Serotypes and susceptibility of *Streptococcus pneumoniae* strains isolated from children in Mexico

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**Abstract**

**Objective.** To identify serotypes and susceptibility of *S. pneumoniae* strains from 48 children with invasive infections and 50 carriers. **Material and Methods.** Typing was performed by the Quellung reaction and susceptibility by Kirby-Bauer and Etest according to CLSI standards. **Results.** Of 31 meningeal strains, serotypes 19F, 3, 6B, 14 and 23F were predominant. Resistance to penicillin and SXT was 16 and 58%, respectively; of 17 invasive non-meningeal strains, serotypes 19F and 3 were predominant and resistance to penicillin and SXT was 0 and 82%, respectively; of carrier strains, serotypes 6A, 6B, 19F and 23F were predominant. **Conclusions.** A 10-valent conjugate vaccine could offer a better coverage for meningeal strains.

Key words: *Streptococcus pneumoniae*; serotyping; vaccines, conjugate; susceptibility; anti-infective agents; child, preschool; child, Mexico

**Resumen**

**Objetivo.** Identificar serotipos y susceptibilidad en cepas aisladas de 48 niños con infecciones invasivas y de 50 portadores. **Material y métodos.** Serotipificación mediante reacción de Quellung y susceptibilidad mediante Kirby-Bauer y E-test. **Resultados.** De 31 cepas meníngeas, predominaron serotipos 19F, 3, 6B, 14 y 23F y la resistencia a penicilina (P) y trimetoprim-sulfametoxazol (SXT) fue de 16 y 58%. En 17 cepas invasivas no meníngeas, predominaron serotipos 19F y 3 y la resistencia a P y SXT fue de 0 y 82%, en cada caso. En portadores predominaron serotipos 6A, 6B, 19F y 23F. **Conclusiones.** La resistencia es similar a otros informes. La vacuna conjugada 10-valente podría ofrecer mejor cobertura para serotipos asociados a meningitis.

Palabras clave: *Streptococcus pneumoniae*; serotipificación; vacunas conjugadas; susceptibilidad; agentes antiinfecciosos; preescolar; niño; México

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Serotypes of Streptococcus pneumoniae strains in Mexico

Streptococcus pneumoniae (Pn) is the leading cause of invasive infections in children, including meningitis, pneumonia, bacteremia, and acute otitis media. It is also carried in the nasopharynx of asymptomatic children and adults. Although 90 serotypes have been described, only a few of these (4, 14, 18C, 23F, 6B, 19A, 19F, 6A, 9V and 9B) have been associated with antimicrobial-resistance and invasive infections in children younger than two years old. The pneumococcal heptavalent conjugated vaccine (Pn-7vcv) was developed based on the seven most prevalent serotypes associated with invasive infections in children from the United States of America (USA). This vaccine had an efficacy of 93.9% in one intent-to-treat analysis of a double blind clinical trial study conducted in the USA. However, several serotypes associated with invasive infections in some Navajo Indian communities in the USA as well as in Saudi Arabia, Egypt, and various countries of Latin America are not included in the Pn-7vcv.

By identifying S. pneumoniae serotypes isolated from patients with invasive infections and carriers, we can explore the adequacy of the current seven-valent pneumococcal conjugated vaccine as well as that of other candidate vaccines containing a greater number of serotypes, such as the eleven-valent conjugate vaccine (recently reduced into a ten-valent one (Pn-10vcv) by elimination of serotype 3). This will help to determine if available conjugate vaccines are adequate or if specific vaccines need to be formulated for use in Latin American countries.

An additional issue in the treatment of patients with invasive pneumococcal infections is the increasing prevalence of penicillin (minimum inhibitory concentration ≥ 2 µg/mL) and multi-drug resistant strains. In Mexico, a previous report demonstrated that resistance to penicillin, erythromycin and trimethoprim-sulfamethoxazole was elevated (20.8, 24.5 and 58.9%, respectively). The aims of the present study were to identify capsular serotypes and antimicrobial susceptibility profiles of S. pneumoniae strains isolated from children with invasive infections and from healthy nasopharyngeal carriers (NPC). We also determined the percent of potential coverage of the serotypes included in the Pn-7vcv and Pn-10vcv.

