Invasive *Streptococcus pneumoniae* Infection in Latin American Children: Results of the Pan American Health Organization Surveillance Study

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Protein-polysaccharide conjugate vaccines against *Streptococcus pneumoniae* promise to be an effective public health intervention for children, especially in an era of increasing antimicrobial resistance. To characterize the distribution of capsular types in Latin America, surveillance for invasive pneumococcal infection in children ≤5 years of age was done in six countries between February 1993 and April 1996. Fifty percent of 1,649 sterile-site isolates were from children with pneumonia, and 52% were isolated from blood. The 15 most common of the capsular types prevalent throughout the region accounted for 87.7% of all isolates. Overall, 24.9% of isolates had diminished susceptibility to penicillin: 16.7% had intermediate resistance and 8.3% had high-level resistance. Three customized vaccine formulas containing 7, 12, and 15 capsular types were found to have regional coverages of 72%, 85%, and 88%, respectively. This study emphasizes the need for local surveillance for invasive pneumococcal disease prior to the development and evaluation of protein-polysaccharide conjugate vaccines for children.

Acute respiratory tract infection (ARI) accounts for >4 million childhood deaths in the developing world each year [1]. Seventy percent of all ARI-related deaths are due to pneumonia, of which *Streptococcus pneumoniae* is the most frequent bacterial cause [2, 3]. There are few data describing the epidemiology of invasive *S. pneumoniae* infection in Latin America, although several studies [4–6] confirm that the pathogen is the most frequent bacterial cause of childhood pneumonia in this region.

The burden of pneumococcal illness in developing countries and the global emergence of antibiotic-resistant *S. pneumoniae* [7] make prevention by immunization an appealing control strategy. The currently available 23-valent polysaccharide vaccine, however, is poorly immunogenic in children <2 years of age, who remain disproportionately affected by pneumococcal disease. A new generation of protein-polysaccharide conjugate vaccines is being developed to address this problem [8].

The optimal capsular-type composition for a protein-polysaccharide conjugate pneumococcal vaccine has been determined for the United States [9] and Europe [10]. Limited data from developing countries suggest that the capsular-type distribution of *S. pneumoniae* is different from that in the developed world [11, 12]. Determination of the regional pneumococcal-type distribution and assessment of differences in type prevalence between countries are crucial antecedents to the development and use of any protein-polysaccharide conjugate vaccines in Latin America.

To address this issue, the Pan American Health Organization, as part of the Sistema Regional de Vacunas (SIREVA) initiative, developed a network of national reference laboratories to conduct region-wide, prospective surveillance of invasive pneumococcal infections in children in Latin America, with laboratory and epidemiological support provided by the Canadian National Centre for Streptococcus (NCS; Edmonton, Alberta) and the Bureau of Infectious Diseases, Laboratory Centre for Disease Control, Health Canada (Ottawa). The network’s specific objective was to determine the relative prevalence of capsular types of *S. pneumoniae* causing invasive disease, particularly pneumonia, in children ≤5 years of age in Latin America.

**Methods**

Study coordinators in Argentina, Brazil, Colombia, Chile, Mexico, and Uruguay recruited >70 hospitals in 31 cities to collect sterile-site pneumococcal isolates from children. Hospi-
tals were selected if they served a pediatric patient population and had clinicians and microbiologists willing to participate. Investigators attempted to select hospitals from different geographic regions within each country.

Clinicians collected sterile-site specimens, including blood and cerebrospinal, pleural, peritoneal, or articular fluid, from patients ≤5 years of age who presented with meningitis, either sepsis or bacteremia without an infectious focus, arthritis, peritonitis, or signs fulfilling the World Health Organization’s clinical criteria for pneumonia [13]. Patients were excluded from the study if they had been ill for >15 days or if they had laryngeal stridor or croup. Nasopharyngeal swab specimens, bronchial aspirates, and specimens from bronchoalveolar lavage were excluded. Pneumonia was considered the primary diagnosis if another clinical syndrome was also present. When pneumococci were isolated from more than one specimen type, only one was listed; blood was considered the primary source, followed by CSF.

Representatives from each national reference laboratory met at the NCS before the study began to review laboratory methodology detailing standard procedures for the isolation and identification of S. pneumoniae [14]. Serotyping, according to the Danish nomenclature system, was done by Quellung reaction with use of 12 pooled antisera [15] and selected factor sera (Statens Seruminstitut, Copenhagen). Isolates that national reference laboratories were unable to type were sent to the NCS for evaluation. Isolates found to be nontypeable at the NCS were referred to the Statens Seruminstitut.

