Surveillance for Invasive *Streptococcus pneumoniae* Disease among Hospitalized Children in Bangladesh: Antimicrobial Susceptibility and Serotype Distribution

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**Background.** Vaccines offer the prospect of primary disease prevention of pneumococcal disease in childhood. For introduction of such vaccines in developing countries, information about disease epidemiology is necessary.

**Methods.** We evaluated antimicrobial susceptibility and serotype distribution of invasive *Streptococcus pneumoniae* disease in children aged <5 years in a network of 7 hospitals in Bangladesh from May 2004 through May 2007.

**Results.** Of 17,969 blood cultures and 3765 cerebrospinal fluid cultures, 139 yielded *S. pneumoniae* isolates; 94 were from meningitis cases, 13 were from pneumonia cases, and 32 were from sepsis cases. Among the children with positive culture results, 73% were aged <12 months and 90% were aged <24 months. Complete resistance against penicillin, chloramphenicol, and cotrimoxazole was found in 0%, 6%, and 32% of isolates, respectively. Of the 37 serotypes observed, the predominant serotypes were 2 (17%), 1 (12%), 14 (7%), 5 (6%), 7F (6%), 45 (7%), and 12A (4%). Serotypes differed between meningitis cases and nonmeningitis cases, especially for serotype 2 (25% of meningitis cases vs. 0% of pneumonia cases; P < .001). The 7-, 10-, and 13-valent vaccines would cover 20% (95% confidence interval [CI], 13%–27%), 43% (95% CI, 35%–51%), and 50% (95% CI, 42%–58%) of these cases of invasive pneumococcal disease overall, with higher coverage of nonmeningitis cases, compared with meningitis cases (7-valent coverage, 23% vs. 18%; 10-valent coverage, 55% vs. 38%; 13-valent coverage, 66% vs. 42%).

**Conclusions.** High levels of nonsusceptibility to cotrimoxazole and susceptibility to penicillin suggest that penicillin may be a drug of choice for treatment of invasive pneumococcal disease. Although serotype distribution is diverse, with changes over time and differences between syndromes observed, implementation of use of the currently available 10- or 13-valent vaccines would have a substantial impact on pneumococcal disease in Bangladesh.

*Streptococcus pneumoniae* is a predominant cause of pneumonia and meningitis, causing >800,000 deaths in children aged <5 years worldwide [1, 2]. In addition to death, meningitis frequently leads to chronic sequelae and incurs substantial direct and indirect costs [3, 4]. *S. pneumoniae*, as an etiology of pneumonia, goes largely undetected, because blood cultures lack sensitivity for detection of the organism and because most patients with pneumonia are not bacteremic [5]. The problem is worsened by the lack of laboratory facilities to isolate this fastidious organism, and that, in turn, discourages clinicians from obtaining blood and CSF specimens from patients with pneumonia and meningitis. Although pneumonia is recognized as the predominant cause of death in children aged <5 years in developing countries, these above-mentioned factors lead to underestimation of pneumococcal as the cause of severe pneumonia [6]. Similarly, the burden of pneumococcal meningitis is also not well appreciated, although the disease is found to be common when good laboratory practices are employed. [7, 8].

Strategies for prevention of pneumococcal disease and of the deaths and complications that it causes in-
clude case management with appropriate antimicrobial therapy and immunization against predominant serotypes. To formulate appropriate strategies, it is important to have current data on the drug resistance and serotype distribution of this organism. Specifically, for GAVI Alliance–eligible countries, it is increasingly important to assemble country- or region-specific serotype data to facilitate local decision making about vaccine introduction, to establish vaccine-formulation eligibility for advance market commitment mechanism support [9], and to inform manufacturers about future vaccine development. Previous reports from Bangladesh showed significant differences from other countries with respect to age distribution, antibiotic-susceptibility patterns, and serotype prevalence [10, 11]. However, these reports were mostly of meningitis cases at a single hospital in Dhaka, the capital of Bangladesh. Thus, these reports may not accurately represent the country or the disease spectrum. We established a multicenter surveillance network consisting of 7 hospitals, to evaluate antimicrobial susceptibility and serotype distribution of invasive *S. pneumoniae* strains among hospitalized children aged <5 years in Bangladesh. Furthermore, the prevalence of serotypes during May 2004–May 2007 was compared with previous data on serotypes from Bangladesh, to assess the stability of specific serotypes over time.

**METHODS**

**Setting.** A network of 7 hospitals, 6 urban and 1 rural, was established in Bangladesh (figure 1). The hospitals are located in 3 different districts of the country, with varied infrastructures and capacities. Two of the hospitals (Shishu Shasthya Foundation and Kumudini Women’s Medical College and Hospital) did not have a microbiology laboratory, and others had severely limited capacity and resources. The microbiology department of Dhaka Shishu Hospital (DSH) was the reference laboratory for the whole surveillance network, in addition to its role as a site laboratory. As reference laboratory, DSH supported the establishment of a microbiology laboratory at the Shishu Shasthya Foundation and the Kumudini Women’s Medical College and Hospital, which would work independently.

