene blue solution. The capsule becomes apparently swollen, sharply delineated and refractile.

Isolation of Pneumococci: - The sputum, blood, CSF, fluid aspirated from middle ear etc are inoculated on blood agar plates and incubated at 37 ^{0}C for 24 hours under 5-10 % co_2**.**

Demonstration of capsular polysaccharide: - It can be demonstrated in the blood, urine & CSF by counterimmunoelectrophoresis.

Other tests: - Antibodies can be demonstrated by agglutination, precipitation. Indirect haemagglutination, indirect FA test and Radioimmuneassay can be employed.

 # Prophylaxis: -

Immunity is type specific and associated with antibody to the capsular polysaccharide. The existence of some 90 serotypes makes a complete polyvalent vaccine impracticable.

A polyvalent polysaccharide vaccine representing the capsular antigens of 23 most prevalent serotypes is being used, which is stated to give 80-90 %

protection. It is not meant for general use, but only in persons at enhanced risk of pneumococcal infection such as those with absent or dysfunctional spleen, sickle cell disease, chronic renal, lung, heart and liver diseases, diabetes mellitus and immunodeficiencies including HIV infection.

It is not recommended in children under 2 years of age and those with lymphoreticular malignancies and immunosuppressive therapy.

 # Treatment: -

The antibiotic of choice is parenteral penicillin in serious cases and amoxycillin in milder ones, provided the infecting strain is penicillin sensitive. Many penicillin resistant strains are also resistant to other antibiotics like erythromycin and tetracyclin.

A third generation cephalosporin is indicated in such cases. Vancomycin is to reserved for life threatening illnesses with highly resistant strains.

Now a day's sparfloxacin, ofloxacin, are used.

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iii) ***Mycobacterium tuberculosis***

 # Introduction: -

Mycobacteria are slender rods that sometimes show branching filamentous forms resembling fungal mycelium. In liquid cultures they form a mold-like pellicle. Hence the name **'mycobacteria',** meaning fungus like bacteria. They do not stain readily, but once stained resist decolourisation. With dilute mineral acids. Mycobacteria are therefore called **'Acid Fast Bacilli' or AFB.**

They are aerobic, nonmotile, noncapsulated and nonsporing. Growth is generally slow. The genus includes obligate parasites, opportunistic pathogens and saprophytes.

The first member of this genus to be identified was the lepra bacillus (leprosy causing) discovered by Hansen in 1868. Koch (1882) isolated mammalian tubercle bacillus and proved its causative role in tuberculosis by satisfying Koch's postulates.

Tuberculosis in humans was subsequently shown to be caused by 2 types of the bacillus—

-- The human and bovine types, designated ***Mycobacterium tuberculosis and Mycobacterium bovis*** respectively.

Several mycobacteria, distinct from human or bovine tubercle bacilli, which have been isolated on occasion from human pathological material, have been grouped together under the loose term **'atypical mycobacteria'.** Unlike tubercle bacilli, which are strict parasites, atypical mycobacteria occur in soil, water and other environmental sources.

 # Morphology: -

- ➔ *M. tuberculosis* is a straight or slightly curved rod, about 3 µm x 0.3 µm, occurring singly, in pairs or as small clumps. The size depends on condition of the growth, and long filamentous, club shaped and branching forms may be sometimes seen.
- ➔ *M. bovis* is usually straighter, shorter and stouter.
- ➔ Tubercle bacilli have been described as Gram-positive, though strictly speaking this is not correct, as after staining with basic dyes they resist decolourisation by alcohol.
- ➔ When stained with carbol fuchsin by the Ziehl-Neelsen method or by fluorescent dyes (auramine O, rhodamine), they resist decolourisation by 20 % sulphuric acid and absolute alcohol for 10 minutes (acid and alcohol fast). Hence these are called as **Acid Fast Bacilli (AFB).** Acid fastness is due to the presence of an unsaponifiable wax **(mycoloic acid)** in the cells.
- ➔ Beaded or barred forms are frequently seen in *M. tubeculosis*, but *M. bovis* stains more uniformly.
- ➔ Electron micrographs of thin sections show that the thick cell wall is composed of 3 layers enclosing a trilaminar plasma membrane.
- ➔ Spheroplasts are formed when grown in the presence of lysozyme. L-froms are also seen.

