RESEARCH ARTICLE

Nasopharyngeal Carriage of *Streptococcus pneumoniae* among Children in relation to Pneumococcal Conjugate Vaccination during Infancy

Shormin M[1], Khandaker M[2], Ferdouse F[3]

Abstract :

Background: Nasopharyngeal carriage of Streptococcus pneumoniae is thought to be a risk for invasive pneumococcal diseases. Detection and monitoring of pneumococcal carriage and distribution of serotype is important to assess the impact and effectiveness of pneumococcal vaccine programs. Diversity among the serotypes and changes in the prevalence of serotypes over time are challenges in designing the ultimate pneumococcal vaccines. **Objectives:** To determine the predominant serotypes and to establish the trend of serotype changes so that the vaccination against predominant serotypes can be evaluated after its introduction. **Materials and Methods:** The study was conducted in the department of microbiology of Dhaka Medical College Hospital (DMCH).200 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.S. pneumoniae were isolated and identified by culture, Gram staining and biochemical test and PCR. **Result:** Out of 200 nasopharyngeal swabs, 92 (46%) were positive by PCR. Out of 200 children, 90 (45%) were received pneumococcal conjugate vaccine (PCV) and 110 (55%) were not vaccinated. Among vaccinated children, 4 (16%) S. pneumoniae were detected in fully vaccinated children and 25 (38.46%) S. pneumoniae were detected in partially vaccinated children by PCR. Among not vaccinated children, 63 (57.27%) S. pneumoniae were detected by PCR. The difference between fully vaccinated and non-vaccinated proportion is statistically significant ($p<0.001$) and the difference between fully vaccinated and partially vaccinated proportion is statistically significant ($p< 0.001$).Out of 4 fully vaccinated PCR positive cases, detected serotypes were 34F (1) and 35B (1) and 6B (1) among 3 children. Out of 25 PCR positive partially vaccinated children, detected serotypes were 34F (7), 35B (3), 6B (3), 14(3), 23(2) among 18 children. Out of 63 PCR positive not-vaccinated children, detected serotypes were 3 (3), 4 (1), 6A (11), 6B (7), 7F (1), 14 (4), 18C (1), 19A (2), 19F (2), 23F (3), 34F (8), 35B (8) among 51 children. **Conclusion:** This study showed detection of serotypes of S. pneumoniae from nasopharyngeal swabs in children may help to predict the shift in serotypes replacement following pneumococcal conjugate vaccination.

Keywords: Streptococcus pneumoniae (SPN), Invasive pneumococcal diseases (IPD), Pneumococcal conjugate vaccine, Vaccine type (VT), Non-vaccine type (NVT).

SMAMC Journal, 2024; 10(1):37-42

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

1. Dr. Moonmoon Shormin, MBBS, M. Phil, Assistant Professor, Department of Microbiology, Shaheed Monsur Ali Medical College, Dhaka
2. Dr. Mahmuda Khandaker, MBBS, M. Phil, Associate Professor and Head, Department of Microbiology, Shaheed Monsur Ali Medical College, Dhaka
3. Dr. Faria Ferdouse, MBBS, M. Phil, Associate Professor, Department of Microbiology, Shaheed Monsur Ali Medical College, Dhaka.

