

## Nasopharyngeal carriage, antibiogram & serotype distribution of *Streptococcus pneumoniae* among healthy under five children

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**Background & objectives:** Information related to nasopharyngeal carriage of *Streptococcus pneumoniae* among healthy children is scanty in India. This prospective study was undertaken to determine the presence of asymptomatic nasopharyngeal colonization, assess serogroups/types (SGT) and drug resistance of *S. pneumoniae* in children below five years of age.

**Methods:** A total of 109 male and 81 female children in the age group of three months to five years belonging to different socio-economic classes were enrolled. They were recruited across all age groups from those attending paediatric OPD of a tertiary care and research centre for immunization program. Fifty three isolates identified as pneumococci were tested for their antimicrobial susceptibility pattern by Kirby-Bauer's disc diffusion and E-Test methods. Serotyping was performed by detection of the quelling reaction with specific antiserum.

**Result:** The pneumococcal carriage rate in the study population was 27.9 per cent. The isolation rate was associated with age being higher (49.2%) in smaller children (3-12 months) and among male (62.2%). The most prevalent SGTs were 19 followed by 10, 14 and 7; 21 per cent of isolates belonging to serotype 10 (n=7) were 11 (n=4) were not covered in any of the conjugate vaccines currently available in Indian market. Resistance to co-trimoxazole, tetracycline, penicillin and erythromycin was observed in 91 per cent (n=48), 36 per cent (n=19), 17 per cent (n=9) and 9 per cent (n=5) isolates, respectively. All the penicillin resistant isolates were found to be intermediately resistant by E-Test. Multidrug resistance was observed in 19 per cent (n=10) isolates.

**Interpretation & conclusions:** High level of antibiotic resistance was present in *S. pneumoniae* isolated from healthy children below age five. A pneumococcal conjugate vaccine with the prevailing SGTs would help to reduce the pool of antibiotic resistant pneumococci. Continued surveillance of serotypes and tracking susceptibility pattern of *S. pneumoniae* will help to introduce appropriate vaccination protocols.

**Key words** Antibiotic resistance - nasopharyngeal carriage - serotypes - *Streptococcus pneumoniae*

*Streptococcus pneumoniae* is a leading cause of life threatening infections in children in developing countries. It kills at least one million children under the age of five every year, >70 per cent of these deaths are in developing countries. The burden of the disease in youngest and oldest sections of the population is more, in both developed and less developed countries<sup>1</sup>. *S. pneumoniae* is part of the commensal flora of the upper respiratory tract, together with *Moraxella cattarrhalis*, *Haemophilus influenzae*, *Neisseria meningitis*, *Staphylococcus aureus* and various haemolytic streptococci it colonizes the nasopharyngeal (NP) niche<sup>1</sup>. Though colonization with pneumococci is mostly asymptomatic, it remains the common cause of community-acquired pneumonia (CAP), bacterial meningitis, bacteraemia, and otitis media<sup>1</sup>. In addition, pneumococcal carriage is believed to be an important source of horizontal spread of this pathogen within community.

Serotype distribution and resistance to antibiotics among nasopharyngeal carriage isolates vary by country, age group, type of cohort, vaccine policies and change over time<sup>1</sup>. Studies have documented that pneumococcal diseases originate from nasopharyngeal colonization with homologous serotypes. Acquisition of a new serotype may lead to an invasive disease, with a risk of 15 per cent in the first month after new acquisition, and the children may act as a reservoir for the dissemination of new serotypes to others<sup>1</sup>. The pneumococcal conjugate vaccines have greatly reduced frequency of invasive disease, and has also resulted in reduction of drug resistance among the vaccine serotypes<sup>2</sup>. But several non-vaccine type strains have mutated to become more successful colonizers and are re-emerging as pathogens. Surveillance of serotype distribution of colonizing isolates in children will serve as an indicator of invasive disease, antibiotic resistance trends and usefulness of vaccine.

Initially pneumococcal strains were universally susceptible to penicillin which remained the drug of choice. Strains resistant to penicillin and multi drugs emerged during 1970s and 1980s and spread globally<sup>3</sup>. This study was carried out to determine nasopharyngeal carriage rate, serotype prevalence and *in vitro* antimicrobial resistance of *S. pneumoniae* in children during their first five years of life.

### Material & Methods

A total of 190 children between the age group of 3 months and <5 yr were enrolled prospectively

during December 2010-December 2011 from those attending outpatients of the department of Paediatrics, Kempegowde Institute of Medical Sciences Hospital and Research Centre, Bangalore, Karnataka, India for immunisation programme. The children belonging to different socio-economic classes were randomly recruited from the paediatric immunization centre. For the study one child per family was included.

*Nasopharyngeal specimen collection:* Nasopharyngeal specimens were obtained at the time of enrolment as per the technique recommended by World Health Organization<sup>4</sup>. Samples were collected using sterile miniature polyester tipped swabs on flexible aluminium shaft (Hi-Media Laboratories, Mumbai, India).

