

## Serotypes and antibiotic susceptibility of *Streptococcus pneumoniae* isolates causative of invasive diseases in Mexican children

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### Abstract

**Introduction:** *Streptococcus pneumoniae* is a worldwide leading cause of morbidity and mortality, while susceptibility towards penicillin and macrolides can be less than 50% in many regions.

**Methodology:** A total of 150 isolates of *S. pneumoniae* causative of invasive diseases in children were characterized, of which 24.6% had a fatal outcome.

**Results:** The most prevalent serotypes were 19F, 6B, 23F and 14. Resistance to penicillin, erythromycin (mostly of macrolide-lincosamide-streptogramin resistance phenotype) or trimethoprim-sulfamethoxazole was found in more than 40% of the isolates, but no resistance phenotype appeared linked to lethality. Serotype 3 isolates, which were seldom resistant, had a twofold lethality rate compared to the total sample.

**Conclusion:** Serotyping could provide a better outcome-predicting tool than susceptibility testing. The seven-valent vaccine does not include the most prevalent serotypes found in Mexico.

**Key Words:** *Streptococcus pneumoniae*; pediatric invasive disease; serotypes; antibiotic resistance

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### Introduction

*Streptococcus pneumoniae* (Spn) is a leading cause of morbidity and mortality associated with infectious diseases worldwide. Local and systemic infections caused by Spn include otitis media and sinusitis as less serious but very common illnesses, as well as bacteremia, pneumonia, sepsis and meningitis [1], all serious bacterial infections. Furthermore, the incidence of penicillin and macrolide pneumococcal resistance has increased so substantially that many regions of the world now report less than 50% of clinical isolates as being fully susceptible. The use of a seven-valent pneumococcal conjugate vaccine (including capsular antigens from serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) has reduced the prevalence of invasive pneumococcal disease [2]; however, this vaccine is not widely used in Mexico.

In this report, the clinical course of pneumococcal invasive disease (PID) was correlated with serotype/serogroup and antimicrobial resistance

patterns in a large pediatric population between 2002 and 2005.

### Methodology

#### Strains

A total of 150 strains considered causative of PID were isolated from hospitalized children under 16 years of age and analyzed. Patients were mostly male (56.6%) and 70% were ≤ two years of age (median age was 15 months). They were hospitalized at the Instituto Nacional de Pediatría, a large teaching hospital in Mexico City, and were included in an institutional surveillance of antibiotic resistance immediately after recording a positive culture of blood, cerebrospinal or pleural fluid, or other normally sterile fluid. Only one isolate per patient was included. Strains were isolated and characterized by standard microbiological techniques and serotyped by the Quellung reaction, with pools and type/group-specific antisera (Statens Serum Institut, Copenhagen,

Denmark). All strains were freeze dried and stored at room temperature for further characterization.

#### *Antibiotic susceptibility testing*

Susceptibility towards penicillin (PEN), cefotaxime (CEF), erythromycin (ERY), chloramphenicol (CLM) and trimethoprim-sulfamethoxazole (SXT) was tested by serial dilution in liquid media. Further testing of clindamycin and tetracycline susceptibility of ERY-resistant strains was performed by disk diffusion. All tests were conducted and interpreted following CLSI guidelines [3].

## Results

### *Serotype prevalence*

Serotypes 19F, 6B, 23F and 14 were the most prevalent, accounting for more than 55% of the strains. A total of 23 of the least common serotypes a comprised 25% of the sample (Table 1).

**Table 1.** Pneumococcal serotype prevalence

Serotype	Number of isolates (%)
19F	34 (22.7)
6B	24 (16.0)
23F	13 (8.7)
14	12 (8.0)
3	8 (5.3)
9V	6 (4)
15C	5 (3.3)
19A	5 (3.3)
35B	5 (3.3)
Other*	38 (25.3)

\*Serotypes included in this category (containing 1-2 isolates each) were 4, 18A, 18C, 18F, 6A, 1, 2, 10, 13, 15, 16, 17, 20, 22, 34, 68, 10A, 15A, 15F, 7C.

### *Antibiotic susceptibility*

Minimal inhibitory concentrations (MIC) are shown in Table 2. MIC<sub>90</sub> values PEN, ERY and SXT were at or above resistance breakpoints (and at the intermediate breakpoint for CEF). SXT MIC<sub>50</sub> was also above the resistance breakpoint, and PEN MIC<sub>50</sub> was above the intermediate breakpoint. Interpretation of MIC values as resistance/intermediate prevalences is also shown in Table 2. Interestingly, resistance to SXT, ERY and PEN was above 40%, and PEN non-susceptible strains comprised as little as one third of this sample (according to CLSI guidelines before 2008 [4]). On average, these strains were resistant to 1.6 of the drugs tested.

### *Macrolide resistance phenotypes*

A total of 65 strains (43%) had ERY MIC at or above 1 µg/mL. Of these, two-thirds were also resistant to clindamycin (MLS phenotype). MLS strains had higher ERY MICs (16 µg/mL or above, compared to an average of 8.7 µg/mL for ERY-resistant, clindamycin-susceptible [M phenotype] strains) and were more frequently resistant also to tetracycline (88% resistant, 4% intermediate, compared to 42% resistant, 16% intermediate for M-phenotype strains). Serotype distribution among MLS/M groups showed no significant differences.

### *Antibiotic resistance and lethality*

One quarter (24.6%) of the strains was isolated from patients who had fatal outcomes. Very slight differences were noticed in fatality rates associated with individual antibiotic resistance phenotypes: endpoints were 38.5% for CEF-resistant isolates that came from deceased patients, whereas 21.9% came from ERY-resistant deceased patients. The multi-drug resistance average was also slightly higher for isolates linked to fatal outcomes: 1.68. However, it is worth highlighting that three out of four penta-resistant strains came from patients who died.