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]. S. pneumoniae is a bacterium that colonizes the nasopharynx of human and main source is person to person transmission[3,4]. S. 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 S. pneumoniae which are collectively called invasivepneumococcal disease (IPD)[5]. Ninety-two capsular serotypes of S. pneumoniae exist, and the prevalence of serotypes differs according to age, region, and time of the surveillance. The 92 serotypes differ in virulence; a minority of serotypes is involved in most of invasive pneumococcal diseases and antimicrobial resistances. Serotypes are classified into vaccine serotype (VT) and nonvaccine serotype (NVT). Vaccine serotype means a serotype included in PCV7 (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F), PCV10 (serotypes 1, 5, and 7F added to PCV7), PCV13 (serotypes 3, 6A, and 19A added to PCV10), and PPSV23 (serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F added to PCV13, except for 6A). Nonvaccine serotype is the serotype, which is not covered by PCV7, PCV10, PCV13, and PPSV236.According to a literature review, serotypes 1, 4, 5, 7F, 8, 12F, 14, 18C, and 19A are more likely to cause IPD. Although serotypes 1 and 19A are the predominant causes of invasive pneumococcal pneumonia, serotype 14 remains one of the most common etiologic agents of non-bacteremic pneumonia in adults, even after 7-valent pneumococcal conjugate vaccine (PCV7) introduction.The distribution of serotypes detected in Bangladesh has changed over time. From 1992 through 2007, serotypes 1, 2 and 5 have emerged as predominant serotypes, and serotypes 12F and 15B have become less common7. This natural emergence of certain serotypes indicates that conclusions about post-vaccination "serotype replacement" need to be made with caution. For example, emergence of serotype 2 in a study 7 might have been interpreted as replacement if its emergence had occurred after vaccine introduction,which could have led to serious public health concerns and a corresponding reduction in the use of the vaccine and thus might have jeopardized the benefits of vaccination. It will be important to monitor whether emergence serotypes disappear over time or remain as a predominant serotype. Because these serotypes consider as a potential candidate for inclusion in future pneumococcal vaccine formulations.Whilst vaccination has had success in reducing the burden of disease caused by vaccine-type S. pneumoniae8,9ongoing surveillance is important to monitor trendsin serotype distribution as the benefits of vaccination could be offset by increased rates of pneumococcal disease caused by non- vaccine serotypes 10.

Materials and Methods :

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 skimmilk-tryptone-glucose-glycerol (STGG) medium and transported to the laboratory. The NPS-STGG specimens were thawed at room temperature (25°C) and vortexed for 10-20 seconds. Then specimens were inoculated on blood agar using one loop (10µl) of sample. The plates were streaked and incubated at 37°C for 24 hours with CO2 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 hemolysis was seen around the colonies. Colonies are Catalase negative andGram-positive diplococci were seen which were ovoid or lanceolate in shape.The isolates with presumptive identification were confirmed by optochin

sensitivity test, bile solubility test and PCR. Serotyping by PCR The primer cpsA were used for targeted highly conserved gene that exists in all capsular loci thus far characterized. Thirty eight specific primers were used for targeted genes specific for serotypes 1, 2, 3, 4 , 5, 6A, 6B, 7F, 9V, 11A, 12F, 14, 18C, 19F, 19A, 23F, 33F, 34F and 35B. Of these serotypes 1, 2, 3, 4 , 5, 6A, 6B, 7F, 9V, 11A, 12F, 14, 18C, 19F, 19A, 23F,33F were vaccine type (VT) and serotypes 34F, 35B were non vaccine (NVT) type. Vaccine serotype means a serotype included in PCV7 (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F), PCV10 (serotypes 1, 5, and 7F added to PCV7), PCV13 (serotypes 3, 6A, and 19A added to PCV10), and PPSV23 (serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F added to PCV13, except for 6A. Nonvaccine serotype is the serotype which is not covered by PCV7, PCV10, PCV13, and PPSV23. An initial screening of the nasopharyngeal swabs were done by the primer cpsA to identify the pneumococci by monoplex PCR. Then primers that are serotype specific were used for serotyping by multiplex PCR with positive and negative controls (Figure 1).

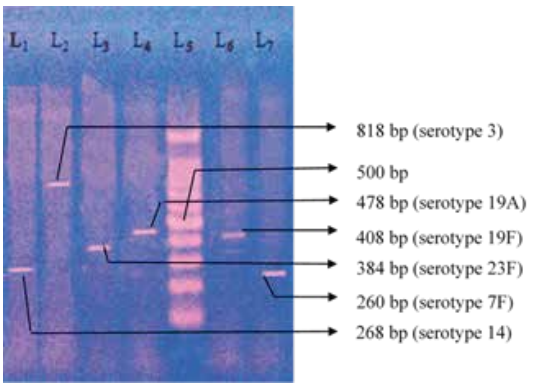

Figure-1: Photograph of gel electrophoresis of detected serotypes

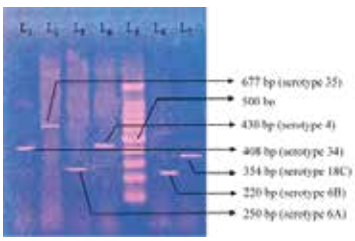

Figure-2: Photograph of gel electrophoresis of detected serotypes

Results :

Total 200 under five children were tested, among them, 92 (46%) were positive by PCR(Figure:3).

