Notes

16

PNEUMOCOCCUS

16.1 INTRODUCTION

Pneumococcus, earlier known as Diplococcus pneumoniae (as it occurs in pairs) is now called Streptococcus pneumoniae in 1974 because it is related to Streptococcus (growth in chains in liquid media). Pneumococci normally inhabit the mucosa of the upper respiratory tract which is kind of the natural habitat of these bacteria. Healthy adults are carriers (approximately 40–70 %) of Pneumococci. Most of Pneumococcal diseases are endogenous infections i. e from the mucosa of respiratory tract the pneumococci invade the carrier host and cause disease.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the classification of Pneumococcus;
- describe the morphological characteristics of Pneumococcus;
- discuss the biochemical and other specific characteristics;
- explain mechanism of virulence, pathogenicity;
- enumerate the diseases caused by Pneumococcus;
- culture and identify Pneumococcus from specimen;
- describe the vaccines available to prevent Pneumococcal infections;
- discuss the vaccine schedule, doses and when to give vaccine.

16.2 HISTORY

Louis Pasteur and George Sternberg independently discovered Pneumococci in 1888.However, the relationship between Pneumococci and pneumonia was discovered in 1886 by Fraenkel and Weichselbaum. Organism was named as *Diplococcus pneumoniae* because of its paired cocci appearance in Gram stained smear from sputum. However, later it was found that this organism is related to Streptococci as explained earlier so the organism was named *Streptococcus pneumoniae*.

Frederik Griffith in 1928 demonstrated a phenomenon called "transformation" wherein he injected a mixture of non-virulent Strept. pneumoniae and killed virulent Strept. pneumoniae in mice and found that the mice died due to infection with virulent pneumococci. Later 1944) it was found that the DNA of killed pneumococci in the mixture entered the non virulent pneumococci and transformed them into virulent pneumococci. This phenomenon was named transformation and it marked the beginning of molecular genetics.

16.3 CLASSIFICATION

Pneumococcus belongs to the kingdom bacteria. The classification is given below:

Class: Bacilli;

Order: Lactobacillales;

Family: Streptococcaceae;

Genus: Streptococcus;

Species: Streptococcus pneumoniae;

Serotypes: I, II, III and heterogeneous group IV (More than 90 different serotypes are recognized in this group).

16.4 MORPHOLOGY

Pneumococci are Gram-positive, slightly elongated, oval to lanceolate-shaped diplococci (0.5 and 1.25 micrometers in diameter), usually occur in pairs or short chains surrounded by a thick capsule. One end of the Pneumococcus is broad and the other end is pointed giving it the typical lanceolate shape. The broad end of the cocci in pair is in apposition and pair of cocci is surrounded by a big capsule. The capsule is most apparent in smears made from exudates (patient sample), capsule is usually lost in culture.

16.5 CULTURAL CHARACTERISTICS

Pneumococci are fastidious organisms to grow i. e. they require enriched medium (blood agar) to grow. The optimum temperature for growth is 37° C (range is 25-42° C) and pH is 7.8. Pneumococci grow better in an atmosphere with 5-10% CO_2 (culture plates kept in candle jar and incubated).

Specimen is cultured on Blood agar and Chocolate Agar and plates are incubated as above. Plates are examined for growth after 18 hrs and more. The colonies on Blood agar are alpha –hemolytic, dome shaped, mucoid (smooth, shiny). The mutants without capsules produce colonies with a rough surface ("R" form). Smooth (S) to Rough (R) variation can occur on repeated culture.

Under anaerobic conditions colonies may be surrounded by clearing of medium, beta haemolysis (due to oxygen labile haemolysin) instead of green discolouration –the alpha haemolysis. Streptococcus pneumoniae is a very fragile bacterium, contains within itself the enzymatic (autolysin- autolytic enzyme, Lyt A) ability to disrupt and to disintegrate the cells. The physiological role of this autolysin is to cause the culture to undergo a characteristic autolysis that kills the entire culture when grown to stationary phase. Bile salt enhances autolysis.

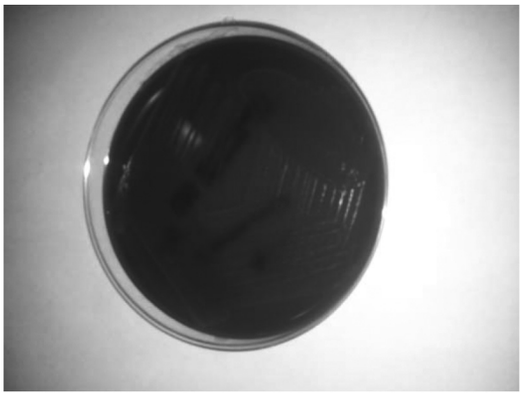


Fig. 16.1: Blood agar plate showing alpha haemolysis (greenish colouration) typical of Pneumococci.

