Streptococcus pneumoniae serotype prevalence and antibiotic resistance among young children with invasive pneumococcal disease: experience from a tertiary care center in South India

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Abstract

Introduction **We performed a study to describe the clinical profile, antimicrobial susceptibility and prevalent serotypes of pneumococcal isolates from children with suspected invasive pneumococcal disease (IPD) admitted to a tertiary care hospital in South India.**

Methods **Hospitalized children, ≤ 5 years with fever (>38 °C); increased respiratory rate or neurological symptoms were recruited, (as part of the Alliance for Surveillance of Invasive Pneumococci – ASIP – project) from January 2011 to March 2013. Identification of pneumococcal isolates from blood or cerebrospinal fluid samples was done by routine culture methods. Isolates were analysed for antimicrobial susceptibility, and confirmed by serotyping (using Quellung's test) and multiplex PCR.**

Results **Out of the 171 samples received in the lab, 17 grew pneumococci identified by standard methods. Fourteen of them were confirmed by multiplex PCR. Maximum recruitment was observed during the months of January and February (36.4%, 28.6%). The average age of affected subjects was 21 months. The common clinical presentation was pneumonia (42.8%). Two isolates belonging to the 19F and 19B serotypes were resistant to penicillin (on Etest). The observed serotype distribution was 6B and 19F (2 each), and 1, 2, 6A, 9V, 10A, 14, 15A, 19B, 21, 35F (1 each). The overall fatality rate was 14.3% (n=2); the *S. pneumoniae* isolates from these two patients belonged to the non-vaccine serotype 19B and serotype 19F and demonstrated *in vitro* resistance to penicillin and erythromycin.**

Conclusion **Our study demonstrates the presence of serotypes not covered by available vaccines in a pediatric cohort. Emergence of drug resistant *Streptococcus pneumoniae* may by associated with severe clinical outcomes.**

Keywords **Non-vaccine serotype, *Streptococcus pneumoniae*, vaccine coverage, drug sensitivity, India**

Introduction

Streptococcus pneumoniae is a leading cause of severe bacterial infections and is responsible for substantial morbidity and mortality rates especially in developing countries. Worldwide, annually 1.1 million children die under the age of five years from pneumococcal infections and nearly all of these deaths occur in children from low and lower-middle income countries.[1] Antimicrobial resistance among *S. pneumoniae* has emerged as a problem The emergence of drug resistant *S. pneumoniae* (DRSP), resistant to important antimicrobials such as penicillins, cephalosporins and macrolides, is a problem of

Received: 23 November 2016; revised: 27 February 2017; accepted: 09 April 2017.

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Note: This study was presented in part at the 9th International Symposium on Pneumococci and Pneumococcal Diseases, Hyderabad, India, 9-13 March 2014 (Abstract no. ISPPD-0394).

Published June 2017

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

global concern that has made treatment of the disease most difficult.[2-4] A systematic review has revealed that only 7 serogroups (1, 5, 6A, 6B, 14, 19F, 23F) are the most common globally in the pediatric age group.[5] Studies have demonstrated that vaccination of children under 2 years of age can reduce the incidence of invasive pneumococcal disease.[6] In India, commonly prevalent serotypes are 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9A, 9N, 9V, 10A, 12F, 14, 15B, 18C, 19A, 19F and 23F, with serotypes 1 and 5 accounting for 30% of invasive pneumococcal disease (IPD) among the non-vaccinated population.[7]

In order to combat the increase in resistance and disease prevalence, many conjugate vaccines (PPSV23, PCV-7, PCV-10, PCV-13) have been introduced and tested, but no single vaccine covers all the 90 known pneumococcal serotypes.[8] The serotypes included and the proteins used for conjugation[9,10] are shown in Table 1. With the availability of conjugate pneumococcal vaccines for infants and young children, surveillance for invasive pneumococcal disease among children with ages below 5 years remains important as it allows the detection of populations that may not have received the vaccines and monitoring of the incidence of non-vaccine serotypes-induced disease. In the present study, we evaluated the clinical profile, antimicrobial susceptibility and prevalent serotypes of pneumococcal isolates from children with suspected IPD admitted to a tertiary care hospital in South India.