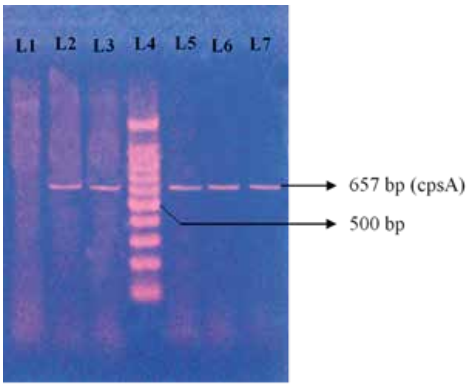

Figure-3: Photograph of amplified cpsA gene of Streptococcus pneumoniae.

Among 92 PCR positive nasoph aryngeal swabs, 70 (76.09%) were serotypes positive and 22 (23.91%) were serotype negative (Figure-4).

Results of serotyping of S. pneumoniae by PCR from nasopharyngeal swabs (N=92)

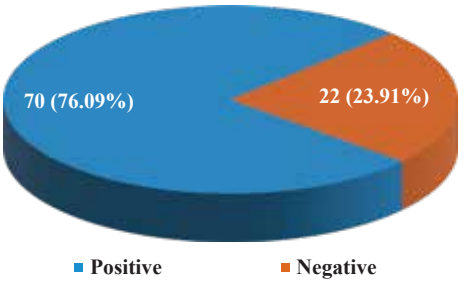

Figure-4: Proportion of detected serotype positive and negative nasopharyngeal swabs by PCR

Out of 200 children, 90 (45%) were received pneumococcal conjugate vaccine (PCV) and 110 (55%) were not vaccinated. Among vaccinated children, 4 (16%) S. pneumoniae were detected in fully vaccinated children and 25 (38.46%) S. pneumoniae were detected in partially vaccinated children by PCR. Among not vaccinated children, 63 (57.27%) S. pneumoniae were detected by PCR (Table 1). The difference between fully vaccinated and non-vaccinated proportion is statistically significant ($p<0.001$) and the difference between fully vaccinated and partially vaccinated proportion is statistically significant ($p< 0.001$). Out of 4 fully vaccinated PCR positive cases, serotypes

proportion is statistically significant (p< 0.001). Out of 4 fully vaccinated PCR positive cases, serotypes 34F (1)and 35B (1) and 6B (1) were detected among 3 fully vaccinated children. Out of 25PCR positive partially-vaccinated childrenserotypes 34F (1) and 35B (1) and 6B (1) among 18 children. Out of 63 PCR positive not-vaccinated children, serotypes were detected in 51 children and the detected serotypes were 3 (3), 4 (1), 6A (11), 6B (7), 7F (1), 14 (4), 18C (1), 19A (2), 19F (2), 23F (3), 34F (8), 35B (8)[table 1].

Table-I: Distribution of S. pneumoniae among study population in relation to vaccinationstatus (N=200)

Vaccination status	PCR positive, n (%)
Fully vaccinated*	4(16.00)
Partially vaccinated**	25(38.46)
Not vaccinated	63(57.27)
Total	92

*Vaccinated with 3 doses of PCV

**Vaccinated with 1 or 2 doses of pneumococcal conjugate vaccine (PCV)

Table-II: Distribution of detected serotypes among vaccinated, partially vaccinated and non-vaccinated children (N=92)

Vaccination status	Detected serotypes(n)
Fully vaccinated(N=4)	***34F(1), *35B (1), 6B (1)**
Partially vaccinated(N=25)	***34F(7),* 35B(3),6B (3), 14 (3), 23F(2)**
Not-vaccinated (N=63)	**3(3), 4(1), 6A(11), 6B(7), 7F(1), 14(4), 19A(2), 19F(2), 23F(3), *34F(8), *35B(8)**

N= Total no.PCR positive sample

N =Total no. of positive sample

VT=3, 4, 6A, 6B, 7F, 14, 18C, 19A, 19F, 23F.

