



Antimicrobial Susceptibility Pattern of *Streptococcus pneumoniae* among Healthy Carrier Children under Five Years Old attended at Outpatient Department of Largest Teaching Hospital in Bangladesh

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Abstract

Background: Over the past several decades, antimicrobial resistance in *Streptococcus pneumoniae* has dramatically increased worldwide. **Objectives:** The purpose of the present study was to detect and monitor the antibiotic susceptibility pattern of *Streptococcus pneumoniae* among under five years old children as carrier. **Methodology:** The cross-sectional study was conducted in the Department of Microbiology of Dhaka Medical College Hospital (DMCH), Dhaka, Bangladesh from July 2016 to June 2017 Data were collected among 200 under five children from Pediatric OPD of Dhaka Medical College Hospital. *Streptococcus pneumoniae* were isolated and identified by culture, Gram staining and biochemical test. Antimicrobial susceptibility test for *S. pneumoniae* was performed by disc-diffusion method. **Results:** Out of 200 nasopharyngeal swabs, 67(33.50%) isolates were positive by culture. Among 67 isolated *Streptococcus pneumoniae*, all the isolates were susceptible to penicillins, cephalosporins, glycopeptides and carbapenems. Among the isolated *Streptococcus pneumoniae*, 92.53% isolates were susceptible to ciprofloxacin, 52.16% isolates were susceptible to erythromycin and azithromycin. *Streptococcus pneumoniae* showed very low susceptibility to gentamicin (11.94%) and co-trimoxazole (23.88%). **Conclusion:** This study has shown the antimicrobial susceptibility pattern of *Streptococcus pneumoniae* in children as carriers. [Bangladesh Journal of Infectious Diseases, June 2021;8(1):12-17]

Keywords: Antimicrobial agent; susceptibility; *Streptococcus pneumoniae*; invasive pneumococcal diseases (IPD); pneumococcal vaccine

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Introduction

Streptococcus pneumoniae is a major cause of pneumonia, meningitis, and other invasive diseases resulting in high mortality and morbidity among children under the age of five, particularly in lower income countries¹. *Streptococcus pneumoniae* is a bacterium that colonizes the nasopharynx of human and main source is person to person transmission². *Streptococcus pneumoniae* colonization is often asymptomatic but may cause overt infections. Community-acquired pneumonia (CAP) and infections of normally sterile sites (pleural fluid, cerebrospinal fluid and blood) are the most common infections by *Streptococcus pneumoniae* which are collectively called invasive pneumococcal disease (IPD)³. It is spread by respiratory droplets and children are the main source of transmission to adults. Universally, carriage rates are highest in young children (40.0 to 60.0%), compared with older children (12.0%), adolescents (6.0% to 10.0%) and adults (3.0% to 4.0%) pathogenesis of invasive pneumococcal disease (IPD) begins with nasopharyngeal (NP) colonization that proceeds, often through local infection, to blood stream invasion⁴.

Antimicrobial resistance in *Streptococcus pneumoniae* remains a serious concern worldwide, particularly in Asian countries⁵. The disease burden of pneumococcal infections has increased due to widespread emergence of antimicrobial resistance in many countries during the past few decades⁶. Some reports have documented very high prevalence rates of beta-lactam and macrolide resistance in *Streptococcus pneumoniae* in Asian countries^{7,8}. In addition, multidrug resistance (MDR) has also been very much prevalent in Asian countries which is much higher than those in other parts of the world⁹. Multidrug resistance to β -lactam antibiotics, macrolides and fluoroquinolone is an emerging problem and complicating the management of CAP¹⁰. In recent years rapid penicillin resistance has been increasing. *Streptococcus pneumoniae* resistant to penicillin and macrolides is a public health concern in Bangladesh and some other Asian countries. Infections caused by resistant microbes fail to respond to treatment, resulting in prolonged illness and greater risk of death. Treatment failures also lead to longer periods of infectivity, which increase the numbers of infected people moving in the community and thus expose the general population to the risk of contracting a resistant strain of infection¹¹.