Table 1. Serotypes contained in conjugate pneumococcal vaccines

Type of vaccine	Serotypes in vaccine
Pneumovax (PPSV23)	1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F
Prevenar (PCV7)	4, 6B, 9V, 14, 18C, 19F and 23F
Synflorix (PCV10)	1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F
Prevenar 13 (PCV13)	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F

PPSV – pneumococcal polysaccharide vaccine; **PCV** – pneumococcal conjugate vaccine.

Methods

A prospective, observational study was carried out at our tertiary-care hospital, between January 2011 and March 2013, as one of the centers participating in a network (Alliance for Surveillance of Invasive Pneumococci – ASIP). The study site, St. John's Medical College Hospital, Bengaluru, Karnataka, India caters to local as well as to referred cases from neighboring states like Andhra Pradesh and Tamil Nadu.[11] Hospitalized children aged less than 5 years, who had fever defined as a temperature above 38 °C, with cough, and/or fast breathing or signs of meningitis were screened, and their caregivers were invited to participate in the study. Informed consent was obtained from parents or guardians of children. Further details on all the inclusion and exclusion criteria and methods for data collection are published elsewhere.[12] The study was reviewed and approved by the Institutional Ethical Review Board (Ref. No.44/2010; dated 10 March 2010) of the institution and coordinating sites.

Depending on the primary site of infection, specimens (blood, cerebrospinal fluid (CSF), or pleural fluid) were collected under aseptic precautions, in a standard blood culture bottle (Bactec Peds Plus/F; Becton Dickinson, MD, USA) and were incubated at 35 °C in BACTEC 9240 instrument (Becton Dickinson). Inoculated bottles were monitored for positive culture signals, which were subsequently processed by plating on to trypticase soy agar (Himedia, Mumbai, India) supplemented with 5% sheep blood, chocolate agar plates and incubated in a candle jar.[7] Suspected *S. pneumoniae* colonies were identified by α-hemolysis, typical colonial morphology, presence of diplococci on Gram stain, optochin sensitivity (Taxo P disks, Becton Dickinson) and confirmed by testing for bile solubility.[13] All isolates were confirmed again by multiplex PCR,[14] serogrouped/typed with Quellung antisera (at Christian Medical College and Hospital, Vellore, India) obtained from Statens Serum Institute (Copenhagen, Denmark). Antibiotic susceptibility was assessed through the Kirby-Bauer disc diffusion method (Himedia) and through determining the

minimum inhibitory concentration (MIC) using the Etest method (bioMérieux, Capronne, France). Clinical Laboratory Standards Institute criteria for zone size and MIC values were used to classify isolates as susceptible, intermediate, or resistant.[15] Standard reference strains of *S. pneumoniae* (ATCC 49619), *Haemophilus influenzae* (ATCC 49247), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used to validate the in-house blood agar, chocolate agar and Mueller Hinton blood agar plates (Himedia) for quality and performance. Multiplex real time polymerase chain reaction was done to detect the genes encoding the pneumococcal pneumolysin using primers shown in Table 2. Molecular capsular typing was done using the oligonucleotide primers designed to target serotypes 1, 3, 4, 5, 6A/B, 7F, 7C, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B/C, 16F, 17F, 18, 19A, 19F, 20, 22F, 23F, 31, 33, 34, 35B, 35F using primer pairs sequences published earlier.[14,16] The results were compiled in an excel spreadsheet, and statistical analyses were performed using SPSS v 16.0 (Chicago, USA). Non-parametric data were analyzed by the Mann-Whitney U test and clinical variables were compared using Chi-square test. All tests were done using two-sided P-values and a value of <0.05 was considered as significant.