 # Cultural characteristics: -

- ➔ The bacilli grow slowly, the generation time in vitro being 14-15 hours. Colonies appear in about 2 weeks and may sometimes take up to 8 weeks.
- ➔ Optimum temperature is 37 ^{0}C and growth does not occur below 25 ^{0}C or above 40 ^{0}C.
- ➔ Optimum pH is 6.4-7.0.
- ➔ *M. tuberculosis* is an obligate aerobe, while *M. bovis* is microaerophilic on primary isolation, becoming aerobic on subculture.
- ➔ *M. tuberculosis* grows luxuriantly in culture as compared to *M. bovis* which grows sparsely. They are therefore termed 'eugonic' and 'dysgonic' respectively.
- ➔ The addition of 0.5 % glycerol improves the growth of *M. tuberculosis,* but has no effect on or may even impair the growth of *M. bovis.*
- ➔ Sodium pyruvate helps the growth of both types.
- ➔ Human tubercle bacilli do not grow in presence of P-nitrobenzoic acid, unlike other nonchromogens.
- ➔ Tubercle bacilli are highly susceptible even to traces of toxic substances like fatty acids in culture media. The toxicity is neutralised by serum albumin or charcoal.
- ➔ Koch originally grew this bacillus on heat coagulated bovine serum.
- ➔ Several media, both solid and liquid have been described. The solid media contain egg **(Lowenstein-Jensen, Petragnini, Dorset),** blood **(Tarshis),** serum **(Loeffler)** or potato **(Pawlowsky).**
- ➔ The solid medium most widely used for routine culture is **Lowenstein-Jensen (L J)** medium without starch, as recommended by the International Union Against Tuberculosis **(IUAT).** This consists of coagulated hens' egg, mineral salt solution, aspargine and malachite green. Malachite green acts as selective inhibiting agent to other bacteria.

- ➔ Liquid media described include Dubo's, Middlebrook's, Proskauer and Beck's, Sula's and Sauton's media. These are not used for routine cultivation, but are used for sensitivity testing, chemical analysis and preparation of antigens and vaccines.
- ➔ On solid media, *M. tuberculosis* forms dry, rough, raised, irregular colonies with a wrinkled surface. They are creamy white, becoming yellowish or buff coloured on further incubation. They are tenacious and not easily emulsified. *M. bovis* colonies, in comparison are flat, smooth, moist, white and break up easily when touched.
- ➔ In liquid media without dispering agents the growth begins at the bottom, creeps up the sides and forms a prominent surface pellicle, which may extend along the sides above the medium. Diffuse growth is obatined in Dubos' medium containing Tween-80 (Sorbitan monooleate). Virulent strains tend to form long serpentine cords in liquid media, while avirulent strains grow in a more dispersed manner.

 # Resistance: -

- ➔ Mycobacteria are not especially heat resistant, being killed at 60 ^{0}C in 15-20 minutes. Survival is influenced by the material in which they are present.
- ➔ Cultures may be killed by exposure to direct sunlight for 2 hours, but bacilli in sputum may remain alive for 20-30 hours.
- ➔ Bacilli in droplet nuclei may retain viability for 8-10 days under suitable conditions.
- ➔ Cultures remain viable at room temperature for 6-8 months and may be stored up to 2 years at –20 ^{0}C.
- ➔ Tubercle bacilli are relatively resistant to chemical disinfectants, surviving exposure to 5% phenol, 15% H_2SO_4, 3% HNO_3, 5% Oxalic acid, 4% NaOH.
- ➔ Are destroyed by tincture of iodine in 5 minutes and by ethanol in 2-10 minutes.
- ➔ 80% ethanol has been recommended as a disinfectant for skin, rubber gloves and clinical thermometers. It sterilises pieces of cloths in 10 minutes.

