Distribution of Serotypes and Antimicrobial Resistance of *Streptococcus Pneumoniae* in a Children’s Hospital in Nepal

Sherchand JB1, Joshi AR2, Gauchan P3, Amatya J4

1Prof. Jeevan Bdr Sherchan, MSc Trop.Med. PhD (U.K.); Professor, Dept of Microbiology and Chief of Health Research Laboratory. 2Ashish Raj Joshi (MSc Medical Microbiology), College of Science and Technology, Kalimati, 3Punita Gauchan; (MSc Medical Microbiology) Research Officer: Tribhuvan University Institute of Medicine, Health Research Laboratory, Department of Clinical Microbiology, Maharajgunj, Kathmandu, Nepal, 4Jyoti Amatya; (MSc Medical Microbiology), Assistant Professor in Microbiology: College of Science and Technology, Kalimati, Nepal.

**Address for Correspondence:** Prof J.B. Sherchand. E-mail: itdrc@healthnet.org.np

**Abstract**

**Introduction:** *Streptococcus pneumoniae* in low number is a part of normal nasopharyngeal and oropharyngeal flora of many healthy persons and also children, which generally remains harmless unless provoked by viral infections such as common cold, influenza etc. In this situation, pneumococcus is secondary pathogen but may be primary pathogen in immunocompromised people. Bacterial colonization of nasopharynx starts immediately after birth without development of disease. **Objectives:** The study was conducted to determine the prevalence of nasopharyngeal colonization with *S pneumoniae*, to determine their antibiotic susceptibility pattern and to determine distribution of different serotypes. **Methods:** Hospital based prospective study was conducted from February 2007 to September 2007. All the specimens were inoculated into 7% sheep blood agar and incubated in 5 – 10% CO₂ atmosphere at 37 °C for 24 hours. *S pneumoniae* was identified by colony morphology, Gram’s stain, opochin susceptibility test and bile solubility test. Antibiotic susceptibility test was performed by modified Kirby-Bauer disc diffusion method. The *in vitro* susceptibility of the Oxacillin resistant isolates to Penicillin was determined by the E-test. Serotypes were determined by bacterial co-agglutination using the Pneumotest Kit (Statens Serum Institut, Denmark) with 12 pooled antisera. **Results:** In the study, 34.6% of the children were found to be nasopharyngeal carriers of *S pneumoniae* among which 50.8% were male and 49.2% were female. Cephotaxime (100%), Chloramphenicol (100%) and Erythromycin (98.5%) were most effective antibiotic against *S pneumoniae* and the least effective was Cotrimoxazole (60%). 15.4% were found to be resistant to Oxacillin. On performing E-test of Oxacillin resistant strains against Penicillin, all were found to be susceptible to Penicillin. The isolated strains were found to belong to 16 different serotypes whereas 8% were Non-typeable. **Conclusions:** Carriage rate is similar both in male and female. Penicillin resistance has not arisen in *S pneumoniae* isolated from nasopharynx which is causing problem world wide.

**Key words:** Children, Nasopharyngeal Swab, *S pneumoniae*, Serotype, E-test

---

**Introduction**

Respiratory tract infection is the major health problem in developing countries. Infection of the respiratory tract is the most frequent and important cause of short term illness in the population. It is frequently the first infection to occur after birth, and too often the final illness before death1.

Although, majority of these respiratory tract infections are viral in origin, secondary bacterial infection is also common, particularly in malnourished and very young. Resident bacteria of upper respiratory tract such as *S pneumoniae, H influenzae, S pyogenes* are the most common causative agents of respiratory tract infections. *S pneumoniae* is chiefly involved in infection of upper and lower respiratory tract. Some important respiratory tract infections that are caused by *S pneumoniae* are sinusitis, otitis media, bronchitis, pneumonia etc2.

Approximately 2.6 million children under 5 years of age die annually of pneumonia predominantly in
the developing world; approximately one half of these
deaths are attributable to *S. pneumoniae* either solely
or in conjunction with a viral respiratory infection,
malnutrition or HIV infection1.

People usually carry pneumococci without
symptoms, but under certain circumstances, this carriage
can also contribute to respiratory or even systemic
disease. Infection usually occurs in a person already
colonized with *S. pneumoniae*, when the bacteria invade
into the patient’s body. Several factors have considerable
impact on pneumococcal carriage and its rates4-5.