Table 2. Primers and probes used for real time PCR

Primer or probe	Nucleotide sequence	Working stock conc (µM)
F373	ACGCAATCTAGCAGA TGAAGCA	2.5
R424	TCGTGCGTTTTAATTC CAGCT	2.5
Pb400i	TGCCGAAAACGC"T"T GATACAGGGAG 5`Cy5;3`SpC6;"T"BHQ2	2.5

Results

During the study period, from January 2011 to April 2013, 171 children admitted with suspected IPD as bacterial sepsis, pneumonia and meningitis were enrolled in the study. Samples collected included 164 blood culture samples, 3 CSF, 2 both (blood & CSF), and 2 pleural fluid samples. Seventeen *S. pneumoniae* were identified by routine laboratory methods. Of them, 14 were confirmed by the reference laboratory at Christian Medical College Vellore, resulting in a *S. pneumoniae* isolation rate of 8.2% among children with suspected IPD. Only one isolate per patient was included. The 14 isolates included in this analysis were collected from sterile body sites, as follows: blood (12), pleural fluid and CSF (1 each). The children with *S. pneumoniae*-proven IPD were in the age range of 5-58 months with median age of 16.5 months (IQR 12-24; p=0.084) (Table 3). Among all cases, 57% were male (p=0.229; OR=0.51) and 78.6% were ≤ two years of age (p=0.971; OR=0.98).

All fourteen children had fever with an average duration of 6.4 days at presentation (p=0.903), and a range from 2 to 15 days. The most common clinical manifestation was pneumonia (fever >38 °C + tachypnea) in 92.9% of cases (13/14). Common symptoms were breathlessness 42.9% (6/14), drowsiness 42.9% (6/14), seizures 28.6% (4/14), and vomiting in 21.4% (3/14). Antibiotic treatment received prior to the present hospital admission was reported among 21.4% (3/14) of cases by the parents and guardians of the patients. Physical examination showed respiratory distress in 42.9% (6/14) of cases, chest retraction 21.4% (3/14), auscultatory crepitations 35.7% (5/14) and meningeal signs 14.3% (2/14).

Radiological features of the chest suggested consolidation and infiltration in 35.7% (5/14) of the cases. The final diagnosis included pneumonia or lower respiratory tract infection (LRTI) in 35.7% (5/14) of patient; sepsis or bacteremia in 35.7% (5/14), and 2 each with meningitis and nephrotic syndrome with sepsis.

Serotyping showed the presence of 12 different serotypes from 14 children. The most frequent serotypes causing IPD among children <5 year in our study were 6B and 19F (2 each), followed by serotypes 1, 2, 6A, 9V, 10A, 14, 15A, 19B, 21 and 35F (1 each). These serotypes represented 41.7% of the serotypes included in PCV-10, 50% of the serotypes included in PCV-

Table 3. Statistical analysis of general characteristics and risk factors for colonization with *S. pneumoniae* in children

