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Pneumococcal Carriage Recovered from Healthy Children and Their Possible Association with Some Risk Factors in Outpatient Department of a 1000 bedded Tertiary Care Hospital at Dhaka City

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Abstract

Background: Detection and monitoring of nasopharyngeal carriage of Streptococcus pneumoniae is important to assess the impact and effectiveness of pneumococcal vaccine programs. **Objective:** The aims of this study were to assess the nasopharyngeal colonization rate, investigate some of the risk factors for nasopharyngeal colonization with S. pneumoniae from healthy children. **Methodology:** The study was conducted in the department of microbiology of Dhaka Medical College Hospital (DMCH). Data were collected among 200 under five healthy children in different age group (13 months to 36 months), from different socioeconomic status with cramped housing condition from Pediatric OPD of Dhaka Medical College Hospital. S. pneumoniae were isolated and identified by culture, Gram staining, biochemical test and polymerase chain reaction (PCR). **Result:** Out of 200 nasopharyngeal swabs, 67 (33.50%) were found to be carriers positive by culture and 92(46%) by PCR. The carrier rate was higher among 13 months to 36 months, low and middle socio-economic groups and among with cramped housing condition. **Conclusion:** In conclusion various factors may affect the nasopharyngeal colonization with S. pneumoniae including early age of life, different socio-economic and living condition. [*Bangladesh Journal of Infectious Diseases, December 2019; 6(2):48-52*]

Keywords: Nasopharyngeal colonization; *Streptococcus pneumoniae*; risk factors; invasive pneumococcal diseases; 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 countries[1]. The World Health Organization estimated that there are nearly one million deaths each year in children younger than five years of age and one child under five years of age dies because of pneumococcal pneumonia in every 20 seconds[2]. *Streptococcus pneumoniae* is a bacterium that colonizes the nasopharynx of human and main source is person to person transmission[3].

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[4]. 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 invasion[5]. Although almost all children become colonized with *Streptococcus pneumoniae* repeatedly during the first few years of life, a very small fraction of these acquisitions results in invasive disease. Colonization rates are higher (>50.0%) in situation of overcrowding such as in day-care centers, orphanages, slums and in indigenous population[6].

The aims of the study were to assess the nasopharyngeal colonization rate as well as to investigate the risk factors for colonization with *Streptococcus pneumoniae* for continuous monitoring of *Streptococcus pneumoniae* carriage for future pneumococcal vaccination program to prevent pneumococcal diseases.

Methodology

This cross sectional study was conducted in the Department of Microbiology of Dhaka Medical College Hospital (DMCH), Dhaka, Bangladesh. 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. The NPS-STGG specimens were inoculated on blood agar using one loop (10µl) of sample. The plates were streaked into four quadrants and incubated at 37^0 C for 24 hours with CO_2 atmosphere inside a candle jar. 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 alfa hemolysis was seen around the colonies. *Streptococcus pneumoniae* were isolated and was identified. Gram positive diplococci were seen which were ovoid or lanceolate in shape as well as catalase negative. The isolates with presumptive identification were confirmed by optochin sensitivity test and bile solubility test. PCR was also done directly from nasopharyngeal swabs. Samples preserved in STGG medium were brought out from freeze and kept them at room temperature to demoisture and samples were vortexed to make a homogenous suspension. Then removed the swab sticks and the vortexed specimens were taken into two micro centrifuge tubes, labeled proper centrifuged at 10,000 X g for 10 minutes and the supernatant was discarded. The deposit was used as pellet for PCR. The micro centrifuge tubes containing pellet were kept at -20℃ until DNA extraction. During DNA extraction, two hundred micro litter of lytic buffer was mixed with the sample pellets and vortexed until mixed well. Then the tubes were incubated at 60℃ for 3 hours. After incubation, tubes were kept in heat block at 100℃ for 10 minutes for boiling. Then the tubes were immediately placed on ice for 5 minutes. After that the tubes were centrifuged at 4℃ at 14000 X g for 10 minutes. Finally supernatant was taken using micropipette and used as template DNA for PCR. This DNA was kept at -20℃ for future use[7]. The primer *cpsA* were used for targeted highly conserved gene that exists in all capsular loci thus far characterized[8].

Figure I: STGG media and STGG media with swab

Results

A total number of 200 cases under five children were tested and among them, 67(33.50%) cases were positive for *Streptococcus pneumoniae* by culture and 92 (46%) were positive by PCR (Table 1).

Table 1: Culture and PCR for *Streptococcus pneumoniae* from nasopharyngeal swabs (n=200)

Methods	Frequency	Percent
Culture positive	67	33.5
PCR positive	92	46.0
Not Positive	41	20.5

Out of 40 children in one month to 12 months age group, *Streptococcus pneumoniae* carriage was 13(32.50%) in culture and 21(52.50%) in PCR. Among 65 children of 13 months to 36 months age group, *Streptococcus pneumoniae* carriage was 29(44.62%) in culture and 38(58.46%) in PCR. Out of 95 children in 37 months to ≤ 60 months age group, *Streptococcus pneumoniae* carriage was 25(26.32%) in culture and 33 (34.74%) in PCR (Table 1).

Table 2: Results of Culture and PCR in Different Age Groups (n=200)

Age Group	Culture Positive	PCR Positive
1 to 12 months	13(32.50%)	21(52.50%)
13 to 36 months	29(44.62%)	38(58.46%)
37 to ≤ 60 months	25(26.32%)	33(34.74%)
Total	**67**	**92**

Out of 63 children of low income group, *Streptococcus pneumoniae* carriage was 24(38.10%) in culture and 33(52.38%) in PCR. Among 129 children of middle income group, *Streptococcus pneumoniae* carriage was 42(32.56%) in culture and 58 (44.96%) in PCR. (Table 3).