**Patients.** Children aged <5 years who fulfilled eligibility criteria for pneumonia, severe pneumonia, very severe disease, or meningitis, as described elsewhere (see table 1 in the article by Naheed et al. [13] in this supplement), and who had a blood and/or CSF culture specimen obtained were recruited after the written consent was obtained from their parents or legal guardian.

**Specimens.** Blood samples (2–4 mL) and CSF samples were collected as part of the routine clinical practice of patient care and were processed for culture in the microbiology laboratories at the respective sites. Sample collection for culture was done around the clock, and most of the CSF specimens were inoculated in real time. Specimens collected at night were processed by the clinical pathology department. In case of delay at the ward or pathology department, the specimens were kept at room temperature.

**Laboratory procedures.** All media were centrally prepared at the reference laboratory at DSH and were sent to the site laboratories every week. Blood and chocolate agar plates were made from blood agar base (Oxoid) with 5% sheep blood. CSF specimens were plated directly onto the media. Blood specimens were aseptically obtained, were inoculated in trypticase soy broth (Oxoid) with 0.25% sodium polyethanol sulfonate (Sigma), and were incubated at 37°C for 7 days. Inoculated broths were subcultured on blood and chocolate agar plates on days 2, 3, and 5. At 3 sites—DSH, Shishu Shasthya Foundation, and Kumudini Women’s Medical College and Hospital—whenever a contaminated blood culture was documented, the case was reviewed by the respective laboratory to identify the possible reason(s) for the contamination as a quality check of the entire process, from blood collection to final plating. Contam-
Methods, American Type Culture Collection (ATCC) strains of \textit{S. pneumoniae} were further confirmed by susceptibility to optochin (5 \(\mu\)g). Strains with a zone of inhibition >14 mm were considered to be \textit{S. pneumoniae}, whereas those with a zone of inhibition of 9–13 mm were subjected to a bile solubility test [14].

**Quality assurance.** To ensure the quality of microbiological methods, American Type Culture Collection (ATCC) strains of \textit{S. pneumoniae} (ATCC 49619), \textit{Escherichia coli} (ATCC 25922), \textit{Haemophilus influenzae} (ATCC 49247), and \textit{Staphylococcus aureus} (ATCC 25923) were cultured on plates, to observe their proper growth on each batch of respective media, before any clinical specimen was cultured. To ensure the standardization of site laboratories and their coordination with the reference laboratory, training of laboratory personnel from each site was arranged at the beginning of the study. In addition, refresher orientations of the staff were conducted every year. For quality assurance of site laboratories, blinded blood culture bottles, spiked with known organisms, were sent to study sites every 4 months. All isolates from the site laboratories were transported to DSH for confirmation of identification and for performance of antibiogram and serotyping.

**Antimicrobial-susceptibility testing.** \textit{S. pneumoniae} strains isolated at the site laboratories were screened by the respective laboratory for resistance to penicillin, ampicillin, ceftriaxone, cotrimoxazole, chloramphenicol, and ciprofloxacin by the disk-diffusion method as described by Jorgensen et al. [15], and results were reported to the clinician. When the strains arrived at DSH, antibiogram was repeated, and strains that were nonsusceptible, on the basis of Clinical and Laboratory Standards Institute guidelines [16] for disk diffusion, were subjected to the E test for determination of MIC values. E tests were performed on Mueller-Hinton agar (Oxoid) supplemented with 5% sheep blood. Inocula were prepared in Mueller-Hinton broth by direct suspension of pneumococcal colonies grown overnight on sheep blood agar to a density that matched a 0.5 McFarland opacity standard tube [16].

**Serotyping.** Pneumococcal strains were serotyped by the capsular-swelling procedure (the Quellung reaction) with type-specific antipneumococcal omni, pool, type, or group, and factor sera (Statens Serum Institute) [10, 17]. ATCC strains 6314, 6301, and 10341 and Johns Hopkins University strains 9, 23, and 4 (kindly provided by Prof. Mark Steinhoff) were used as known control strains. Nontypeable \textit{S. pneumoniae} strains were screened out with use of omni serum at the first step of serotyping. A random selection of 36 strains of 24 different serotypes was tested further at the Microbiology Laboratory of Oxford University (United Kingdom), and typing results were identical at both laboratories.

