Pneumonia

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Streptococcus pneumoniae serotype distribution in Bangladeshi under-fives with community-acquired pneumonia pre-10-valent pneumococcal conjugate vaccination

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Abstract

Background *Streptococcus pneumoniae* is the most frequent causative pathogen of bacterial pneumonia in children worldwide. Bangladesh introduced the 10-valent pneumococcal conjugate vaccine (PCV10) in their national immunization program for infants in 2015. We assessed its potential coverage in under-fives with community-acquired pneumonia (CAP) in the years *before* PCV10 was introduced.

Methods A total of 1502 childhood pneumonia cases (< 5 year olds living in the urban section Kamalapur, Dhaka) were enrolled between 2011 and 2013. Acute phase and late (convalescent) serum samples were collected from 1380 cases. Serotype-specific pneumococcal antibody concentrations were measured using a 25-plex immunoassay panel. Pneumococcal CAP was diagnosed based on a serotype-specific pneumococcal antibody response.

Results *S. pneumoniae* was serologically identified as causative pathogen in 406/1380 (29%) cases. The five most prevalent serotypes were (in descending order) 11A, 22F, 3, 2 and 19F. Based on the percentage of pneumonia cases associated with PCV10 vaccine types, the potential PCV10 coverage was 29% (116/406).

Conclusions In almost a third of the studied cases *S. pneumoniae* was identified as a causative pathogen. Because of the characteristics of the immunoassay, this might well be a gross underestimation. Nevertheless, the potential PCV10-coverage was low. Given the high serotype diversity, the region might benefit greatly from a higher-coverage PCV or recombinant protein vaccine.

Keywords Pneumonia, Pneumococcal, Serotypes, Children, Bangladesh

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Background

Streptococcus pneumoniae is the most frequent bacterial cause of community-acquired pneumonia (CAP) in children worldwide [1]. The burden of pneumococcal disease is especially high in young children from developing countries [2]. Bangladesh is situated in a region where the vast majority of the world's severe and fatal pneumonia cases in young children occur, exemplified by an incidence of 130.3 episodes/1000 children (95% uncertainty interval (UI) 106.5-157.5) versus a global incidence of 107.7 episodes/1000 children (95% UI 87.5–130.9) and 44.6 (95% UI 35.5–56.3) in high-income countries [3, 4].

With support of the GAVI Alliance, Bangladesh has implemented the 10-valent conjugate vaccine (PCV10; Synflorix®) in its childhood national immunization program as of March 2015, with an expected vaccination coverage of >90% [5]. PCV10 offers protection against 10 pneumococcal serotypes: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. Pneumococcal conjugate vaccines (PCV) have been developed for the prevention of severe pneumococcal disease in children, especially invasive pneumococcal disease (IPD). Given the limited resource setting of Bangladesh, including human resources for health services [6], the additional benefit of PCV on pneumococcal CAP in children, in addition to its effects on IPD, might be enormous [7].

The pneumococcal capsular polysaccharide is the single most important trigger to the host immune response, which is serotype specific with limited cross-reactivity [8, 9]. Based on differences in the composition of the pneumococcal polysaccharide capsule, over 90 serotypes have been identified. Antibodies against the polysaccharide capsule are protective against IPD and form the basis of the available pneumococcal vaccines [10].

The vast majority of adult pneumococcal CAP is due to non-invasive (or non-bacteremic) infection [11, 12]. Although less is known for early childhood pneumonia in high disease burden settings in low- and middle-income countries, this appears to hold true for these cases as well [13, 14]. Most studies conducted in Bangladesh have focused on the serotype distribution in IPD [15–17]. Very limited data is available on the contribution of *S. pneumoniae* and the pneumococcal serotype distribution in children with CAP, especially non-bacteremic pneumonia [18].

Serological multiplex analyses of anti-capsular antibodies have been shown useful in identifying the pneumococcal serotype distribution in adults hospitalized with bacteremic and non-bacteremic CAP [19, 20]. The aim of the present study was to evaluate the proportion of *S. pneumoniae* and its serotype distribution in children <5 years from Bangladesh with CAP, before PCV10 was introduced, based on serotype-specific pneumococcal antibody dynamics.

Methods

Study design and participants

Children between 1 and 59 months old from the urban field site of Kamalapur, Dhaka that were diagnosed with pneumonia were enrolled. This study was locally managed by the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr, b), which has conducted pneumonia disease burden surveillance and intervention trials at this site since 1998 [18, 21–23]. Kamalapur is divided into seven geographical strata and 450 geographical clusters, each consisting of ~100 households. All children <5 years old residing in selected clusters were kept under active morbidity surveillance, after parents provided informed written consent. The Research Review and Ethical Review Committees of the icddr, b approved the study.

Data collection, clinical characteristics and definitions

During weekly home visits, vital signs and signs of respiratory illness were evaluated based on a structured calendar questionnaire used by the study site for respiratory and febrile illness surveillance since 2004. All study participants with signs of respiratory and/or febrile illness were initially referred to the onsite study clinic within 24–48 h of start of illness signs. Definitive CAP diagnosis was made by the study physician based on standard clinical case definitions, as previously described [18, 21]. Definitions of CAP and severity of illness can be found in Supplementary Material 1. Multiple enrolments of one child were allowed, but only after it had recovered from a prior episode of CAP with at least 7 disease free days between two illness episodes. Each unique CAP episode is hereafter referred to as a 'case'. Available outcome measures were: duration of illness (in days) and outcome of illness (full recovery, recovery with disabilities, continues to be ill, lost to follow-up, deceased). Full definitions of outcomes are included in Supplementary Material 1.

For the current study, two serum samples were collected and stored at the field clinic at 4 °C, and transported twice daily in a cool box at 4° −8 °C to the clinical microbiology laboratory of Dhaka and stored at -20 °C. The first sample was collected on day 1 at the time of CAP diagnosis (acute phase). The second sample was obtained during a post-recovery follow-up visit (convalescent phase; no sooner after seven disease free days after the last signs of illness, but typically between 2 and 6 weeks post illness resolution depending on child availability). Later all samples were aliquoted and stored at -80 °C. Aliquots were sent to the St. Antonius Hospital in Nieuwegein, The Netherlands, for batch analyses.