*NVT=34F, 35B

Discussion

S. pneumoniae is a common cause of respiratory infections requiring hospitalization in young children worldwide with increasing rates of antibiotic resistance[11].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 infection[12]. 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 antibiotics[13]. 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. In the present study, a total of 200 nasopharyngeal swabs were processed. Out of 200 nasopharyngeal swabs,92(46%) nasopharyngeal carriage of S. pneumoniae was detected by PCR. A study in Switzerland reported that carriage rate was 51.6% by PCR[13]. Another study reported 69% carriage rate in Netherland by PCR[14]. All the results of the previous studies showed higher carriage rate in contrast to the present study. The reasons might be due to different geography15 . Besides in the present study data were enrolled from healthy, urban children.In the present study, carriage rate was significantly higher in non-vaccinated children than fully-vaccinated children (p<0.001) and also significantly higher in partially-vaccinated children than fully-vaccinated children (p<0.001). The present study showed that out of 70 detected serotypes positive nasopharyngeal swabs, different serotypes were more prevalent in non-vaccinated children and partially vaccinated children than fully-vaccinated children.In non-vaccinated and partially vaccinated groups both VT and NVT serotypes were more prevalent whereas in fully

vaccinated children NVT serotypes were more prevalent. The findings of the present study might be due to the fact that introduction of PCV reduced the carriage rate in vaccinated children and reduced carriage of VT pneumococci accompanied by an increased NVT 16. Some previous studies analyzed the impact of pneumococcal vaccination on S. pneumoniae carriage rate and serotype distribution [17,18,19]. They reported that overall carriage rate in residents decreased following vaccination, carriage rates of all age group were lower at first and second post vaccination surveys than at the pre vaccination surveySome previous studies showed that the prevalence of vaccine type (VT) carriage decreased after PCV vaccination[17,19]. Some studies [20,21] reported that reduction in carriage of VT pneumococci due to PCV vaccination is often accompanied by an increase in the carriage of non-vaccine type (NVT) pneumococci and to a lesser extent, an increase in the incidence of IPD caused by NVT pneumococci.

Conclusion :

This study suggests that, pneumococcal conjugate vaccine has the potential to reduce pneumococcal carriage and the number of serotypes belonging to vaccine serotypes. Currently, routine vaccination of all children with the pneumococcal conjugate vaccine is our best strategy for reducing the burden of early-child-hood pneumococcal diseases.

References :

