# Susceptibility and serotypes of *Streptococcus pneumoniae* isolates in invasive pneumococcal disease: a study from Kerala, South India

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## SUMMARY

Invasive pneumococcal disease (IPD) is a major burden causing significant mortality and morbidity. This study was conducted to ascertain the magnitude of the problem of drug resistance, the pneumococcal serotypes that are prevalent in our area, and whether current pneumococcal vaccines are able to cover the prevalent serotypes adequately. A retrospective study was done by reviewing the microbiology registry of our hospital. Details of patients whose blood, cerebrospinal fluid (CSF) or any other sterile fluid grew *S. pneumoniae* between the period January 1, 2016 and December 31, 2019 were collected. Identification and susceptibility testing were done by Vitek2 as per CLSI 2008 guidelines. Serotyping was attempted for 39 isolates. Fifty-five pneumococcal isolates in blood and CSF were identified over four years from 51 patients, of whom nine belonged to the paediatric age group. Among 55 isolates, 50 were isolated from blood, four had growth of pneumococci in both blood and CSF, and one had growth in CSF alone. Overall non-susceptibility to penicillin was noted in 11 isolates, and 10 isolates were non-susceptible to ceftriaxone. Common serotypes isolated were 9V, 19F, 23F and 6 B. The most common clinical presentation was pneumonia followed by sepsis and meningitis. Five of the 51 patients succumbed to the illness. Penicillin susceptibility among pneumococcal isolates in IPD was 80% and susceptibility to ceftriaxone was 82%. This observation reiterates the view that vancomycin must be added to the empiric therapy of suspected IPD. Most of the identified serotypes are covered by current pneumococcal vaccines, highlighting the pivotal role of pneumococcal vaccine in prevention of IPD.

*Keywords*: Invasive Pneumococcal Disease, *Streptococcus pneumoniae*, pneumococcal vaccine.

## INTRODUCTION

Pneumococcal disease is a huge health burden and causes significant mortality and morbidity related to bacteremia, meningitis and pneumonia. World Health Organization (WHO) estimates deaths per annum due to invasive pneumococcal disease (IPD) to be around 1.6 million [1, 2]. More than 50% of the deaths occur in the paediatric age, specifically <5 years of age. Most of these deaths occurs in economically underprivileged areas of Asia and Africa [1]. Invasive pneumococcal disease is thus a major cause of vaccine preventable deaths especially in the paediatric age group. Pneumococcal vaccines have significantly reduced mortality due to IPD in areas with significant vaccine coverage. Currently available pneumococcal vaccines cover majority of serotypes

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causing IPD. Ten-valent pneumococcal conjugate vaccine (PCV10) contains ten of serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F while 13-valent PCV (PCV13) contains pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The polysaccharide PPSV23 contains 23 serotypes as follows: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F. In India poor pneumococcal vaccination coverage is a major concern and is compounded by growing penicillin resistance [3].

In this study from Kerala, South India, we looked at the problem of penicillin and ceftriaxone resistance in *S. pneumoniae*, the clinical profile of patients with IPD, pneumococcal serotypes that are prevalent in this area and adequacy of coverage of these serotypes by available pneumococcal vaccines.

## MATERIALS AND METHODS

Invasive pneumococcal disease is due to infection by *S. pneumoniae* in normally sterile sites like blood, CSF, joint fluid, pleural (but not sputum) or peritoneal fluid. Data of those patients whose blood, CSF or any other sterile specimen which grew *S. pneumoniae* between the period January 1, 2016 and December 31, 2019 were included and analyzed. A retrospective study was done by reviewing electronic microbiology registry for pneumococci growth in any of the sterile specimen, and details of patient who had IPD, were collected from the medical records. Identification and susceptibility of the pneumococcal isolate was done by Vitek2, bioMérieux SA, F-69280 Marcy I'Etoile, France. Susceptibility report was based on the revised CLSI breakpoints (2008) for parenteral penicillin (resistant ≥8 µg/ml for non-meningeal isolates and ≥0.12 µg/ml for meningeal isolates). Pneumococcal serotyping was performed by Quellung test, agglutination was viewed by phase contrast microscope (Pneumotest, Statens Serum Institute, Denmark).

