Original Article

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Microbiology Section

Circulating Serotypes and Trends in Antibiotic Resistance of Invasive *Streptococcus Pneumoniae* from Children under Five in Bangalore

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ABSTRACT

Background: Globally, *Streptococcus pneumoniae* is estimated to be responsible for 1 to 2 million deaths annually, in extremes of age. Serotypic distribution of *pneumococci* varies with age, time, and geographical area. Limited data is available on serotypic prevalence and antimicrobial susceptibility patterns of *pneumococci* in India.

Aim: To assess resistance trends to different groups of antimicrobials and serotypic prevalences of invasive *pneumococci*.

Settings and Design: A prospective, hospital based study was conducted for two years, at a tertiary care medical college hospital in south Bangalore. Forty invasive pneumococcal isolates from children who were ≤5 years, with a clinical and radiological diagnosis of invasive pneumococcal disease, (IPD) were evaluated.

Methods: Qualitative typing/grouping was performed by doing the capsular reaction test (Neufeld test). Antimicrobial susceptibility was tested by Minimum Inhibitory Concentration method using automated microdilution procedure.

Results: The predominant invasive pneumococcal serotypes were serogroups/types (SGTs) 6 (25%) and 14 (17.5%). 35%, 77.5% and 15% of isolates were resistant to Penicillin, Trimethoprim/Sulfamethoxazole (TMP-SMX) and Ceftriaxone respectively. Intermediate and high level resistances to penicillin were seen in 22.5% and 12.5% of *S. pneumoniae* isolates correspondingly. Multidrug resistance was observed in 20% of strains.

Conclusion: This study reported presence of high level drug resistance in invasive pneumococcal isolates which were obtained from children. The serogroup/type distribution in our study and those in other Indian studies were not even. This calls for monitoring of resistance and mapping of serotype distribution.

Keywords: *Streptococcus pneumoniae*, Invasive pneumococcal disease, Serotypes, Drug resistance

INTRODUCTION

Streptococcus pneumoniae kills at least one million children who are under the age of five years, every year, globally. More than 70% of the deaths are seen in developing countries [1]. A recent UNICEF publication estimated that 410,000 children who were under age of 5 years died of pneumonia each year in India [2] and data showed that an estimated 25% of all child related deaths in India were caused by pneumonia [3]. Inspite of the availability of antimicrobial therapy and introduction of pneumococcal vaccines, *S. pneumoniae* continues to be a major cause of morbidity and mortality worldwide [4].

Pneumococci cause diseases which range from acute otitis media and sinusitis, to more severe pneumonia, septicaemia and meningitis [5]. *S. pneumoniae* have been divided into more than 94 serogroups/types (SGTs) on the basis of the immunochemistry of their capsular polysaccharides. Virulence has been noted in certain serotypes, which accounts for a majority of invasive infections [6]. Serotypic distribution, which causes invasive infections, varies by age, time, place and socio economic conditions of the patient. [6] Among children, serotypes 6,14,9 and 23 account for a majority of infections which have been reported in the west, as well as in India [7,8].

Drug resistant *pneumococci* have emerged as a problem of global concern.[9] Penicillin has been the drug of choice for the treatment of pneumococcal infections, but the increasing number of reports on penicillin resistant *pneumococci* (PRP) throughout the world makes it essential to determine the prevalence of PRP regionally [10,11]. Moreover, the PRP has been reported to harbour resistance to other antimicrobial classes, thus making the treatment much more difficult [12]. Multidrug resistance (MDR) of *pneumococci* was first reported in 1977 in South Africa and subsequently in Europe [13] and United states [14]. Since then, low levels of MDR have been reported from few regions of India.

Most of the studies have utilized disk diffusion and agar dilution techniques for antimicrobial susceptibility testing, The disk diffusion method is cheap and easy to perform, but it offers only qualitative results. The dilution technique is time consuming and labour intensive [15]. Hence, an MIC determination is necessary, to confirm and to assess the degree of resistance [16].

This study was conducted to assess the prevalence of resistance to different groups of antibiotics in invasive *S. pneumoniae* and to verify the presence of MDR. Serogroups/types (SGTs) were identified and correlated with drug resistance.

MATERIAL AND METHODS

Study Site and Study Period

This prospective, active study was carried out in a tertiary care teaching hospital and research centre in Bangalore, from Feb 2009 to Feb 2011. *S. pneumoniae* which were isolated from children who were 28 days to ≤5 years old, who had IPD, were included in the study.

