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Article

Streptococcus pneumoniae, a pathogen of childhood pneumonia: an evaluation of isolated serotypes for vaccine effectiveness

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Abstract: The burden of serious pneumococcal disease is the heaviest in the developing world that focuses on its diagnostic importance. The incidence of pneumonoccoal disease in different regions of the world is not caused by some specific serotypes or serogroups of pneumonocci rather they are randomly distributed in population. A total number of 412 nasopharyngeal swab samples were cultures between January 2010 and December 2012. All the isolates were serotyped by using chessboard modification of quellung methods. A total of 102 *S. pneumoniae* isolates were found. The distribution among age groups shows that age groups 15 years and over 60 years are more frequent. The most common serotypes were 20 (17.6%), 33(16.7%), 6 (15.7%), 19 (14.7%) and 23 (10.8%) types. The addition of a pneumococcal vaccine (PCV) covering the prevalent serotypes in the immunization program could be useful for reducing the burden of pneumococcal diseases.

Keywords: *Streptococcus pneumonia*; serotyping, childhood pneumonia

1. Introduction

Streptococcus pneumoniae (pneumococcus) is the leading cause of childhood pneumonia in children accounting 6.3 million deaths among children younger than five years in 2013, a total approximately 950,000 deaths worldwide (Liu *et al*., 2015; WHO, 2015). Moreover, as common bacterial pathogen in humans, it causes infections like pneumonia, otitis media and meningitis, often associating with bacteremia (Bridy-Pappas *et al*., 2005; Herva *et al*., 2006; Wardlaw *et al*., 2006). Approximately ninety percent of deaths by pneumococcal infections occur in the developing world (O'Brien *et al*., 2009).

S. pneumoniae a childhood pathogen with 90 serotypes exists as part of normal nasopharyngeal flora and nasopharyngeal carriage is considered as the main reservoir of pneumococcus (O'Brien *et al*., 2008). Acquisition of nasopharyngeal carriage is the first step toward pneumococcal disease (Gray *et al*., 1980; Sleeman *et al*., 2005; Syrjänen *et al*., 2005). A child typically encounters several different serotypes during the first year of life and with increasing age pneumococcal carriage decreases with a change of colonizing serotypes as seen in adults (Abdullahi *et al*., 2008; Granat *et al*., 2007; Hill *et al*., 2008). The incidence of pneumococcal diseases in different regions of the world is not caused by some specific serotypes rather they are randomly distributed in population. Appropriate vaccination and treatment with antibiotics reduces morbidity and mortality caused by pneumococcal infections. The success of vaccination depends on the disease causing serotypes circulating in the community.

The prevalence of disease causing capsular serotypes is variable in countries with different levels of protection by pneumococcal vaccine and capsular serotypes are monitored internationally from the beginning of their use as vaccine antigens (Bogaert *et al*., 2004) Burden of serious pneumococcal diseases is the heaviest in developing world (Greenwood, 1999) and different serotypes often have variable virulence and carriage properties (Shouval *et al*., 2006; Smith *et al*., 1993) that focus on the essential need of serotype study of pneumococci.

At the beginning, pneumococcal vaccine was prepared using cell surface capsular polysaccharides from the infecting pneumococcal serotypes (Daniels *et al*., 2016) and to increase the efficacy, the carbohydrate-protein conjugate vaccines were introduced that was based on serotyping of pneumonia causing streptococci (Bridy-Pappas *et al*., 2005). The World Health Organization (WHO) recommends the use of the conjugate vaccine in routine immunizations given to children. Pneumococcal capsular polysaccharides are of various serotypes, protein based conjugate vaccines may give more protection against pneumococcal disease.

The pneumococcal conjugate vaccines have different coverage of the disease causing invasive serotypes of *Streptococcus pneumoniae*. Frequency of pneumococcal carriage is highest in developing countries including Bangladeshi families (Erästö *et al*., 2010).