## RESULTS

Fifty-five pneumococcal isolates in blood and CSF were identified over 4 years from 51 patients who had IPD. All patients whose CSF culture was done also had blood cultures done. All patients were hospitalized, and majority were adults (84%), while nine belonged to pediatric age group (age <14 years), of which 8 were <5 years of age. Males were more and accounted for 56.4% of cases. Eighty seven percent of patients were from Kerala, rest were from neighboring state of Tamil Nadu. Among 55 pneumococcal isolates, 50 were blood isolates and 5 were CSF isolates. Though the clinical presentation was varied, 50 patients had bacteremia and, among them, 34 had pneumonia, 11 had sepsis syndrome, 4 had meningitis and 1 had spondylodiscitis (Figure 1). Among the 5 patients with meningitis 4 had both blood and CSF growing pneumococci, while one had growth only in CSF.

Overall non-susceptibility (including resistance and intermediate susceptibility) to penicillin was noted in 11 isolates (20%), 4 were resistant and 7 had intermediate susceptibility. Ten isolates were non-susceptible (3 resistant, 7 intermediate susceptibility) to ceftriaxone. Among the 5 CSF isolates 3 were resistant to penicillin, and 3 had intermediate susceptibility to ceftriaxone. All the isolates were uniformly susceptible to vancomycin, levofloxacin and linezolid. Clindamycin, erythromycin, co-trimoxazole, tetracycline resistance was noted to be as high as 44%, 74.5%, 87.3% and 74.5% respectively. Multidrug resistant (MDR)

**Table 1** - Percentage coverage of pneumococcal isolates by vaccines.

| *SEROTYPE* | *Frequency* | *PCV 10* | *PCV 13* | *PPSV 23* |
|---|---|---|---|---|
| | 1 | Y | Y | Y |
| 14 | 1 | Y | Y | Y |
| 15B | 3 | N | N | Y |
| 17F | 1 | N | N | Y |
| 18C | 1 | Y | Y | Y |
| 19A | 2 | N | Y | Y |
| 19F | 5 | Y | Y | Y |
| 23F | 3 | Y | Y | Y |
| 1 | 1 | N | Y | N |
| 6B | 4 | Y | Y | Y |
| 8 | 1 | N | N | Y |
| 9V | 6 | Y | Y | Y |
| Total | 31 | 22 | 25 | 30 |
| Percentage coverage by vaccine | | 71 | 80.6 | 97 |

isolates (resistance to ≥3 antibiotics) accounted for 63.6% (Figure 2).

Among the 9 patients belonging to pediatric age group, 8 presented as pneumonia and one as sepsis syndrome. All 9 isolates were from blood. Penicillin and ceftriaxone non-susceptibility in this age group were noted in 3 samples and the pattern was as follows; penicillin resistance and ceftriaxone intermediate susceptibility in one isolate, penicillin sensitive but ceftriaxone intermediate susceptibility was noted in the second isolate and penicillin intermediate susceptibility but resistance to ceftriaxone was noted in the third isolate. Similar to adults, all isolates were susceptible to vancomycin, levofloxacin and linezolid. High percentage of resistance was noted for erythromycin, clindamycin, tetracycline and co-trimoxazole.

Among the 51 cases, initial empiric antibiotic therapy included ceftriaxone monotherapy in 22 cases, ceftriaxone in combination with vancomycin or macrolide in 15 cases and carbapenem with or without vancomycin in 10 cases, co-amoxiclav in combination with quinolones in 4 cases. There was no correlation between antibiotic choice and

