

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

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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, optochin 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 infection³.

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 rates^{4,5}.

The bacteria *S pneumoniae* carried in the nasopharynx of children reflect the infection causing strains currently circulating in the community. So studies of the prevalence of different pathogens and their resistance patterns can provide useful indications for more rational therapeutic and preventive strategies. The nasopharyngeal 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 nasopharyngeal 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.

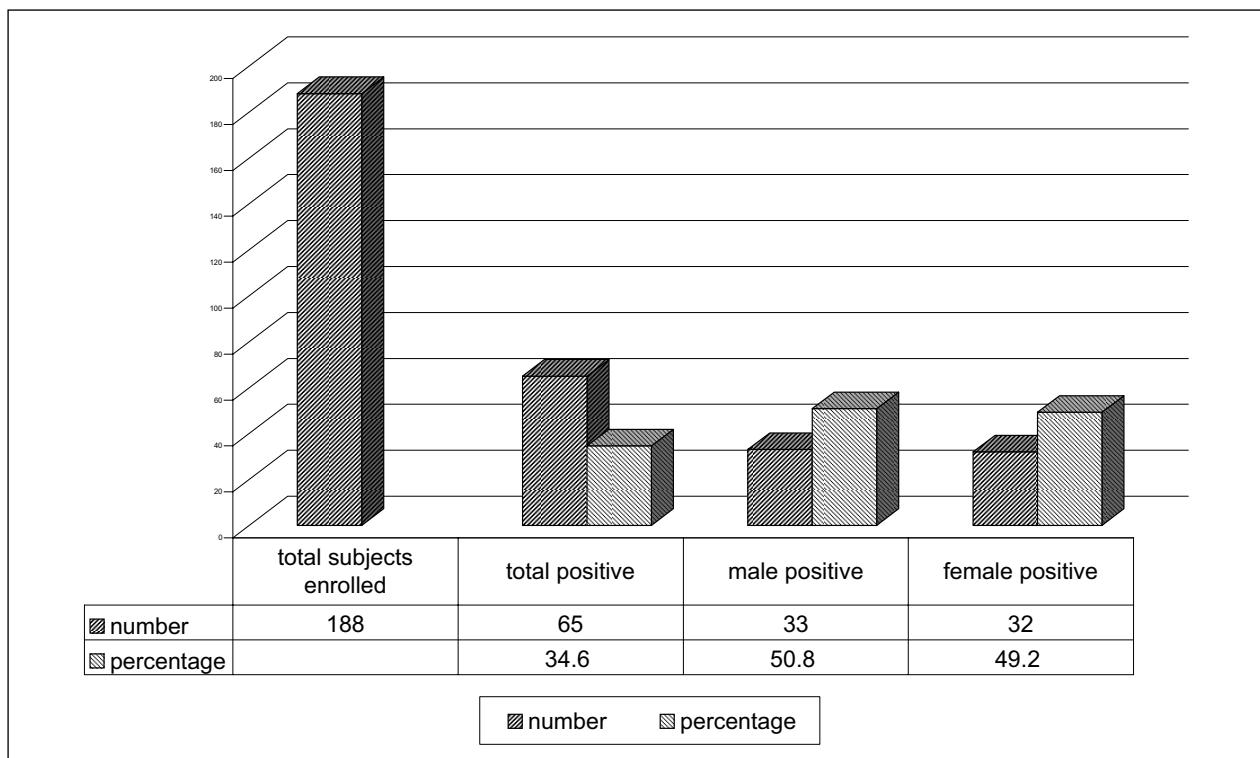
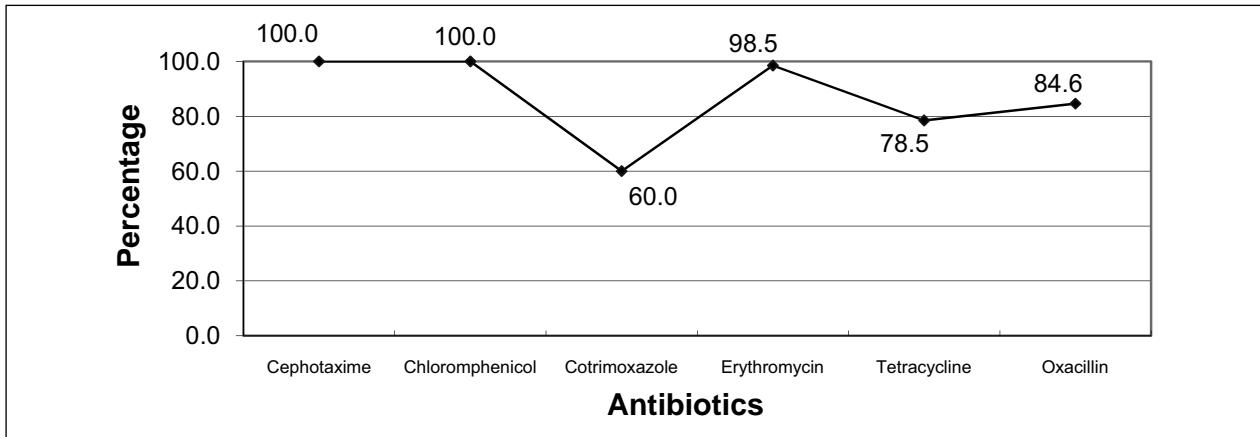


Fig. 2: Showing antibiotic susceptibility pattern of nasopharyngeal pneumococcal isolates.



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

Serotypes	Number	Percentage
19	12	18%
6	12	18%
15	6	9%
14	5	8%
23	4	6%
11	4	6%
20	3	5%
7	3	5%
3	3	5%
12	2	3%
18	1	2%
17	1	2%
10	1	2%
9	1	2%
8	1	2%
5	1	2%
Non-Typeable	5	8%
Total	65	100%

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*⁸. In addition to this, two studies in Pakistan had found Cotrimoxazole to be ineffective in one third of patients of pneumoniae 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 treatment⁹.

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), conjugative 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*⁸.

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 failure⁹.

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 children¹².

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.

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