Penicillin resistance and serotyping of *Streptococcus pneumoniae* in Latin America

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INTRODUCTION

*Streptococcus pneumoniae* (*Strep. pneumoniae*) remains as an important aetiological agent of pneumonia worldwide. Children under the age of two, and those with predisposing conditions (such as malnutrition), are most prone to be infected. Penicillin is important in the treatment of pneumonia and its low cost favours its use in the developing world. Unfortunately, the efficacy of penicillin is threatened by the emergence of resistant strains. The rate of severe infections is much higher in developing than in developed countries and more than 50% of children in developing countries are colonised before they are 6 months old.¹

Pneumococci are not intrinsically resistant to penicillin but, once resistance is acquired by a virulent clone, it can spread to other countries and continents in a relatively short time.² Penicillin-resistant strains were first discovered in 1965, when two strains were identified in Boston. Further resistant strains were reported in Australia in 1967 and New Guinea in 1971. By the late 1980s, penicillin-resistant pneumococci had spread throughout the world. In Latin America, resistance to penicillin was first verified in Mexico in 1981.³ Since then, other authors have reported occurrences in other countries, mostly in the late 1980s and early 1990s.
ASSESSING RESISTANCE

Penicillin resistance in Strep. pneumoniae is quantitative, i.e. the level of resistance is determined using the minimum inhibitory concentration (MIC). The dilution test and the E-test, which are used to determine the MIC in developed countries, are not widely available in Latin America. However, the recommended two-step strategy allows relatively unsophisticated laboratories to perform a screening test (agar diffusion with oxacillin discs) and this is complemented by the determination of MIC in laboratories that are able to do so. It is known that resistance of Strep. pneumoniae to beta-lactam antibiotics is the result of changes in penicillin-binding proteins (PBP), and is not related to beta-lactamase production.

PENICILLIN RESISTANCE AND SEROTYPING: A BRIEF REVIEW

Several studies on susceptibility to penicillin and prevalence of serotypes have been carried out in Latin America. However, prevailing conditions, such as a large geographic area and population, along with peculiar risk factors arising from the fact that two-thirds of children and adolescents live in poverty, allow for only partial knowledge of the current situation.

Due to limited resources, it is difficult to implement an efficient continuous surveillance system of susceptibility to penicillin and the prevalence of serotypes. For instance, in Brazil, which has a surface area close to that of Europe, only one reference laboratory can perform routine serotyping. As relatively few data are available, it is difficult for health authorities to recommend an appropriate empirical antibiotic therapy for a specific region, or even to estimate the efficacy or the impact of the current heptavalent conjugate vaccine on the community.

Penicillin resistance is increasing in Latin America. A comparison of data obtained in the years 1994 and 1998 showed a statistically significant distribution of highly resistant pneumococci in five out of six countries analysed. In 1993, the Regional System for Vaccines (SIREVA-Vigia) of the pan-American Health Organization (PAHO) designed a study to identify the Strep. pneumoniae capsular types that cause invasive diseases in Latin American children under the age of five. The aim of the project was to determine the relative prevalence of capsular types and antimicrobial susceptibility of Strep. pneumoniae that cause invasive diseases, especially pneumonia, in children in Argentina, Brazil, Chile, Colombia, Mexico, and Uruguay. This information should provide reliable data for the formulation of an adequate vaccine for use in Latin America.

As pneumonia due to Strep. pneumoniae with diminished susceptibility to penicillin (DSP) can be treated with penicillin, we will focus our review on the prevalence rate of high-level resistant strains recovered from invasive diseases.

Argentina

With a break-point for the MIC ≥20 µg/mL, the proportion of penicillin-resistant strains verified in studies conducted from 1993 to 2005 ranged from 9.9% to 21.5%. Since the break-point for highly penicillin-resistant pneumococci ≥ 40 µg/mL was recently proposed, few invasive isolated strains were classified as having that penicillin MIC.