Most clinical isolates of pneumococci undergo lysis mediated by autolysin between18-24 hours after culture under optimal conditions. Autolysis changes the colony character from plateau-type morphology to colony with lysed/ depressed center.

16.6 BIOCHEMICAL AND SPECIFIC IDENTIFICATION CHARACTERISTICS

Pneumococcus is an aerotolerant anaerobe and ferments many sugars. Hiss's serum sugars are used for fermentation reaction. Pneumococci hydrolyze inulin

and this test is used to differentiate Pneumococci from Streptococci. Pneumococci are oxidase and catalase test negative. They do not display an M protein like some other Streptococci.

The specific characteristics of Pneumococci include bile solubility, optochin sensitivity and Quellung phenomenon or Capsule swelling reaction. Let us discuss these one by one.

16.6.1 Bile Solubility Test

A few drops of 10% sodium deoxycholate solution are added to 1 ml of overnight broth culture of pneumococci. Clearing of broth culture is seen within few minutes due to lysis of pneumococci. Other method to do this test is to place a loopful of 10% deoxycholate solution on the colony of pneumococci on blood agar-the lysis of colony is seen within few minutes. The test is used to differentiate Pneumococci from other alpha haemolytic streptococci like Strept viridans.

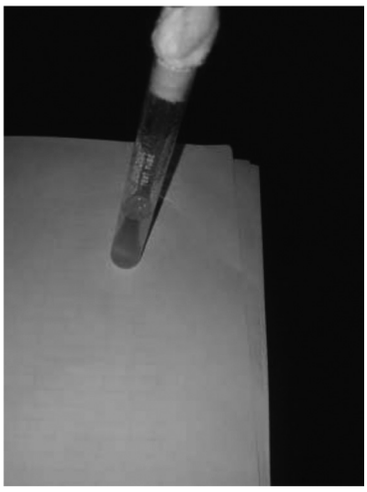


Fig. 16.2: Bile solubility test-showing clearing of turbidity due to destruction of Pneumococci.

16.6.2 Optochin Sensitivity Test

Optochin discs (5 mg ethyl hydrocuprein hydrochloride) are available commercially. Blood agar plate is inoculated with Pneumococci; the disc is placed in the center of the plate and is incubated in a CO_2 incubator overnight. The plate is examined the next day and the zone of inhibition around the disc is measured. An inhibition zone of 15 mm or more means the organism is sensitive to optochin. The test differentiates Pneumococci from other alpha haemolytic streptococci.

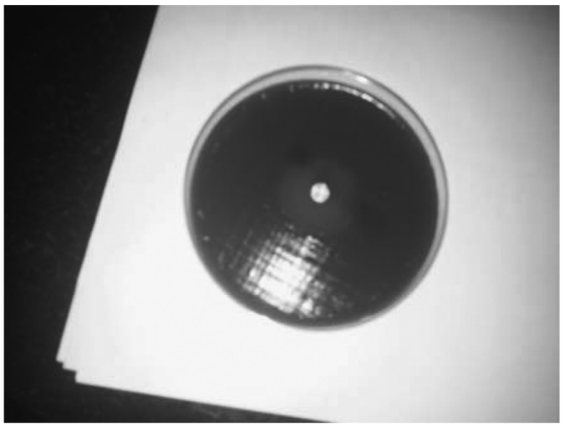


Fig. 16.3: Blood agar plate showing zone of inhibition of Pneumococcal growth around optochin disc.

16.6.3 Quellung Test or Capsule Swelling Reaction

This test can be performed on specimen like sputum or on plate showing mixture of organisms. The specimen or material is mixed with a drop of Pneumococcal polyvalent antiserum ; smear is made and stained. The Pneumococcal capsule appears to be swollen. Another way to do the test is mix one loopful of bacterial suspension/specimen with one loopful of polyvalent or type specific anti serum and a drop of methylene blue staining solution. Mix all three and examine under microscope. Highly refractile swollen capsule surrounding Pneumococci will be seen in the presence of specific antiserum. This test is used to identify and serotype Pneumococci.