1. Normark B, Tuomanen EI. The pneumococcus: Epidemiology, microbiology, and pathogenesis. Cold Spring Harb Perspect Med, 2013; 3: e010215.
2. World Pneumonia Day. Universal Access for Pneumonia Prevention and Care, 2014.
3. Gray BM, Converse GM, Dillon HC. Epidemiologic studies of Streptococcus pneumoniae in infants: acquisition, carriage, and infection during the first 24 months of life. J Infect Dis, 1980; 142: 923-933.
4. Pasinato A, Indolfi G, Marchisio P, Valleriani C, Cortimiglia M, Spanevello V, et al. Nasopharyngeal carriage in children pneumococcal serotype distribution in 1315 nasopharyngeal swabs from a highly vaccinated cohort of Italian children as detected by RT-PCR. Vaccine, 2014; 32: 1375–1381.
5. Lang ALS, McNeil SA, Hatchette TF, Elsherif M, Martin I, Jason J,etal. Detection and prediction of Streptococcus pneumoniae serotypes directly from nasopharyngeal swabs using PCR. J Med Microbiol, 2015; 64: 836-844
6. Kim SH, Chung DR, Song JH, Wang H. Changing Trends in Antimicrobial resistance and serotypes of Streptococcus pneumoniae isolates in Asian countries: an Asian Network for Surveillance of resistant Pathogens (ANSORP) Study. AAC, 2012; 56: 1418-1426.
7. Saha SK, Naheed A, Arifeen SE, Islam S, Emran HA, Amin R. Surveillance for invasive Streptococcus pneumoniae diseases among hospitalized children in Bangladesh: Antimicrobial susceptibility and serotype distribution. Clin Infect Dis, 2009; 48: 75-81.
8. Huss A, Scott P, Stuck AE, Trotter C, Egger M. Efficacy of pneumococcal vaccination in adults: a meta-analysis. CMAJ, 2009; 180: 48–58.
9. Bonten MJ, Huijts SM, Bolkenbaas M, Webber C, Patterson S, Gault S, et al. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. N Engl J Med, 2015; 372: 1114–1125.
10. Zuccotti G, Mameli C, Daprai L, Garlaschi ML, Dilillo D, Bedogni G, et al. Serotype distribution and antimicrobial susceptibilities of nasopharyngeal isolates of Streptococcus pneumoniae from healthy children in the 13-valent pneumococcal conjugate vaccine era. Vaccine, 2014; 32: 527–534.
11. Resti M, Moriondo M, Cortimiglia M, Indolfi G, Canessa C, Becciolini L, et al. Community acquired bacteremic pneumococcal pneumonia in children: diagnosis and serotyping by real time polymerase chain reaction using blood samples. Clin Infect Dis, 2010; 51: 1042–1049.
12. Shahriar M, Madak M, Haque A, Kabir S, Saha MR. Current status of antimicrobial sensitivity pattern of Streptococcus pneumoniae strains collected from clinical sources in Dhaka, Bangladsesh. S J Pharm Sci, 2010; 3: 59-62.

13. Wyllie AL, Wijmenga AJ, Marlies A, Groot JA. Molecular surveillance of nasopharyngeal carriage of Streptococcus pneumoniae in children vaccinated with conjugated polysaccharide pneumococcal vaccines. Sci Rep, 2016; 6: 238-242.
14. Brugger SD, Hathaway LJ, Muhlemenn K. Detection of Streptococcus pneumoniae strain colonization in the nasopharynx. J Clin Microbiol, 2009; 47: 1750-1756.
15. Giebink GS. The preven of pneumococcal diseases in children. N Engl J Med, 2001; 345: 1177-1183.
16. Gebre T, Tadesse M, Aragaw D, Feye D, Mekonennen M, Byene BH, et al. Nasopharyngeal Carriage and Antimicrobial susceptibility patterns of Streptococcus pneumoniae among children under five in Southwest Ethiopia. Children, 2017; 4: 27-34.
17. Richard AA, DeAntonio R, Hill PC, Roca A, Usuf E, Hoet B, et al. Carriage of Streptococcus pneumoniae and other respiratory bacterial pathogens in low and lower-middle income countries: A systemic review and meta-analysis. PLoS One, 2014; 9: e103293.
18. Roca A, Bottomley C, Hill PC, Bojang A, Egere U, Antonio M, et al. Effect of age and vaccination with a pneumococcal conjugate vaccine on the density of pneumococcal nasopharyngeal carriage. Clin Infect Dis, 2012; 55: 816-824.
19. Cheung YB, Zaman SM, Nsekpong ED, Van Beneden CA, Chris A, Adegbola RA, et al. Nasopharyngeal carriage of Streptococcus pneumoniae in Gambian children who participated in a 9-valent pneumococcal conjugate vaccine trial and in their younger siblings. Pediatr Infect Dis J, 2009; 28: 990-995.
20. Akinsola AK, Ota MO, Enwere GC, Okoko BJ, Zaman SM, Martin O, et al. Pneumococcal antibody concentrations and carriage of pneumococci more than 3 years after infant immunization with a pneumococcal conjugate vaccine. PLoS One, 2012; 7: 1050.
21. Isaacman DJ, Mclntosh ED, Reinert R. Burden of invasive pneumococcal disease and serotype distribution among Streptococcus Pneumoniae isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. Int J Infect Dis, 2010; 14: l97-209.
22. Flasche S, Slack M, Miller E. Long term trends introduce a potential bias when evaluating the impact of the pneumococcal conjugate vaccination programme in England and Wales. Euro Surveill, 2011; 16: 168 -198.