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Original Research Article

Streptococcus Pneumoniae: Identification, Antibiogram and Serotypes from Clinical Isolates: A Hospital Based Descriptive Study

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Abstract:

Background and Objectives: *Streptococcus pneumoniae* infection is a serious problem worldwide. It is a common cause of pneumonia, meningitis, and septicemia, and the case fatality rate remains high. Globally, India has the highest number of deaths caused by pneumococcal infections among children below 5 years of age, partly because of its large population. The incidence, severity, and mortality of the disease depend on host factors such as age, underlying disease, co morbid conditions, and immunosuppression, but also on the properties of the organism. The present study was conducted to know the extent of Pneumococcal infections in tertiary care hospitals, their antibiogram, serotyping and how many strains were covered by existing Pneumococcal vaccines

Methods: A total of 51 consecutive *Streptococcus pneumoniae* isolates were collected and analyzed in this study. All isolates of *Streptococcus pneumoniae* were identified by Standard phenotypic methods from all clinical samples obtained Study duration of Two years. They were analyzed for MIC of Penicillin by E-Test method, Antibiogram by Kirby Bauer disc diffusion method and Serotyping by Multiplex PCR (Molecular method) was done in Department of Microbiology, PMCH Patna.

Conclusion: The present study gave an insight regarding the extent of Pneumococcal infections in tertiary care hospitals, their antibiogram, serotyping showed diverse strains many of which were covered by existing Pneumococcal vaccines. However considering the occurrence of other serotypes though rarely which were not covered by immunization, there is a need for constant monitoring regarding Pneumococcal serotypes and also more studies of this type to be included in clinical Bacteriology research Programs.

Keywords: C-Reactive Protein **CSF:** Cerebrospinal Fluid **DNA:** Deoxyribonucleic Acid. Acute Otitis Media.

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Introduction

Streptococcus pneumoniae is the leading cause of morbidity and mortality worldwide especially among extremes of age and people with underlying disease [1]. Until 2000, Streptococcus pneumoniae infections caused 100,000-135,000 hospitalizations for pneumonia, 6 million cases of otitis media, and 60,000 cases of invasive disease, including 3300 cases of meningitis. Incidence of sterile-site infections showed geographic variation from 21 to 33 cases per 100,000 population. Death occurs in 14% of hospitalized adults with invasive disease [2]. Disease Burden in India, Pneumonia is the single most important cause of death among children in the post-neonatal period, contributing as much as 27.5% of total under-five mortality It appears that about 12-35% of childhood pneumonias are caused by Streptococcus pneumoniae [3]. Around 123,000 to 164,000 children <5 years die annually from pneumococcal pneumonia [4]. Neurologic sequelae and/or learning disabilities can occur in meningitis patients. Hearing impairment can result from recurrent otitis media [2] The rates of pneumococcal infections are reported to be highest among young children and elderly. Acute respiratory infections in children younger than 5 years are the leading cause of childhood mortality in the world, accounting for about 20% of childhood deaths. Most of these deaths are caused by pneumonia. Streptococcus pneumoniae remains the most common cause of childhood community acquired pneumonia. WHO estimates that Streptococcus pneumoniae causes 1,612,000 deaths annually worldwide, of which 716,000 are in children under 5 years of age.2 Approximately, 26% of these deaths occur in the Asia Pacific region mainly in South- East Asia. [4,5] A total of 564 adults (age range 22–100 years, median age 45 years) were enrolled in the IBIS study over a period of 10 years. Of these, 304 participants had laboratory confirmation of Streptococcus pneumoniae from normally sterile body fluids. The overall case-fatality rate of IPD in adults was 30%. [6] The 94

serotypes of Streptococcus pneumoniae differ in virulence, level of antibiotic resistance, and prevalence. Once colonized some serotypes might never cause disease while others may cause disease rapidly. Across the world the serotypes that are common in one geographic region may vary when compared to regions that are distant. [7,8]