INTEXT QUESTIONS 16.1

1. Process of converting non circular pneumococci into virulent organism is .....
2. Pneumococci are gram ..... and ..... cocei occuring in pairs.
3. Puenmococi are ..... organisms.
4. ..... serum sugar are used for fermentation reaction.
5. ..... test is used for differectiating pneumococci from Streptococi.
6. Pneumococci are oxidase and catalase .....

16.7 ANTIGENS

The outermost structure is capsule made of polysaccharide. This polysaccharide diffuses into medium and into host tissues during infection and is called "specific soluble substance" (SSS). Capsule plays an important role in virulence which we will discuss later.

On the basis of type of polysaccharide Pneumococci are classified into:

- Type I;
- Type II;
- Type III;
- Heterogenous group IV. This group has more than 90 different serotypes.

Other antigens include somatic "C" carbohydrate antigen and the nucleoprotein. The antigen "C" is used to detect C reactive protein, a beta globulin which is raised in sera of patients of pneumonia and other diseases where there is inflammation and breakdown of tissue.

16.8 VIRULENCE AND PATHOGENICITY

Pneumococci are normally present in naso-pharynx of humans; may become invasive and spread to the surrounding organs like sinuses, middle ear, respiratory tract and meninges to cause infections of these organs. Pneumococci produce some weak toxins like haemolysin and leucocidin which are not virulent; Pneumococci produce a virulent toxin named pneumolysin. Pneumolysin damages cell membrane, is cytotoxic and may activate complement . This combined with the anti-phagocytic property of the capsular polysaccharide; all contribute in pathogenesis of infections and diseases caused by Pneumococci.

Autolysin of Pneumococci lyses the bacteria present in tissues and the bacterial products released on lysis may also cause harm to tissues and thus may be involved in causing disease.

So you see the capsule protects the pneumococci from phagocytosis and is the most important determinant of pneumococcal virulence. Un encapsulated variants are not capable of causing disease. Other potential virulence factors include pneumolysin and probably bacterial products released on lysis of bacteria as already indicated.

16.9 INFECTIONS AND DISEASES CAUSED BY STREPT PNEUMONIAE

There are certain factors which may predispose to pneumococcal infections. These include: primary cardiopulmonary diseases, primary respiratory viral infections (e.g., influenza), extirpation of the spleen (splenectomy) and/or some complement system defects.

Pneumococci can cause from simple infections like sinusitis to serious, invasive type of pneumococcal infections (septicemia and meningitis). Respiratory infections including pneumonia are most commonly caused by pneumococci.

Notes

The various infections caused by Pneumococci are listed below;

- Sinusitis;
- Otitis media
- Mastoiditis;
- Lobar pneumonia;
- Bronchopneumonia;
- Acute exacerbation of chronic bronchitis;
- Joint infections;
- Endocarditis;
- Meningitis;
- Bactreamia;
- Septicaemia;
- Abscesses in organs following septicaemia.
- Conjuctivitis

The common symptoms of respiratory Pneumococcal infection are cough, high fever, difficulty in breathing, rapid breathing and pain in the chest area. The signs include headache, fatigue, muscle ache, nausea and vomiting. Laboratory diagnosis for Pneumococci and treatment should be carried out in all suspected cases of infection with Pneumococci.

16.10 LABORATORY DIAGNOSIS AND IDENTIFICATION OF PNEUMOCOCCI

Clinical diagnosis of an infection is easy; however, to decide whether the infection is caused by Pneumococci, we have to do the aetiological diagnosis. For this purpose the appropriate sample is collected and processed as detailed below to detect Pneumococci.

Laboratory diagnosis of pneumococcal infection is done as below:

- Collect the appropriate sample from clinically suspected cases of pneumococcal disease;
- The appropriate sample in respiratory infection is sputum; otitis media-pus/ aspirate from middle ear; blood in case of septicaemia; CSF from a case of meningitis and so on;
- Perform gram staining on smear prepared from the sample;
- Examine the smear microscopically and look for typical Lancet shaped Gram positive diplococcic surrounded by a thick capsule;