The bacteria *S. pneumoniae* carried in the naso-
pharynx of children reflect the infection causing strains
currently circulating in the community. So studies of
the prevalence of different pathogens and their resist-
tance patterns can provide useful indications for more
rational therapeutic and preventive strategies. The naso-
pharyngeal carriage of *S. pneumoniae* in young children
has been related to the development of disease and the
spread of the pathogen and it has been found to belong
to a limited number of serotypes that are also some of
the most common cause of invasive pediatric diseases6.
Considering these facts, the current study was carried
out.

**Methods**

A total of 188 children of age between 2 months
and 5 years attending out patient department of Kanti
Children’s Hospital were included in the study after taking
consent from guardian of a child and also from the child
whenever possible. Nasopharyngeal swab specimens
were collected using specifically designed pediatric
sized swab of thin flexible aluminum shaft tipped with
Dacron polyester (Copan Diagnostics Corona, Ca USA)
and transported to Health Research Laboratory, Institute
of Medicine, Tribhuvan University Teaching Hospital
in Skim milk tryptone glucose glycerin (STGG). In the
laboratory, the specimens were vortexed and inoculated
on blood agar supplemented with 7% sheep blood and
incubated at 37 °C for 24 hours in 5 – 10% CO₂
atmosphere. *S. pneumoniae* was identified by colony
morphology, Gram’s stain, optochin susceptibility test
and bile solubility test. Antibiotic susceptibility test
was performed by modified Kirby-Bauer disc diffusion
method on Mueller-Hinton Agar supplemented with
7% sheep blood and interpreted according to National
Committee for Clinical Laboratory Standards (NCCLS)
recommendation. E-test of Oxacillin resistant strains of
pneumococci was performed against Penicillin by using
E-test strip of Penicillin (AB Biodisk North America,
Inc) and interpreted according to NCCLS. Serotyping of
the isolated pneumococci was done by coagglutination
method using Pneumotest kit with 12 pooled antisera.
it was based on capsular reaction due to interaction
between pneumococcal capsular polysaccharide and its
homologous antibody.

**Results**

Of 188 specimens investigated for *S. pneumoniae*,
65 (34.6%) showed the growth of *S. pneumoniae*. Out of
the 65 isolates, 33 (50.8%) were male and 32 (49.2%) were female (Figure 1). Gender wise distribution of na-
sopharyngeal carriage of *S. pneumoniae* was statistically
not significant (P> 0.05).

**Fig. 1:** Showing children enrolled and gender wise distribution of *S. pneumoniae* among them.
Fig. 2: Showing antibiotic susceptibility pattern of nasopharyngeal pneumococcal isolates.

Cephotaxime, Chloramphenicol and Erythromycin were found to be most effective drugs against the isolates. Cotrimoxazole was the least effective drug. 15.4% of the isolates were found to be Oxacillin resistant (Figure 2).

On performing E-test of Oxacillin resistant strains against Penicillin, Minimum Inhibitory Concentration (MIC) of Penicillin was found to lie below 2 μg/ml indicating these isolates to be susceptible to Penicillin.

The isolated strains of *S. pneumoniae* were found to belong to 16 different serotypes whereas 8% of the isolates were Non-Typeable. Serotypes 19, 6, 15 and 8 were the most common (Table 1).

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>12</td>
<td>18%</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>18%</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>9%</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>8%</td>
</tr>
<tr>
<td>23</td>
<td>4</td>
<td>6%</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>6%</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>5%</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>5%</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>5%</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>3%</td>
</tr>
<tr>
<td>18</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>17</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>Non-Typeable</td>
<td>5</td>
<td>8%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>65</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

**Table 1: Showing distribution of different serotypes of the isolated *S. pneumoniae*.**

Discussion

Out of 188 nasopharyngeal swab specimen processed, 65 (34.6%) of them showed growth of *S. pneumoniae*. Although a comparison of pneumococcal carriage rates between studies is difficult due to variable methodological factors, the pneumococcal carriage rate was a little bit lower in this study when compared to the study carried out by Todar in which, nasopharyngeal colonization with pneumococci was found in 40% of the cases7.