Risk factors that contribute towards increased rates of Streptococcus pneumoniae infection have been demonstrated to include (but are not limited to): concurrent/preceding viral respiratory infection, lack of healthcare services, overcrowding, exposure to indoor pollution, lack of pneumococcal immunization, poor sanitation, and inadequate nutrition. [9,10] Penicillin has been the drug of choice for treatment of pneumococcal infections but the increasing number of reports of penicillin resistant pneumococci (PRP) throughout the world makes it essential to determine the prevalence of PRP regionally. Moreover the PRP has been reported to harbour resistance to other antimicrobial classes making the treatment much more difficult. [9,10] Identification of the prevailing serotypes among invasive and non invasive strains of Streptococcus pneumoniae and their susceptibility to penicillin will add more information to the existing data from India especially from South India. [11]

Objectives

- To identify Streptococcus pneumoniae by standard phenotypic methods.
- To determine the antibiogram by Kirby Bauer disc diffusion method and Minimum Inhibitory Concentration (MIC) to Penicillin by E-test.
- To determine the serotype of isolates by molecular method (Multiplex PCR)

Materials and Methods

A total of 51 consecutive Streptococcus pneumoniae isolates were collected and analyzed in this study. All isolates of Streptococcus pneumoniae were identified by Standard phenotypic methods from all clinical samples obtained Study duration of Two years. They were analyzed for MIC of Penicillin by E-Test method, Antibiogram by Kirby Bauer disc diffusion method and Serotyping by Multiplex PCR (Molecular method) was done in Department of Microbiology, PMCH Patna**.**

Inclusion Criteria: All isolates of Streptococcus pneumoniae identified by Standard phenotypic methods from all clinical samples obtained, included in the study.

Exclusion Criteria: Repeat isolates from the same patient, from the same site (sample) were not included in the study.

Colony Morphology: On blood agar, typical colonies may be observed - round, flat, smooth, translucent often with central pitting (Carrom coin appearance or Draughtman's appearance) and they produce a zone of alpha (green) hemolysis, which differentiates Streptococcus pneumoniae from the group A (beta hemolytic) streptococcus, but not from commensal alpha hemolytic (viridans) streptococci which are co-inhabitants of the upper respiratory tract. Streptococcus pneumoniae is a very fragile bacterium and contains within itself the enzymatic ability to disrupt and to disintegrate the cells. Colonies initially appear with plateau-type morphology, and then start to collapse in the centers when autolysis begins**. Smear**: Streptococcus pneumoniae are Gram-positive, lanceolate-shaped cocci (elongated cocci with a slightly pointed outer curvature). Usually, they are seen as pairs of cocci (diplococci), but they may also occur singly and in short chains Other tests:

Optochin Test: Streptococcus pneumoniae strains are sensitive to the chemical optochin (ethyl hydrocupreine hydrochloride). Optochin sensitivity allows for the presumptive identification of alpha-hemolytic streptococci as Streptococcus pneumoniae, although some pneumococcal strains are optochin-resistant. Other alpha- hemolytic streptococcal species are optochin-resistant **.**

Bile Solubility Test: The bile (sodium deoxycholate) solubility test distinguishes Streptococcus pneumoniae from all other alpha-hemolytic streptococci. Streptococcus pneumoniae is bile soluble whereas all other alpha-hemolytic streptococci are bile resistant. Sodium deoxycholate (2% in water) will lyse the pneumococcal cell wall . The antibiotic-impregnated disc absorbs moisture from the agar and antibiotic diffuses into the agar-medium. Mueller-Hinton agar with 5 % horse/sheep blood is the preferred medium for antibiotic sensitivity testing. The rate of extraction of the antibiotic from the disc is greater than the rate of diffusion. As the distance from the disc increases, there is a logarithmic reduction in the antibiotic concentration. The extent of antimicrobial diffusion is affected by the depth of the agar. Visible growth of bacteria occurs on the surface of the agar where the concentration of antibiotic has fallen below its inhibitory level for the test strain. The time required to reach the critical cell mass (4-10 hr) is characteristic of each species but is influenced by medium composition and incubation time. The point at which the critical cell mass is reached appears as a circle of bacterial growth, with the middle of the antibiotic disc forming the centre of the circle. The concentration of the diffused antibiotic at the interface of growing and inhibited bacteria approximates to the MIC obtained in the dilution tests.