Serotype-specific pneumococcal antibody measurement by multiplex immune assay

Serotype-specific pneumococcal antibodies were measured in paired serum samples. The multiplex immunoassay was performed on a Luminex platform (Luminex Corporation, Austin, TX). This method has been described in detail elsewhere [20] and is added in Supplementary Material 1. A 25-plex immunoassay panel was used including the 10 serotypes covered by PCV10 (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F), and 15 additional serotypes selected based on literature on IPD serotype distribution in children living in South-East Asia (2, 3, 6A, 8, 9N, 10A, 11A, 12A, 12F, 15B, 19A, 20, 22F, 33F and 45) [15–18]. Serotypes 12A and 45 are not covered by the 007sp reference serum, hence concentrations are given in arbitrary units (AU/ml).

Extensive information on selection of sera, details about the assay and our approach of antibody analyses are included in Supplementary Material 1.

Pneumococcal pneumonia definition

Cases with a serotype-specific antibody response were defined as with pneumococcal CAP. A positive immune response was defined as at least a 2-fold increase in serotype-specific antibody concentration between the first and second serum sample and a concentration at convalescence of ≥0.2 µg/ml (≥0.2 AU/ml for serotype 12A and 45). This convalescence concentration was chosen as it was well within the range of the lower end of the standard curves for all serotypes. The fold increase in antibody concentration against a given single pneumococcal serotype had to be at least two times greater than the fold increase against any other serotype, with exception of a 2-fold increase for serotypes within the same serogroup (e.g. 6A/6B), a scenario in which the serotype with the highest fold increase was regarded as infecting serotype. To prevent false positive and negative results, cases whose convalescent serum was drawn < 14 days or > 100 days after diagnosing CAP were excluded.

Based on the methods described above, patients were categorized as having pneumococcal CAP or non-pneumococcal CAP.

Conventional microbiological diagnostics

Blood cultures were performed using pediatric fastidious antimicrobial neutralization (FAN®, bioMérieux, France) bottles at the time of study enrollment. Bottles were sent twice daily (within 4 h of collection) to the clinical microbiology laboratory at the icddr, b for culture by BactAlert 3D (bioMérieux, France). Unfortunately, pneumococcal isolates were not available for serotyping. In addition, nasopharyngeal washes (NPWs) were done. The NPW was then placed in viral transport medium (containing Dulbecco's Modified Eagle Medium), stored and transported at 4 °C to the icddr, b virology laboratory within 8 h. The obtained nasopharyngeal wash samples were tested for the presence of influenza virus A or B components using multiplex RT-PCR.

Data analyses

Analyses were stratified by disease severity (non-severe vs. severe and very severe CAP) or age group (1–17 months vs. 18–60 months of age). The 18 months cut-off was based on the evidence that newborns and infants up to the age of 18–24 months are less capable of producing antibodies to bacterial capsular polysaccharides which may increase susceptibility for infections with pneumococci in cases below 18 months old [24–26].

Statistics

Descriptives were stated as number (%) and continuous data were presented as mean (standard deviation (SD)) or median (interquartile range [IQR]). Differences in proportions between serotype groups were tested with $\chi 2$ tests. Pearson correlation coefficients (PCC) were calculated to determine correlation between anti-polysaccharide antibodies specific for different serotypes. A p-value < 0.05 was considered to represent a statistically significant difference. Microsoft® Office Excel for Windows (version 2010) and SPSS software (version 22.0) were used for statistical analyses.

Results

All-cause pneumonia

Between October 2011 and December 2013, two unique serum samples were obtained from 1502 cases. The median time between collection of the acute phase and convalescent serum was 25 [IQR 19–41] days. This group consisted of 815/381/142/164 children that participated either once (54%), twice (25%), three times (10%) or >3 times (11%), respectively, with a maximum of 7 enrolments (one child). The 122 cases with a delta < 14 days (n = 41, 2.7%) or > 100 days (n = 81, 5%) between the early and late sample were excluded from further analyses (unless stated otherwise).

The mean age of the remaining 1380 cases was 20.2 months (median 17.2 months, [IQR 10.6–27.0]). The majority of cases (1274/1380, 92%) were diagnosed with non-severe pneumonia. 105/1380 (8%) cases met the criteria of severe CAP. One child had very severe CAP. Baseline characteristics, conventional microbiological test results and outcomes of illness, are shown in Table 1.

Proportion of streptococcus pneumoniae

The definition of pneumococcal CAP was met by 406/1380 (29%) cases. These 406 cases had a positive immune response for a specific pneumococcal serotype thereby representing the proportion of pneumococcal

Table 1 Baseline characteristics, results from conventional diagnostic tests and outcomes

Baseline characteristics ($n=1380$)	
Age (months (SD))	20.2 (13.1)
Pneumonia by severity	
Non-severe	1274 (92%)
Severe	105 (7.6%)
Very severe	1 (< 0.1%)
Blood culture isolates ($n=1358$)	
Coagulase-negative Staphylococcus species*	14
Salmonella typhi	7
Streptococcus pneumoniae	4
Streptococcus species	3
Micrococcus species	3
Moraxella species	3
Pseudomonas species	3
Acinetobacter species	2
Enterococcus species	2
Campylobacter species	1
Neisseria meningitidis	1
Nasopharyngeal wash influenza virus PCR	
Type A – H1N1	18 (1.3%)
Type A – H3N2	22 (1.6%)
Type B	10 (0.7%)
No sample collected	22 (1.6%)
Negative	1308 (95%)
Outcomes	
Non-severe pneumonia (n = 1274)	
Duration of illness (days)	6 [IQR 4–9]
Outcome of Illness	
Recovered	966 (76%)
Recovered with disability	291 (23%)
Continues to be ill	1 (0.1%)
Deceased	0
Lost to follow-up	16 (1.3%)
Severe pneumonia (n = 106)	
Duration of illness (days)	8 [5–13]
Outcome of Illness	
Recovered	83 (78%)
Recovered with disability	22 (21%)
Continues to be ill	0
Deceased	0
Lost to follow-up	0
Very severe pneumonia ($n=1$)	
Duration of illness (days)	6
Outcome of Illness	Recovered with disability

CAP. It was not possible to draw conclusions regarding the immune responses in 103 cases (7%), e.g. due to a response against multiple serotypes or indistinct variations in antibody dynamics. No immune response was detected in 871 (62%) cases. Supplementary Fig. 1 shows examples of antibody dynamics of each of the three aforementioned scenarios.