In order to combat the increasing incidence of resistance as well as increasing disease prevalence,

pneumococcal vaccines (PCV-7, PCV-10, PCV-13, PPV-23) have been made available as preventive tools. These vaccines constitute those strains that cause 80% of the invasive pneumococcal disease (IPD) and are resistant to antibiotics¹². WHO-GAVI (World Health Organization & Global Alliance for Vaccines and Immunization) alliance has approved 3 conjugate vaccines e.g. PCV-7, PCV-10, and PCV-13 for use in children. These vary in the serotypes contained and the proteins used for conjugation. The introduction of these vaccines in the United States and Western Europe has decreased the incidence of vaccine strain associated invasive pneumococcal disease significantly. Whilst vaccination has had success in reducing the burden of disease caused by vaccine-type *Streptococcus pneumoniae*, ongoing surveillance is important to monitor trends in frequency of children as carrier and their exposure to antibiotic favors the selection of drug-resistant pneumococcal carriers. Different antimicrobial susceptibility pattern of *Streptococcus pneumoniae* also provide supportive implications for the proper treatment of *Streptococcus pneumoniae* induced infections. The purpose of the present study was to detect and monitor the antibiotic susceptibility pattern of *Streptococcus pneumoniae* among under five years old children as carrier.

Methodology

Study Settings and Population: The cross-sectional study was conducted in the Department of Microbiology of Dhaka Medical College Hospital (DMCH), Dhaka, Bangladesh from period of July 2016 to June 2017. Nasopharyngeal swabs were collected from healthy children aged one month to less than five years who attended the outpatient department of DMCH for routine immunization, child growth monitoring and nutritional advice. Nasopharyngeal swabs were collected, labeled and placed immediately in one ml of skim milk-tryptone-glucose-glycerol (STGG) medium and transported to the laboratory.

Ethical Clearance: This protocol was approved by Research Review Committee (RRC) of Microbiology Department of Dhaka Medical College, Dhaka in Bangladesh. Informed written consent was obtained from the parents or legal guardians of each child before sample collection.

Culture and Isolation: The NPS-STGG specimens were inoculated on blood agar using one loop (10 μ l) of sample. The plates were streaked into four quadrants and incubated at 37⁰ C for 24 hours with CO₂ atmosphere inside a candle jar.

Identification of *Streptococcus pneumoniae*:

Small, smooth and transparent colonies were seen on blood agar plate. Colonies were low convex, tiny and they became flattened centrally showing the 'draughtsman form'. A narrow zone of α hemolysis was seen around the colonies. Gram positive diplococci were seen which were ovoid or lanceolate in shape. Catalase negative. The isolates with presumptive identification were confirmed by optochin sensitivity test and bile solubility test (Table 1).

Table 1: Identification of *Streptococcus pneumoniae* and its difference with Viridans *Streptococcus*

Characteristic	<i>Streptococcus pneumoniae</i>	Viridans <i>Streptococcus</i>
Colonies	Flattened, draughtsman	Convex
Effect on BA	Narrow zone of alpha haemolysis	Narrow zone of alpha haemolysis
Optochin Sensitivity	Positive	Negative
Bile solubility	Positive	Negative

Antimicrobial Susceptibility Test: All *Streptococcus pneumoniae* isolates were tested for antimicrobial susceptibility by Kirby-Bauer modified disc-diffusion technique following CLSI guidelines (CLSI, 2015). The antimicrobial discs were used according to the standard antibiotic panel for isolated organisms. Antibiotic discs were obtained from commercial source (Oxoid Ltd, UK). Oxacillin (1 μ g/disc) was used for amoxicillin, ampicillin, amoxicillin-clavulanate, ceftriaxone, cefotaxime, cefuroxime and imipenem. Other antimicrobial disc were erythromycin (15 μ g/disc), azithromycin (15 μ g/disc), gentamicin (10 μ g/disc), co-trimoxazole (25 μ g/disc), vancomycin (30 μ g/disc), ciprofloxacin (5 μ g/disc). Mueller-Hinton with 5.0% sheep blood agar plate was used for antimicrobial susceptibility test by disc diffusion method. A representative of each batch of disc was tested with WHO reference strains of *Staphylococcus aureus* ATCC25923 to verify their optimum performance. The test with the antimicrobial discs was repeated at least three times for individual isolate and the mean of the results was recorded.

Statistical Analysis: Statistical analyses were performed with SPSS software, versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were summarized in terms of the mean, standard deviation

and number of observations. Categorical or discrete data were summarized in terms of frequency counts and percentages.