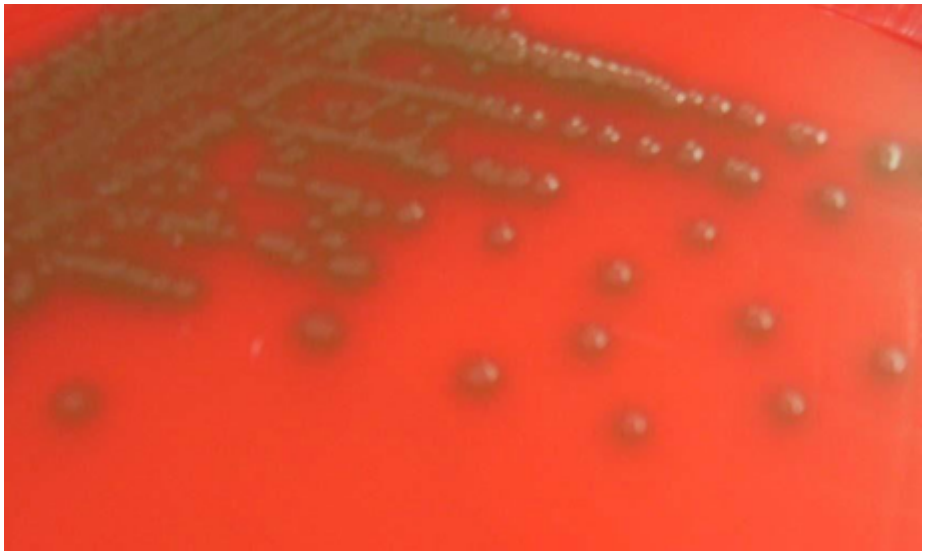

Figure 1: α-lytic glistening draughtsman colonies on 5 % sheep BA

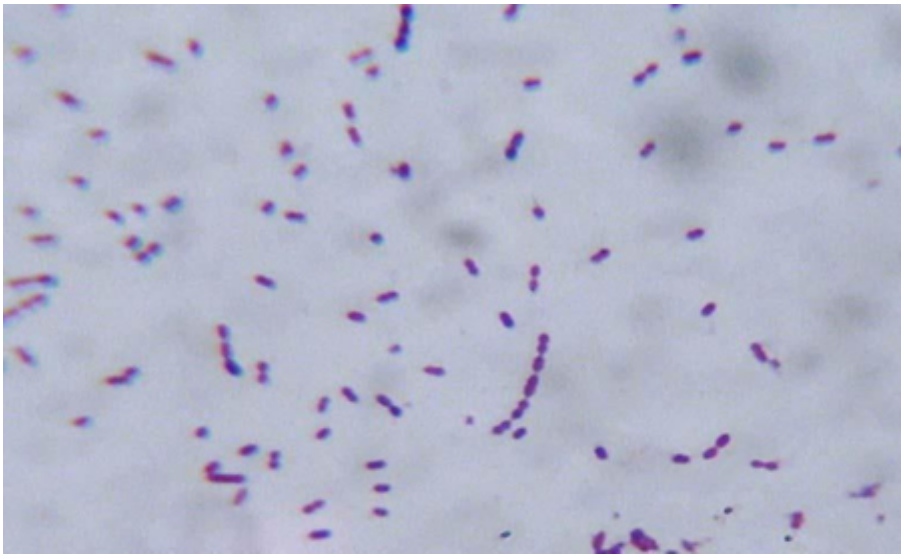

Figure 2: Gram positive lanceolate diplococci

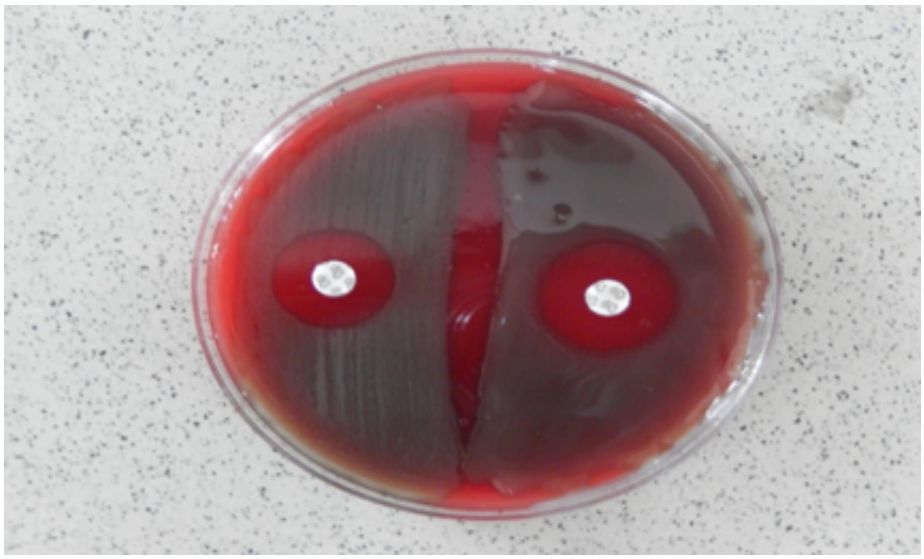

Figure 3: Optochin sensitivity test of 2 isolates. Both isolates are sensitive

Figure 4: Bile solubility test showing clearance in 'T' (test).

Results

A total of 51 consecutive Streptococcus pneumoniae isolates were collected and analyzed in this study. All isolates of Streptococcus pneumoniae were identified by Standard phenotypic methods from all clinical samples obtained.

Table 1:

	Number	Percentage
Male	33	65%
Female	18	35%
Total	51	

Different clinical samples

Table 2:

Samples	Males	Females	Total Numbers
Sputum	16	09	25
Blood	10	05	15
Pleural fluid	01	02	03
Ear discharge	01	01	02
BAL	02	01	03
Tracheal trap	03	00	03

maximum number of Streptococcus pneumoniae was isolated from respiratory secretions [Sputum, pleural fluid, Broncho- alveolar lavage (BAL), Tracheal trap], followed by Blood, and ear discharge.

Clinical condition

Table 3:

Clinical condition	Numbers
Respiratory condition (COPD, Asthma, Bronchitis)	21(41%)
Immunocompromised status (HIV, Diabetes, Cancer)	16(31%)