Twenty-two of 406 cases (5%) had severe pneumococcal CAP and 384 (95%) non-severe pneumococcal CAP. The prevalence of pneumococcal CAP was slightly higher in cases 18–60 months old (213/662, 32%) compared to those 1–17 months old (27%, 193/718; $p=0.03$). Comparing cases with pneumococcal CAP and non-pneumococcal CAP, there were no significant differences in duration of illness (7.3 days (SD 5.4) vs. 7.6 days (SD 5.8) or the rate of recovery with disabilities (95/406 (23%) vs. 219/974 (22%)).

Ten out of 406 (3%) cases with pneumococcal CAP had a positive blood culture, in only two of which *S. pneumoniae* was isolated. These coincided with a humoral immune response against serotypes 1 and 8 (no conventional typing performed). It is noteworthy that two out of the four cases yielding a positive blood culture from which *S. pneumoniae* was isolated did not fulfill the serological case definition criteria. The remaining conventional microbiological test results are shown in Table 1.

Pneumococcal serotype distribution

Figure 1 shows the serotype distribution in cases with pneumococcal CAP. All 25 serotypes included in the assay were identified at least once. The three most frequently identified serotypes were 11A ($n=45$, 11%), 22F ($n=35$, 9%) and 3 ($n=34$, 8%). The most prevalent serotype potentially covered by PCV10 was serotype 19F ($n=26$, 6%). Based on the identified serotypes, the potential PCV10-coverage was 29% of the 25 serotypes included in panel.

No large differences in serotype distribution was found between the two age group (see Fig. 2). The potential PCV10-coverage in 1–17 months was 29% vs. 28% in 18–60 months ($p=0.85$). Notable differences were the higher proportion of serotype 2 and 3 in cases 1–17 months old (20/193 (10%) vs. 11/213 (5%); $p=0.05$ and 21/193 (11%) vs. 13/213 (6%), $p=0.08$, respectively), whereas the proportions of serotype 9N and 33F were higher in cases 18–60 months of age (13/213 (6%) vs. 5/193 (2.6)%, $p=0.09$, and 14/213 (7%) vs. 5/193 (3%), $p=0.06$, respectively). The number of cases with (very) severe CAP was too low ($n=1$) to identify differences in serotype distribution vs. non-severe CAP ($n=384$) (data not shown).

Pneumococcal antibody concentration dynamics

The distribution of serotype-specific antibody concentrations in early and late serum from cases with pneumococcal CAP and other cases is shown in Table 2. In-depth analyses regarding intra-assay correlations of early and late serotype-specific antibody concentrations, and cross-reactivity and specificity can be found in Supplementary Material 1, Supplementary Tables 1 and 2, and Supplementary Figs. 2 and 3. Especially serotype 12A and

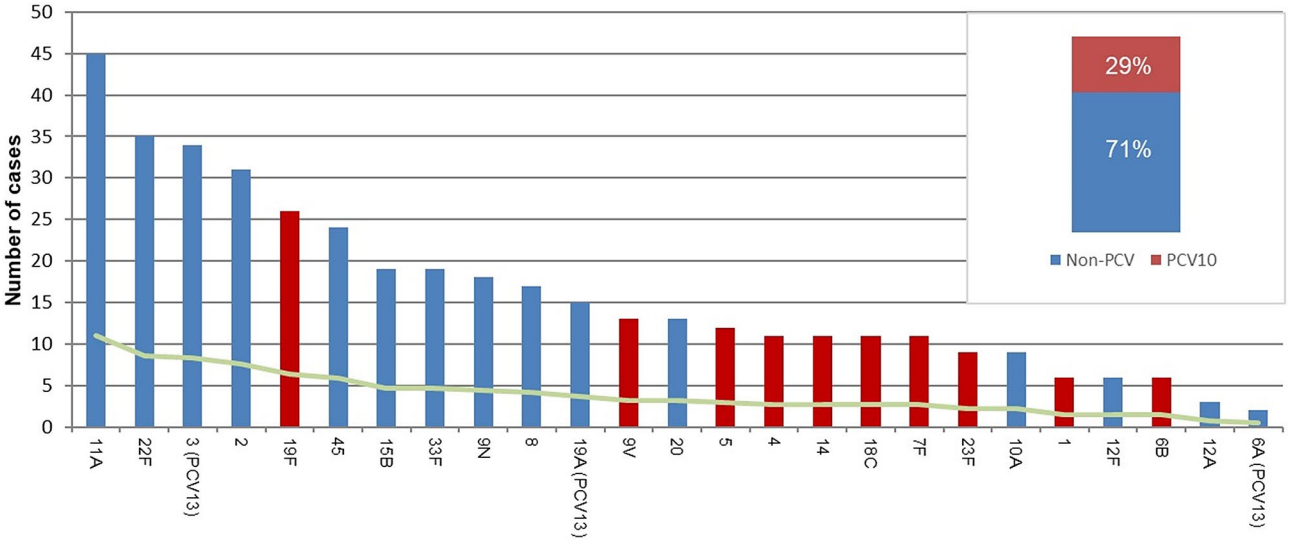

Fig. 1 Distribution of serotypes causing pneumococcal CAP based on serotype-specific antibody responses. Bars indicate the number of cases, the line shows the proportion. Proportion of PCV10 coverage is shown in the right upper corner