- Do slide agglutination test by mixing a drop of the CSF/aspirate, etc. with a drop of commercially available polyvalent and/or locally prevalent serotype specific antiserum to detect the presence of specific soluble substance (SSS) in the specimen which points to Pneumococcal serotype causing infection and treatment can be started right away;
- Culture the sample on blood agar and chocolate agar plates, incubate in 5-10 % CO_2, at 37° C overnight (18 hrs);
- Examine the plates for growth, in case of Pneumococci typical colonies surrounded by greenish discolouration due to alpha haemolysis will be seen as described above;
- Prepare a smear from the plate, do the Gram staining and examine for typical Gram positive diplococcic;
- Carry out the bile solubility test, optochin sensitivity test and inulin fermentation test to confirm the identity of *Strept pneumonia*;
- Carry out the Latex slide agglutination test by mixing a drop of the culture suspension with a drop of commercially available polyvalent or locally prevalent serotype specific antiserum helps to confirm the serotype of Pneumococci causing infection;

16.11 RESISTANCE

Pneumococci are sensitive to heat (52°C) and commonly used antiseptics. It is difficult to maintain Pneumococci for long in culture. Pneumococci in the lab can be maintained by culture on semisolid blood agar and by lyophilization.

16.12 EPIDEMIOLOGY AND PROPHYLAXIS

16.12.1 Epidemiology

The reservoir of Pneumococci is the healthy human carriers and patients suffering from pneumococcal infections. Pneumococcal infections are endemic and occur in all seasons, more frequently at extremes of ages, in the elderly and small children. Infections are more common during the outbreaks of respiratory viral infections like influenza. Pneumococcus causes secondary infections in patients suffering from influenza. Outbreaks of Pneumococcal pneumonia can occur in overcrowding and closed communities.

The incidence of infection also depends on the prevalent serotype of Pneumococcus. Type 3 is the most virulent so if it is prevalent in the community then there may be more infections.

Notes

16.12.2 Prophylaxis

Pneumococcal Vaccine is used for prevention of pneumococcal infections in extremes of ages; individuals with chronic lung, heart and renal diseases; individuals with non/dysfunctional spleen, celiac disease and so forth. Two types of Pneumococcal vaccines are available and used. These are polysaccharide and conjugated pneumococcal vaccines.

The purified polysaccharide vaccine (PPV 23) is a 23 valent vaccine containing the serotypes - 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F. This vaccine is poorly immunogenic in children below the age of 2 years, has low immune memory, does not reduce nasopharyngeal carriage and does not provide herd immunity. Efficacy is around 70 % only in the high-risk population. The dose is 0.5 ml administered subcutaneous/intramuscularly. Vaccine is safe with occasional local side effects.

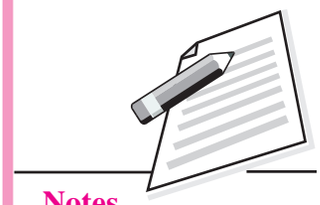
To improve the immunogenicity and efficacy of pneumococcal vaccine polysaccharide conjugate vaccines (PCV) was developed by conjugating Pneumococcal polysaccharide with different proteins.

INTEXT QUESTIONS 16.2

1. ..... of capsule plays a major role in the virulence of the organism.
2. Pneumococci produce virulence toxin known as .....
3. ..... Text is used to identify the seotype of pneumococci.
4. Pneumococci are named inhabitat of .....

WHAT HAVE YOU LEARNT

- Pneumocci are gram-positive, slightly elongated, oval to lanceolate shaped diplococci, ocuring in pairs or short chains surronded by capsule
- Pnuemococci are fasitidious organism
- Speciemn is cultured on Blood agar and Chocolate Agar
- Pneumococcus ferments many sugars. Pneumocci hydrolyze inulin, oxidase & catalase test negative
- Spccific characteristics of pneumococcus are bile solubility, optochin sensitivity & Quellung phenomenon or capsule swelling reaction
- Capsule is made of Ploysaccharide which is called as Specific Solubel Substance (SSS)



- Pneumococci are sensitive to heat and commonly used antiseptics
- Pneumococcal vaccine is used for prevention of pneumococcal infections.

TERMINAL QUESTIONS

1. Enumerate the biochemical and other tests used to differentiate Strept pneumoniae from other Streptococci.
2. Describe Quellung test.
3. Describe optochin sensitivity test.
4. Briefly describe the mechanism of pathogenesis of pneumococcal infection.
5. Enumerate the diseases caused by Strept. Pneumonia.
6. Briefly describe the steps in lab diagnosis of infections caused by Strept pneumonia.
7. Discuss the types of pneumococcal vaccines available. Describe the dosing schedule and frequency

ANSWERS TO INTEXT QUESTIONS

16.1

1. Transformation
2. Positive; diplo
3. Fastidions
4. Hiss's
5. Inculin
6. Negative

16.2

1. Sepcific source substance
2. Penumolysin
3. Quellung
4. Upper respiratory