Appropriateness of a Pneumococcal Conjugate Vaccine in Brazil: Potential Impact of Age and Clinical Diagnosis, with Emphasis on Meningitis

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The distribution of pneumococcal serotypes in Brazil was analyzed by age group and clinical diagnosis, using data obtained during 20 years of national surveillance. Serotypes 1 and 5 remained among the main serotypes in all age groups, increasing in frequency with age. Serotype 14 was prevalent among children, whereas serotypes 3 and 4 were most prevalent among the adult population. The potential impact of the 7- and 9-valent conjugate vaccines on children up to age 5 years with severe pneumococcal diseases was 58.2% and 73%, respectively; the highest coverage of the 7-valent vaccine for pneumonia was achieved for children aged 7 months to 2 years (70%), whereas, for meningitis, it was observed for children aged 7 months to 5 years (58.6%). The use of conjugate vaccine may be of potential benefit by reducing the childhood sequelae and mortality of pneumococcal infection in Brazil.

Streptococcus pneumoniae is part of the normal microbial flora of the human nasopharynx and is a major bacterial pathogen causing infection of the respiratory tract, bacteremia, and meningitis in children and in elderly persons in both industrialized and developing countries [1, 2]. It is estimated that 1.6–2.2 million children die each year in the world from acute respiratory infections [3]. Pneumonia is the single most important cause of child death in the world, and pneumococcus remains the most common cause of childhood community-acquired pneumonia [4].

Since 1999, with the introduction of the Haemophilus influenzae conjugate vaccine into the Brazilian immunization program, pneumococcus has persisted as the second most common cause of meningitis, exceeded in frequency only by Neisseria meningitidis, with an annual incidence rate of 10 cases/100,000 children up to age 1 year and an overall case-fatality rate of 27.5% (National Centre for Epidemiology, National Foundation for Health, Ministry of Health of Brazil; unpublished data). In the past 2 years, the Brazilian Ministry of Health has introduced the pneumococcal 23-valent capsular polysaccharide vaccine, with the aim of decreasing the morbidity and mortality rates of bacteremic pneumococcal disease in elderly persons [5, 6]. A 7-valent vaccine that includes the capsular serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F was recently licensed. Its formulation is based on the most common invasive serotypes causing infections in the United States and European countries [7]. In Brazil, because of the high
price of this vaccine, it has been accessible only in the private sector and, thus, only to a small part of the population [8].

The distribution of pneumococcal serotypes may vary according to geographic region, time, clinical presentation, and age of patients, as has been shown by several reports [2, 9–11] and recently confirmed by Hausdorff et al. [12, 13]. In this regard, Di Fabio et al. [14] found a distinctive profile of pneumococcal serotypes in Latin American children, compared with those in other countries. So far, few publications have addressed the age distribution of pneumococcal serotypes in Latin America. We analyze here the distribution of pneumococcal serotypes in Brazil by age group and clinical diagnosis, using data from the national surveillance of *S. pneumoniae* over a 20-year period. Our objective was to assess the appropriateness of the conjugate vaccine for preventing pneumococcal invasive disease in Brazil and to gather an epidemiological baseline to measure further the impact of the conjugate vaccine.

**MATERIALS AND METHODS**

**Surveillance of *S. pneumoniae***. The Adolfo Lutz Institute (IAL), a Public Health Laboratory located in São Paulo, is the national reference center for meningitis and *S. pneumoniae* in Brazil. Isolates from invasive pneumococcal diseases recovered by the epidemiological survey network come from public and private hospitals and public health laboratories in different geographic regions in the country. The Brazilian isolates are systematically sent to the IAL for serotyping and other tests. Between 1977 and 2000, the IAL received 4858 invasive strains of *S. pneumoniae*, 33% of which were isolated before 1992; most of them were from the southeastern and northeastern regions of the country. In 1993, when the Regional System for Vaccients (SIREVA)–Vigia program for Latin America [15] was established in Brazil, in agreement with the Ministry of Health, the surveillance of *S. pneumoniae* was gradually extended to other regions, leading to the recovery of a larger number of isolates. Among the 4858 isolates, there were 826 from patients with pneumonia, 3714 from patients with meningitis, and 318 from patients with other clinical syndromes (bacteremia, arthritis, and abdominal abscess). Pneumonia isolates were recovered from blood (60%) or pleural fluid (40%), whereas the majority of meningitis isolates were recovered from cerebrospinal fluid (96.4%). Data on age were available for 4120 (84.8%) patients. Multiple isolates of the same type from different clinical sites in the same patient were treated as a single isolate. Most patients were hospitalized with community-acquired infections.