Bacterial Isolates

A total of 40 *S. pneumoniae* isolates were analyzed. Invasive isolates of *S. pneumoniae* were recovered from clinical specimens of normally sterile body sites such as, blood (n=36), CSF (n=3) and

pleural fluid (n=1). The demographic, clinical, radiological and other investigational details of children was noted and scrutinized.

Identification, Serotyping and Antimicrobial Susceptibility Tests

Specimens (blood, CSF and pleural fluid) were inoculated into BACTEC Peds Plus/F blood culture bottles and they were incubated at 35°C in BACTEC™ 9050 instrument (Becton Dickinson, Baltimore, USA) within 2 hours of their collections. The bottles were continuously agitated in the automated culture system for maximum recovery of organisms. Positive cultures were flagged by an indicator light and an audible alarm. The positive cultures were subcultured immediately onto sheep blood agar plates and the plates were incubated in a 5% CO_2 incubator.

S. pneumoniae isolates were identified on the basis of colony morphology, gram staining, susceptibility to optochin and bile solubility tests, which were done by standard methods [17].

The strains were serotyped by the Quellung reaction according to the manufacturer's recommendations. Test kits were obtained from Staten's Serum Institute, Copenhagen, Denmark.

Antibiogram was performed by microdilution procedure using automated Siemens Microscan WalkAway® system with synergy panels (Siemens healthcare diagnostics Ltd, Frimley Camberley, UK). Standardized inoculum was prepared as per the manufacturer's protocol. The antimicrobials which were tested were Penicillin, Eryt hromycin,Levofloxacin,Trimethoprim/Sulfamethoxazole(TMP-SMX), Vancomycin and Ceftriaxone. Strains were defined as Multidrug resistant if they showed resistance to three or more different groups of antibiotics.

MIC values of ≤0.06µg/ml defined *Pneumococci* as susceptible to penicillin, those of 0.12-1µg/ml defined them as Intermediately or relatively resistant and those of ≥2µg/ml defined them as highly resistant to oral penicillin V. For penicillin, parenteral (meningitis) doses of ≤0.06µg/ml and ≥0.12µg/ml could predict susceptibility and resistance respectively. MICs of ≤2µg/ml and ≥8µg/ml were the breakpoints for penicillin parenteral (nonmeningitis) [18].

All *S. pneumoniae* isolates which were identified in the local laboratory were confirmed by the Quintiles Central Laboratory at Singapore for quality control and assurance checks.

Ethics

This study was conducted in accordance with applicable laws and regulations. It was approved by the institutional review board and independent ethics committee. The subject's parent(s) or legal guardian(s) completed written informed consent process.

STATISTICAL ANALYSIS

The statistical software, SPSS, v11.0 was used for the analysis of the data and for generation of figures and tables.

RESULTS

Demographic Details

S. pneumoniae strains were isolated from children who were 28 days to ≤5 years old, who had IPD, who were living in south Bangalore region. Male to female ratio was 2.07:1 (Male= 27, 67.5% and Females= 13, 32.5%). Clinically, 25 (62.5%) patients were suffering from pneumonia, 11(27.4%) were suffering from bacteraemia, 4 (10%) were suffering from meningitis.

Serotype Distribution and Antibiogram

Forty pneumococcal strains were distributed among 11 SGTs [Table/Fig-1]. Four serotypes, 6 (n=10, 25%), 14 (n=7, 17.5%), 18 (n=5, 12.5%) and 5 (n=5, 12.5%), in order of prevalence, accounted for 67.5% (27/40) of all isolates. Serotypes 6 and 14 were the dominant types. Serotype 6 was most common in the age group of 28 days-20 months and type 18 was common in the age group of 21-40 months. There were differences in the distributions of serotypes among the different age groups. Serotypes 3, 4, 9 and 10 were seen only in 28 days-20 months age group and serotype 15 was seen only in age group of 41-60 months.

Penicillin resistance was seen in 14(35%) isolates. An intermediate penicillin resistance was seen in 9(22.5%) isolates who belonged predominantly to serotype 6 (n=6). A high level of penicillin resistance was seen in 5(12.5%) isolates who belonged largely to serotype 14 (n=3) [Table/Fig-2]. One isolate of serotype 6, which was obtained from pleural fluid, was highly resistant to penicillin. Resistance was not seen in any of the isolates which were recovered from CSF (n=3, serotypes 3, 6 and 14). The MIC range for penicillin was 0.016-4µg/ml.

