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# Surveillance of invasive in *Streptococcus pneumoniae* in Argentina 1994–2007: Changes in serotype distribution, serotype coverage of pneumococcal conjugate vaccines and antibiotic resistance

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**Abstract**. *Streptococcus pneumoniae* is a prevalent cause of invasive diseases in children, justifying continuous surveillance programs such as by the SIREVA group (Pan American Health Organization). The aim of this study was to determine the serotype distribution of *S. pneumoniae* causing invasive disease in children $< 6$ years old, the serotype coverage of the pneumococcal conjugate vaccine 7-valent (PCV7), 10-valent (PCV10) and 13-valent (PCV13), and antibiotic resistance, from 1994 to 2007. During this period, 2205 invasive *S. pneumoniae* were included in the study. Although 49 different capsular types were identified, 12 serotypes accounted for 86% of all isolates. These were prevalent throughout the study period with serotype 14 predominating. Penicillin non-susceptible *S. pneumoniae* was detected in 33.2% of all isolates. The coverage of PCV7, PCV10 and PCV13 from 2004 to 2007 for children $< 2$ years old was 51.7%, 72.4% and 84.5%, respectively. The data demonstrates a decline in serotype 14, and an increase in serotypes 1 and 19A in the study period. Resistance to penicillin and trimethoprim-sulfamethoxazole decreased, while resistance to erythromycin increased. These results demonstrate the need for the introduction of a conjugate pneumococcal vaccine and continuing surveillance to monitor changes in serotypes distribution and antimicrobial resistance.

Keywords: Invasive pneumococcal diseases, *S. pneumoniae*, epidemiology, infectious diseases

## 1. Introduction

*Streptococcus pneumoniae* is a prevalent cause of invasive diseases in children. The World Health Organization (WHO) has estimated that in 2005, *S. pneumoniae* was responsible for the deaths of 1.6 million children less than 5 years old, most of whom were less than 2 years of age [1].

About 18,000 children less than 6 years old die annually of invasive pneumococcal disease (IPD) in Latin American and Caribbean countries [2]. Differences exists in the distribution of capsular serotypes in terms of geographic area, patient age, and source of infec-

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tion is the subject of such continuous surveillance programs [3,4] according to the Regional System for Vaccines (SIREVA) group of the Pan American Health Organization [5,6].

The goal of this study was to determine the serotype distribution of *S. pneumoniae* causing invasive disease in Argentinean children $< 6$ years old, and the potential of serotype coverage of the pneumococcal conjugate vaccines 7-valent (PCV7), the 10-valent (PCV10) and the 13-valent (PCV13), as well as resistance to antibiotics. PCV7 covers serotypes 4, 6B, 9V, 14, 18C, 19F and 23F; PCV10 covers PCV7 and serotypes 1, 5 and 7F; and PCV13 PCV10 plus serotypes 3, 6A and 19A.

## 2. Materials and methods

*S. pneumoniae* was isolated from invasive infections in children $<$ 6 years of age that were admitted to 37 hospitals in 17 provinces and the city of Buenos Aires, Argentina, from 1994 to 2007. Samples were obtained from blood, cerebrospinal fluid, pleural fluid, and other sterile sites (peritoneal fluid, synovial fluid, lung biopsy, brain biopsy and vitreous humor). Isolates were sent to the National Reference Laboratory INEI-ANLIS "Dr Carlos G. Malbrán", where capsular serotyping and antimicrobial susceptibility testing were carried out. Serotyping was based on the Neufeld-Quellung reaction using antisera produced by the Statens Serum Institute (Copenhagen, Denmark). Minimal inhibitory concentration (MIC) was performed using the Clinical and Laboratory Standards Institute (CLSI) agar dilution method. MICs were interpreted following (CLSI 2007) guidelines [7] (breakpoints for penicillin: susceptible $\leqslant 0.06$ $\mu$g/mL, intermediate 0.12–1 $\mu$g/mL and resistant $\geqslant 2$ $\mu$g/mL). Those isolates with MIC $\geqslant 0.12$ $\mu$g/mL were considered penicillin non-susceptible. External quality assurance was done by the Adolfo Lutz Institute (Sao Paulo-Brazil) [8]. Data were analyzed using EpiInfo and WHONET 5.4 (WHO) and $P < 0.05$ was considered statistically significant.