In descending order, serotypes 14, 5, 1, 6A/6B, 7F, 9V, 19F, 16F, and 23F were responsible for 89.3% of 505 isolates from invasive infections in children younger than 5 years from 1994 to 1996. In 2002 there were few changes in the serotype prevalence, with serotype 14 representing 34.4% of the total sample, followed by 5 (13.9%), 1 (9.4%), 6A/6B (9.1%), 7F (4.4%), 9V (3.1%), 23F (2.9%), 18C (2.7%), 19A (2.7%), 19F (2.5%), 9N (1.8%); the last nine serotypes represented 82.6% of 1650 Strep. pneumoniae isolates from invasive diseases. The proportion of serotypes 5 and 1 was significantly smaller (11.1% vs 20.1%; P < 0.001) in the <2 years group. In 2004 continual surveillance showed a similar pattern; only 23F and 18C increased moderately.

Brazil

In Brazil, the first report of an intermediate penicillin-resistant strain of Strep. pneumoniae was documented in the city of São Paulo in 1988. During the 1990s, the prevalence of resistance (MIC ≥20 µg/mL) – according to many different well-standardised surveillance studies (predominantly from the south-east region of the country) – was under 5%. As were the results from two regional studies of invasive isolates from children and adolescents. However, on average, resistance among invasive pneumococcal isolates in 1998 was 1.9%, whereas in 2003 it was 7.2% and the risk of having a resistant isolate in 2003 was almost four-times the risk in 1998. According to the surveillance studies, penicillin-resistant isolates with serotypes 6B, 14 and 23F predominate in Brazil.

However, data collected nationally might not reflect the epidemiology of a region of the country. For instance, in Minas Gerais, one of the largest states in the Brazilian federation, a survey that analysed 502 strains of Strep. pneumoniae found a susceptibility rate of 88.2%, using the disc diffusion method.

According to a study in 1993 that investigated strains isolated in Brazil, serotypes 1, 5, 6A, 6B, 9V, 14, 19A, 19F, and 23F were responsible for 77.7% of 360 strains isolated in Brazil, serotypes 1, 5, 6A, 6B, 9V, 14, 19A, 19F, and 23F were responsible for 89.3% of 505 invasive pneumococcal isolates from invasive infections in children younger than 5 years from 1994 to 1996. In 2002 there were few changes in the serotype prevalence, with serotype 14 representing 34.4% of the total sample, followed by 5 (13.9%), 1 (9.4%), 6A/6B (9.1%), 7F (4.4%), 9V (3.1%), 23F (2.9%), 18C (2.7%), 19A (2.7%), 19F (2.5%), 9N (1.8%); the last nine serotypes represented 82.6% of 1650 Strep. pneumoniae isolates from invasive diseases. The proportion of serotypes 5 and 1 was significantly smaller (11.1% vs 20.1%; P < 0.001) in the <2 years group. In 2004 continual surveillance showed a similar pattern; only 23F and 18C increased moderately.

According to a study in 1993 that investigated strains isolated in Brazil, serotypes 1, 5, 6A, 6B, 9V, 14, 19A, 19F, and 23F were responsible for 77.7% of 360 strains isolated from children with invasive infections in three different cities. In the period 1993–1999, serotypes more frequently involved in invasive infections in Brazilian children were 14 (24.9%), 6A/6B (15.3%), 1 (9.8%), 18C (7.4%), 5 (6.7), 23F (5.2%), 19F (4.5%), and 9V (4.4%). It is noteworthy that the prevalence of serotype 14 increased from 18.7% in 1994 to 29.3% in 1999.
Chile

The first occurrence of pneumococcal resistance in Chile was reported in 1987. From 1994 to 2003, the rate of high-level resistant pneumococci varied between 5% and 18.8%. No differentiation was performed between intermediate and highly resistant isolates in another study in which these two categories accounted for 31.3% of all 99 strains.

A study conducted from 1989 to 1993 showed that serotypes 1 and 5 were identified in approximately 30% of hospitalised patients. However, in a study by Gherardi et al, the 68 multidrug-resistant isolates analysed from 1994 to 1999 were represented by serotypes 19F, 14, 23F, and 6B/6A (1 isolate). More recently, serotypes 1, 3, 5, 6A, 18C, and 7F showed a similar distribution in southern countries (Chile, Argentina, and Uruguay). In northern latitudes there was a decrease in the prevalence of types 1 and 5 – both uncommon in North America – and an increase in serotype 23F, one of the seven main existing serotypes in the United States.

Colombia

In three studies conducted separately – the first one of 324 isolates from 1994 to 1996, the second one of 623 isolates from 1994 to 1998, and the last one from 1997 to 2001 – high resistance ranged from 3.1% to 25%.