**Definition of isolates.** Isolates were labeled as meningitis strains if they were isolated in CSF specimens or in blood specimens only from patients who had a CSF leukocyte count \(\geq 10\) leukocytes/mm\(^3\). Blood isolates were labeled as pneumonia strains if they were isolated in specimens from patients with pneumonia or severe pneumonia. All other isolates that did not fit the definition of pneumonia or meningitis strains or a suspected meningitis strain with a CSF leukocyte count <10 leukocytes/mm\(^3\) were considered to be sepsis strains. Strains isolated from both blood and CSF specimens were considered to be a single meningitis isolate. The serotypes of the isolates in this study were sorted and ranked according to prevalence and were compared with those identified in 2 previous studies from DSH alone [10, 11].

**Data management.** Laboratory report forms were entered in EpiData and were analyzed by Epi Info, version 6.02. \(P\) values were calculated by the \(\chi^2\) test with the StatCalc calculator of Epi Info. The 95% CIs were calculated using an online calculator [12].

**Ethics.** This project was approved by the ethics review committee of the Bangladesh Institute of Child Health, DSH, and the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B).

**RESULTS**

From May 2004 through May 2007, blood cultures were performed for 17,969 patients, and CSF cultures were performed for 3765 patients. \textit{S. pneumoniae} strains were isolated from 139 patients, including 94 isolates (11.2%) in 842 CSF specimens from patients with meningitis who had leukocyte counts \(\geq 10\) cells/mm\(^3\), 13 (0.3%) in 4305 specimens from patients with pneumonia, and 32 (0.3%) in 12,822 specimens from patients with sepsis. Of the patients with isolates, 64% of those with meningitis strains and 61% of those with pneumonia strains were male.

Pneumococcal isolation was most common among young children (figure 2); 55% of patients with isolates were aged \(\leq 6\) months, 76% were aged \(\leq 12\) months, and 90% were aged \(\leq 24\) months. Patients with meningitis strains were younger than those with sepsis strains (median age, 4 vs. 14 months; \(P<.001\)). Indeed, 66% of all meningitis isolates were from children aged \(< 6\) months, compared with 22% of sepsis strains (figure 2). Likewise, 54% of patients with pneumonia were aged \(< 6\) months, with a median age of 5 months. Of 139 strains, 4%, 4%, and 6% were nonsusceptible to penicillin, ciprofloxacin, and chloramphenicol, respectively, whereas 72% were nonsusceptible to cotrimoxazole. The antimicrobial-nonsusceptibility rates were similar for meningitis and pneumonia isolates. Nonsusceptibility to ciprofloxacin (6 strains) and penicillin (5 strains) was only at the intermediate level (MIC range for ciprofloxacin, 1.5–3.0 \(\mu\)g/mL; MIC range for penicillin, 0.064–0.380 \(\mu\)g/mL).
We serotyped 137 strains because 2 strains were lost during transport at the initial stage of the study. We detected 37 different serotypes, with a predominance of serotypes 2 (17%), 1 (12%), 14 (7%), 5 (6%), 7F (6%), 45 (7%), and 12A (4%), which comprised 59% of all isolates (figure 3). Serotype distribution of isolates from meningitis and nonmeningitis cases differed, especially for serotype 2 (24% of meningitis cases vs. 0% of nonmeningitis cases; $P < .001$).

Comparing the serotype distribution in this study with those in previous studies conducted at 1 of the network hospitals (DSH), serotype 2, which was not found in the 1992–1995 study [10] and ranked sixth in the 1999–2000 study [11], emerged as the predominant type during May 2004–May 2007. In the present study, serotypes 1 and 5 ranked second and fourth, compared with rankings of seventh and eight in 1992–1995 and fifth and third in 1999–2000, respectively. On the other hand, serotype 14 was ranked third most common in 1992–1995 and also in the present study, but it ranked 10th in the 1999–2000 study (table 1). Although the previous studies were confined to a single hospital (DSH), that hospital yielded 43% of the strains identified by this surveillance network. Distribution of serotypes from the present study indicates that the currently available and forthcoming 7-, 10-, and 13-valent pneumococcal conjugate vaccines would cover 20% (95% CI, 13%–27%), 43% (95% CI, 35%–51%), and 50% (95% CI, 42%–58%), respectively, of cases of invasive pneumococcal disease overall. When evaluated by syndrome, vaccine coverage is greater for pneumonia cases than for meningitis cases—33% vs. 18% for 7-valent vaccine, 58% vs. 38% for 10-valent vaccine, and 75% vs 42% for 13-valent vaccine. Vaccine coverage for sepsis was 19% for 7-valent vaccine, 53% for 10-valent vaccine, and 63% for 13-valent vaccine (figure 4).

**DISCUSSION**

Invasive pneumococcal disease was found to be most prevalent early in life, with 48% of patients aged ≤6 months and 72% aged ≥12 months. This age distribution is younger than in industrialized countries but is similar to those of other developing countries [18, 19], as well as among blacks in the United States [20], which indicates the appropriateness of intervention with conjugated vaccine as part of the schedule of the national immunization program of Bangladesh, given at ages 6, 10, and 14 weeks.