All isolates were screened for reduced susceptibility to penicillin with use of a 1-μg oxacillin disk on Mueller-Hinton agar supplemented by 5% sheep blood. The MIC of penicillin was determined by broth microdilution [16]. Susceptibility to penicillin was defined by an MIC of ≤0.06 μg/mL, intermediate susceptibility by an MIC of 0.12–1.0 μg/mL, and high-level resistance by an MIC of ≥2.0 μg/mL. S. pneumoniae ATCC 49619 was included as a control strain for each testing batch. A laboratory quality-control program was established by the NCS, through which 10% of the isolates were sent to the NCS for confirmation and, regularly, a panel of five unknown isolates were submitted to the national reference laboratories for characterization, typing, and antimicrobial-resistance testing.

Standardized epidemiological information was collected on all children from whom S. pneumoniae was isolated. Data were analyzed with Epi-Info software, version 6.04b (Centers for Disease Control and Prevention, Atlanta). The Mantel-Haenszel χ² test was used to compare proportions. Odds ratios and 95% confidence intervals were determined with the Statcalc feature of Epi-Info.

Three vaccine formulas were evaluated to determine the optimal number of capsular types that, if included in a vaccine designed for children, would provide uniform protection across the region. Types 6A and 6B were included together as group 6 because of the high degree of cross-protective antibody induced by each for the other [17]. The vaccine formulas were Vac7, Vac12, and Vac15, containing the most frequently isolated types 7, 12, and 15, respectively.

Differences between countries in the age and presenting diagnosis of patients were identified as factors that could confound type distribution. To address this, a stratified analysis with use of Mantel-Haenszel odds ratios and 95% confidence intervals was used to compare the proportion of subjects in each country covered by the three products. Uruguay was used as an internal standard for intercountry comparison because it had the highest coverage with each of the vaccine formulas. Any country with a proportion of coverage that did not differ significantly from Uruguay’s could be confident of having coverage that approached this region-high level.

Results

Between February 1993 and April 1996, 1,649 isolates of S. pneumoniae were recovered from children in Argentina (n = 424), Brazil (n = 364), Colombia (n = 323), Chile (n = 198), Mexico (n = 171), and Uruguay (n = 169). Forty-five hospitals each contributed ≥10 isolates. Children <24 months of age and those ≤6 months of age were the source of 65.3% and 27.5% of all isolates, respectively (figure 1). Among 1,622 isolates from children whose sex was reported, 58.4% were from males. A primary diagnosis was reported for 1,621 patients. Pneumonia was identified in 817 patients (50.4%), meningitis in 528 (32.5%), sepsis or bacteremia without an infectious focus in 182 (11.2%), and other diagnoses in 94 (5.8%). S. pneumoniae was isolated from the blood of 856 patients (51.9%), the CSF of 448 (27.2%), the pleural fluid of 288 (17.5%), and other sterile sites in 57 (3.5%).

Eleven isolates from Uruguay and one isolate from Argentina were not received by the respective national reference laboratories for serotyping and were not included in the analysis of
vaccine coverage. There were 61 different capsular types among a total of 1,637 fully factored isolates. Seven isolates (0.3%) were nontypeable. The 16 most prevalent regional types are shown in table 1, by decreasing order of frequency. These 16 types comprised 87.7% of isolates.

The results of antimicrobial susceptibility testing were reported for 1,635 isolates. Diminished susceptibility to penicillin (DSP) was identified in 408 isolates (24.9%), including 274 (16.7%) with intermediate susceptibility and 134 (8.2%) with high-level resistance. The proportion of all isolates with DSP ranged from 47.3% in Mexico to 12.1% in Colombia. High-level resistance was greatest in Mexico (21.3% of all isolates) and lowest in Brazil (1.4%).

The association between age and infection by pneumococci with DSP was examined. After stratification for country of origin, children <24 months of age were more likely to be infected by pneumococci with DSP (26.2%) than were older children (22.7%; OR = 1.56; CI, 1.20–2.03; P < .001). DSP was seen among 26 different capsular types, of which types 14, 23F, 6B, 19A, and 19F accounted for 87.5% (figure 3).

Figure 2. Proportion of isolates with diminished susceptibility to penicillin recovered from children with invasive pneumococcal disease, per country (■ = penicillin-susceptible isolates; □ = isolates of intermediate susceptibility; ▲ = isolates with high-level resistance).