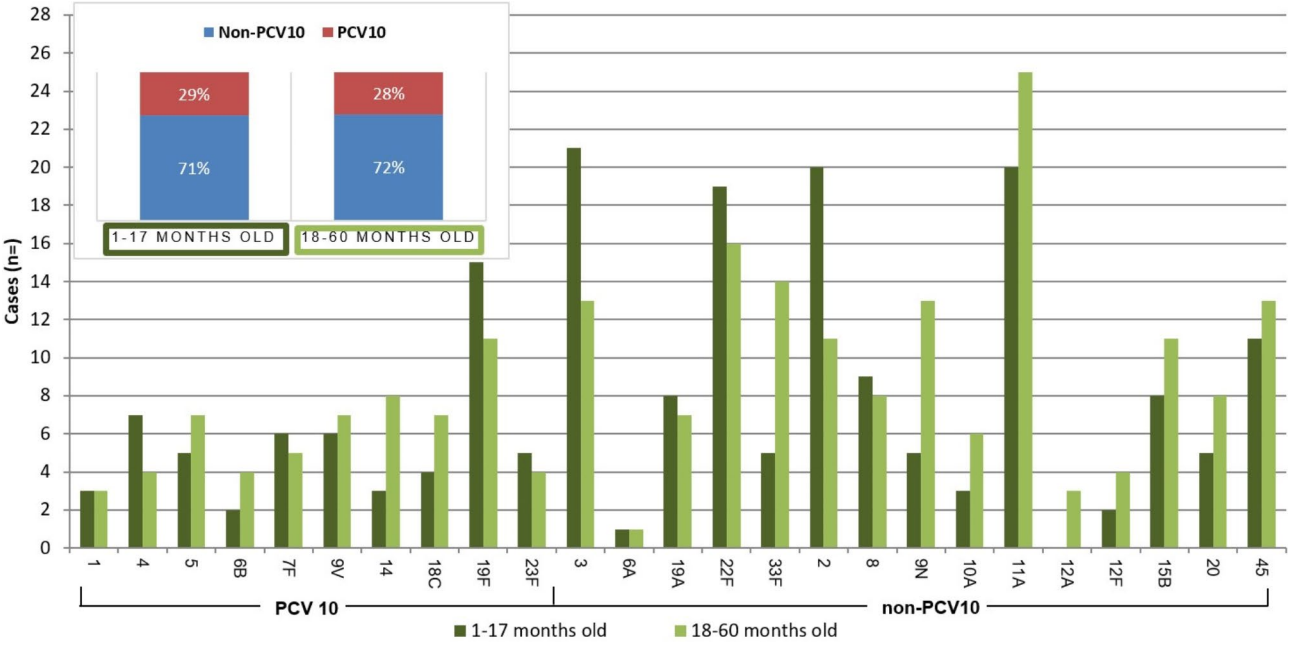

Fig. 2 The overall serotype distribution based on serotype-specific antibody responses stratified by age group. Bars indicate the number of cases. Proportion of PCV10 coverage is shown in the left upper corner

12F (PCC=0.70) and 6A and 6B (PCC=0.66) correlated well. In contrast, the correlation between 19A and 19F was low (PCC=0.54). Noteworthy was the correlation of the biologically unrelated serotype 8 vs. serotype 12A and 12F (PCC=0.63).

Discussion

By assessing serotype-specific pneumococcal antibody dynamics in serum, we were able to establish the contribution of *S. pneumoniae* in 29% of almost 1400 childhood cases with CAP in Dhaka, Bangladesh. Our study was conducted in an urban low-income section of Dhaka, Bangladesh the years before introduced PCV10 in 2015. The most striking finding, based on these unique data from under-fives with CAP, was that only 29% of pneumococcal CAP was caused by a serotype potentially covered by PCV10. By our best knowledge, this is the first study to report on the serotype-specific pneumococcal contribution in, predominantly non-bacteremic, childhood pneumonia cases.

Table 2 Distribution of serotype-specific antibody concentrations in pneumococcal CAP cases and cases with non-pneumococcal CAP