Table 3: *Streptococcus pneumoniae* among Study Population in Different Socio-Economic Groups (n=200)

Socio-economic groups	Culture Positive	PCR Positive
Low income	24(38.10%)	33(52.38%)
Middle income	42(32.56%)	58(44.96%)
High income	1 (12.50%)	1(12.50%)
Total	**67**	**92**

Among 117 cases, 32 (27.35%) were culture positive and 37 (31.62%) were PCR positive with family size below five. Out of 83 children, 35 (42.17%) were culture positive and 55 (66.27%) were PCR positive with family size above five. Out of 95 cases, 48 (50.53%) were culture positive and 64 (67.37%) were PCR positive with one room in their house. Among 105 cases, 19 (18.10%) were culture positive and 28 (26.67%) were PCR positive with two or more rooms in their house (Table 4).

Table 4: Culture and PCR Positivity among Study Population in Relation to Crowding Status (n=200)

Crowding Factors	Culture Positive	PCR Positive
Family Size		
<5 (N= 117)	32(27.35%)	37(31.62%)
≥5 (N= 83)	35(42.17%)	55(66.27%)
Total	**67**	**92**
Number of Room		
1	48(50.53%)	64(67.37%)
≥2	19(18.10%)	28(26.67%)
Total	**67**	**92**

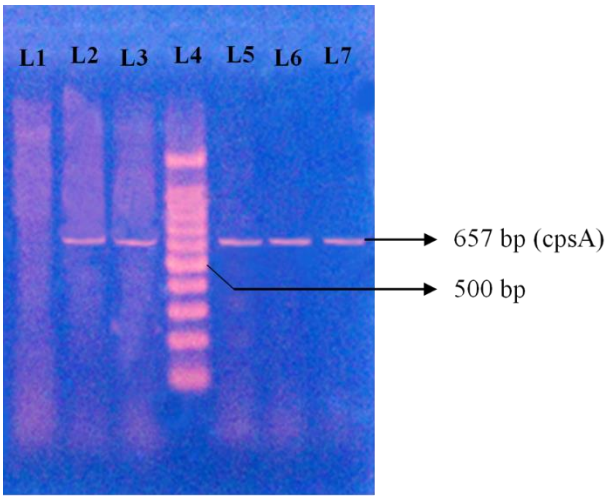

Figure II: Photograph of amplified *cpsA* gene of *Streptococcus pneumoniae*; Lane 1: negative control with DNA of *Staphylococcus aureus* ATCC25923; Lane 2, 3, 5, 6, 7: amplified DNA of 657 bp of *cpsA* gene; Lane 4: hundred bp DNA ladder

Discussion

Various factors may affect the nasopharyngeal colonization with *S, pneumoniae.* Various demographic characteristics had been described to associated with an increase in nasopharyngeal carriage of *Streptococcus pneumoniae* including young children[9,10] family size[11,12], less number of room in house[13,14], low socio-economic

condition[15,16]. In the present study, all of the aforementioned demographic characteristics had been described to be associated with an increase in *Streptococcus pneumoniae* carriage. A study in Ehtiopia[13] reported that *Streptococcus pneumoniae* showed an age-related downward trend. Children belonging to the age group of < 3 years were the most colonized, followed by those 3-5 years of age. The decline in *Streptococcus pneumoniae* carriage rate associated with increasing age may reflect the gradual acquisition of mucosal immunity and reduction of exposure. In the present study, carriage rare was more detected in those children who had > 5 members. In a study[13] reported that, children living with siblings (family size >5) showed significantly higher *Streptococcus pneumoniae* carriage compared with children who do not have any siblings. This finding was similar to reports from other studies[17,18] and from the present study. This is of great relevance because exposure to other children during childhood, especially to younger siblings, had been clearly associated with an increased risk for invasive and noninvasive pneumococcal diseases[13]. Moreover, a study by Assefa et al[13] also reported significantly higher *Streptococcus pneumoniae* nasopharyngeal carriages rates among children who lived with their family in a house with only one room and other children. This result is consistent with the present study. A study reported that in low and lower-middle income population, the prevalence of pneumococcal carriage was high in young children in contrast in upper-middle and high income population (93.4% vs 58%)[19]. This finding is similar with the present study. *Streptococcus pneumoniae* carriage was more associated with low socio-economic condition, reasons might be due to low socioeconomic status causes poor, overcrowding living conditions and also causes poor nutritional status and poor nutrition lead to lower immunity which prone to colonization of *Streptococcus pneumoniae*.

Streptococcus pneumoniae detection rate from nasopharyngeal swab was higher by PCR than culture which might be due to the fastidious nature of the organism and low load of the organism with co-colonization that can result in false negative results. PCR can detect bacterial DNA even if the numbers of organism is too low to grow in culture[20] and can detect from non-viable organisms after treatment with antibiotics[21].

Conclusion

Detection rate of nasopharyngeal carriage of *Streptococcus pneumoniae* was relatively more including with various factors may affect the nasopharyngeal colonization with *S. pneumoniae* using both culture and PCR. *Streptococcus pneumoniae* is a commensal species of the human upper respiratory tract, local or systemic infections are preceded by colonization, and colonized individuals also serve as a reservoir in the community, *Streptococcus pneumoniae* is a major etiological agent of non-invasive infections like acute otitis media (AOM) and severe invasive diseases like community acquired pneumonia (CAP), meningitis and bacteremia. So, surveillance programs should be carried out continuously to monitor the risk factors in order to crub the problem of developing pneumococcal diseases by early stage vaccination among these risk groups.

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