 # Biochemical reactions: -

Sr. No.	**Biochemical test**	***M. tuberculosis***	M. bovis	**Atypical mycobacteria**
1	**Niacin test** Egg medium culture + 10% Cyanogen bromide + 4% aniline Canary yellow colour	Niacin is formed	Not formed	Positive in few
2	Aryl sulphatase test Organisms grown in a medium containing 0.001 M tripotassium phenolphthalien disulphate + 2N NaoH ⟶ Pink colour	Aryl sulphatase not formed	Negative	Positive
3	Neutral red test Ability to bind neutral red in alkaline buffer solution	Able to bind	Able to bind	Not able to bind
4	**Catalase-peroxidase** 5 ml test culture + mixture of H_2O_2 & 0.2% catechol Effervescence indicates catalase production &	Weakly catalase positive Peroxidase positive	Weakly catalase positive Peroxidase positive	Strongly catalase positive Peroxidase negative

	browning indicates peroxidase			
5	**Amidase tests** Ability to split amides E.g. Acetamide, Benzamide	Negative	Negative	Positive in some strains
6	**Nitrate reduction test**	Positive	Negative	Positive in some strains

 # Pathogenesis: -

The essential pathology of tuberculosis consists of the production of a characteristic lesion called as **'tubercle'** in infected tissues.

'Tubercle' is an avasular granuloma composed of a central zone containing giant cells with or without caseation necrosis, surrounded by epithelial cells and a peripheral zone of lymphocytes & fibroblasts.

Tubercle bacilli **do not produce toxin.** The various components of the bacillus have been shown to possess different biological activities, which may influence the pathogenesis, allergy and immunity in the disease.

- ➔ The cell wall induces resistance to infection, causes delayed hypersensitivity.
- ➔ Tuberculoprotein can elicit the tuberculin reaction.
- ➔ Polysaccharide induces immediate hypersensitivity.
- ➔ Lipids cause the accumulation of macrophages and neutrophils.
- ➔ Phosphatides induce the formation of tubercles.

Tuberculosis lesions are of 2 types—

I) Exudative
II) Productive

I) Exudative lesions: -

It is an acute inflammatory reaction with accumulation of oedema fluid, polymorphonuclear leucocytes and monocytes around the bacilli. The lesion may heal by resolution, lead to necrosis of the tissues or develop into productive type.

II) Productive lesions: -

It is predominantly cellular, composed of a number of tubercles, which may enlarge, coalesce, liquefy & undergo caseation.

The fate of tubercle bacilli entering the body is influenced by a variety of factors—

➔ The dose, virulence and mode of entry of bacillus
➔ The age, resistance and hypersensitivity of the host

Tubercle bacilli enter the body commonly by inhalation, less often by ingestion and rarely by inoculation into the skin.

There are mainly 2 types of tuberculosis—

I) Pulmonary tuberculosis
II) Extrapulmonary tuberculosis

I) Pulmonary tuberculosis: -

In this type, lungs are affected. When tubercle bacilli are inhaled, they lodge in the pulmonary alveoli, where they are promptly phagocytosed by alveolar macrophages. But instead of being killed, the bacilli multiply intracellularly and eventually disrupt the phagocyte. Phagocytes with ingested bacilli may even act as vehicles transporting the infection to different parts of the body. Intracellular multiplication of the bacillus is interrupted only with the development of specific cellular immunity, which sets in about 6-8 weeks after infection.

In children, primary infection leads to the ‘primary complex’. This consists of a sub pleural focus of tuberculous pneumonia in the lung parenchyma usually found in the lower lobe or the lower part of the upper lobe.

The adult type of tuberculosis is generally due to reactivation of the primary infection. The adult type of pulmonary lesion may heal by resorption, fibrosis and occasionally calcification or progress to chronic fibrocaseous tuberculosis with tubercle formation, caseation, cavitation & shedding of tubercle bacilli in sputum. **(Open tuberculosis).** Rarely an acute rapidly fatal infection may occur in adults.