Characteristics		All cases n=171 (%)	*S. pneumoniae* positive n=14 (%)	*S. pneumoniae* negative n=157 (%)	Statistical test results
Age, months	Median (IQR)	12 (5,24)	16.5 (11.75, 29.25)	12 (5,24)	U=791.5, p=0.083, r=0.146
	Mean (±SD)	17.22 (±15.0)	22.5 (±15.64)	16.74 (±14.86)	
Gender					OR=0.51; 95%CI=0.17-1.53
	Male	111 (64.9)	7 (50.0)	104 (66.2)	z-score=1.203
	Female	60 (35.1)	7 (50.0)	53 (33.8)	χ(1)=1.489; p=0.222
Age, years					OR=0.97; 95%CI=0.25-3.70
	≤2	135 (78.9)	11 (78.6)	124 (79.0)	z-score=0.036
	2-5	36 (21.1)	3 (21.4)	33 (21.0)	χ(1)=0.001; p=0.971
Fever duration, days	Median (IQR)	5 (3,7)	4.5 (3,10.5)	5 (3,7)	U=1077, p=0.903, r=0.903
	Mean (±SD)	6.5±6.1	6.4±4.6	6.5±6.2	
Cough	Present	151 (88.3)	11 (78.6)	140 (89.2)	χ(1)=1.399; p=0.238;
	Absent	20 (11.7)	3 (21.4)	17 (10.8)	OR=0.45; 95%CI=0.11-1.76
Cough, significant	Yes	93 (61.6)	4 (36.4)	89 (63.8)	χ(1)=3.191; p=0.074;
	No	58 (38.4)	7 (63.6)	51 (36.4)	OR=0.33; 95%CI=0.09-1.17
Breathlessness	Yes	92 (53.8)	6 (42.9)	86 (54.8)	χ(1)=0.734; p=0.391;
	No	79 (46.2)	8 (57.1)	71 (45.2)	OR=0.62; 95%CI=0.21-1.87
Drowsiness	Yes	58 (33.9)	6 (42.9)	52 (33.1)	χ(1)=0.544; p=0.461;
	No	113 (66.1)	8 (57.1)	105 (66.9)	OR=1.51; 95%CI=0.49-4.59
Seizures	Yes	30 (17.5)	4 (28.6)	26 (16.6)	χ(1)=1.282; p=0.258;
	No	141 (82.5)	10 (71.4)	131 (83.4)	OR=2.01; 95%CI=0.59-6.92
Respiratory distress	Yes	119 (69.6)	6 (42.9)	113 (72.0)	χ(1)=5.15; p=**0.023;**
	No	52 (30.4)	8 (57.1)	44 (28.0)	OR=0.25; 95%CI=0.08-0.77
Chest retraction	Yes	89 (52.0)	3 (21.4)	86 (54.8)	χ(1)=5.73; p=**0.017;**
	No	82 (48.0)	11 (78.6)	71 (45.2)	OR=0.23; 95%CI=0.06-0.84
Auscultation - bronchial breath sounds	Yes	33 (19.3)	1 (07.1)	32 (20.4)	χ(1)=0.98; p=0.321
	No	138 (80.7)	13 (92.9)	125 (79.6)	OR=0.30; 95%CI=0.04-2.3
Crepitations	Yes	126 (73.7)	5 (35.7)	121 (77.1)	χ(1)=11.34; p**<0.001**
	No	45 (26.3)	9 (64.3)	36 (22.9)	OR=0.17; 95%CI=0.05-0.52
Meningeal signs	Yes	22 (12.9)	2 (14.3)	20 (12.7)	χ(1)=0.03; p=0.868;
	No	149 (87.1)	12 (85.7)	137 (87.3)	OR=1.14; 95%CI=0.24-5.48
Any antipyretics taken 24 hours/any antibiotics taken 3 days before sample collection		33 (19.3)	3 (21.4)	30 (19.1)	χ(1)=0.04; p=0.833;
		138 (80.7)	11 (78.6)	127 (80.9)	OR=1.16; 95%CI=0.30-4.39
Duration of hospital stay	Median (IQR)	8 (4,12)	8 (5,7)	8 (4,12)	U=929, Z=-0.12, p=0.904, r=0.031
	Mean±SD	9.35±8.0	8.8±9.9	9.39±7.9	
Chest X-ray	Abnormal	129 (75.4)	8 (57.1)	121 (77.1)	χ(1)=2.755; p=0.097
	Normal	42 (24.6)	6 (42.9)	36 (22.9)	OR=0.39; 95%CI=0.13-1.22

CI – confidence interval; **OR** – odds ratio; **SD** – standard deviation; **IQR** –interquartile range.

13 vaccine and 66.7% of the serotypes included in PPV-23. All the serotypes were from children diagnosed to have pneumonia except for serotypes 6B, 14 and 19F which were from children diagnosed with meningitis. Non-vaccine serotypes (serotypes 15A, 19B and 21) were commonly found in children under 2 years of age (OR=0.17; 95%CI=0.007-4.033; χ(1)=2.121; p=0.145).