Material and Methods

This cross-sectional study was performed between March 2000 and May 2005. Protocol was approved by the IMSS Local Research and Ethics Committee (No. F 2005-1302-022). Informed consent was obtained from the parents of the children attending day-care centers before taking nasopharyngeal cultures. S. pneumoniae strains were isolated both from cultures taken in children with invasive infections seen in a tertiary reference children’s hospital and from nasopharyngeal cultures of healthy non-vaccinated children attending day-care centers in the metropolitan area of Guadalajara, Mexico, and used for comparative purposes.

All the strains were identified by conventional microbiological procedures. Antimicrobial susceptibility to chloramphenicol (C; 30 µg), vancomycin (Va; 30 µg), erythromycin (E; 15 µg), and trimethoprim-sulfamethoxazole (STX; 1,25:23.75) (BBL Becton Dickinson, Sparks USA) was determined by disc diffusion (Kirby-Bauer) in Mueller-Hinton agar with 5% sheep blood, and interpreted according to the criteria of the Clinical Laboratory Standards Institute (CLSI), USA (document M100 S16, 2006). Susceptibility to penicillin (P) was determined by Etest strips (AB BIODISK Solna, Sweden) only for strains isolated from invasive infections, and they were interpreted according to the CLSI criteria. For strains isolated from the cerebrospinal fluid (CSF) of children with meningitis, strains were considered resistant to penicillin if the MIC ≥ 2 µg/mL. S. pneumoniae strain ATCC 49619 was used for quality control.

Serotyping was performed in the Infectious Disease Laboratory of the Texas Children’s Hospital of Houston, Texas with the Quellung reaction, using sera produced by the Statens Seruminstitut, Copenhagen, Denmark.

Results

Forty-eight S. pneumoniae strains were recovered from the children with invasive infections. Another 50 strains were isolated from the nasopharynx of the healthy non-vaccinated children.

Invasive strains were isolated from the children with an age range of 1-12 years (mean= 4.7 ± S.D. 4.3); 55% were males. Non-invasive strains were isolated from NPC of similar age range (2 to 13 years, mean= 5.5 ± SD 2.9) and gender distribution.

Of the 48 invasive strains (INV), 31 (65%) were recovered from the CSF of patients with meningitis, and seventeen (35%) from other normally sterile sites; 11 from middle ear effusion in acute otitis media patients, one from a patient with mastoiditis; two from blood cultures of pneumonia patients; two from patients with peritonitis and one from acute endophthalmitis.

The serotypes isolated from 31 children with meningitis were: 1, 3, 4, 6A, 6B, 7F, 9V, 11, 14, 19A, 19F, 23F, 31, and non-typeable. From those, serotype 19F was the most frequently isolated (16%) followed by 3 (13%), 6B (13%), and 14 (13%). Twelve serotypes accounted for...
90% of the isolates. The proportion of serotypes included in both the Pn-7vcv and the Pn-10vcv was 58 and 65%, respectively (Table I).

The S. pneumoniae serotypes isolated from each of the non-meningitis invasive infections were: acute otitis media (19F, 3, 11, 19A, 29-35-42, and non-typeable); pneumonia (19F, 35); peritonitis (19F); endophthalmitis (6A), and mastoiditis (3). Serotype 19F was the most frequently isolated (29%), followed by serotype 3 (18%). Nine serotypes accounted for 88% of the isolates. The proportion of serotypes included in both the Pn-7vcv and the Pn-10vcv was 29% (table I).

Twenty-one serotypes were isolated from 50 carriers: 3, 6A, 6B, 7, 10, 11, 14, 15, 17, 18A, 18C, 19A, 19F, 23A, 23F, 28, 29-34-35-42, 29-35-42, 35B, 6-29-35-42, and non-typeable. Of these, the most prevalent serotypes were: 6B (18%); 19F (16%); 23F (8%) and 6A (8%). Nineteen serotypes accounted for 90% of the isolates. The proportion of serotypes included in both the Pn-7vcv and the Pn-10vcv was 48% (table I).