Results

Total 200 under five children were tested, among them, 67(33.50%) were positive for *Streptococcus pneumoniae* by culture (Table 2).

Table 2: Results of culture of *Streptococcus pneumoniae* from nasopharyngeal swabs (n=200)

Culture Results	Frequency	Percent
Positive	67	33.5
Negative	133	66.5
Total	200	100.0

Out of 200 nasopharyngeal swabs, 67(33.50%) isolates were positive by culture. Among 67 isolated *Streptococcus pneumoniae*, all the isolates were susceptible to penicillins, cephalosporins, glycopeptides and carbapenems. Among the isolated *Streptococcus pneumoniae*, 92.53% isolates were susceptible to ciprofloxacin, 52.16% isolates were susceptible to erythromycin and azithromycin. *Streptococcus pneumoniae* showed very low susceptibility to gentamicin (11.94%) and co-trimoxazole (23.88%) (Figure 1).

Discussion

Streptococcus pneumoniae is a bacterium that colonizes in the nasopharynx of human and most common cause of pneumonia and other invasive pneumococcal diseases (IPD). The capsular polysaccharide, a well-known virulence factor and the serotype determinant of pneumococci that prevents opsonization and phagocytosis of *Streptococcus pneumoniae*. Pneumococcal capsular vaccines protect against disease and nasopharyngeal carriage due to the serotypes included in the vaccine formulations. Nevertheless, surveillance of pneumococcal carrier prevalence with antimicrobial susceptibility patterns is very important since the serotypes responsible for invasive disease can change over time¹³. To reduce the very high mortality associated with *S. pneumoniae* in children we need protocols that are simple but effective. In this present study, 13 different antimicrobial agents were tested on isolated *Streptococcus pneumoniae* to study antimicrobial susceptibility pattern. Among 67 isolated *Streptococcus pneumoniae* 100% were susceptible to penicillin, amoxicillin and ampicillin.

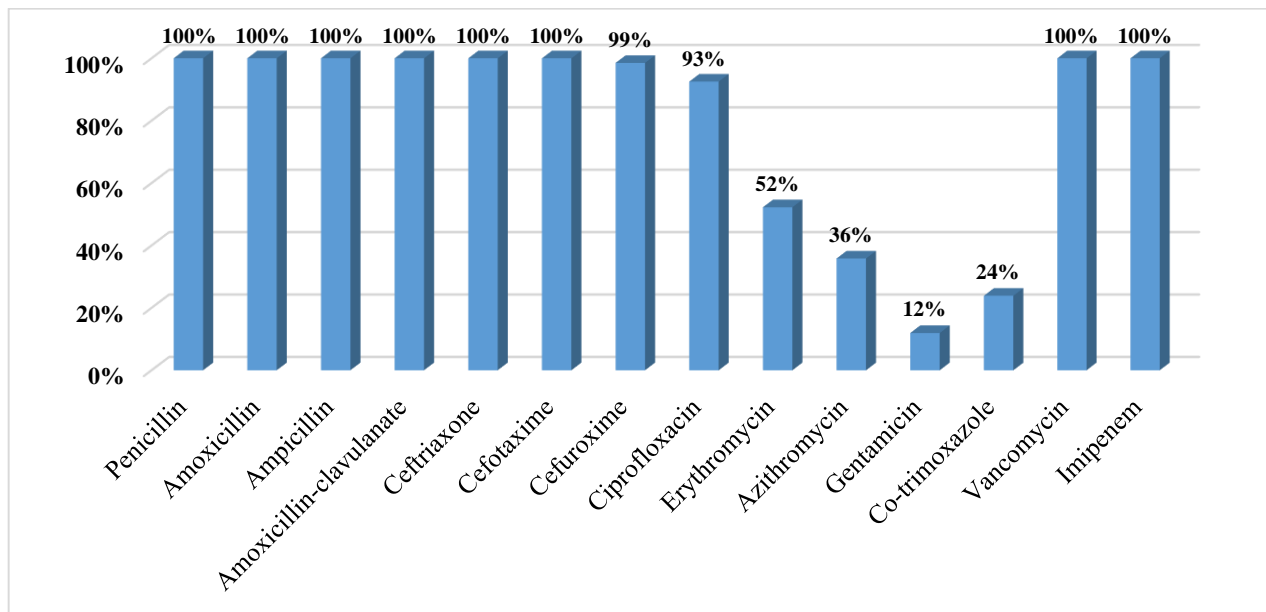


Figure I: Sensitivity pattern of isolated *Streptococcus pneumoniae* from nasopharyngeal swabs (n=67)