All of the isolates were sensitive to chloramphenicol and cefotaxime (Table 4). There was no difference in the antibiotic resistance

Table 4. Serotypes and antimicrobial resistance of 14 *Streptococcus pneumoniae* strains causing invasive disease and isolated from children aged <5 years.

Serotype	Age, month	Total number of isolates	Number of isolates resistant to:				
			Penicillin	Chloramphenicol	Erythromycin	Co-trimoxazole	Cefotaxime
1	15	1	0	0	0	1*	0
2	12	1	0	0	0	1*	0
6A	58	1	0	0	1	0	0
6B	8, 20	2	0	0	1	2*	0
9V	24	1	0	0	0	0	0
10A	11	1	0	0	0	0	0
14	17	1	0	0	0	1	0
15A[a]	16	1	0	0	0	1*	0
19B[ab]	12	1	1	0	1	0	0
19F[b]	5, 48	2	1	0	2	1	0
21[a]	24	1	0	0	0	0	0
35F	45	1	0	0	0	1*	0
Total		**14**	**2**	**0**	**5**	**8**	**0**

[a]Non-vaccine serotype; [b]serotype caused death; *intermediate resistance.

pattern between vaccine serotypes (PCV10 & PCV13) and non-vaccine serotypes. Of the total 14 isolates, the highest rate of resistance was to trimethoprim-sulfamethoxazole (co-trimoxazole 57.1%, 2/14 resistant and 6/14 intermediate resistant) followed by erythromycin 35.7% (5/14) and penicillin 14.3% (2/14, one each of 19F and 19B). One isolate of 19F serotype was multidrug resistant (resistant to penicillin, erythromycin and co-trimoxazole) and was isolated from a 5 months old diagnosed with meningitis. The overall fatality rate was 14.3% (2/14) and was due to a 19B isolate (resistant to penicillin and erythromycin, non-vaccine serotype) and a multidrug resistant serotype 19F isolate.

Discussion

Our prospective study demonstrates the burden of invasive pneumococcal disease and distribution of diverse serotypes of *S. pneumoniae* among young children in Southern India. Notably, this is the first report of the presence of serotype 15A in pediatric invasive pneumococcal disease in India. The most common isolates in our study were 19F and 6B, a similar observation to that from an earlier report by Balaji et al., 2015 from South India.[4] The occurrence of non-vaccine (21.4%) serotypes (i.e., that are not included in PCV10, PCV-13 and PPV-23) is also consistent with the observations of other studies, reporting rates of 25.4%[4] and 27%.[17] The emergence of non-vaccine serotypes might presumably be a result of the introduction of recent clones or even a result of capsular switching of existing stain or by many single genetic changes.[18-20] This baseline information would be important in regions where the population is relatively naïve to the vaccine.

Our results indicate the presence of 12 different pneumococcal serotypes; currently licensed vaccines (PCV10, PCV13 and PPV23) cover only 41%, 50% and 67% of the circulating serotypes, respectively. The documented presence of these non- vaccine serotypes (15A, 19B and 21)

would need to be addressed to ensure appropriate coverage by the vaccine in our region. Replacement by non-vaccine serotypes has been documented in surveillance studies following widespread use of pneumococcal vaccines.[21,22] However, detection of penicillin and erythromycin resistance in non-vaccine serotype 19B isolates is of particular concern as resistance to this serotype was described previously only once in Japan[23] and is now documented widely.[5] Appropriately designed community-based studies for surveillance of IPD and its associated circulating serotypes are required to demonstrate the changes from baseline data (i.e., prior to vaccination).