**Identification and serotyping***. At the local level, species identification of isolates was performed by standard methods. At the IAL, identification of *S. pneumoniae* was confirmed by Gram stain, colonial morphology on blood agar media, susceptibility to optochin (5-μg disks), and bile solubility [16]. The isolates were kept lyophilized. Pneumococci were serotyped by Quellung reaction with serum obtained from the Statens Seruminstitut [17]. Before 1993, all of the strains were serotyped at the World Health Organization Collaborating Center for Reference and Research on Pneumococci (University of Pennsylvania School of Medicine, Philadelphia). Thereafter, the strains from children aged <6 years were serotyped at the IAL.

**Data analysis***. All information related to each isolate was recorded in a data file by using EpilInfo software (version 6.04d; Centers for Diseases Control and Prevention). An exploratory analysis was first done to investigate possible temporal variations in the distribution of the 14 (75th percentile) most frequent serotypes isolated during the 2 periods, 1977–1989 and 1990–2000. The rationale for choosing these periods took into account the epidemiological characteristics of pneumococcal infections [12]. Because no marked differences were noted in the most frequently isolated serotypes during the 2 periods, we proceeded to a pooled data analysis of serotypes, considering the entire study period as a whole. The patients were grouped by age: 7–24 months, >2–5 years, 0–5 years, 6–49 years, and ≥50 years. The groups 6–19 and 20–49 years were merged, because no statistically significant differences in the distribution of the predominant serotypes between these 2 age groups could be detected. The frequency of serotypes for infants up to age 29 days was also investigated. We did not find any predominance or disappearances of serotypes, compared with older children. Exploratory data analysis was applied to identify patterns of age distribution according to different serotypes. Box-plot techniques were used to display the level, spread, and symmetry of the age distribution within each pneumococcal serotype. The coverage of the 7- and 9-valent (serotypes in the 7-valent plus serotypes 1 and 5) vaccines with the respective 95% confidence intervals (CIs) was estimated for each age group as the number of isolates belonging to the serotypes included in the vaccines divided by the total number of isolates in the respective age group. Prevalence rates and 95% CIs were estimated for each serotype by age group and clinical diagnosis (meningitis or pneumonia). Differences in the estimated prevalence of serotypes were considered to exist when the 95% CIs did not overlap. The data analyses were carried out by using the statistical packages EpilInfo and SPSS (version 9.0; SPSS).

**RESULTS**

Overall, 4858 isolates of *S. pneumoniae* were obtained, 63 serotypes were identified, and 25 strains were not typeable. The most prevalent serotypes (74.4%) associated with invasive pneumococcal diseases in Brazil are displayed in table 1. Serotypes 8, 9N, 11A, 12F, 15B, 17F, 18A, 23A, 23B, and 24F were identified at rates of 1%–2% each, whereas serotypes 9A, 11B,
### Table 1. Ranking of serotypes of *Streptococcus pneumoniae* isolated from invasive diseases, by age groups and clinical diagnoses, Brazil, 1977–2000.