Nephrotic syndrome	5(10%)
Renal failure	7(14%)
Otitis media	2(4%)

serotypes encountered in this study not covered by Pneumococcal conjugate (PCV-7, PCV-10, PCV-13) and Polysaccharide vaccines (PSV-23)

Table 4:

PCV-7	PCV-10	PCV-13	PSV-23
1	2	2	6A
2	6A	13	13
6A	13	21	15A
13	21	15A	19B
21	15A	19B	21
15A	19B	33F	35F

19B	33F	35F	-
33F	35F	-	-
35F	-	-	-

That coverage rate of PCV-7, PCV-10 and PCV-13 are 33.34%, 41.6% and 50% respectively. Coverage rate of PSV-23 is 58.34% (PCV- Pneumococcal conjugate vaccine, PSV-Pneumococcal Polysaccharide vaccine) These are the serotypes not covered by the current vaccines but are found in our study

The common serotypes infecting patients in age group 45-60 years are 6B, 11, 15A isolated from sputum sample. The common isolates infecting patients above 61 years are two isolates of serotype 1 and two isolates of serotype 19F isolated from sputum sample. The other serotypes were 3, 6B, 7F (isolated from pleural fluid), 9V, 11D, 16F (isolated from ear discharge), 23F, 33F. The coverage rate of PCV-7, PCV-10, PCV-13 are 33.34%, 50%, 58.34% and coverage rate of PSV-23 is 66.67%. These are the serotypes not covered by the current vaccines but are found in our study