Figure 3. Proportions of the 16 most frequently isolated pneumococcal capsular types with diminished susceptibility to penicillin (□ = susceptible isolates; □ = isolates of intermediate susceptibility; and ■ = isolates with high-level resistance).
Table 2. Coverage by proposed vaccine formulas in each country participating in the study.

<table>
<thead>
<tr>
<th>Vaccine formula</th>
<th>All of Latin America</th>
<th>Argentina</th>
<th>Brazil</th>
<th>Chile</th>
<th>Colombia</th>
<th>Mexico</th>
<th>Uruguay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vac7*</td>
<td>71.7</td>
<td>71.9</td>
<td>72.8</td>
<td>68.7</td>
<td>74.3</td>
<td>61.4</td>
<td>78.5</td>
</tr>
<tr>
<td>Vac12</td>
<td>84.6</td>
<td>83.7</td>
<td>86.3</td>
<td>84.8</td>
<td>86.7</td>
<td>72.5</td>
<td>91.8</td>
</tr>
<tr>
<td>Vac15</td>
<td>87.7</td>
<td>88.4</td>
<td>88.7</td>
<td>87.4</td>
<td>89.8</td>
<td>75.4</td>
<td>92.4</td>
</tr>
</tbody>
</table>

* Vac7 includes types 14, 6, 1, 5, 23F, 19F, and 19A.

³ Vac15 includes types 14, 6, 1, 5, 23F, 19F, 19A, 9V, 7F, 18C, 3, and 4.

controlling for country of origin (OR = 1.54; CI, 1.19–1.99; P = .001).

The overall coverage of Vac7, which included the seven most prevalent regional types, was 71.7%, ranging from 61.4% in Mexico to 78.5% in Uruguay (table 2). Compared with the coverage in Uruguay, there were no significant differences in the proportion of coverage within Argentina, Brazil, Chile, or Colombia, as demonstrated by 95% confidence intervals that overlap by 1.0. Mexico had significantly fewer subjects covered by Vac7 than were covered in Uruguay (OR = 0.43; CI, 0.24–0.87; P = .03).

A second vaccine formula, incorporating the 12 most frequently isolated types, increased vaccine coverage significantly throughout Latin America, from 71.7% to 84.6% (table 2) (OR = 2.17; CI, 1.82–2.58; P < .001). The proportion of coverage by Vac12 in each of the countries, compared with coverage in Uruguay, was significantly less in both Argentina (83.7%; OR = 0.47; CI, 0.24–0.85; P = .01) and Mexico (72.5%; OR = 0.22; CI, 0.10–0.51; P = .0006).

The third vaccine, Vac15, was created by adding types 9N, 16F, and 15B. The region-wide coverage of Vac15 (87.7%) was significantly higher than that of Vac12 (OR = 1.38; CI, 1.13–1.70; P = .001). Mexico was the only country that continued to have significantly fewer children covered by Vac15 than in Uruguay (OR = 0.23; CI, 0.09–0.55; P = .001).

The proportion of coverage by vaccine for all children <2 years of age was compared with the coverage for children aged 2–5 years (table 3). There were fewer study patients in Mexico <2 years of age (30.4%) than in the five other countries (mean, 69.3%; range, 65.6% to 74.6%). When the analysis was controlled for country of origin, there were no significant differences between the two age groups in proportion of coverage by any of the vaccine formulas. Vaccine coverage of isolates with DSP was 91.7% with Vac7, 94.4% with Vac12, and 95.6% with Vac15.

Discussion

The development of the SIREVA network of laboratories represents a major effort by international, national, and local investigators to improve the regional infrastructure necessary for surveillance of infectious diseases [18–22]. This study, based on the work of the SIREVA network, describes the first prospective multicountry surveillance for invasive S. pneumoniae infections in Latin America.

The study’s main finding was the identification of types 14, 6, 5, 1, 23F, 19F, 19A, 9V, 7F, 18C, 3, 4, 16F, 9N, and 15B as the most prevalent in the region, accounting for 87.7% of isolates obtained. We identified the most prevalent capsular types, taken exclusively from sterile sites in a population of children, half of whom presented with lower respiratory tract infection. In addition, isolates were received from a large number of hospitals throughout the region, the majority providing ≥10 isolates.