**Figure 1** - Clinical presentation of IPD.

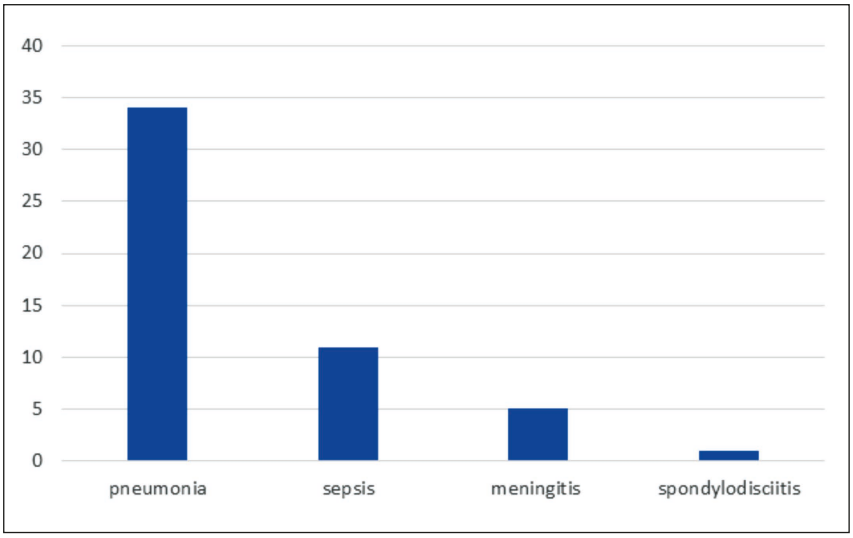
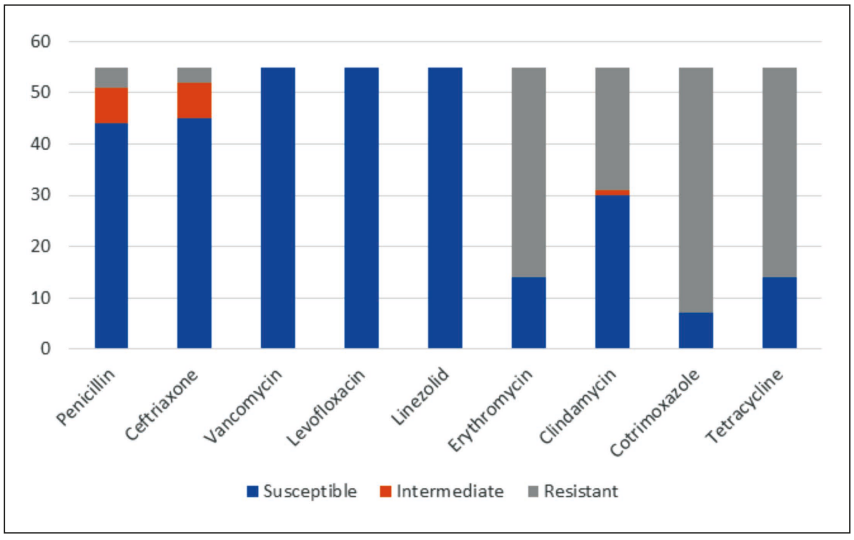



**Figure 2** - Antibiotic susceptibility of pneumococcal isolates (number of isolates).



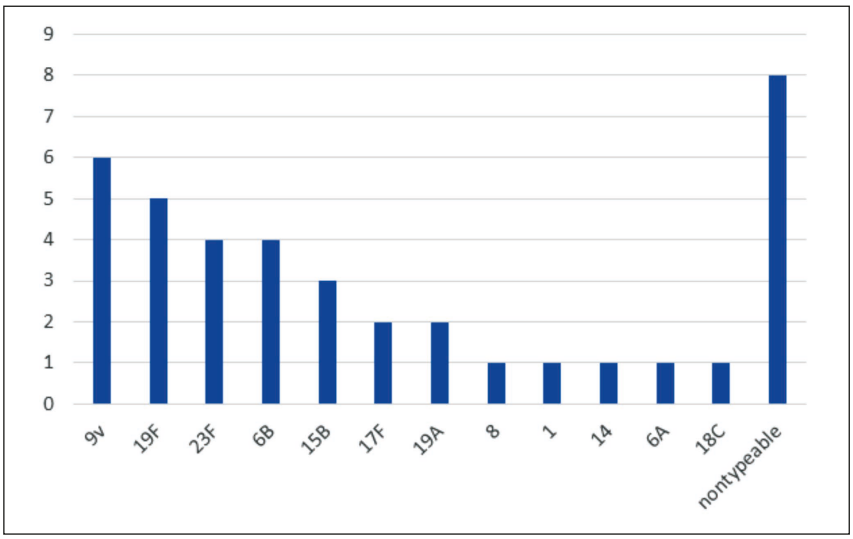



**Figure 3** - Frequency of pneumococcal serotypes.

outcome. After the culture reports, antibiotics were de-escalated in most patients to ceftriaxone. Among 51 patients, 5 patients died, of whom 4 had sepsis syndrome and 1 had pneumonia syndrome. Majority had good outcomes, while one patient was discharged at request against medical advice, and no follow-up was possible. No mortality was noted among pediatric patients.

Among 55 isolates, serotyping was performed in 39 isolates, of which 8 were non-typeable. Common isolates noted were 9V (6 isolates), 19F (5 isolates), 23F and 6 B (4 isolates each) and depicted in Figure 3. Overall, the isolates covered by PCV10, PCV13 and PPSV23 were 71%, 80.6% and 97% respectively. Among the 9 isolates from pediatric patients, serotype was not done for one, and in the 8 who were serotyped, 2 were non-typeable. Among the 6 serotypes identified, 4 were covered by PCV 10 and all 6 by PCV 13. Two among the nine pediatric cases had received previous vaccination and surprisingly both were infected by vaccine strain.