### *Antibiotic resistance and lethality differs on serotypes*

Resistance patterns among the five most prevalent serotypes were very different. Serotypes 14, 19F and 6B were very similar to the sample average; however, there was much less CEF resistance among 19F strains, and much less CML resistance among 6B strains. In contrast, 23F strains were much more resistant than the average (except for ERY), whereas strains of serotype 3 were much less resistant, particularly towards CML and CEF. On the other hand, 19F and 6B strains were much less associated with fatal outcomes than the sample average, but those of serotype 3 were twice as likely to be linked to patient deaths, compared to the average (Table 3).

## Discussion

Pneumococcal serotypes that cause invasive infections in Mexican children are not precisely those included in the seven-valent conjugate vaccine. Serotypes 19F, 6B, 23F and 14 constitute more than half the isolates, and serotype 9V amounts to 4%; however, serotypes 4 and 18C are of very low prevalence (1.3% each), whereas serotype 3, which amounts to 5%, is not included in the vaccine.

Serotypes 14 and 23F were previously reported to be the most prevalent among penicillin-susceptible isolates from sterile-site infections in Mexican children [5]. Anti-pneumococcal vaccination is not a common practice in Mexico.

of mortality, penicillin and cephalosporin resistance were not associated with increased mortality [10].

Macrolide resistance, which comprised 40% of the isolates, is mostly of the MLS phenotype, a high-level resistance, with MICs of at least 16 µg/mL. It is

**Table 2.** Minimal inhibitory concentrations (µg/mL) and resistance prevalence (%).

	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Resistant	Intermediate susceptibility*
PEN †	0.015-16	0.5	4	41 (9)	26 (3)
CEF	0.015-16	0.25	2	9	25
ERY	0.015->16	0.125	>16	43	3
CML	0.015-16	0.25	2	13	0
SXT	0.03->16	8	16	58	8

\*CEF breakpoints for non-meningeal isolates.

† according to CLSI breakpoints before 2008, resistant ≥2 µg/mL, intermediate 0.12-1 µg/mL; in parentheses, according to new CLSI breakpoints for non-meningeal isolates, intravenous administration, resistant ≥8 µg/mL, intermediate 4 µg/mL [4].

**Table 3.** Resistance prevalence and lethality correlation to serotype\*

Serotype	PEN	ERY	CML	CEF	SXT	MR †	Lethal
3	0.61	0.59	0	0	0.43	0.47	2
14	1.02	1.56	0.62	1.89	1.72	1.46	0.32
19F	1.37	0.67	1.15	0.33	1.36	1.41	1.52
23F	2.24	1.26	5.31	4.22	1.59	2.16	1.52
6B	1.02	1.56	0.31	0.89	1.29	1.22	0.32

\* The frequency of resistant isolates to each antibiotic among each serotype, and the frequency of lethal outcome among each serotype (expressed as the ratio group/total) is shown.

† MR is the average of resistances.

Antibiotic resistance among pneumococci isolated from invasive infections in Mexican children is highly prevalent; Mexico has the highest Spn penicillin-resistance prevalence in Latin American countries [6]. This is likely the consequence of extensive antibiotic abuse, ranging from inadequate medical prescription to self-prescription practices that are possibly a result of the over-the-counter availability of antibiotics [7,8]. However, antibiotic resistance traits do not seem to be linked to an increased lethality of the infection process. Similar results have been observed previously; for instance, penicillin resistance does not seem to be related to penicillin treatment failure of pneumonia in children (22% failure among bearers of susceptible strains, 18% failure for resistant strains [9]). In another report, although mortality of adult patients with pneumonia was 38% when causative pneumococci were penicillin-resistant versus 24% for penicillin-sensitive isolates, when adjusting for other predictors

often linked to tetracycline resistance, as previous studies have reported [11]. This phenotype could be more likely linked to therapeutic failure [12]; however, overall ERY-resistant strains were not appreciably associated with fatal outcomes in this sample; in fact, the opposite was true, although in a non-significant way. The prevalence of macrolide resistance was not significantly different among serotypes, nor was the resistance phenotype. Interestingly, there are reports of serotype 19F being the most common among macrolide resistant isolates, and of MLS<sub>B</sub> being the most common phenotype [13]; macrolide resistance was below average among 19F serotype isolates of this study's collection (Table 3).

Serotypes seem to vary in their ability to gather resistance determinants. Strains of serotype 23F showed a high prevalence of resistance, particularly toward CML (which is often plasmid-mediated in other bacterial species) and CEF (which is only chromosomal, but can be horizontally transferred by

transformation). A previous report on serotype 23F Mexican isolates also showed a common multi-resistance profile that included penicillin, CML, SXT and tetracycline (but not ERY) [14]. It might be possible that this serotype has fewer restriction mechanisms for foreign DNA, making it more capable of gaining resistance determinants. On the other hand, serotype 3 is overall much less resistant than the average of this sample; no resistance towards CML or CEF was detected in strains of this serotype. However, serotype 3 is twice as likely to be linked to a fatal outcome, indicating that antibiotic resistance is not necessarily linked to the lethality of pneumococcal invasive infections. Serotype 19A, which now amounts to 3% of isolates, could increase its prevalence if the use of the seven-valent vaccine becomes a common practice, as has been previously reported [15]. For instance, in the United States, where vaccination is common, serotype 19A has a prevalence of 30.5% among isolates from children [16].

Overall, these results indicate that, although antibiotic resistance is importantly increasing among invasive pneumococci from Mexican children, this trend might not be associated with higher lethality. Serotyping could have a much more predictive value than assessing antibiotic susceptibility when evaluating the lethal risk of pneumococcal invasive infections in children.

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