In a study in Bangladesh¹⁴ reported that all the stains of *Streptococcus pneumoniae* were susceptible to penicillin. A study in India¹⁵ and another study by in Nepal¹⁶ also reported that none of their isolated *Streptococcus pneumoniae* were resistant to penicillin. Both the findings of previous studies and the present study are similar. A study reported the changing trend in penicillin sensitive *Streptococcus pneumoniae* (PSSP) in Asian countries. Comparison to the current study, in children, prevalence of PSSP was as low as 0% in China and Iran, while the highest susceptibility rate was seen in Bangladesh, India and Japan (100%)¹⁷.

Several studies had shown geographical differences in the prevalence of resistant serotypes of *Streptococcus pneumoniae*. Children younger than 5 years are more frequently infected with these penicillin resistant strains¹⁸. It had been reported that the spread of penicillin resistant strains of *Streptococcus pneumoniae* in different Asian countries might be derived by the selective pressure created by excessive use, miss use or even easily availability of this agent¹⁹.

Non-susceptibility to penicillin among *Streptococcus pneumoniae* is also widespread in the USA and many countries in Europe²⁰. The emergence and rapid dissemination of antibiotic-resistant pneumococcal clones in area of southern and Eastern Europe, North America, South America and the frequency of children as carriers and their exposure to antibiotics favors the selection of drug-resistant strains²¹.

Several studies have shown geographical differences in the prevalence of resistant serotypes and antimicrobial resistance in Europe²². However, this is not yet a common scenario in Bangladesh and some other parts of the Asian countries. In the present study, penicillins were the most susceptible and effective antimicrobial agent against 100% *Streptococcus pneumoniae*. The reasons might be due to, the distribution of non-resistant serotypes in Bangladesh¹⁸. Besides, as penicillin is a narrow-spectrum, old antimicrobial agent and it is not used as an empirical therapy in case of meningitis and pneumonia. Physicians give preference on broad-spectrum antibiotics in cases of meningitis and pneumonia to cover all organisms. Penicillins are only used after knowing whether organism is sensitive to penicillin or not. In the present study, *Streptococcus pneumoniae* also displayed 100.0% susceptibility to cephalosporins, vancomycin and imipenem. High susceptibility to these antimicrobial agents had been observed in many previous studies²³⁻²⁵.

All the results of previous studies and the results of the present study are similar. In the present study higher susceptibility was found. The reasons might be due to the fact that these antimicrobial agents are injectable and more expensive, so without proper indication and physician recommendation, use of these drugs in children is less common, moreover in the present study data were collected from OPD, where indiscriminate use of this category of drug is not available.

In the present study, 11.94% to 52.16% *Streptococcus pneumoniae* displayed antimicrobial susceptibility to gentamicin, co-trimoxazole, azithromycin and erythromycin. High antimicrobial resistance to these antimicrobials has been observed in many studies²⁶⁻²⁷. Regarding susceptibility pattern of *Streptococcus pneumoniae* to these common antibiotics was observed a decreasing trend. The antibiotic resistance in Bangladesh and developing countries commonly occurs due to inappropriate antibiotic use, over-prescribing and inappropriate prescribing, unethical practices of health professionals, unqualified drug sellers offer alternative drugs when the prescribed drugs are not available²⁸.

There are some limitations of this study. However, this is not yet a common scenario in Bangladesh. All susceptible strains were collected from only healthy carrier not from diseased children who received antibiotics before collection of specimens.

Conclusion

In conclusion all isolated *Streptococcus pneumoniae* are susceptible to penicillins, cephalosporins, glycopeptides and carbapenems which has implications for treatment and in replacement/unmasking following PCV introduction. However, existing and forthcoming pneumococcal vaccines can have meaningful and substantial impact on antimicrobial susceptibility pattern among healthy children. It is important to continue surveillance for antimicrobial susceptibility among younger children who are potential pneumococcal carrier and for the treatment of various IPD with common antimicrobial agents.

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