## 3. Results

Two thousand two hundred and five *S. pneumoniae* isolates were obtained. Fifty seven percent of the individuals they were obtained from were males and 66.8% children $<$ 2 years old. The annual distribution of isolates (n) was: 1994 (282), 1995 (157), 1996 (51), 1997 (88), 1998 (140), 1999 (165), 2000 (143), 2001 (121), 2002 (115), 2003 (170), 2004 (225), 2005 (181), 2006 (202) and 2007 (165). The monthly distribution increased during the cold months (June to October) and reached a peak in September. *S. pneumoniae* was isolated from blood (47.4% of isolates), cerebrospinal fluid (26.5%), pleural fluid (22.8%) and others (3.3%). The patient's clinical diagnosis were pneumonia (56.7% of patients), meningitis (26.2%), bacteremia (8.3%), fever without focus (4.3%) and others (4.5%). A predominance of meningitis and bacteremia vs. pneumonia was observed in the first year of life: 62.4% and 61.6% vs. 32.9% ($P < 0.0001$). However, in children $\geqslant 1$ year old, pneumonia was more frequent than meningitis and bacteremia: 67.1% vs. 37.6% and 38.4%, respectively ($P < 0.0001$).

Serotyping was performed in 2205 *S. pneumonia* isolates; and 49 serotypes were identified. The most prevalent ones were 14 (31.7%), 5 (13.1%), 1 (10.5%), 6B (6.5%), 7F (4.3%), 19A (3.5%), 18C (3.3%), 9V (3.2%), 23F (2.9%), 19F (2.6%), 6A (2.5%) and 3 (1.5%).

More prevalent serotypes in pneumonia vs. meningitis were serotype 14 (37.0% vs. 25.3%), odds ratio (OR) 1.50 (95% confidence interval [CI]: 1.28–1.77), $P <$ 0.0001; serotype 1 (12.7% vs. 7.6%), OR 1.59; (95% CI: 1.14–2.21), $P = 0.005$ and serotype 9V (3.9% vs. 2.1%), OR 1.86 (95% CI: 1.0–3.47), $P = 0.04$. By contrast, more prevalent serotypes in meningitis vs. pneumonia were serotype 18C (7.5% vs. 1.3%), OR 6.43 (95% CI: 3.54–11.69), $P < 0.0001$; serotype 7F (5.9% vs. 3.6%), OR 1.86 (95% CI: 1.18–2.92), $P = 0.006$ and serotype 23F (4.0% vs. 1.8%), OR 2.29 (95% CI: 1.29–4.08), $P = 0.003$ (Table 1).

More prevalent serotypes in pneumonia vs. bacteremia were serotype 14 (37.0% vs. 26.6%), OR 1.62 (95% CI: 1.20–2.18), $P < 0.001$ and serotype 1 (12.7% vs. 4.7%), OR 2.96 (95% CI: 1.61–5.54), $P = 0.0002$. By contrast, more prevalent serotypes in bacteremia vs. pneumonia were serotype 18C (4.3% vs. 1.3%), OR 0.29 (95% CI: 0.13–0.66), $P < 0.001$; serotype 6A (4.3% vs. 2.0%), OR 0.45 (95% CI: 0.22–0.97), $P =$ 0.04; serotype 19A (5.8% vs. 3.0%), OR 0.51 (95% CI: 0.27–0.98), $P = 0.04$ and serotype 23F (4.0% vs. 1.8%), OR 0.46 (95% CI: 0.21–1.01), $P = 0.05$ (Table 1).

Serotypes 14 and 6B were more prevalent in children $<$ 2 years old: (36.4% vs. 22.5%), OR 0.49 (95% CI: 0.40–0.61), $P < 0.0001$ and (7.9% vs. 3.5%), OR: 2.44 (95% CI: 1.53–3.94), $P < 0.0001$, respectively and serotypes 1 and 5 were significantly less prevalent in

Table 1
Distribution of *Streptococcus pneumoniae* serotypes according to main clinical diagnosis ($n = 2102$) and age groups ($n = 2205$), period 1994–2007

| Serotype | Clinical diagnosis | | | Age groups | |
|---|---|---|---|---|---|
| | Meningitis n (%) | Pneumonia n (%) | Bacteremia n (%) | $<$ 2 years n (%) | 2 to 5 years n (%) |
| 1 | 44 (7.6) | 158 (12.7) | 13 (4.7) | 80 (5.4) | 150 (20.5) |
| 3 | 5 (0.9) | 21 (1.7) | 3 (1.1) | 17 (1.2) | 15 (2.0) |
| 4 | 6 (1.0) | 7 (0.6) | 2 (0.7) | 13 (0.9) | 4 (0.5) |
| 5 | 66 (11.4) | 185 (14.8) | 32 (11.5) | 150 (10.2) | 138 (18.8) |
| 6A | 13 (2.3) | 25 (2.0) | 12 (4.3) | 40 (2.7) | 16 (2.2) |
| 6B | 33 (5.7) | 78 (6.3) | 26 (9.4) | 117 (7.9) | 26 (3.5) |
| 7F | 34 (5.9) | 45 (3.6) | 11 (4.0) | 62 (4.2) | 32 (4.4) |
| 9V | 12 (2.1) | 49 (3.9) | 9 (3.2) | 48 (3.3) | 23 (3.1) |
| 14 | 146 (25.3) | 461 (37.0) | 74 (26.6) | 536 (36.4) | 165 (22.5) |
| 18C | 43 (7.5) | 16 (1.3) | 12 (4.3) | 49 (3.3) | 24 (3.3) |
| 19A | 20 (3.5) | 38 (3.0) | 16 (5.8) | 60 (4.1) | 19 (2.6) |
| 19F | 17 (2.9) | 25 (2.0) | 10 (3.6) | 42 (2.9) | 16 (2.2) |
| 23F | 23 (4.0) | 23 (1.8) | 11 (4.0) | 51 (3.5) | 12 (1.6) |
| Others | 115 (19.9) | 116 (9.3) | 47 (16.9) | 207 (14) | 93 (12.7) |
| Total | 577 | 1247 | 278 | 1472 | 733 |