Discussion

Streptococcus pneumoniae is a human pathogen and is a common cause of Invasive diseases (sepsis, meningitis) and respiratory tract infections (pneumonia) and other diseases such as otitis media, peritonitis, conjunctivitis. It can also be found as a colonizer in nasopharynx of asymptomatic carriers. [1,3,4] Streptococcus pneumoniae has been defined on basis of phenotypic identification methods. In Gram's stain it appears as Gram positive diplococci that sometimes form short chains. Colony morphology on 5% sheep blood agar is smooth glistening colonies and show draughtsman appearance. Streptococcus pneumonaie is optochin sensitive, inulin fermentative and bile soluble.[12,13]There is paucity of information regarding pneumococcal disease burden and obtaining this data is fraught with challenges: [8,11]

Diagnosis requires appropriate specimen collection and microbiology laboratory investigations. Low yield even when appropriate specimens are obtained because of prior antibiotic treatment. It is estimated that 30% to 80% of children in Asian countries have been exposed to antibiotics before diagnostic evaluation, which can be overcome partly by the use of latest culture techniques like BACTEC. Procedure) and quality tested media reagents should be used. [12] Identification of Streptococcus pneumoniae is a major challenge. Awareness and training are required to differentiate Streptococcus pneumoniae from other Alpha-haemolytic Streptococci. Identification based on Morphology (Gram's stain and culture) is incomplete and inconclusive, it should be accompanied with the panel of Biochemical tests (Optochin susceptibility, Inulin fermentation and Bile solubility testing) for which the Microbiology laboratories should follow a sound SOP (Standard operating In this study, a total number of 51 Streptococcus pneumoniae isolates were isolated from different clinical samples such as sputum, blood, pleural fluid, tracheal trap, broncho-alveolar lavage , ear discharge from both paediatric and adult age group. This study shows that out of 51 isolates, 33(65%) were obtained from males were as 18(35%) from females. Our study also shows that 15 out of 51(29%) isolates belonged to paediatric age group and 17 out of 51(33%) isolates belonged to age group above 61 years of age. [13] This study shows that Streptococcus pneumoniae infections are more common in extremes of age group. Multiple antimicrobial resistance is seen Streptococcus pneumoniae. Resistance is mainly seen for Penicillin, Erythromycin, Tetracycline, Co-trimoxazole. As Penicillin is the drug of choice for Streptococcus pneumoniae infections for many years, susceptibility testing was not indicated. Isolation of Penicillin Resistant Pneumococci (PRP) in 1967 and many studies from different part of the world subseqently have reported increasing emergence of PRP. In this present study, Penicillin susceptibility was tested for all 51 isolates by MIC for penicillin by E-test method, all the isolates were sensitive for parenteral Penicillin (all readings were ≤ 2). India has the lowest incidence of penicillin-resistant Streptococcus pneumoniae (PRP) among the Asian countries. [5] A gradual increase in the intermediate resistance to penicillin (IRP) has been documented in India since 1995, The rate of pneumococcal resistance to Co-trimoxazole has increased significantly in India, from 21.8% to 61.7% between 1996-2002. A high rate of resistance (46%) to Co-trimoxazole was observed in our study as compared to that which was seen in other studies done in India. Alarming levels of Co-trimoxazole resistances raise the question as to whether WHO recommendations on use of Co-trimoxazole as first line of treatment of choice in upper respiratory tract infections needs to be revised, based on local data

Conclusion

Streptococcus pneumoniae infection is a serious problem worldwide. It is a common cause of pneumonia, meningitis, and septicemia, and the case fatality rate remains high. Globally, India has the highest number of deaths caused by pneumococcal infections among children below 5 years of age, partly because of its large population. The incidence, severity, and mortality of the disease depend on host factors such as age, underlying disease, comorbid conditions, and immunosuppression, but also on the properties of the organism.

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