Children < 3 months of age, with a history of antibiotics in recent past (4 weeks) and recent hospitalization were excluded. Children from household with adult member suffering from acute lower respiratory tract infection or with a history of taking pneumococcal vaccines were also excluded.

The study protocol was approved by the hospital ethical committee. Informed written consent from parents or guardians was obtained at the time of enrolment.

*Microbiological methods:* The swabs were streaked immediately on to a 5 per cent sheep blood agar plate. The plates were incubated overnight at 35°C in a carbon dioxide incubator. Isolation of *S. pneumoniae* was confirmed by Gram staining, optochin sensitivity and bile solubility tests<sup>4</sup>. Serogrouping and serotyping by the Quellung reaction was done from the pure culture directly after isolation, using the pneumotest plus kit. (Statens Serum Institute, Copenhagen, Denmark).

*Antimicrobial susceptibility testing:* Susceptibility testing of *S. pneumoniae* was done on Muller-Hinton agar with 5 per cent sheep blood. The antibiotics tested were penicillin, erythromycin (15µg), Co-trimoxazole (25 µg), cefotaxime (30 µg), ciprofloxacin (5 µg) and tetracycline (30 µg). The 1µg oxacillin disc was used to screen for penicillin susceptibility. Multi drug resistant *S. pneumoniae* defined as resistant to penicillin and two or more non β-lactam agents such as macrolides, co-trimoxazole or tetracycline were analysed in the study.

Minimum inhibitory concentrations (MIC) for penicillin were determined using E-strips (Hi-media, Mumbai, India) *S. pneumoniae* quality control strain ATCC 49619 was included along with the tests.

**Statistical analysis:** One proportion Z-test was used to find the significance of percentage of isolation of pneumococcal carriage. The statistical softwares SPSS 15.0 and Stata 8.0 were used for the analysis of data.

### Result

Pneumococci were isolated from 53 of the 190 children tested (109 male, 81 female), with a carriage rate of 27.9 per cent. The carriage of pneumococci was found to be higher in male children (34/109, 30.3%) than in female (20/81, 24.7%). Carriage rate of pneumococci (Table I) was highest in 3-12 months group, followed by 1-3 year group, and the lowest in the 3-5 years group.

The SGT isolates were 1 (n=4), 3 (n=6), 6 (n=2), 7 (n=6), 9 (n=2), 10 (n=7), 11 (n=4), 14 (n=6), 18 (n=4) and 19 (n=11), the most common being SGT 19. Two isolates were non-vaccine type (21, 24). Six SGT (19, 10, 6, 7, 3, 14) comprised 79.2 per cent of pneumococcal isolates.

Nine (16.98%) of the 53 isolates were resistant to oxacillin. The MIC of penicillin was in the range of 0.125-0.5µg/ml indicating that all were intermediately resistant to penicillin. All the oxacillin resistant isolates were also resistant to co-trimoxazole.

Highest degree of resistance among the six drugs was seen for co-trimoxazole, (n=48, 90.6%) (Table II). Number of resistant isolates to tetracycline, erythromycin, ciprofloxacin and cefotaxime were 19, 5, 5 and 2, respectively; 41.5 per cent of isolates were resistant to one antibiotic, 28.3 per cent to two antibiotics and 18.8 per cent to three or more antibiotics. Two among the multi drug resistant (MDR) isolates were resistant to all the six drugs tested.

### Discussion

As the highest frequency of pneumococcal colonization and highest crowding index are found in young children, this group is the most important

**Table I.** Age distribution and percentage of pneumococcal isolation

Age	No. of cases	No. of isolation	Percentage of isolation
3-12 months	96	21	49.2
1-3 yr	76	27	35.5
3-5 yr	18	5	27.8
Total	190	53	27.9

**Table II.** Drug resistance pattern

Numbers	Drugs tested	% resistance
Single	Co-trimoxazole	41.5
Double	Co-trimoxazole + tetracycline	20.7
	Penicillin + co-trimoxazole	7.5
Multiple	Penicillin + co-trimoxazole + tetracycline	3.7
	Penicillin + co-trimoxazole + ceftriaxone	1.8
	Erythromycin + co-trimoxazole + tetracycline	3.7
	Co-Trimoxazole + tetracycline + ceftriaxone	3.7
	Penicillin + co-trimoxazole + tetracycline + ceftriaxone	1.8
	Erythromycin + co-trimoxazole + tetracycline + ceftriaxone	3.7

reservoir for horizontal dissemination of the pathogen within the community<sup>3</sup>. Therefore, strategies to prevent pneumococcal disease need to focus on prevention of nasopharyngeal colonization especially in children. Industrialized countries like Israel, Finland have reported nasopharyngeal carriage rates ranging from 43-90 per cent<sup>5,6</sup>. Studies conducted in various regions of India have found similar carriage of 6.5-83 per cent<sup>7-9</sup>. There are many factors that are independent determinants for nasopharyngeal colonization like ethnicity, age, environmental features, season and antibiotic usage practices. Some of the identified specific risk factors for carriage establishment are the presence of children in the household, day-care attendance, overcrowded living conditions, upper respiratory tract infections and rural residence<sup>10,11</sup>. The carriage rate in our study was higher than the results reported from Lucknow (20.2%)<sup>7</sup>. There was a progressive decrease of carriage rate with increasing age of children similar to other reports<sup>12-14</sup>.