Resistance was most common to TMP-SMX [Table/Fig-3]. 77.5% (n=31/40) of isolates were resistant to TMP-SMX, which predominantly belonged to SGTs 14 (n=5) and 6 (n=6). Among these isolates, high level and intermediate level resistances to TMP-SMX were observed in 42.5% (n=17/40) and 35% (n=14/40) isolates respectively.

Ceftriaxone and Erythromycin resistant strains accounted for 15% and 12.5% strains, who belonged predominantly to SGTs 14 and 6, in that order. All the Erythromycin strains were highly resistant. Resistance to different antimicrobials, with serotypic distribution, has been represented in [Table/Fig-4].

Minimal resistance (5%) was observed to Levofloxacin. As was expected, Vancomycin was the antibiotic to which all the isolates were susceptible.

Serotypes	28days-20 months			21-40 months			41-60 months			Total
	Blood	CSF*	PF†	Blood	CSF	PF	Blood	CSF	PF	
1	1	-	-	1	-	-	1	-	-	3 (7.5%)
3	-	1	-	-	-	-	-	-	-	1 (2.5%)
4	1	-	-	-	-	-	-	-	-	1 (2.5%)
5	3	-	-	1	-	-	1	-	-	5 (12.5%)
6	7	1	-	1	-	-	-	-	1	10 (25%)
9	2	-	-	-	-	-	-	-	-	2 (5%)
10	1	-	-	-	-	-	-	-	-	1 (2.5%)
14	5	1	-	1	-	-	-	-	-	7 (17.5%)
15	-	-	-	-	-	-	1	-	-	1 (2.5%)
18	2	-	-	3	-	-	-	-	-	5 (12.5%)
19	3	-	-	1	-	-	-	-	-	4(10%)
Total	25 (62.5%)	3 (7.5%)	-	8 (20%)	-	-	3 (7.5%)	-	1 (2.5%)	40 (100%)

[Table/Fig-1]: Age, Specimen and serotype distribution of 40 *S. pneumoniae* isolates
*Cerebrospinal fluid; †Pleural fluid

Multidrug Resistance

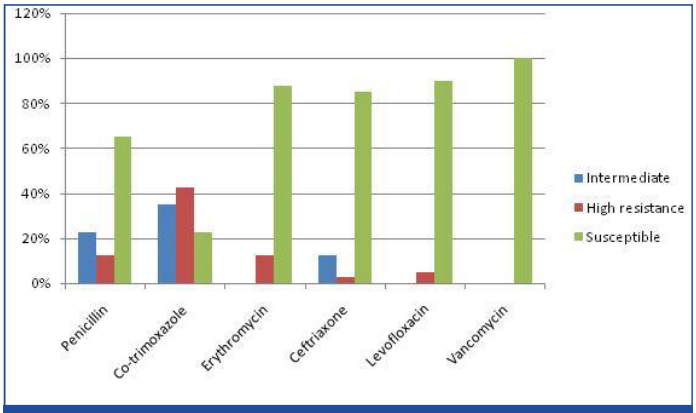
Multiple resistance was observed in 20% (n=8) of the strains. Penicillin, TMP-SMX and Ceftriaxone was the most common combination, which accounted for 62.5% (n=5/8). Intermediate and high penicillin resistances were observed in 3 and 5 isolates correspondingly. MDR strains belonged to SGTs 6 (n=3), 14 (n=3) and 9 (n=2), in descending order.

DISCUSSION

Reports on increasing resistance to the commonly used antibiotics and the possible prevention of life threatening infections by using vaccines, monitoring of serotype prevalence and susceptibility pattern of *S. pneumoniae* have assumed great significance [19].

Serotypes	IPD Isolates with Penicillin resistance			
	No. of Isolates	S*	I†	R‡
1	3	3	-	-
3	1	-	1	-
4	1	1	-	-
5	5	4	1	-
6	10	3	6	1
9	2	-	1	1
10	1	1	-	-
14	7	4	-	3
15	1	1	-	-
18	5	5	-	-
19	4	4	-	-
Total	40 (100%)	26 (65%)	9 (22.5%)	5 (12.5%)

[Table/Fig-2]: Penicillin resistance and serotype distribution in IPD isolates
*Susceptible; †Intermediate resistance; ‡High Resistance



[Table/Fig-3]: Drug resistance pattern of *S. pneumoniae*

The serotypic distribution varies with geographical area, disease, age, socioeconomic conditions, season, vaccine usage and type of cohort [6]. Data on serotypic prevalence and antibiotic susceptibilities of invasive *S. pneumoniae* isolates in children who are ≤5years of age are limited in India.