Antibiotic resistance among *S. pneumoniae* is an emerging problem.[3,4] The heightened incidence and spread of *S. pneumoniae* has emphasized the need for accurate diagnosis of the infection. The World Health Organization recommended the use of penicillin, ampicillin, or co-trimoxazole for the treatment of children with pneumonia.[21] In the present study, we found high co-trimoxazole resistance (14.3% resistant; 42.9% intermediate resistant) followed by erythromycin (35.7%) and penicillin (14.3%) resistance. Different studies have reported varied rates of resistance to co-trimoxazole, ranging from 56% to 74%.[5,24] Although in the present study co-trimoxazole (57.1%) resistance was found to be lower compared to other studies from India that reported rates of 96.4%,[4] 74.2%,[17] and 61.7%,[25] or from neighboring countries like such as Bangladesh >76%[26] or Sri Lanka 73.9%,[27] the emergence of co-trimoxazole resistance among *S. pneumoniae* is a cause for concern.[3] While India has a low incidence of penicillin resistant *S. pneumoniae*, recently Balaji et al. have shown an increase in the trend of penicillin resistant IPD serotypes from 1.3% in 1999 to 5.2% in 2015.[4,7] This trend is different in different regions in India.[28] This study showed a higher rate (14.3%) of penicillin resistance, which is similar to an earlier report from Bengaluru (12.5%).[24] Two isolates belonging to serotypes 19F and 19B, found to be resistant to penicillin in this study, were reported to be serotypes sensitive to penicillin in previous studies in Indian children.[7,24] The isolates resistant to penicillin were also resistant to erythromycin. This finding is worrisome, as the emergence of such isolates can pose a significant problem in terms of treatment of pneumococcal meningitis, where penicillin is the antibiotic of choice, and could be due to inadequate antibiotic stewardship.[3] Resistance to erythromycin (35.7%) was consistent with other Indian studies (30%[4,24] and 27.8%[24]). One isolate, identified as 19F serotype associated with meningitis in a 5 months old child, was found to be multidrug resistant, i.e., resistant to penicillin, erythromycin and co-trimoxazole. Nasal carriage has been documented in many preschool children, as well as carriage of multiple serotypes with variable susceptibility patterns. Exposure to antibiotics commonly used in the treatment of upper respiratory tract infections could result in primary resistance among the circulating *S. pneumoniae* strains.[3]

Our study is limited in that this data is from a single hospital, and may not represent the burden in the community and the antimicrobial resistance pattern of the region. The sample size is small and does not allow us to draw conclusions regarding the overall serotype prevalence in the region.

Conclusions

In conclusion, we have reported the serotype distribution of *S. pneumoniae* strains involved in pediatric IPD and the diversity in serotypes compared to other regions. Two deaths documented in this study had isolates of non-vaccine *S. pneumoniae* serotypes; one was resistant to penicillin and erythromycin and cultured from blood, and the other was multidrug resistant and cultured from the CSF. The emergence of drug resistant pneumococci and non-vaccine serotypes is likely to enhance the complexity of treatment and prophylaxis.

Authors' contributions statement: AM designed the study and provided overall supervision for all participating centers; AS provided guidance with respect to the clinical perspective of the study; BSK acquired laboratory and clinical data, analyzed it, and drafted the manuscript; SN contributed to the laboratory component of the study protocol, supervision of the laboratory data, analysis of data, drafting and

finalizing the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

Funding: This study was a part of the national study (Alliance for Surveillance of Invasive Pneumococci – ASIP) funded by GlaxoSmithKline, Biologicals, India.

Acknowledgments: The authors thank the children and their caregivers who participated in this study. We also thank all the clinical research coordinators Mr. Jalagandeeswaran, Ms. Salome Mary Thomas, Ms. Anu Krishnan and Ms. Venus Muddana for their overwhelming efforts and overall involvement in the ASIP study.

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Please cite this article as:

Nagaraj S, Kalal BS, Manoharan A, Shet A. *Streptococcus pneumoniae* serotype prevalence and antibiotic resistance among young children with invasive pneumococcal disease: experience from a tertiary care center in South India. GERMS 2017;7(2):78-85. doi: 10.18683/germs.2017.1112