	Acute phase concentrations (µg/ml)		Convalescent concentrations (µg/ml)	
Serotype	**Non-pneumococcal CAP cases**	**Pneumococcal CAP cases**	**Non-pneumococcal CAP cases**	**Pneumococcal CAP cases**
	Median [IQR], number of cases	Median [IQR], number of cases	Median [IQR], number of cases	Median [IQR], number of cases
1 (PCV10)	0.04 [0.02–0.07], *n* = 1496	0.08 [0.05–0.12], *n* = 6	0.03 [0.02–0.06], *n* = 1496	1.12 [0.62–1.66], *n* = 6
2	0.08 [0.02–0.33], *n* = 1471	0.1 [0.04–0.27], *n* = 31	0.07 [0.02–0.3], *n* = 1471	0.67 [0.34–1.68], *n* = 31
3	0.26 [0.13–0.69], *n* = 1468	0.21 [0.13–0.39], *n* = 34	0.23 [0.12–0.59], *n* = 1468	2.03 [0.86–9.09], *n* = 34
4 (PCV10)	0.01 [0.01–0.02], *n* = 1491	0.02 [0.01–0.06], *n* = 11	0.01 [0-0.02], *n* = 1491	0.92 [0.6-3], *n* = 11
5 (PCV10)	0.05 [0.02–0.12], *n* = 1490	0.09 [0.02–0.14], *n* = 12	0.05 [0.02–0.13], *n* = 1490	0.55 [0.45–0.76], *n* = 12
6A	0.01 [0.01–0.03], *n* = 1500	0.11 [0.06–0.16], *n* = 2	0.01 [0.01–0.03], *n* = 1500	2.41 [1.45–3.37], *n* = 2
6B (PCV10)	0.01 [0-0.02], *n* = 1496	0.03 [0.01–0.1], *n* = 6	0.01 [0-0.02], *n* = 1496	2.2 [0.79–4.11], *n* = 6
7F (PCV10)	0.05 [0.03–0.13], *n* = 1491	0.15 [0.07–0.31], *n* = 11	0.05 [0.02–0.12], *n* = 1491	2.56 [1.3–4.62], *n* = 11
8	0.11 [0.05–0.28], *n* = 1485	0.15 [0.11–0.2], *n* = 17	0,11 [0.04–0.29], *n* = 1485	0.95 [0.54–7.36], *n* = 17
9N	0.06 [0.02–0.17], *n* = 1484	0.09 [0.05–0.19], *n* = 18	0.05 [0.02–0.18], *n* = 1484	0.61 [0.28–1.35], *n* = 18
9V (PCV10)	0.02 [0.01–0.07], *n* = 1489	0.01 [0.01–0.01], *n* = 13	0.02 [0.01–0.06], *n* = 1489	1.41 [0.54–1.94], *n* = 13
10A	0.03 [0.01–0.08], *n* = 1493	0.07 [0.03–0.09], *n* = 9	0.03 [0.01–0.08], *n* = 1493	0.75 [0.51–1.42], *n* = 9
11A	0.1 [0.03–0.49], *n* = 1457	0.06 [0.04–0.15], *n* = 45	0.09 [0.03–0.45], *n* = 1457	0.97 [0.39–3.99], *n* = 45
12A	0.01 *AU/ml* [0.01–0.03], *n* = 1499	0.05 *AU/ml* [0.05–0.09], *n* = 3	0.01 *AU/ml* [0.01–0.03], *n* = 1499	0.31 *AU/ml* [0.17–0.34], *n* = 3
12F	0.06 [0.03–0.15], *n* = 1496	0.11 [0.1–0.14], *n* = 6	0.06 [0.03–0.15], *n* = 1496	0.5 [0.42–0.58], *n* = 6
14 (PCV10)	0.02 [0.01–0.11], *n* = 1491	0.08 [0.04–0.33], *n* = 11	0.01 [0.01–0.08], *n* = 1491	1.7 [0.55–2.64], *n* = 11
15B	0.04 [0.02–0.14], *n* = 1483	0.08 [0.02–0.12], *n* = 19	0.04 [0.02–0.12], *n* = 1483	0.56 [0.38–1.01], *n* = 19
18C (PCV10)	0.03 [0.01–0.09], *n* = 1491	0.12 [0.04–0.19], *n* = 11	0.02 [0.01–0.08], *n* = 1491	0.93 [0.58–3.65], *n* = 11
19A	0.07 [0.02–0.3], *n* = 1487	0.1 [0.04–0.15], *n* = 15	0.06 [0.02–0.24], *n* = 1487	1.16 [0.73–1.92], *n* = 15
19F (PCV10)	0.14 [0.04–0.83], *n* = 1476	0.07 [0.06–0.16], *n* = 26	0,12 [0.04–0.63], *n* = 1476	4.69 [1.61–10.37], *n* = 26
20	0.02 [0.01–0.07], *n* = 1489	0.1 [0.03–0.2], *n* = 13	0.02 [0.01–0.06], *n* = 1489	0.44 [0.37–0.73], *n* = 13
22F	0.02 [0.01–0.1], *n* = 1467	0.03 [0.02–0.11], *n* = 35	0.02 [0.01–0.09], *n* = 1467	1.74 [0.53–7.88], *n* = 35
23F (PCV10)	0.02 [0.01–0.06], *n* = 1493	0.04 [0.02–0.08], *n* = 9	0.02 [0.01–0.05], *n* = 1493	4.48 [1.73–7.03], *n* = 9
33F	0.03 [0.02–0.09], *n* = 1483	0.06 [0.04–0.11], *n* = 19	0.03 [0.01–0.09], *n* = 1483	0.44 [0.28–1.18], *n* = 19
45	0.03 *AU/ml* [0.02–0.1], *n* = 1478	0.06 *AU/ml* [0.03–0.09], *n* = 24	0.03 *AU/ml* [0.01–0.1], *n* = 1478	0.36 *AU/ml* [0.27–0.71], *n* = 24

PCVs have been developed based on epidemiological serotype distribution data from Western countries and have had a positive impact on occurrence of IPD, covering the most prevalent serotypes before the vaccine was implemented in these countries [27]. Given the high burden of CAP among children in the South-East Asia region, Bangladesh could highly benefit from a vaccine that covers serotypes causing non-bacteremic CAP. The high pneumonia burden was reflected in our study by the fact that many children participated ≥ 2 times in little over two years.

Active IPD surveillance started in Bangladesh with support from GAVI's PneumoADIP to assess the burden of IPD (not limited to pneumonia) among children < 5 years old [28]. Studies were conducted in rural and urban areas and enrolled hospitalized and/or non-hospitalized children [15–18]. The top 3 most prevalent serotypes from these three studies combined consisted of serotype 1, 2, 5, 6B, 14 and 19A. All were included in our panel, however, only serotype 2 was found in the top ten of most prevalent serotypes in our study limited to childhood cases with CAP. Noteworthy, among the top 10 causative serotypes most frequently observed in our study, which are not encompassed by PCV10, the more recently introduced 20-valent conjugate vaccine covers an extra set of six serotypes (namely serotype 11A, 22F, 3, 15B, 33F and 8), while investigational vaccines could even further increase coverage (e.g. PCV24 which includes serotypes 2 and 9N) [29]. However, given the high serotype diversity observed in Bangladesh and neighboring countries [30, 31], next to ongoing replacement disease in countries with years of experience with PCVs, a recombinant protein vaccine aimed at offering serotype-independent protection seems optimal [32], and may render the risk for serotype replacement redundant [33].

The wide variation in serotypes likely reflects the geographical diversity between studies and difference in type of disease [17]. Bacteremic pneumonia and meningitis may be mediated by different pneumococcal serotypes than non-bacteremic disease due to a higher propensity of specific serotypes to invade the bloodstream [15]. Also, serotype diversity of nasopharyngeal carriage in the region is diverse, and can vary per geographic location [34, 35]. This is supported by the study of Safari et al.. in Southeast Asia, which investigated the nasopharyngeal carriage of *S. pneumoniae* among children < 5 years of age in two regions of Indonesia prior to PCV introduction. Carriage of serotype 11A proved to be the

most common non-vaccine serotype in only one of these regions (whereas 22F was found only sporadically in either region) [34]. Next to regional differences in serotype distribution, virulence of serotypes such as 11A may be lower. Our study methodology involved active surveillance including weekly home visits, which allows early detection of milder cases. Consequently, the majority of pneumonia cases in our study classified as non-severe. This could explain why antibodies against serotype 11A were most prevalent versus the low prevalence of serotype 1 (e.g.) in our study, compared to the Bangladeshi PneumoADIP centers focusing on children with IPD [36–39].