SGTs involved in invasive infections in our study were dominated by type 6, followed by SGTs 14 and 18. The study of Kanungo and Rajalakshmi [19] which was conducted at Pondicherry in 2001 and a multicentre hospital surveillance study [8] which was done in 1999, have reported SGTs 6,1,23 and 6,1,19 respectively as the most prevalent isolates in the age group of ≤5years. Serotype 6 which is the most common serotype in developed countries [20], was also the most common one which was seen in this study, which accounted for 25% of all invasive isolates, which was in concurrence with other reports [19,8]. Reports from developing countries like Brazil [21] (SGTs 14,1 and 5), Bangladesh [22] (SGTs 7,12 and 14) and Nepal [23] (SGTs 1, 5 and 4) have shown a different pattern of invasive serotypic distribution. In these studies, serotype 6 was not the predominant isolate in young children (≤5 years). This brought into focus the different serotype prevalence patterns which were based on geography.

There are differences in the prevalence and the rank order of serotypes which are involved with invasive disease, depending on age groups. In young children who were 28 days-20 months old, *S. pneumoniae* type 6 was the most common one, whereas type 18 was predominant in the 21-40 months age group. However, we did not notice any significant difference in the distribution of serotype 1 across the age groups. Other Indian studies [19,8] have identified serotype 1 as the second most prevalent isolate (19% and 14%), which differed from our findings (7.5%). Serotype 1 which has largely disappeared from many developed countries, is also disappearing in this region of the country.

With the introduction of serotype specific, polyvalent, conjugate vaccines in India, determination of the major serotypes which cause invasive pneumococcal infections in different regions of India is of public health importance. 10 valent and 13valent Pneumococcal conjugate vaccines which are presently available in Indian market, contain 73% and 82% SGTs of our study respectively. Our study indicated that two SGTs, 10 and 15 were not covered by both vaccines.

The increase in the incidence and the spread of antibiotic resistant *pneumococci* has placed great emphasis on prompt and accurate recognition of pneumococcal resistant patterns. Our data clearly suggest an increase in the absolute number of the strains with reduced susceptibilities to penicillin in south Bangalore. Among 40 invasive pneumococcal strains, 14 (35%) had reduced susceptibility

SGT	No. of Isolates	Penicillin			TMP-SMX*			Ceftriaxone			Erythromycin			Levofloxacin			Vancomycin		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
1	3	3	-	-	-	3	-	3	-	-	3	-	-	3	-	-	3	-	-
3	1	-	1	-	-	-	1	1	-	-	1	-	-	1	-	-	1	-	-
4	1	1	-	-	-	-	1	1	-	-	1	-	-	-	-	1	1	-	-
5	5	4	1	-	-	-	5	5	-	-	5	-	-	5	-	-	5	-	-
6	10	3	6	1	4	5	1	8	1	1	7	-	3	10	-	-	10	-	-
9	2	-	1	1	-	-	2	2	-	-	1	-	1	1	-	1	2	-	
10	1	1	-	-	-	1	-	1	-	-	1	-	-	1	-	-	1	-	-
14	7	4	-	3	2	2	3	4	3	-	6	-	1	7	-	-	7	-	-
15	1	1	-	-	-	1	-	1	-	-	1	-	-	1	-	-	1	-	-
18	5	5	-	-	2	1	2	4	1	-	5	-	-	5	-	-	5	-	-
19	4	4	-	-	1	1	2	4	-	-	4	-	-	4	-	-	4	-	-
Total	40	26	9	5	9	14	17	34	5	1	35	-	5	38	-	2	40	-	

[Table/Fig-4]: IPD S. pneumoniae isolates- Serogroup/types and their antibiogram
*Trimethoprim/Sulfamethoxazole.

to penicillin (12.5% with high resistance and 22.5% with intermediate resistance). Analysis done, of studies done in India revealed that decreased susceptibilities to penicillin ranged from 1.3% to 18.3%. [8,28,19,24,11] Goyal et al., [11] (2.3%) and Chawla et al., [25] (4%) have reported low prevalences of high resistance to penicillin in *S. pneumoniae* in Delhi and Karnataka respectively. Increase in the percentage of strains with high resistance for penicillin (12.5%) in the present study was a matter of concern, as it could result in the spread of resistant strains to other locations. These strains could lead to higher rates of treatment failures and an increased economic burden. Therapeutic problems which are linked to this prevalence are compounded by the frequency of cross resistance to many other antibiotics.