Treatment of infections due to *S. pneumoniae* has become a complicated global problem due to antibiotic resistance. Antibiotic resistance patterns found among carriage specimens provide a rough estimate of those found among invasive isolates<sup>15</sup> which indicates that pneumococcal carriage studies may be used for monitoring resistance patterns<sup>16</sup>. The risk factors for development of antibiotic resistance

in pneumococci are use of antibiotic particularly at low levels for prolonged periods, easy availability over the counter and wider, indiscriminate use in the hospital as well as in the community. The emergence of resistance to penicillin in 1980's led to the increased use of macrolides, flouroquinolones and other drugs<sup>17</sup>. While these antibiotics provided effective alternate therapy for *S. pneumoniae* infections, selective pressure associated with the widespread use of these antibiotics, has resulted in resistance to various classes of antimicrobial agents.

In our study, the penicillin resistance rate was found to be 16.9 per cent and all isolates were intermediately resistant. Penicillin resistant pneumococci in various studies from India have been reported to be from 0-15.4 per cent<sup>7,11,17,18</sup>. In these studies, high level penicillin resistant strains were not detected. Studies in urban areas of Southeast Asia have found a much higher proportion of penicillin resistance, sometimes up to 60 per cent<sup>19</sup>. Increasing emergence of intermediate resistance of *S. pneumoniae* as observed in the present study is of great concern as it can cause spread of resistant strains in the community. This may lead to higher rate of treatment failure, additional cost of therapy and extra days of hospitalization. Moreover, it is also known that strain showing penicillin resistance can have genes responsible for resistance to other antibiotics<sup>15</sup>.

Excessive consumption, inappropriate and overuse of co-trimoxazole are possibly the contributory factors for high resistance in our study. Hence the empirical use of co-trimoxazole is not justified in this region. The nasopharyngeal isolates also demonstrated resistance to other antimicrobial agents like tetracycline, erythromycin, ciprofloxacin and cefotaxime.

Multi drug resistant *S. pneumoniae* is increasingly being reported from many parts of the globe<sup>19</sup>. The first MDR strain of *S. pneumoniae* was reported in 1977 from Johannesburg<sup>14</sup>. Penicillin susceptibility is an important marker for the presence or absence of a multi drug resistant phenotype. Strains with reduced susceptibility to penicillin are usually cross-resistant to other antibiotics also<sup>14</sup>. Prolonged carriage and rapid re-acquisition provide an increased chance of exposure to antibiotics and thus may be important selective factor in predisposing to antibiotic resistance<sup>21</sup>. In our study, 19 per cent isolates belonged to multi drug resistance group. Majority of our multi resistant isolates showed profile of resistance to penicillin, co-trimoxazole and tetracycline, and were found to be associated with four serotypes 6, 9, 14 and 19.

In the current study the predominant capsular serotype encountered was 19. A study conducted at Delhi also identified serotype 19 as the most common<sup>9</sup>. Other centres in India have identified serotypes 6 and 33 as the commonest highlighting the need to conduct prevalence studies in various regions<sup>17,22</sup>. In a study recently conducted in our centre it was observed that most prevalent serotypes in invasive pneumococcal disease in children below five years were 6, 14, 18, 5, 1, 3, 19, 9, 4, 10 and 15 in descending order; 81 per cent of SGTs found in nasopharyngeal carriage were also present in invasive pneumococcal group. Therefore, it can be concluded that most types of invasive *S.pneumoniae* are those found in nasopharynx. This finding is compatible with the concept that the types of *S.pneumoniae* that cause disease in man are usually derived from normal human flora<sup>21,23</sup>.

With the emergence of resistant pneumococcal strains specifically MDR and their isolation from healthy carriers is a reason for consideration of active immunization. Immunization with a conjugate pneumococcal vaccine has been reported to result in reduced carriage rate of pneumococcal serotypes included in the vaccine<sup>24</sup>. Further studies and analysis of the serotypes of nasopharyngeal isolates of different regions will provide useful information on the applicability of these conjugate pneumococcal vaccines.

The limitation of the study was its short duration and small number of children included from those attending paediatric OPD for immunization program.

In conclusion, a high rate of drug resistance was found in colonized *S. pneumoniae* in healthy children. A diversity was seen among the serotypes compared to other regions. Data obtained emphasize need to control the use of antibiotics and introduction of vaccines. The increasing penicillin and multidrug resistance has important clinical implications.

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