In an earlier study in Dutch adults hospitalized with CAP the same immunoassay as used in the current study had a sensitivity of 42-45% to detect pneumococcal pneumonia in comparison to extensive microbiological testing [19, 20]. If this sensitivity holds true for childhood pneumonia, the true pneumococcal proportion of 29% found in our study might be more than twice as high. On the other hand, with the lack of a gold standard, it is not possible to be sure that each CAP episode with a serotype-specific antibody response in the current study is indeed caused by *S. pneumoniae.* Many children are colonized with pneumococci, which may cause an immune response in parallel to infection with another respiratory pathogen. Moreover, many children did have high antibody concentrations against multiple serotypes, which likely is a reflection of living in close contact with relatives leading to higher pneumococcal carriage rate and density. Though two-point serology testing may be diagnostically impractical, our findings do indicate its usefulness in providing a good level of evidence of the pneumococcal serotype distribution, also in the absence of a true gold standard for identification of pneumococcal pneumonia. A follow-up study of similar design in this setting post vaccine exposure, including conventional microbiological testing, could shed light on the question of carriage rate, density and infection [40].

The main strength of this study is that we obtained data from an experienced pneumonia surveillance system, which has yielded a unique cohort of 1502 pneumonia cases < 5 years of age. Using a multiplex immunoassay, we were able to map the serotype distribution in childhood cases of non-bacteremic pneumococcal CAP in Bangladesh before the introduction of PCV10. However, there were also several limitations. First, the assayed panel included only 25 serotypes and was therefore unable to identify other serotypes, which might have led to an underestimation of the pneumococcal proportion and PCV10-coverage. Second, the sensitivity of the applied immunoassay is limited compared to conventional diagnostics. However, besides blood cultures, in this setting no other microbiological tests were available, yielding it impossible to confirm *S. pneumoniae* by conventional methods (which is the third limitation). Moreover, due to challenging technical conditions (i.e. temperature, logistics), the < 3% bacteremic cases with pneumococcal CAP might well be an underestimation. Lastly, the lack of a healthy control group is a limitation but this has been overcome by using the antibody measurements in cases with non-pneumococcal CAP (and using measurements of the non-infecting serotypes within cases with pneumococcal CAP) as internal controls.

Conclusions

The proportion of *S. pneumoniae* in childhood cases < 5 years with CAP in Bangladesh was high before introduction of PCV10. Based on serotype-specific pneumococcal antibodies measurement, the potential PCV10-coverage was only 29% of the 25 serotypes assayed. The novel 20-valent and 25-valent PCVs could substantially increase coverage. The serotype distribution differed largely compared to IPD studies from the region and from Western countries. Future research should focus on mapping the serotype-specific burden of disease in the region, aiming towards the development of vaccines directed at protecting children living in this low-income region.

Abbreviations

CAP	community-acquired pneumonia
icddr,b	International Center for Diarrheal Disease Research, Bangladesh
IPD	Invasive pneumococcal disease
IQR	Interquartile range
PCV	Pneumococcal conjugate vaccine
PCR	Polymerase chain reaction
SD	Standard deviation
UI	Uncertainty interval

Supplementary Information

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Supplementary Material 1

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Author contributions

GTR, WAB, HPE, SPM, BMJ and BJMV contributed to the study conception and design. DG was the primary study coordinator, overseeing training, conduct, and data management; DA was responsible for local laboratory activities, including sample collection. BJ and TAL performed the laboratory experiments. BM supervised the laboratory experiments. SMTV, BM and TAL analysed the data. SMTV and BM drafted the manuscript. All authors reviewed and approved the final manuscript.

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Data availability
The datasets utilized and/or analyzed in the present study can be obtained from the corresponding author upon a reasonable request.