Penicillin resistance was associated with a lower age being higher in age group of 28 days-20 months (78.5%), followed by that of 21-40 months (21.5%). These findings were concurrent with the observations of the ANSORP study [4]. Harbouring of *S. pneumoniae* in greater numbers in nasopharynx, frequent exposure to antimicrobial agents are the important risk factors for high percentage of penicillin resistance in children.

Studies [26,27] done in different parts of the world have shown that the high level penicillin resistant strains belonged primarily to SGTs 6, 19, 14 and 23 and that those with intermediate resistance belonged to SGTs 19 and 14. Lalitha et al., [28] have reported that the intermediate penicillin resistant strains of *S. pneumoniae* from the surveillance programme belonged predominantly to SGTs 14 and 19 and that SGT 1 was most prevalent in Kanungo's and Rajalakshmi's study [19]. Information related to SGT pattern of high level penicillin resistance from India is scant [8,19]. In the study done by us, high level penicillin resistant strains (n=5, 12.5%) mainly belonged to SGT 14 (n=3, 60%) and intermediate resistant strains (n=9, 22.5%) belonged largely to SGT 6 (n=6, 66.6%). This underlines the need for a constant surveillance and reporting of the findings [8,19].

In this study, 10-valent PCV and 13-valent PCV covered 80% and 100% SGTs of the penicillin resistant strains respectively, thus indicating the potential usefulness of the vaccination in Bangalore population. Since *Pneumococci* have the genetic capacity to switch serotypes by horizontal transfer, recombination or other genetic events, knowledge on the frequencies of these serotypic exchanges is important, to predict the long term efficacy of vaccines.

TMP-SMX has been widely used for upper respiratory tract infections, because of its broad coverage, synergetic effects and low cost. TMP-SMX functions by inhibiting dihydrofolate reductase and dihydropteroate synthase. Alteration of these genes by mutations or acquisition of exogenous genes, leads to resistance. The rate of pneumococcal resistance to TMP-SMX has increased significantly in India [24,19,25,11], from 21.8% to 61.7% between 1996-2002. A high rate of resistance (77.5%) to TMP-SMX was observed in our study as compared to that which was seen in other studies done in India. Alarming levels of TMP-SMX resistances raise the question as to whether WHO recommendations on use of TMP-SMX as first line of treatment of choice in upper respiratory tract infections needs to be revised, based on local data.

85.7% (n=12) of penicillin resistant strains (intermediate resistance n=7, high resistance n=5) had co-resistance to TMP-SMX. All the 6 strains which were resistant to Ceftriaxone and all the 5 which were resistant to Erythromycin were also resistant to TMP-SMX. The emergence of such strains is of particular concern in the treatment of pneumococcal meningitis, because ceftriaxone is an important component of the combination regimens which are used for pneumococcal meningitis [4].

Multidrug resistance was observed in 8 strains, all of which had reduced susceptibilities to penicillin. Penicillin resistance is an important marker for multidrug resistant phenotypes. Each of high penicillin resistant strains in our study exhibited multidrug resistance. To the best of our understanding, this is the first report from India, which has described this high resistance profiles of *S. pneumoniae* strains. The increased rate of resistance to these antibiotics can be possibly correlated with the wide use of these antibiotics in communities, because of their dose convenience, cost effectiveness, and also, their easy availability over the counter [23]. This is a matter of great concern, as it results in higher morbidity, mortality.

Our data clearly documents high rate of MDR, changing trend of antibiogram of *S. pneumoniae,* with a distinctive increase in the prevalence rate and resistance level of penicillin and other drugs. The strength of this study lies in its ability in determining different dynamics of serotype-specific IPD and drug resistance in specific age groups. The sensitivity resistant patterns which are identified, will help the clinicians in appropriately planning treatment regimen. Our study indicated that a majority (82%) of IPD cases could be prevented with the use of 13valent PCV.

Due to the diversity in populations in different parts of India and also the living conditions, our findings cannot be generalized. Owing to small number of isolates in this study, there are limitations to our findings, that may reduce the generalizability of our results.

CONCLUSION

The study identified a high prevalence of penicillin resistance and MDR in invasive *S. pneumoniae* among those children who were aged below five years. A continuous surveillance of serotypes and antimicrobial resistance patterns of *pneumococci* in multicentric studies which involve rural and urban areas, is needed. Appropriate antibiotic treatment plans and use of pneumococcal vaccinationa are essential, to decrease morbidity and mortality.

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