Among 65 positive subjects, 50.8% were male carriers and 49.2% were female carriers indicating absence of correlation between gender and nasopharyngeal colonization with pneumococci. The result can be correlated to the findings of Malla *et al* in which 49% were female and 51% were male carriers8.

All the isolated pneumococci were found to be susceptible to Cephotaxime and Chloramphenicol. Erythromycin was also effective drug of choice since only 1.5% were found to be resistant to it. Tetracycline could also be used for therapy since only 9.2% were resistant to it.

Cotrimoxazole showed lowest susceptibility to isolated pneumococci with 40% isolates resistant to it. In a similar study, Cotrimoxazole was the least effective drug against *S. pneumoniae*9. In addition to this, two studies in Pakistan had found Cotrimoxazole to be ineffective in one third of patients of pneumonias and children under age of 1 year were especially susceptible to treatment failure. Cotrimoxazole was recommended by W.H.O. for treatment of infections caused by *S. pneumoniae* due to its lesser side effects, lower cost and easy availability. However, majority of *S. pneumoniae* in South Asia are now Cotrimoxazole resistant raising the question of whether W.H.O. should shift from Cotrimoxazole to more expensive drug for treatment10.

There are several possible mechanisms of development of resistance in pneumococci such as due to single point mutation, transformation (uptake and chromosomal exchange of free DNA from closely related strains or species), conjugal transposons (transfer and genetic incorporation of small segments of DNA during bacterial fusion events) etc10.
Oxacillin, though it is not used in therapy, was used for predicting the resistance of *S pneumoniae* against Penicillin due to its higher resistance to degradation during long term storage and 15.4% of the isolates were found to be Oxacillin resistant. Oxacillin resistance was high in the study when compared to 5.1% in study done by Malla et al.9.

On performing E-test of Oxacillin resistant strains against Penicillin, MIC of two Oxacillin resistant pneumococcal isolates was found to be moderately susceptible and that of remaining eight was found to be susceptible to Penicillin. Thus, none of the isolates were found to be Penicillin resistant. E-test of Oxacillin resistant strains of *S pneumoniae* was performed against Penicillin since disc testing of Oxacillin resistance for *S pneumoniae* is not sufficient to distinguish between complete and partial resistance against Penicillin. Penicillin resistance among pneumococcal isolates in Asia has also emerged and is gradually increasing resulting treatment failure6.

Information on the regional distribution of pneumococcal serotype is essential for the development and use of appropriate pneumococcal vaccine in developing countries11. Determining the serotype of *S pneumoniae* is important as the vaccine production is based on the most common serotypes. The isolated *S pneumoniae* were found to belong to 16 different serotypes which are shown in table 1. Serotype 1 is regarded as the most invasive strain. Serotypes 1, 3, 5, 6, 14, 19 and 23 are considered comprehensive types in invasive pneumococcal infections. Except serotype 1, all serotypes considered comprehensive type in pneumococcal infections were encountered in the study. Serotypes 19, 6, 15, 23, 9, 11, 8, 7, 17, 20 and 22 are chiefly involved in nasopharyngeal colonization in children22.

**Conclusion**

There was found to be no correlation between nasopharyngeal carriage of *S pneumoniae* and gender of children. Cotrimoxazole was found to be the least effective drug for treatment. MIC of Oxacillin resistant strains against Penicillin lied in susceptible and moderately susceptible region indicating absence of Penicillin resistance. Anti pneumococcal polysaccharide vaccine (23-valent) covers all the isolated serotypes of pneumococci. Protein-polysaccharide (Conjugate) vaccine suppresses nasopharyngeal carriage of *S pneumoniae*. Thus, conjugate vaccine immunization followed by polysaccharide vaccine boosting might provide a foundation for life long protection against pneumococcal disease.

**Acknowledgement**

We are thankful to Dr. Chris Coles, Assistant Professor, Johns Hopkins Bloomberg School of Public Health and NNIPS family for their generous support to the study. We would also like to acknowledge staffs of Health Research Laboratory, Institute of Medicine, Tribhuvan University Teaching Hospital and Kanti Children’s Hospital for their cooperation throughout the study.

**References**