Declarations

Ethics approval and consent to participate
The Research Review and Ethical Review Committees of the icddr, b approved the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. O'Brien KL, Baggett HC, Brooks WA, Feikin DR, Hammitt LL, Higdon MM et al. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. The Lancet [Internet]. 2019 Aug 31 [cited 2019 Oct 27];394(10200):757–79. http://www.ncbi.nlm.nih.gov/pubmed/31257127
2. Wahl B, O'Brien KL, Greenbaum A, Majumder A, Liu L, Chu Y et al. Burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000-15. Lancet Glob Health [Internet]. 2018 Jul [cited 2023 Aug 23];6(7):e744–57. http://www.ncbi.nlm.nih.gov/pubmed/29903376
3. UNICEF. Pneumonia claims the lives of the world's most vulnerable children. 2018 [cited 2018 Nov 30]. Pneumonia in Children Under Five - UNICEF DATA. https://data.unicef.org/topic/child-health/pneumonia/
4. Troeger C, Blacker B, Khalil IA, Rao PC, Cao J, Zimsen SRM et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis [Internet]. 2018 Nov [cited 2019 Oct 27];18(11):1191–210. http://www.ncbi.nlm.nih.gov/pubmed/30243584
5. Gavi country factsheet. Bangladesh [Internet]. [cited 2019 Oct 27]. https://www.gavi.org/country/bangladesh/
6. WHO Global Health Workforce Alliance. WHO, World Health O. 2019 [cited 2019 Jan 17]. Bangladesh. https://www.who.int/workforcealliance/countries/bgd/en/
7. Johnson HL, Deloria-Knoll M, Levine OS, Stoszek SK, Freimanis Hance L, Reithinger R et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project. Cohen J, editor. PLoS Med [Internet]. 2010 Oct 5 [cited 2018 Nov 30];7(10):e1000348. https://doi.org/10.1371/journal.pmed.1000348
8. Musher DM, Groover JE, Rowland JM, Watson DA, Struewing JB, Baughn RE et al. Antibody to capsular polysaccharides of Streptococcus pneumoniae: prevalence, persistence, and response to revaccination. Clin Infect Dis [Internet]. 1993 Jul [cited 2018 Nov 30];17(1):66–73. http://www.ncbi.nlm.nih.gov/pubmed/8353248
9. Väkeväinen M, Eklund C, Eskola J, Käyhty H. Cross-Reactivity of Antibodies to Type 6B and 6A Polysaccharides of *Streptococcus pneumoniae*, Evoked by Pneumococcal Conjugate Vaccines, in Infants. J Infect Dis [Internet]. 2001 Sep 15 [cited 2019 Oct 27];184(6):789–93. http://www.ncbi.nlm.nih.gov/pubmed/11517443
10. Daly TM, Hill HR. Use and Clinical Interpretation of Pneumococcal Antibody Measurements in the Evaluation of Humoral Immune Function. Papasian CJ, editor. Clinical and Vaccine Immunology [Internet]. 2015 Feb [cited 2019 Jan 17];22(2):148–52. http://www.ncbi.nlm.nih.gov/pubmed/25520149
11. Fedson DS, Guppy MJ. Pneumococcal vaccination of older adults: conjugate or polysaccharide? Hum Vaccin Immunother [Internet]. 2013 Jun 12 [cited 2019 Jan 17];9(6):1382–4. http://www.tandfonline.com/doi/abs/10.4161/hv.24692
12. Said MA, Johnson HL, Nonyane BAS, Deloria-Knoll M, O'Brien KL, Team for the AAPBS. Estimating the Burden of Pneumococcal Pneumonia among Adults: A Systematic Review and Meta-Analysis of Diagnostic Techniques. Hill PC, editor. PLoS One [Internet]. 2013 Apr 2 [cited 2018 Feb 2];8(4):e60273. https://doi.org/10.1371/journal.pone.0060273
13. Pneumonia Etiology Research for Child Health (PERCH) Study Group. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. Lancet [Internet]. 2019 Aug 31 [cited 2023 Aug 23];394(10200):757–79. http://www.ncbi.nlm.nih.gov/pubmed/31257127
14. Brooks WA, Zaman K, Goswami D, Prosperi C, Endtz HP, Hossain L et al. The Etiology of Childhood Pneumonia in Bangladesh: Findings From the Pneumonia Etiology Research for Child Health (PERCH) Study. Pediatr Infect Dis J [Internet]. 2021 Sep 1 [cited 2023 Aug 23];40(9S):S79–90. http://www.ncbi.nlm.nih.gov/pubmed/34448747
15. Saha SK, Naheed A, El Arifeen S, Islam M, Al-Emran H, Amin R et al. Surveillance for invasive Streptococcus pneumoniae disease among hospitalized children in Bangladesh: antimicrobial susceptibility and serotype distribution. Clin Infect Dis [Internet]. 2009 Mar 1 [cited 2018 Nov 30];48 Suppl 2(s2):S75-81. https://academic.oup.com/cid/article-lookup/doi/https://doi.org/10.1086/596544
16. Arifeen SE, Saha SK, Rahman S, Rahman KM, Rahman SM, Bari S et al. Invasive Pneumococcal Disease among Children in Rural Bangladesh: Results from a Population-Based Surveillance. Clinical Infectious Diseases [Internet]. 2009 Mar 1 [cited 2019 Jan 3];48(s2):S103–13. http://www.ncbi.nlm.nih.gov/pubmed/19191605
17. Saha SK, Hossain B, Islam M, Hasanuzzaman M, Saha S, Hasan M et al. Epidemiology of Invasive Pneumococcal Disease in Bangladeshi Children Before Introduction of Pneumococcal Conjugate Vaccine. Pediatr Infect Dis J [Internet]. 2016 Jun [cited 2019 Jan 3];35(6):655–61. http://Insights.ovid.com/crossref?an=00006454-201606000-00014
18. Brooks WA, Breiman RF, Goswami D, Hossain A, Alam K, Saha SK et al. Invasive pneumococcal disease burden and implications for vaccine policy in urban Bangladesh. Am J Trop Med Hyg [Internet]. 2007 Nov [cited 2018 Nov 30];77(5):795–801. http://www.ncbi.nlm.nih.gov/pubmed/17984328
19. van Mens SP, Meijvis SCA, Endeman H, van Velzen-Blad H, Biesma DH, Grutters JC et al. Longitudinal analysis of pneumococcal antibodies during community-acquired pneumonia reveals a much higher involvement of Streptococcus pneumoniae than estimated by conventional methods alone. Clin Vaccine Immunol [Internet]. 2011 May [cited 2018 Feb 1];18(5):796–801. http://cvi.asm.org/lookup/doi/https://doi.org/10.1128/CVI.00007-11
20. Vestjens SMT, Wagenvoort GHJ, Grutters JC, Meek B, Aldenkamp AF, Vlaminckx BJM et al. Changes in pathogens and pneumococcal serotypes causing community-acquired pneumonia in The Netherlands. Vaccine [Internet]. 2017 Jul 24 [cited 2018 Feb 1];35(33):4112–8. http://www.ncbi.nlm.nih.gov/pubmed/28668570
21. Brooks WA, Goswami D, Rahman M, Nahar K, Fry AM, Balish A et al. Influenza is a Major Contributor to Childhood Pneumonia in a Tropical Developing Country. Pediatr Infect Dis J [Internet]. 2010 Mar [cited 2018 Dec 19];29(3):216–21. http://www.ncbi.nlm.nih.gov/pubmed/20190613
22. Abdullah Brooks W, Erdman D, Terebuh P, Klimov A, Goswami D, Sharmeen AT et al. Human metapneumovirus infection among children, Bangladesh. Emerg Infect Dis [Internet]. 2007 Oct [cited 2018 Dec 19];13(10):1611–3. http://www.ncbi.nlm.nih.gov/pubmed/18258022
23. Abdullah Brooks W, Terebuh P, Bridges C, Klimov A, Goswami D, Sharmeen AT et al. Influenza A and B infection in children in urban slum, Bangladesh. Emerg Infect Dis [Internet]. 2007 Oct [cited 2018 Dec 19];13(10):1507–8. http://wwwnc.cdc.gov/eid/article/13/10/07-0368_article.htm
24. Rijkers GT, Sanders EAM, Breukels MA, Zegers BJM. Infant B cell responses to polysaccharide determinants. Vaccine [Internet]. 1998 Aug 1 [cited 2018 Dec 18];16(14–15):1396–400. https://www.sciencedirect.com/science/article/pii/S0264410X9800098X
25. Breukels MA, Zandvoort A, Rijkers GT, Lodewijk ME, Klok PA, Harms G et al. Complement Dependency of Splenic Localization of Pneumococcal Polysaccharide and Conjugate Vaccines. Scand J Immunol [Internet]. 2005 Apr [cited 2018 Dec 18];61(4):322–8. https://doi.org/10.1111/j.1365-3083.2005.01584.x
26. Mitchell R, Kelly DF, Pollard AJ, Trück J. Polysaccharide-specific B cell responses to vaccination in humans. Hum Vaccin Immunother [Internet]. 2014 [cited 2018 Dec 18];10(6):1661–8. http://www.ncbi.nlm.nih.gov/pubmed/24632599