<table>
<thead>
<tr>
<th>Age group, clinical diagnosis (no. of isolates)</th>
<th>1st (no. of isolates)</th>
<th>2nd (no. of isolates)</th>
<th>3rd (no. of isolates)</th>
<th>4th (no. of isolates)</th>
<th>5th (no. of isolates)</th>
<th>6th (no. of isolates)</th>
<th>7th (no. of isolates)</th>
<th>8th (no. of isolates)</th>
<th>9th (no. of isolates)</th>
<th>10th (no. of isolates)</th>
<th>11th (no. of isolates)</th>
<th>12th (no. of isolates)</th>
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<td>0–5 years</td>
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<td>Meningitis (n = 1547)</td>
<td>14 (10.1)</td>
<td>6B (10.1)</td>
<td>18C (8.7)</td>
<td>5 (7.0)</td>
<td>6A (6.1)</td>
<td>23F/19F (5.3)</td>
<td>1 (5.0)</td>
<td>9V (3.1)</td>
<td>19A (2.8)</td>
<td>4 (2.0)</td>
<td>10A (1.9)</td>
<td>3 (1.7)</td>
<td>7F (1.7)</td>
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<td>Pneumonia (n = 644)</td>
<td>14 (38.6)</td>
<td>1 (14.9)</td>
<td>5 (8.7)</td>
<td>6B (5.9)</td>
<td>19A/23F (4.0)</td>
<td>6A (3.9)</td>
<td>9V (3.3)</td>
<td>18C (2.3)</td>
<td>3 (2.2)</td>
<td>19F/4 (1.5)</td>
<td>7F (0.6)</td>
<td>10A (0.3)</td>
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<tr>
<td>Others (n = 135)</td>
<td>14 (28.5)</td>
<td>6B (10.2)</td>
<td>1/19F (9.5)</td>
<td>5/23F (7.3)</td>
<td>18C/19A (4.4)</td>
<td>3/6A (2.2)</td>
<td>9V/7F (1.5)</td>
<td>4 (0.7)</td>
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<td>6–9 years</td>
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<td>Meningitis (n = 1321)</td>
<td>1 (9.7)</td>
<td>3 (7.1)</td>
<td>18C (6.1)</td>
<td>23F (4.5)</td>
<td>6A/6B (4.4)</td>
<td>4 (3.5)</td>
<td>19F (3.4)</td>
<td>5 (3.2)</td>
<td>10A (2.9)</td>
<td>7F (2.5)</td>
<td>9V (2.3)</td>
<td>19A (1.1)</td>
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<tr>
<td>Pneumonia (n = 106)</td>
<td>1 (18.9)</td>
<td>9V (7.5)</td>
<td>3 (6.6)</td>
<td>5/14/19A (5.7)</td>
<td>23F (3.8)</td>
<td>4/6B/7F/18C (2.8)</td>
<td>6A/10A (1.9)</td>
<td>19F (0.9)</td>
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<tr>
<td>Others (n = 99)</td>
<td>1 (15.4)</td>
<td>14 (14.4)</td>
<td>4/6B (7.7)</td>
<td>5/9V (6.7)</td>
<td>19A (5.8)</td>
<td>3 (4.8)</td>
<td>7F/23F (3.8)</td>
<td>10A (2.9)</td>
<td>19F (1.9)</td>
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<tr>
<td>Meningitis (n = 198)</td>
<td>3 (20.2)</td>
<td>10A (6.1)</td>
<td>4/19F/23F (5.0)</td>
<td>5/14/7F/18C (4.0)</td>
<td>1 (3.5)</td>
<td>6A (3.0)</td>
<td>6B (2.5)</td>
<td>9V (2.0)</td>
<td>19A (0.5)</td>
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<td>Pneumonia (n = 45)</td>
<td>14 (13.3)</td>
<td>3 (11.1)</td>
<td>1/6B/9V (6.7)</td>
<td>19F (4.4)</td>
<td>4/6A/18C/19A (2.2)</td>
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<tr>
<td>Others (n = 25)</td>
<td>14 (22.2)</td>
<td>6/6B/19F (11.1)</td>
<td>6A (7.4)</td>
<td>4/5/9/23F (3.7)</td>
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<td>Total* (n = 4858)</td>
<td>14 (15.6)</td>
<td>1 (9.4)</td>
<td>6B (7.3)</td>
<td>5 (6.1)</td>
<td>18C (6.0)</td>
<td>6A (5.0)</td>
<td>3 (4.9)</td>
<td>23F (4.7)</td>
<td>19F (4.4)</td>
<td>9V (3.2)</td>
<td>4 (2.9)</td>
<td>19A (2.5)</td>
<td>10A (2.4)</td>
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* Seven hundred thirty-eight isolates with no information on age are included.
Figure 1. Distribution of age related to each prevalent serotype in Brazil. Box length represents the interquartile ranges of age. Top and bottom of the boxes are 25th and 75th percentiles, respectively. Lines that cross the boxes indicate median values of the age distribution. Central vertical lines (whiskers) that extend from the boxes to the highest and lowest rates indicate the 95% and 5%, respectively, of the age distribution. Circles (extreme values correspond to cases with age values >3 box lengths from the upper or lower edge of the box) or asterisks (outlier values correspond to cases with age values 1.5–3 box lengths from the upper or lower edge of the box) indicate the individual values outside whiskers. Numbers above serotypes indicate no. of isolates per serotype.

28F, 33A, 33B, 35A, 35B, 35C, 36, and 48 were isolated only once during the whole study period. Isolates belonging to serotype 2 (n = 12) were not identified after 1987. The mean and median ages of the 4120 patients for whom data were available were 13 and 3 years, respectively. Forty-six percent of strains were isolated from children up to age 2 years, whereas 6.5% were from elderly adults (aged ≥50 years). For those with pneumonia, the mean and median ages were 8 and 2 years, respectively, whereas for isolates from spinal fluid, the corresponding values were 14 and 5 years.