II) Extrapulmonary tuberculosis: -

Rarely the primary infection may lead to haematogenous spread (through blood) and the development of—

- ➔ Miliary tuberculosis
- ➔ Meningitis (Brain TB)
- ➔ Lesions in different organs such as the spleen, liver and kidneys.

 # Epidemiology: -

Tuberculosis is an ancient disease. It has been for many centuries the most important of human infections, in its global prevalence, devastating morbidity and massive mortality. It has been called **'the captain of all the men of death'.**

Its prevalence increased greatly following the industrial revolution with rapid urbanisation and overcrowding. With improvements in the standards of living, its incidence has come down in the developed countries. This disease is called as **'barometer of social welfare'.**

There are about 20 million open cases of tuberculosis in the world of which 70-8-% are in the poor nations. Some 3 million people die of tuberculosis every year. About 4 to 5 million new cases of open tuberculosis arise each year.

In India, 8-9 million active cases are present of which 2-3 millions are sputum positive. The annual mortality rate from the disease is estimated to be 60-80 per 1 lakh of the population.

There is high prevalence of both infection and active disease in the developing countries. Practically everyone is infected by the age of 20 & the infected rates are high as 10-15% in the school. Mortality is high in infants and in children below 5 years.

Low socio-economic status and malnutrition are important predisposing factors. Dusty occupations, doctors, nurses and laboratory workers are prone to develop the disease.

The major source of infection is the 'open' human case shedding the bacillus in the sputum. The bacilli remain viable for weeks in dust. Inhalation of such dust is the main mode of infection. **The bovine tubercle bacillus responsible for intestinal, granular, bone and joint** tuberculosis is transmitted through milk from infected animals.

 # Laboratory diagnosis: -

It is done by—

I) Demonstration of the bacillus in the lesions by microscopy
II) Isolation of bacillus in culture
III) Transmission of infection to experimental animals
IV) Demonstration of hypersensitivity to tuberculoprotein

I) Demonstration of the bacillus in the lesions by microscopy: -

➔ Sputum from pulmonary tuberculosis, Cerebrospinal Fluid (CSF) from tuberculous meningitis, Urine from renal tuberculosis, specimen from intestine etc. is taken and smear is prepared on clean glass slide, it is dried, heat fixed and stained by the Ziehl-Neelen technique.
➔ The smear is flooded with Carbol fuchsin and gently heated to steaming for 5-7 minutes.
➔ The slide is then washed with water and decolourised with 20% H_2SO_4 till no more stain comes off and then with 95% ethanol for 2 minutes or by 3% HCL + 95% ethanol mixture.
➔ Then counter stain methylene blue or malachite green is added for 1 minute.
➔ Then smear is washed, air dried and observed under oil immersion lens.
➔ Acid Fast Bacilli (AFB) are seen as bright red rods.

II) Isolation of bacillus in culture: -

➔ Cultures are very sensitive for detection of tubercle bacilli and may be positive with as few as 10-100 bacilli / ml of sputum.

- ➔ The material is inoculated into at least two bottles of 'Lowenstein-Jensen' medium. Then incubated at 37 ^{0}C for 4 days (for rapid growing mycobacteria) and for 8-12 weeks for slow growing mycobacteria.
- ➔ Then smears from colonies are stained and examined for routine purposes.
- ➔ A slow growing, nonpigmented, niacin positive, acid fast bacillus is *M. tuberculosis.*
- ➔ Confirmation is obtained by detailed biochemical studies.

III) Transmission of infection to laboratory animals: -

- ➔ The concentrated material is inoculated intramuscularly into the thigh of 2 healthy guinea pigs about 12 weeks old.
- ➔ The animals are weighed before inoculation and at intervals thereafter.
- ➔ Progressive loss of weight is an indication of tuberculosis.
- ➔ One guinea pig is killed at 4 weeks and autopsy is done. If no evidence of tuberculosis is noticed, the other is killed after 8 weeks and autopsy is done.
- ➔ In autopsy, an infected animal will show the following features--

--- A caseous lesion at the site of inoculation, enlarged lymph nodes and glands, enlarged spleen, tubercles in the peritoneum, a few tubercles may be seen in the lungs. Kidneys are not affected. Then diagnosis of tuberculosis in the guinea pig has to be confirmed by demonstration of acid-fast bacilli in the lesions.