## DISCUSSION

Invasive pneumococcal disease is a life-threatening infection, requiring prompt diagnosis and effective treatment. In our study 84% of the patients were adults. Majority of previous studies done in pediatric IPD focus on an age group of less than 5 years which is the most vulnerable age group for IPD related mortality and morbidity. Among the 9 pediatric patients in our study, 8 were less than 5 years of age.

Most of our patients presented with pneumonia (61.8%), followed by sepsis syndrome. Four had meningitis and one had spondylodiscitis. Eight out of 9 paediatric patients presented with pneumonia syndrome. This is similar to the observations in most of the previous studies in which pneumonia (58%) was the most common presentation followed by meningitis (31%) [4].

Among 55 isolates, 50 were blood isolates, 4 had growth of pneumococci in both blood and CSF, and one had growth in CSF alone. Applying the CLSI 2008 criteria for penicillin susceptibility for meningeal and non-meningeal isolates non-susceptibility (including resistance and intermediate susceptibility) to penicillin and ceftriaxone were noted in 11 isolates (20%) and 10 isolates (18%) respectively. Among the 5 CSF isolates, 3 were resistant to penicillin while intermediate susceptibility to ceftriaxone was noted in 3 isolates, which is a finding of great concern. Very high levels of penicillin resistance (43.7%) and ceftriaxone resistance (14.9%) have been reported in a large Indian study including 830 invasive pneumococcal isolates from Vellore by Verghese VP et al [5].

In our study group, the pediatric population commonly presented as pneumonia (8 patients), one as sepsis syndrome, and all the isolates were from blood. Penicillin and ceftriaxone resistance was noted in 1 isolate each, intermediate susceptibility to ceftriaxone was noted in 2

blood isolates, which has clinical relevance. As the pediatric age group is less represented and there were only 9 isolates, larger data may be needed to understand the prevalence of penicillin and ceftriaxone resistance among this population. Penicillin resistance noted in the study by Verghese VP et al was higher than the current study, ranging from 27.3% in 16 years or older to as high as 59.7% in those <5 years of age. Cefotaxime non-susceptibility in the same age group ranged between 10.4% for older children and 18% for <5 years old. Another important finding noted in their study was a steady increase in resistance to drugs during the study period 2008 to 2016, from 9.5% to 42.8% for penicillin and 4.7% to 28.5% for cefotaxime [5]. In another multi-centre study from India done in almost similar period (2011-2015) among 361 IPD patients, penicillin non-susceptibility was seen in only 8% [6]. In the study by Balaji et al, out of the 114 *S. pneumoniae* isolates studied, 5.2% of the isolates were non-susceptible to penicillin and only 0.8% was non-susceptible to cefotaxime while high degree of resistance was noted for co-trimoxazole (96.4%) and erythromycin (70%) [3].

In our study, all the isolates were susceptible to vancomycin, levofloxacin and linezolid. Clindamycin, erythromycin, co-trimoxazole, tetracycline resistance was noted as high as 44%, 74.5%, 87.3%, and 74.5% respectively. Multidrug resistant (MDR) isolates (which exhibit resistance to ≥3 antibiotics) accounted for 63.6%. In the study by Manoharan et al., co-trimoxazole, erythromycin and chloramphenicol resistance occurred in 66%, 37% and 9% respectively. Surprisingly multidrug resistance was noted only in 9% of the patients [6]. In another study done from 2011 to 2013 from South India, in 17 blood culture positive 14.3% isolates were resistant to penicillin [7]. In another South Indian study done by Kiran Chawla, et al., in non-IPD population, the prevalence of penicillin-resistant pneumococci and multidrug-resistant strains among *S. pneumoniae*, isolated from 50 respiratory specimens was 4% while 10% showed intermediate susceptibility [8].

There is a large variation in resistance to penicillin and third generation cephalosporins among different countries and it depends on the clinical breakpoints (MIC cut-off) used. Majority of the previous studies have been done in pediatric population. In a population-based surveillance study on IPD from rural Bangladesh from 2004 -2007, Arifeen et al noted that among patients of <5 years of age, penicillin resistance was 85%, but ceftriaxone was uniformly sensitive (100%) [9]. High co-trimoxazole resistance was also noted (76%). In a study from Sri Lanka done during 2005-2007 in a population of children less than 5 years of age, more than 90% were penicillin resistant, and resistance to third-generation cephalosporins was as high as 48% [10]. In Asian Network for Surveillance for Resistant Pathogens (ANSORP) study by Kim SH et al, the prevalence rate of penicillin resistance was 57.5% in meningeal isolates, after applying the revised CLSI breakpoints for penicillin [11]. Penicillin resistance in pneumococcal infection is a growing concern especially in pediatric population of <5 years of age [12]. In another study from China in 2016, data from 10 paediatric hospitals was analyzed. 6132 *S. pneumoniae* isolates were studied, in which majority were from children younger than 5 years of age (85.1%). The resistance rates of *S. pneumoniae* to penicillin and ceftriaxone were 1.4% and 18.1% respectively in CSF isolates. The resistance rates of *S. pneumoniae* to clindamycin, erythromycin, tetracycline, and co-trimoxazole were 95.8%, 95.2%, 93.6%, and 66.7%, respectively [12]. In the ANSORP study, the overall rate of MDR in pneumococcal isolates was 59.3%, with the highest MDR rate being 83.3% in China, followed by Vietnam (75.5%), South Korea (63.9%), Hong Kong (62.2%), and Taiwan (59.7%). All MDR strains were resistant to at least one of the macrolides tested [11].