After 5 years of introducing 7-valent Pneumococcal conjugate vaccine (PCV7), pneumococcal infection rate returned to pre-vaccine stage due to most frequent other 6 serotypes that were not included in PCV7 (Singleton *et al*., 2007) After introducing more effective PCV13 vaccine that included those 6 serotypes of pneumococci, it was found that the only serotype 35B was responsible for increasing infection (Shouval *et al*., 2006). Thus the serotype replacement became the part of searching effective vaccine development. Efficacy of vaccines depends on the distribution and stability of the disease-causing pneumococcal serotypes in the population. Recurrence of invasive serotypes in population focuses the importance of isolation, identification and serotyping. Study of serotype stability in population is a potential factor for vaccine efficacy trial.

A limited number of hospital based reports and a few communities based study in Bangladesh focuses on the need of extensive study of pneumococcal serotypes in large population to identify the existing serotypes for selecting appropriate pneumococcal vaccines. Study concerning isolation of pneumococcal pathogen from human population and identifying their serotypes indicates the justified use of vaccines in our population. The main focus of the present study was to emphasize on isolation, identification and serotyping of pneumococcal isolates.

2. Materials and Methods

The study was conducted for the time period of 2010-2012. The pneumococcal isolates were obtained through several steps (i) sample collection: nasopharyngeal secretion as sample was collected according to the procedures followed in SOP, using calcium-alginate swab and skim milk-tryptone-glucose-glycerol (STGG) medium in screw cap tube in cold box. The collected samples were transported to laboratory within six hours of sampling using insulated box containing cold chargers as mentioned previously (Herva *et al*., 2006), (ii) culturing of samples on 5% sheep blood agar with gentamicin (5 ug/ml) at 36˚C for up to 48 hrs incubation, (iii) observation of characteristic colonial growth for *Streptococcus pneumoniae* and sub-culturing the selected desired colonies for obtaining pure-culture, (iv) identification of the cultured organism *Streptococcus pneumoniae*, was confirmed by alpha-haemolytic colony and optochin resistivity, (v) serotyping of the isolates was performed using chessboard modification of quellung method (Herva *et al*., 2006) with two sets of antisera pools (A-I and P-T) and omniserum (Staten Seruminstitut, Copenhagen, Denmark) (Sørensen, 1993). Penicillin sensitivity was checked by using oxacillin biodisk (1 µg) based on standard microbiological methods described in NCCLS and CLSI guideline (CLSI, 2006). Fresh pure-culture of isolated pathogen was used for sensitivity testing according to Kirby-Bauer method (Bauer, 1966).

3. Results

A total number of 412 nasopharyngeal swab samples were cultured according to SOP and the isolation of pneumococci (PNC) were done. The positive isolates were characteristically identified as *Streptococcus pneumoniae.* The growth positive isolates with alpha-haemolytic colony on gentamicin blood agar and optochin resistivity confirmed the isolates as pneumococci. The tests for quellung reaction with different antisera for the isolates resulted in specific serotypes of *Streptococcus pneumoniae.* From a total of 412 cultured samples, positive pneumococcal growth was found for 102 samples (24.76%) (Table 1). The distribution of the serotypes among different age groups (Table 2) shows that the age groups below 15 years and over 60 years are more susceptible to streptococcal infections. The serotypes found in the study subjects were 6, 10, 11, 14, 15, 19, 20, 23, 33 among which the predominating ones were 6 (15.7%, n=16), 19 (14.7%, n=15), 20 (17.6%, n=18), 23 (10.8%, n=11) and 33 (16.7%, n=17) (Table 3).

Table 1. Pneumococcal samples distribution in different sexes.

	Total Sample	**Male**	**Female**
Total collected	412 (100%)	218 (52.9%)	194 (47.1%)
Positive	102 (100%)	58 (56.9%)	44 (43.1%)

Table 2. Distribution of positive pneumococcal cases in different age groups.

Age group (Years)	**Male /Number (%)**	**Female /Number (%)**
0-15	22 (37.9%)	15 (34.1%)
16-30	12 (20.7%)	8 (18.2%)
31-45	4 (6.9%)	4 (9.1%)
46-60	8 (13.8%)	6 (13.6%)
>60	12 (20.7%)	11 (25.0%)
Total	58 (100%)	44 (100%)

Table 3. Different serotypes of pneumococcal isolates.