None of the 98 strains was resistant to vancomycin or ceftriaxone. From CSF strains, 16% were resistant to penicillin (MIC ≥ 2 µg/mL). Resistance to erythromycin was higher and resistance to SXT was lower than in those from other invasive infections. No resistance to penicillin was detected among the strains isolated from other invasive infections. In NPC, resistance to erythromycin was higher (30%) and resistance to SXT was lower (56%) than in the strains isolated from CSF (23 and 58%, respectively) (table I). Of those strains resistant (including intermediate) to penicillin, chloramphenicol, erythromycin or trimethoprim-sulfamethoxazole, almost half (50, 50, 55, and 52%, respectively) belonged to serotypes included either in the 7th or 10th valent conjugate vaccines.

**Discussion**

It has been reported that Pn-7vcv offers good protection for the USA Caucasian population. However, several authors have demonstrated an inferior coverage of this vaccine for S. pneumoniae serotypes associated with invasive diseases in Saudi Arabia, Egypt, indigenous communities (Navajos) of the southwestern USA, and several Latin American countries. In these cases, Pn-10vcv could provide better coverage for those serotypes associated with invasive infections in children when available.

In this study, the serotypes identified were similar to those described by other authors in Latin American countries, Asia, and Russia, showing a worldwide distribution of certain serotypes. We found that a slightly higher proportion of S. pneumoniae serotypes isolated from children with meningitis (7%) were included in the Pn-10vcv than in the Pn-7vcv. However, no differences in serotypes coverage by the 7-valent or 10-valent conjugate vaccines was found in strains isolated from non-CSF invasive strains and from non-vaccinated NPC. The typing of nasopharyngeal isolates is useful as baseline information for future studies in vaccinated populations, in which we can expect a shift towards and replacement by non-vaccine serotypes.

Although the number of strains reported is limited, they were collected during a four-year period in a tertiary referral hospital that provides medical care to roughly 40% of the children living in a state of 6.9 million

<table>
<thead>
<tr>
<th>Source of strains</th>
<th>Serotypes</th>
<th>Vaccine Serotypes</th>
<th>P (MIC)</th>
<th>E</th>
<th>STX</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pn-7vcv</td>
<td>Pn-10vcv</td>
<td>S</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Meningitis (CSF)</td>
<td>1, 3, 4, 7F, 11, 14, 19A, 19F, 23F, 31. Non-typeable</td>
<td>58</td>
<td>65</td>
<td>36</td>
<td>48</td>
<td>16</td>
</tr>
<tr>
<td>Other Invasive</td>
<td>3, 6A, 11, 19A, 19F, 29, 35, 42. Non-typeable</td>
<td>29</td>
<td>29</td>
<td>47</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>NPC</td>
<td>3, 6A, 6B, 7C, 10, 11, 13, 14, 15, 17, 18A, 18C, 19A, 19F, 23A, 23F, 29, 34, 35B, 42. Non-typeable</td>
<td>48</td>
<td>48</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

P (MIC): Minimal inhibitory concentration of Penicillin
R: Resistant
E: Erythromycin
STX: Trimethoprim-sulfamethoxazole
C: Chloramphenicol
S: Susceptible
I: Intermediate
ND: Not done
7vcv: seven serotypes conjugate vaccines
10vcv: ten serotypes conjugate vaccines
CSF: cerebrospinal fluid
NPC: nasopharyngeal carriers
inhabitants. Our findings show important similarities to the serotypes associated with invasive disease in other Latin American and Asian countries.

The number of non-susceptible strains to penicillin (considering intermediate and resistant strains together) was high (64%) in children with meningitis. We also found a higher percentage of strains with intermediate resistance than that in other reports from Latin American countries. However, all of these strains remained susceptible to vancomycin. The moderate level of resistance to erythromycin and the high level to trimethoprim-sulfamethoxazole found in the present study agree with the reports from other parts of the world suggesting a worldwide spread of certain clones.6,7,14

In conclusion, the number of serotypes isolated from patients with invasive infections and included in the Pn-10vcv was slightly superior than those included in the Pn-7vcv. The number of strains with diminished susceptibility to penicillin, moderate resistance to erythromycin, and high resistance to trimethoprim-sulfamethoxazole could be associated with the abuse of antimicrobial prescriptions or with the intercontinental spread of multi-resistant strains. At least half of the serotypes with resistance to the antimicrobials tested belonged to the ones included in the 7 or 10 valent conjugate vaccines.

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References