The results of antibiotic-susceptibility testing from this multicenter study are similar to those of several previous studies from Bangladesh and the region [8, 11, 21, 22]. Most of the isolates were susceptible to all antibiotics except cotrimoxazole, to which 72% of the isolates were nonsusceptible. High resistance to cotrimoxazole among pneumococci is possibly a result of its widespread use by community health care workers. However, it is difficult to establish the relevance of these in vitro results for in vivo outcomes, because culture-proven cases are very rare, especially in settings where cotrimoxazole is used. However, in vitro evidence from several countries demonstrates high MIC values among the resistant strains [8, 11, 22, 23]. In addition, more clinical treatment failures have been reported with the use of cotrimoxazole, compared with the use of amoxicillin [24, 25]. Indeed, the World Health Organization is recommending that acute respiratory infection treatment programs change the first line of treatment from cotrimoxazole to amoxicillin.

In contrast to cotrimoxazole, nonsusceptibility to penicillin and chloramphenicol remained low, without any significant change in the past decade [11, 21, 25]. In our previous study, a significant difference in chloramphenicol resistance between
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<th>Serotype</th>
<th>No. (%) of study patients with serotype</th>
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<tr>
<td>2</td>
<td>23 (17)</td>
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<tr>
<td>1</td>
<td>16 (12)</td>
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<td>14</td>
<td>9 (7)</td>
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<td>7 (5)</td>
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<td>18C</td>
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<td>19A</td>
<td>4 (3)</td>
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<td>23F</td>
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**NOTE.** NA, not available (only serogroup data were available).

Figure 3. Serotype distribution (bars) among cases of meningitis (93 cases), pneumonia (12 cases), and sepsis (32 cases) and as a cumulative percentage (line) for all cases of invasive pneumococcal disease (IPD).

Although a diverse set of serotypes was isolated, only 7 serotypes (2, 1, 14, 5, 7F, 12A, and 45) accounted for 59% of cases of invasive disease. Except for serotype 14, these prevalent serotypes are rarely isolated in developed countries [20, 26, 27]. The serotype distribution differed from that in previous studies in Bangladesh; serotype 2 was the most common serotype observed among patients with meningitis in the present study (prevalence, 25% vs. 2% of the meningitis cases in previous studies [10, 11, 21]), whereas serotypes 7F and 6 predominated in previous studies. Serotype 2 is infrequently reported from different parts of the world, including Papua New Guinea [28], India [8], and Mali [29], but almost never from developed countries [30]. Serotypes 1 and 5 ranked second and fourth and were equally responsible for meningitis and nonmeningitis cases. These 2 serotypes are commonly found in developing countries in Asia and Africa [8, 29, 31–33] and in Europe [34, 35] but are rarely detected in Japan [36], Korea [37], the United States [20, 27, 38], and Canada [39].

We observed that 17 serotypes accounted for 80% of isolates identified in hospitalized patients with invasive pneumococcal disease in Bangladesh, compared with only 7 serotypes that have the same coverage in the United States [20, 28] and Finland [27], before the introduction of pneumococcal vaccine. However, this difference may be attributed in part to the lower threshold for culture of blood specimens from sick babies in developed parts of the world, and common pretreatment with antibiotics may affect the detection of selective serotypes. In addition, most pneumococcal infections are not culture positive, and the serotypes may vary in their ability to be cultured. Thus, cultured serotype distribution may not reflect true serotype distribution. Furthermore, these patients are ill, hospitalized children and may not represent nonsevere pneumococcal infections treated in an outpatient department or the community. However, conjugate vaccine in other countries was introduced on the basis of the identification of strains isolated in hospitals, and this approach resulted in marked overall reduction in the incidence of invasive pneumococcal disease [40, 41]. Thus, these strains are most likely to represent the epidemiologically important serotypes. Importantly, marked differences between serotypes causing meningitis, sepsis, and pneumonia were reflected in vaccine coverage by the existing 7-valent and forthcoming 10- and 13-valent pneumococcal vaccines. Coverage rates for sepsis and pneumonia cases by the 10- and 13-valent formulations are notably higher than for meningitis cases, as was also observed for population-based pneumococcal pneumonia cases in urban Bangladesh, for which 10- and 13-valent vaccine coverage rates were 59% and
the ultimate pneumococcal vaccine for the Bangladeshi population aged <5 years. However, existing and forthcoming pneumococcal vaccines can have a meaningful and substantial impact on public health in Bangladesh. It is important to continue surveillance for circulating pneumococcal serotypes, to determine the predominant serotypes and to establish the trend of serotype changes without any selective intervention, so that the benefit of vaccination against predominant serotypes can be evaluated after its introduction.

PNEUMOCOCCAL STUDY GROUP

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