Such broad-based participation is likely to reflect the serotype distribution in the region. Although the order of most
frequently isolated individual types may differ slightly between countries, the identification of major intercountry differences in coverage by a specific vaccine product is most important from a public health perspective. A single protein-polsaccharide conjugate pneumococcal vaccine for use in Latin America (unpublished data, Marguerite Lovgren). In the United States in coverage by a specific vaccine product is most important the proportion of coverage by these vaccines among children

Serotype prevalence data were used to compare coverage by three vaccine formulas. This analysis suggests that a 7-, 12-, or 15-valent vaccine covering the most prevalent regional types would achieve similar proportions of coverage in most of the participating countries. In Argentina, Brazil, Chile, and Colombia, there were no statistically significant differences in the proportions of vaccine coverage by Vac7 or Vac15, in comparison with coverage in Uruguay. A statistically significant difference in coverage between Uruguay and Argentina that was observed with Vac12 disappeared with the Vac15 product after type 16F, particularly prevalent in Argentina, was included. Regional coverage improved significantly from 71.7% with Vac7 to 84.6% with Vac12. Although there was a further significant increase in region-wide coverage afforded by Vac15 (87.7%) in comparison with Vac12 coverage, vaccine development costs and issues such as the total protein content of a 15-valent vaccine need to be considered before it can be determined whether the marginal increase (3.1%) in coverage afforded by the 15-valent product is cost-beneficial.

The proportion of coverage in Mexico remained significantly lower than in other countries with all vaccine formulas. The type distribution of invasive pneumococci in Mexico determined in this study may reflect a selection bias. Eighty-nine percent of Mexican isolates were from patients at two hospitals in urban Mexico City, a sample unlikely to reflect invasive types of *S. pneumoniae* throughout the country. These results support the need for more comprehensive pneumococcal-disease surveillance in Mexico prior to evaluating the appropriateness of a commercial vaccine for local use.

The importance of types 1 and 5 (10.7% and 10.6%, respectively) in Latin America have been confirmed in this and other studies [23–25], though they are relatively rare in the developed world [26, 27]. In contrast, many of the prevalent types identified in this study, including 14, 6, 23F, 19F, and 19A, are common in developed regions. When published reports describing pneumococcal-serotype distribution among children in other developing regions were reviewed [28–30], it was found that the proportion of coverage by Vac7 ranged from 44% in Pakistan [31] to 71% in the Gambia [32]. While these differences may reflect true differences in pneumococcal-type prevalence between regions, they may also reflect differences in surveillance methodology, such as sample size and specimen source.

To determine whether the three vaccine formulas would be appropriate in other settings in the Americas, we determined the proportion of coverage by these vaccines among children <5 years of age in the United States [23] and in Canada (unpublished data, Marguerite Lovgren). In the United States (n = 3,884), coverage was 67.1%, 89.6%, and 91.3% with Vac 7, Vac12, and Vac15, respectively. Similar proportions of coverage were seen in Canada (n = 303), with 68.0%, 92.7%, and 94.7%, respectively, covered by the three vaccines. These findings support the use of the Vac12 or Vac15 formulation in all of the Americas.

The finding of DSP among 25% of invasive pneumococcal isolates is compatible with reports from other developing and some developed [33] countries; however, our observed rate of high-level resistance (8.2%) was much greater [29, 30, 34]. This may reflect the presence of a highly resistant type (23F) at two tertiary hospitals in Mexico City. In Mexico, 45.0% of isolates with DSP had high-level resistance, of which 55.6% were of type 23F. When isolates from Mexico were excluded, the region-wide prevalence of isolates with DSP was 22.4%, and the prevalence of high-level resistance was 6.7%. As has been identified in previous reports [35, 36], the majority of isolates (93%) with DSP were confined to groups 6, 9, 14, 19, and 23.

The SIREVA network has provided the first multicountry prospective surveillance for invasive pneumococcal disease in the developing world. It has established the most prevalent pneumococcal types and defined levels of DSP among children with invasive pneumococcal disease in Latin America. It has also identified several vaccine formulas, based on prevalent regional types, that could achieve similar levels of coverage in most participating countries.

Future surveillance programs will need to be designed to obtain better estimates of incidence and burden of illness related to invasive pneumococcal disease. This information will be important to public health officials when evaluating the potential impact of a protein-polsaccharide conjugate vaccine on respiratory disease in their respective jurisdictions. If pneumococcal conjugate-vaccine programs are implemented in Latin America, the SIREVA network will be used to monitor changes in pneumococcal-type distribution and to assess the impact of immunization.

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References