Among the 39 isolates that were serotyped, 8 were non-typeable. Commonest serotypes included 9V (6 isolates), 19F (5 isolates), 23F and 6 B (4 isolates each). Other isolates were 15B (3 numbers), 2 each of 17F and 19A, 1 each of 8, 1, 14, 16A, 18C. The distribution of serotype is shown in Figure 3. PCV10, PCV13 and PPSV23 covered 71%, 80.6% and 97% of the prevalent strains respectively. Among the 6 serotypes identified in pediatric population, 4 were covered by PCV 10 and all 6 by PCV 13. Two of the 9 children had received previous pneumococcal vaccination, and this could probably suggest vaccine failure. In an Indian study conducted at Vellore from 2007 to 2011 as part of an international surveillance program among 244 isolates, serotypes 14, 19F, 6B,

6A, 23F, 9V and 5 were the most common strains [5]. In the study by Molander, the most prevalent 5 serotypes were 1, 5, 19F, 6B, and 14, while the serotype coverage by available vaccines did not appear impressive. Vaccine coverage by PCV7, PCV10, PCV13, PCV15, and pneumococcal polysaccharide vaccine (PPV) PPV23 was 29%, 53%, 64%, 66%, and 73%, respectively [4]. In the study by Balaji et al., the most common pneumococcal serotypes causing invasive infections in children less than five years of age were 14, 19F, 5, 6A and 6B [3].

In a study from Hong Kong done in pediatric age group <6 years of age by Ho et al, among 88 invasive isolates, vaccine coverage by PCV7 was 89.7% [13]. In another study from Sri Lanka by Batuwanthudawe et al in children aged <5 years, the most common serotypes were 19F, 14, 23F, and 6B. Of the serotypes identified, 60% were covered by the available 7-valent conjugate pneumococcal vaccine [10]. In a large study by ANSORP looking at Asian countries, the frequencies of serotypes included in PCV7, PCV10, and PCV13 in invasive isolates were 46.3%, 58.1%, and 78.6%, though it varied between different countries. Compared with the data from a previous ANSORP study done during 2000 and 2001, the PCV7 coverage rate has significantly decreased in Asian countries from 60.5% to 52.4% between 2008 to 2009, while the prevalence of serotype 19A isolates has markedly increased (3% to 8.2% in 2008 to 2009) [11].

Two among the nine pediatric cases had received previous vaccination and surprisingly both were infected by vaccine strain. In a systematic review of literature on pneumococcal vaccine failure, by Oligbu G et al, involving 7584 participants in children aged ≤5 years, a total of 159 vaccine failure cases were identified, representing 2.1% [95% CI: 1.8-2.4%] of the reported IPD cases. The two common serotypes associated with vaccine failure in this review were 19F and 6B. Overall failure with PCV is rare, irrespective of schedule.

## CONCLUSIONS

In this study, the overall non-susceptibility to penicillin was found to be 20%, 4 isolates were resistant (7.2%) while 7 (12.7%) showed intermediate susceptibility. Ten isolates (18.2%) were non-susceptible to ceftriaxone, among which resistance was noted in 3 (5.45%), and intermediate susceptibility in 7 (12.7%). This reiterates that vancomycin must be added in the empiric therapy of suspected IPD till susceptibility reports are available. Vaccine coverage in this study ranged between 80 to 97% depending on the vaccine used, and highlights the pivotal role of pneumococcal vaccine in prevention of IPD. A large multicentre study with a bigger sample size would be needed to further understand the prevalance of pneumococcal vaccine failure.

**Funding**
None

**Acknowledgements**
Dr. K. L. Ravikumar, Prof. of Microbiology, Chief of Central Research Laboratory, KIMS Hospital and Research Center, Bangalore, for his help in serotyping the pneumococcal isolates.

**Conflicts of interests**
None

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