According to table 1, for children up to age 5 years, 9 serotypes (1, 5, 6A, 6B, 9V, 14, 18C, 19F, and 23F) remained the most prevalent during the study period. Serotype 14 was prominent in children, decreasing in frequency for those aged 6–49 years, notably among isolates from meningitis. Serotypes 3 and 4 showed a tendency to increase in frequency with age and were most prevalent among the adult population. Serotypes 1 and 5 remained among the principal serotypes in Brazil, mainly in children with pneumonia. Serotype 1 was also predominant as the cause of meningitis and pneumonia in patients aged 6–49 years, despite its low frequency in the ≥50-year-old age group. Serotype 19A was isolated more frequently from patients with pneumonia, whereas serotype 18C predominated among spinal fluid isolates.

The box plots in figure 1 depict the age distribution for children up to age 5 years within each serotype for meningitis and pneumonia isolates. Among meningitis isolates (figure 1A), the median ages for all serotypes were <1 year (3–8 months). Serotypes 1, 14, 19A, 19F, 5, 6A, and 6B were strikingly found in children aged <2 years, with the 75th percentiles in those up to age 1 year. In contrast, the box plots for serotypes 3 and 9V display a significantly wider range of age without outliers in the distribution. For pneumonia isolates, the box plots for all serotypes, except for serotype 4 (probably because of the small number of isolates), shifted upward, compared with meningitis isolates (figure 1B). The median varied between 12 and 27 months. Serotypes 1, 18C, 19F, 23F, 3, and 5 showed wider dispersion of age, with the 75th percentiles in children aged ≥3 years without outlier values. Remarkable differences in the age distribution of serotypes 1, 5, and 9V were found between meningitis and pneumonia isolates.

The estimated coverage for the 7- and 9-valent conjugate vaccines among children aged 7 months to 5 years was 58.2% (95% CI, 55.7%–60.7%) and 73.0% (95% CI, 70.6%–75.2%), respectively. Figure 2 shows the percentages of strains belonging to 7-valent serotypes isolated from the sterile fluids of patients with pneumonia and meningitis, stratified by age group. For pneumonia isolates in the 7 months to 2 years age group, it could be estimated that 70% (95% CI, 65.2%–74.4%) were 7-valent vaccine serotypes. The potential coverage by the 7-valent vaccine, however, decreased significantly to an average of 29.8 (95% CI, 27.7–32.0) for those age groups ≥2 years (P < .01). For meningitis, lower coverage was observed among children aged 7 months to 2 years (61%; 95% CI, 57.0%–64.9%) and among children aged 7 months to 5 years (58.6%; 95% CI, 55.2–62.0), compared with that for pneumonia (P < .01). An opposite trend was found for the age group ≥2 to 5 years, in which 51.7% (95% CI, 44.8%–58.6%) of meningitis isolates and 29.2% (95% CI, 23.0%–36.0%) of pneumonia isolates were included in the 7-valent vaccine (P < .01). The overall coverage for serotypes included in the 23-valent polysaccharide vaccine
and upper and lower limits of 95% confidence intervals by vertical lines.

was 86.2%, which was without significant difference, compared with 83.2% of coverage for patients aged ≥60 years.

DISCUSSION

Our study includes a large body of data collected during ≥2 decades that are concerned with invasive pneumococcal disease in different regions of Brazil. This nationwide effort resulted in 4858 pneumococcal isolates. The relative frequency of the most prevalent invasive serotypes in Brazil differed somewhat from that reported from other regions of the world [9, 12]. An example is the frequency of serotypes 1 and 5, which increased with age, peaking in older children and adults. Both serotypes 1 and 5 are among the most prevalent isolates causing invasive infection among children in Latin America [14], some African countries [18], Israel [19, 20], and India [21]. Although serotypes 1 and 5 are now relatively uncommon in North America [22–26] and in some European countries [9, 12, 13], prior to the 1970s, they ranked among the serotypes most frequently found in these areas [27–29]. A continuing analysis of serotype distribution may determine whether the epidemiological profile of serotype distribution experienced by developing countries today corresponds to that observed in industrialized countries in the past.