27. Black S, Shinefield H, Fireman B, Lewis E, Ray P, Hansen JR, Northern California Kaiser Permanente Vaccine Study Center Group. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Pediatr Infect Dis J [Internet]. 2000 Mar [cited 2018 Dec 19];19(3):187–95. http://www.ncbi.nlm.nih.gov/pubmed/10749457
28. Team & Approach [Internet]. 2007 [cited 2019 May 19]. PneumoADIP | Team & Approach. https://www.preventpneumo.org/about_us/team_approach/
29. Datta A, Kapre K, Andi-Lolo I, Kapre S. Multi-valent pneumococcal conjugate vaccine for global health: From problem to platform to production. Hum Vaccin Immunother [Internet]. 2022 Nov 30 [cited 2023 Aug 11];18(4):2117949. http://www.ncbi.nlm.nih.gov/pubmed/36239596
30. Kelly DF, Thorson S, Maskey M, Mahat S, Shrestha U, Hamaluba M et al. The burden of vaccine-preventable invasive bacterial infections and pneumonia in children admitted to hospital in urban Nepal. International Journal of Infectious Diseases [Internet]. 2011 Jan [cited 2019 Jan 30];15(1):e17–23. http://www.ncbi.nlm.nih.gov/pubmed/21123100
31. Singh J, Sundaresan S, Manoharan A, Shet A. Serotype distribution and antimicrobial susceptibility pattern in children ≤ 5 years with invasive pneumococcal disease in India – A systematic review. Vaccine [Internet]. 2017 Aug 16 [cited 2019 Jan 30];35(35):4501–9. http://www.ncbi.nlm.nih.gov/pubmed/28711387
32. Brooks WA, Chang LJ, Sheng X, Hopfer R, PPR02 Study Team. Safety and immunogenicity of a trivalent recombinant PcpA, PhtD, and PlyD1 pneumococcal protein vaccine in adults, toddlers, and infants: A phase I randomized controlled study. Vaccine [Internet]. 2015 Aug 26 [cited 2019 May 19];33(36):4610–7. https://linkinghub.elsevier.com/retrieve/pii/S0264410X15008798
33. Pichichero ME. Pneumococcal whole-cell and protein-based vaccines: changing the paradigm. Expert Rev Vaccines [Internet]. 2017 Dec 2 [cited 2019 Oct 27];16(12):1181–90. https://www.tandfonline.com/doi/full/https://doi.org/10.1080/14760584.2017.1393335
34. Safari D, Daningrat WOD, Milucky JL, Khoeri MM, Paramaiswari WT, Tafroji W et al. Nasopharyngeal carriage of Streptococcus pneumoniae among children < 5 years of age in Indonesia prior to pneumococcal conjugate vaccine introduction. PLoS ONE. 2024;19(1 January).
35. Wang J, Qiu L, Bai S, Zhao W, Zhang A, Li J et al. Prevalence and serotype distribution of nasopharyngeal carriage of Streptococcus pneumoniae among healthy children under 5 years of age in Hainan Province, China. https://doi.org/10.1186/s40249-024-01175-7
36. Hausdorff WP, Bryant J, Paradiso PR, Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. Clin Infect Dis [Internet]. 2000 Jan 1 [cited 2019 May 19];30(1):100–21. https://academic.oup.com/cid/article-lookup/doi/https://doi.org/10.1086/313608
37. Hausdorff WP, Bryant J, Kloek C, Paradiso PR, Siber GR. The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formulation and use, part II. Clin Infect Dis [Internet]. 2000 Jan 1 [cited 2019 May 19];30(1):122–40. https://academic.oup.com/cid/article-lookup/doi/https://doi.org/10.1086/313609
38. Brueggemann AB, Peto TEA, Crook DW, Butler JC, Kristinsson KG, Spratt BG. Temporal and Geographic Stability of the Serogroup-Specific Invasive Disease Potential of *Streptococcus pneumoniae* in Children. J Infect Dis [Internet]. 2004 Oct 1 [cited 2019 Oct 27];190(7):1203–11. http://www.ncbi.nlm.nih.gov/pubmed/15346329
39. Baqui AH, McCollum ED, Mahmud A, Roy A, Chowdhury NH, Rafiqullah I et al. Population-based incidence and serotype distribution of invasive pneumococcal disease prior to introduction of conjugate pneumococcal vaccine in Bangladesh. PLoS One [Internet]. 2020 [cited 2023 Aug 14];15(2):e0228799. http://www.ncbi.nlm.nih.gov/pubmed/32053640
40. Baggett HC, Watson NL, Deloria Knoll M, Brooks WA, Feikin DR, Hammitt LL, et al. Density of Upper Respiratory Colonization With Streptococcus pneumoniae and Its Role in the Diagnosis of Pneumococcal Pneumonia Among Children Aged <5 Years in the PERCH Study. Clinical Infectious Diseases [Internet]. 2017 Jun 15 [cited 2018 Feb 7];64(suppl_3):S317–27. Available from: https://academic.oup.com/cid/article-lookup/doi/10.1093/cid/cix100

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