The nine most frequent types (81.0% of the total sample) identified among 324 isolates included serotypes 14 (21.9%), 5 (10.5%), 23F (9.6%), 1 (9%), 6B (9%), 19F (7.1%), 6A (6.2%), 18C (4.9%), and 7F (2.8%).

Mexico

Penicillin-resistant pneumococci were first reported in Mexico in 1981, when nine out of 117 strains (7.7%) recovered from healthy children showed MICs >1.25 μg/mL.

From August 1993 to April 1995, 49 out of 220 isolates (22.2%) obtained from blood; cerebrospinal fluid; bronchial aspirate; or middle ear, peritoneal or joint fluids revealed an MIC of ≥2 μg/mL. A similar resistance rate (23.2%) was observed by others in the period 1995–2001.

Capsular typing showed that serotypes 23F, 14, 6B, 19F, 6A, 19A, 4, 11A, 5, 18C, and 19A were the prevalent serotypes from 1993 to 1999.

Uruguay

An analysis carried out from 1994 to 1998 on 322 invasive isolates showed a resistance rate ranging from 5.6% to 20%. Capsular types 14, 6A/6B, 5, 1, 23F, 19F, 18C, 19A, and 9V represented 82.2% of the isolates. In another paper, the same serotypes were responsible for 80.6% of the 506 invasive isolates studied in the period 1994–2001.

Table 1 summarises the average high resistance rate and the 11 serotypes (6A and 6B were considered as the same serotype) responsible for approximately 80–85% of pneumococcal invasive diseases in the main collaborative international study carried out in six Latin American countries (data represent 1998–1999 figures).

Interestingly, serotypes 14, 5, 6A, 6B, and 7F showed a similar distribution in southern countries (Chile, Argentina, and Uruguay). In northern latitudes there was a decrease in the prevalence of types 1 and 5 – both uncommon in North America – and an increase in serotype 23F, one of the seven main existing serotypes in the United States.

Molecular Epidemiology

The Pneumococcal Molecular Epidemiology Network (PMEN) was established in 1997 to carry out global surveillance of antibiotic-resistant Strept. pneumoniae and currently recognises 26 international clones. The occurrence and dissemination of four different clones showing MIC/C21 mg/mL have been verified in six Latin American countries (Table 2). Relatively few clones are responsible for most infections due to penicillin-resistant pneumococci in Latin America. These clones, however, are widely distributed in different countries (especially clones Spain23F-1 and Spain9V-3) and contribute to the dissemination of resistance. Other clones might be circulating undetected and slowly spreading resistance. These data demonstrate the need for continuous surveillance, based on serotyping and tools of molecular epidemiology, such as pulsed field gel.
electrophoresis and multilocus sequencing typing, to better understand the dissemination of pneumococcal resistance in Latin America.

**PNEUMOCOCCAL RESISTANCE AND CLINICAL PRACTICE**

There are conflicting data regarding the clinical relevance of penicillin resistance. A strain with reduced susceptibility (e.g., MIC > 1.0 \( \mu \text{g/mL} \)) behaves as a susceptible organism when it causes pneumonia but not when it causes meningitis. So the definition of susceptibility might depend on the site infected, although studies on the relevance of Strep. pneumoniae with a MIC of <4 \( \mu \text{g/mL} \) to mortality rates have shown conflicting results.31 Furthermore, there is evidence that a dosage of amoxicillin which achieves sufficient serum concentration ensures efficacy against Strep. pneumoniae with MICs to amoxicillin of 4 \( \mu \text{g/mL} \)–1 in the treatment of pneumonia.31

**FACTORS RELATED TO PENICILLIN RESISTANCE**

There is a statistical correlation between penicillin resistance and the following risk factors: age <2 years;8,32 use of penicillin or ampicillin within 3 months of illness [odds ratio (OR) \( \approx 3.0 \)] and serotype 14 [OR = 6.3; 95% confidence interval (95% CI): 1.7 to 23.3].6 Isolates from male patients were 40% more likely to be resistant than isolates from female patients.4 Non-bacteraemic illnesses seem to be a protective factor (OR: 0.34, 95% CI: 0.14 to 0.84).32 Reduced susceptibility to penicillin was considered to be a reliable marker for the higher probability of multidrug resistance, thus requiring in vitro tests to guide chemotherapy or the choices of parenteral extended spectrum cephalosporins or newer respiratory quinolones.29