IV) Demonstration of hypersensitivity to tuberculoprotein (Tuberculin / Mantoux test): -

It is of limited value only, as it does not differentiate between clinical disease and subclinical infection. A negative tuberculin test often helps to exclude the diagnosis of tuberculosis.

Koch prepared a protein extract of the tubercle bacillus by concentrating and evaporating a 6-8 week culture filtrate of the bacillus grown in 5% glycerol broth. This was called **'Original or Old Tuberculin' (OT).**

Then a purified preparation of the active tuberculoprotein has been standardised by Seibert from cultures grown in semisynthetic medium. This

standardised and stable antigen known as **'Purified Protein Derivative (PPD)'** is now generally used.

Mantoux / Tuberculin test: -

Graded doses of tuberculin ranging from 1 tuberculin unit to 100 or 250 TU, are injected intradermally on the forearm using a tuberculin syringe.

(1TU is equivalent to 0.01 mg OT or 0.00002 mg PPD).

On examination after 48-72 hours, a positive reaction is indicated by oedema and indurations at the site, measuring at least 6 to 10 mm in diameter. Erythema alone is not taken as a positive reaction.

Tuberculin testing may be used as an aid in diagnosing active infection in infants and young children, to measure the prevalence of infection in a community, to select susceptibles for BCG vaccination or as an indication of successful vaccination.

 # Prophylaxis: -

In the prevention of tuberculosis, general measures such as—

- ➔ Adequate nutrition, good housing, and health education are as important as specific antibacterial measures.
- ➔ The latter consists of early detection & treatment of cases, chemoprophylaxis and immunoprophylaxis.
- ➔ Immunoprophylaxis is by intradermal injection of the live attenuated vaccine introduced by **'Calmette & Guerin'** (1921), the **'Bacille Calmette – Guerin' or BCG.**
- ➔ BCG is a strain of *M. bovis* attenuated by 239 serial subcultures in a Glycerin – Bile – potato medium over a period of 13 years.
- ➔ Injection of BCG leads to dissemination & multiplication of the bacilli in different organs with production of small tubercles. Within a few weeks, the bacilli stop multiplying; they survive in the tissues for an indefinite period of time. This self limited infection induces delayed hypersensitivity & immunity.
- ➔ After BCG vaccination, negative recipient is converted to a positive reactor. The immunity has been found to last at least for 10-15 years.
- ➔ BCG does protect against tuberculosis, particularly in infants and children. The protection is not absolute, but the disease in the immunised children

runs a milder course. It is also believed to prevent skeletal, meningeal and miliary forms of tuberculosis to a large extent.

➔ The vaccine is given intradermally over the deltoid. Freeze dried preparations are employed as they are more stable than the liquid vaccine.

➔ Immunization with BCG leads to a stimulation of the reticuloendothelial system. It has been reported to confer some protection against leprosy and leukaemia.

 # Treatment: -

Chemotherapy has revolutionised the management of tuberculosis. The antituberculous drugs employed are—

➔ Rifampicin
➔ Isoniazid (INH)
➔ Pyrazinamide
➔ Streptomycin
➔ Ethambutol
➔ Ethionamide
➔ Thiacetazone
➔ Para Amino Salycyclic acid (PAS)
➔ Cycloserine

The first 4 drugs are bactericidal and others are bacteriostatic. For many years, the standard practice was to give streptomycin, INH and PAS for 2 years or more. Since the introduction of Rifampicin, more rapid cures have become possible and several short-term courses have been devised.

Such courses employed are as follows—

➔ Rifampicin, INH and Ethambutol for 2 months and the last two for a further period of 7 months.
➔ Rifampicin, INH, Pyrazinamide and Streptomycin for 2 months, followed by Rifampicin and INH for further 4 months period.

The major problem in the chemotherapy of tuberculosis is the development of drug resistance. The mechanism of resistance is mutation and selection. Hence resistance can be prevented by simultaneous treatment with two or more drugs.

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