Serotype	**Number (Frequency)**
6	16 (15.7%)
10	7 (6.9%)
11	8 (7.8%)
14	3 (2.9%)
15	7 (6.9%)
19	15 (14.7%)
20	18 (17.6%)
23	11 (10.8%)
33	17 (16.7%)
Total	58 (100%)

4. Discussion

The pneumococcal vaccine was primarily developed for the U.S. and European epidemiological situation that has limited coverage of serotypes causing serious pneumococcal infections in most developing countries (Barocchi *et al*., 2007). This focuses on the serotype based study in the developing countries like Bangladesh. The serotypes found in the present study were 6, 10, 11, 14, 15, 19, 20, 23, 33 among which the predominating ones were 6, 19, 20, 23 and 33. Several vaccines already developed namely PCV7, PCV10, PCV13, PCV15, 20vPnC, PPSV23 etc. which have different ranges of protection coverage against disease causing pneumococcal serotypes. Historically developing a vaccine is a cumbersome process and it takes several years even more than a decade to make it available for the population of developing world (Hammitt *et al*., 2006).

Prevnar PCV7, the first heptavalent pneumococcal polysaccharide conjugate vaccine of seven serotypes of *S. pneumoniae* (4, 6B, 9V, 14, 18C, 19F, and 23F) was able to reduce the infection rate in children under 2 years of age and in unimmunized individuals(Richter *et al*., 2013; Whitney *et al*., 2003) .It showed 98% probability of protection against the strains causing 80% of the pneumococcal disease in infants in the U.S. though it is no longer produced but after 5 years of introducing this vaccine, pneumococcal infection rate returned to pre-vaccine stage (Singleton *et al*., 2007). Prevnar 13 (PCV13), a tridecavalent vaccine contains thirteen pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) (CDC, 2010). PCV13 with six additional strains (1, 3, 5, 6A, 19A and 7F, not included in PCV7) had protection against majority of the remaining pneumococcal infections (FDA, 2010.) and was approved for use replacing the PCV7 (EMA, 2020; EMEA, 2009).

Synflorix (PCV10) a decavalent vaccine contains ten pneumococcal serotypes (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) (GSK, 2009; Pfizer Inc., 2020). The 20-valent pneumococcal conjugate vaccine candidate 20vPnC contains the serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F is supposed to be in market after approval (Merck, 2020) and a pentadecavalent vaccine PCV15 with serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A, 22F, 23F, and 33F is in progress for production (Miller *et al*., 2016). The 23-valent pneumococcal polysaccharide vaccine (PPSV23) was developed with capsular polysaccharides of serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F, and its protection coverage against 80% to 90% infecting pneumococci.

From the above data, variable protection coverage of different pneumococcal vaccines against disease causing invasive serotypes of *Streptococcus pneumoniae* is evident. The above mentioned vaccines also have variable protection coverage against serotypes of present study i.e. 44.4% (serotypes 6, 14, 19, 23) by PCV7, PCV13, PCV10, and likewise 66.7% by PCV15, 88.9% by 20vPnC and 100% by PPSV23.

Efficacy of vaccines depends on the disease-causing pneumococcal serotypes available in the population. Moreover, study of serotype stability in population is a potential factor for vaccine efficacy trial. For selecting appropriate pneumococcal vaccines and justified use, isolation of pneumococcal pathogen from human population and their serotyping are of great concern. The present investigation was conducted with the data of limited number of pneumococcal samples. Moreover, the serotypes of higher frequencies (6, 19, 20, 23 and 33) of the present study are mostly under the coverage of pneumococcal vaccines (PCV15, 20vPnC, PPSV23) it does not reflect the sufficient argument for vaccine use in Bangladesh community.

5. Conclusions

With more reliability, searching pneumococcal serotypes other than those included in present vaccines is important consideration for introducing any vaccine in our population. The study emphasizes on the extensive study with larger population size of the community and hospital settings in different areas of Bangladesh.

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Conflict of interest

None to declare.

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