Moreover, in pneumonias caused by Strep. pneumoniae not susceptible to penicillin, the therapeutic regimen should be similar to that prescribed for susceptible organisms, as this approach does not necessarily result in a worse clinical outcome.5,4,10

**ANTIPNEUMOCOCCAL VACCINATION**

The major limitation of the polysaccharide pneumococcal vaccine is that it is not immunogenic for children younger than 2 years of age, the age group with the highest incidence of invasive pneumococcus disease. In part, this is because it elicits an independent T cell response.33 Pneumococcal conjugate vaccines were developed to overcome this but the number of serotypes is limited. This means that a vaccine that is effective in one region might be less so in another and it is therefore necessary to determine the local/regional epidemiology of invasive pneumococcal disease, especially in developing countries.4

Following a landmark clinical trial demonstrating 89% efficacy against vaccine serotypes, the seven-valent pneumococcal conjugate vaccine (PCV7), containing serotypes 4, 6B, 9V, 14, 18C, 19F e23F, was licensed and introduced in 2000 in the United States for universal administration to children younger than 2 years of age and in children 2–5 years of age with risk factors. These seven types represented 82% of the Strep. pneumoniae strains isolated from invasive infections in northern California.34

The data generated by SIREVA-Vigilia made it possible to estimate the expected coverage of three conjugated vaccines by country and region for pneumonia and meningitis prevention.4 In Latin America, the coverage of the seven-valent formulation was a maximum of 58% for these diseases, because serotypes 1 and 5 were not included in the formulation. The overall percentage would have increased to 76.2 and 80.7% with the nine- and eleven-valent vaccines, respectively. These three different vaccine formulations have good coverage of Strep. pneumoniae penicillin-resistant capsular types; however, type 19A, which is missing in all formulations, was relevant in all Latin American countries with the exception of Colombia.4

In Latin America, the seven-valent vaccine covers 89% of penicillin-resistant pneumococci. However, even if these conjugate vaccines prove to be highly immunogenic, they still might not offer enough protection against all pneumococcal infection.

Some studies suggest that there is some cross-protection for different serotypes belonging to the same serogroup (6A, 9A, 9L, 18B, 19A and 23A).35,36 Dagan et al. showed that carriage rates for penicillin-resistant and non-resistant pneumococci were significantly reduced for vaccine serotypes.37 Additionally, nasopharyngeal carriage of the vaccine-specific serotypes decreased in the group which had received seven-valent or nine-valent formulation.36,37

These results suggest that conjugate vaccines are able to reduce the carriage of the vaccine-related serotypes in

<table>
<thead>
<tr>
<th>Table 2</th>
<th>International clones of penicillin-resistant (MIC ( \geq 2.0 \mu \text{g/mL} )) pneumococci in Latin America</th>
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<tbody>
<tr>
<td>Clone</td>
<td>Minimal inhibitory concentration (( \mu \text{g/mL} ))</td>
</tr>
<tr>
<td>Spain23F-1</td>
<td>2.0</td>
</tr>
<tr>
<td>Spain6B-2</td>
<td>2.0</td>
</tr>
<tr>
<td>Spain9V-3</td>
<td>2.0</td>
</tr>
<tr>
<td>Czech Republic14-10</td>
<td>8.0</td>
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Source: Pneumococcal Molecular Epidemiology Network (www.sph.emory.edu/PMEN)
children as well as decrease the burden of antibiotic-resistant strains.\(^3\) The vaccine could therefore help reduce the dissemination of these bacteria, as well as reducing the need for antibiotics. However, carriage of non-vaccine-specific serotypes was found to be more prevalent, suggesting the possibility of replacing nasopharyngeal by serotypes not included in the vaccine.\(^3,36\) There is agreement that the seven-valent vaccine should be used in children less than 2 years of age with diseases such as cystic fibrosis, asplenia, and immunodeficiency (including HIV).

Although pneumococcal conjugate vaccines present undeniable benefits, the remaining difficulties include: the biochemical issues involved in the construction of the optimal vaccine, and their cost of production and their diminished effectiveness in developing countries. There is a need for different formulations, dosages and combinations of serotypes to cover different populations, age groups and geographical characteristics in Latin America.

**REFERENCES**