Serotype 1 has been considered to be among the more invasive pneumococcal types. It has been suggested that the increased recovery of serotype 1 may be related to diagnostic practices, notably when more severe infections are included [30]. This situation may be the case in Brazil, where the sources of isolates are patients diagnosed with severe infections and the majority of cases of mild pneumonia and bacteremia receive empirical antimicrobial treatment without any microbial diagnosis.

The estimated coverage by the 7-valent conjugate vaccine during childhood (age 7 months to 5 years) invasive pneumococcal diseases in Brazil (58.2%) is lower than that of other countries [12]. Serotypes 4 and 9V, which are included in the vaccine, appear at low frequency in Brazil, accounting for only 5.1% of the isolates. On the other hand, the inclusion of serotypes 1 and 5 in the 9-valent vaccine formulation would improve substantially, by 15%, the potential vaccine coverage for children in Brazil. The highest coverage of the 7-valent conjugate vaccine was achieved for children aged 7 months to 2 years for pneumonia, and, for meningitis, the highest coverage was observed for children aged 7 months to 5 years. Therefore, because the highest incidence of pneumococcal diseases in children occurs from birth to age 2 years, we could expect that children aged 7–24 months would be the most likely to benefit from the 7-valent conjugate vaccine in Brazil.

Serotype 14 ranks as the most common cause of meningitis and pneumonia in children but is less common among adults, including elderly adults. The importance of serotype 14 in childhood infections has already been recognized in Brazil [31–33] and in many other countries [12, 14, 22, 34]. In contrast, infections with serotypes 3 and 4 are distributed more evenly throughout the life span.

Ninety serotypes of pneumococci exhibiting individual differences in their invasiveness have been identified [9, 11]. Although a small number of serotypes is responsible for the preponderance of pneumococcal infection in humans, it is difficult to detect statistical differences in ranking, because a large number of isolates of each serotype would be necessary. Invasive types change in rank order but tend to remain the same over many years. The representativeness of our results on serotype distribution may differ from those studies predominantly of respiratory infection, because our data are composed of a higher number of meningitis isolates than pneumonia isolates and because a substantial proportion of strains was recovered from pleural fluid rather than from blood. The surveillance system for meningitis and pneumonia in Brazil is active and voluntary. Meningitis is a compulsory reportable disease, and hospitals or public health laboratories routinely perform bacterial isolation of cerebrospinal fluid throughout the country. For pneumonia and bacteremia, the collection of blood for diagnostic purposes is not routinely done, because it depends on the doctor’s decision. However, with the introduction of the SIREVA project in Brazil, active surveillance of pneumonia in children was implemented in some medical centers located in different regions of the country.

As a result, the majority of the isolates from our pneumococcal surveillance was associated with severe invasive disease, leading to high case-fatality rates of pneumococcal infection. Considering, however, that the ultimate goal of the conjugate pneumococcal vaccine is to prevent sequelae and deaths, an
assessment of the vaccine’s coverage based on the surveillance of these isolates from cases of meningitis could approximate the potential impact of this intervention in reducing childhood mortality.

It is worth noting that the prevalence of serotypes summarized in the present study was not marked by considerable differences in drug resistance among the several serotypes causing invasive illness, and no significant differences were noted for penicillin resistance rates for invasive pneumococci in Brazil for the past few years. In fact, the incidence of penicillin resistance of pneumococci in Brazil prior to 2000 was lower than that in other countries [14, 35, 36]. The main serotypes associated with penicillin resistance are the same as those infecting children elsewhere (14, 6A/B, 19A/F, and 23F) [37], and, because serotypes 1 and 5 have not been associated with antimicrobial resistance in Brazil [15, 31, 35], the use of 7- or 9-valent conjugate vaccines should be equally effective in covering resistant isolates. In a recent study of meningitis in Salvador, northeast Brazil [38], serotype 18C appeared at low prevalence among 291 pneumococcal isolates (4.8%), and serotypes 1 (<1.0%) and 5 (1.7%) were found at much lower frequencies than those in the present study, which suggests that there are regional differences of serotype distribution.

In Brazil, despite the ongoing pneumococcal serotype surveillance, there is still a need to encourage the collection of isolates from a wider clinical spectrum of pneumococcal infections. In view of the important changes in serotype diversity that have occurred in the past few decades in some countries [24, 27], and particularly with the increasing incidence of resistant strains of S. pneumoniae, monitoring the trends in pneumococcal serotypes will be critical for assessing the appropriateness and possible impact of the current and future vaccines according to the local epidemiological scenario.

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