this age group: (5.4% vs. 20.5%), OR 0.21 (95% CI: 0.15–0.28), $P < 0.0001$ and (10.2% vs. 18.8%), OR 0.50 (95% CI: 0.38–0.65), $P < 0.0001$, respectively (Table 1).

There were no significant changes in serotype distribution between 1994–1999. In order to make it easier to appreciate short-term variations in serotype prevalence and consequently vaccine coverage, we divided the period 2000–2007 into two parts. The comparison between 2000–2003 and 2004–2007 showed statistically significant changes in the frequency of recovery only for serotypes 14, 1 and 19A. The following changes were observed: a decrease in serotype 14 (35.3% to 25.8%, OR 1.37; 95% CI: 1.15–1.63, $P = 0.0004$); an increase in serotype 1 (7.5% to 13.8%, OR 0.54; 95% CI: 0.38–0.77, $P = 0.0005$) and in serotype 19A (2.6% to 5.3%, OR 0.47; 95% CI: 0.24–0.90, $P < 0.01$). Some differences that were not statistically significant were observed for other prevalent serotypes: 5 (12.8% to 13.1%), 6B (7.5% to 5.4%), 7F (3.5% to 4.3%), 9V (3.1% to 3.8%), 23F (3.5% to 2.6%), 6A (2.0% to 2.8%), 19F (2.9% to 2.3%) and 3 (1.5% to 2.3%) (Fig. 1).

Serotype coverage of PCV7, PCV10 and PCV13 during the entire period (1994–2007) were 52.2%, 79.9% and 86.1%, respectively. In children $<$ 2 years old, they were 59.5%, 79.3% and 86.6%, respectively. In children $\geqslant$ 2 years old: 36.8%, 80.5% and 87.3%, respectively. For children $<$ 2 years old with pneumonia, the coverage were 66.9%, 83.3%, and 90.5%, respectively; and for those with meningitis: 50.0%, 76.1% and 81.9%, respectively.

There were changes in serotype coverage in the 2004–2007 periods. The coverage of PCV7, PCV10 and PCV13 for children $<$ 2 years old were 51.7%, 72.4% and 84.5%, respectively. For children $<$ 2 years old with pneumonia 49.8%, 74.2% and 87.6%, respectively; and for meningitis 53.3%, 74.3%, and 80.2%, respectively.

Antimicrobial resistance determination was performed in 2196 out of the 2205 pneumococcal isolates. Penicillin non-susceptibility was observed in 728 of 2196 (33.2%) isolates (intermediate 20.7%, resistant 12.5%). A significant increase in penicillin non-susceptible strains was observed from 1994 to 1996 (17.0% to 43.2%) $P < 0.0001$, and remained almost constant over the following years, and declining to 25.6% in the 2006–2007 periods (Fig. 2). No isolates with MIC to penicillin $> 4$ $\mu$g/mL were detected. Penicillin non-susceptibility was higher in the $<$ 2 years old than in the $\geqslant$ 2 years old groups: 38.5% vs. 22.4% $P < 0.0001$.

Main serotypes associated with penicillin non-susceptibility were 14 (75.3%) 6B (54.9%), 19A (48.7%), 23F (30.1%), 9V (28.2%), 19F (22.4%), 9N (10.3%) and 6A (7.3%). Non-susceptibility to cefotaxime was 13.2% (MIC $\geqslant$ 2 $\mu$g/mL: 1.7%) for meningeal isolates and 3.4% for non-meningeal isolates.

Erythromycin resistance was 8.7% and increased throughout the entire study, from 0% in 1994 to 13.8% in 2007 ($P < 0.0001$). Trimethoprim-sulfamethoxazole resistance was 53.1%, and decreased from 58.7% in 1994–1999 to 54.6% in 2000–2003 and 47.1% in 2004–2007 ($P < 0.0001$). Resistance to tetracycline was 9.0% and chloramphenicol 0.9%, but all isolates were susceptible to vancomycin and ofloxacin.

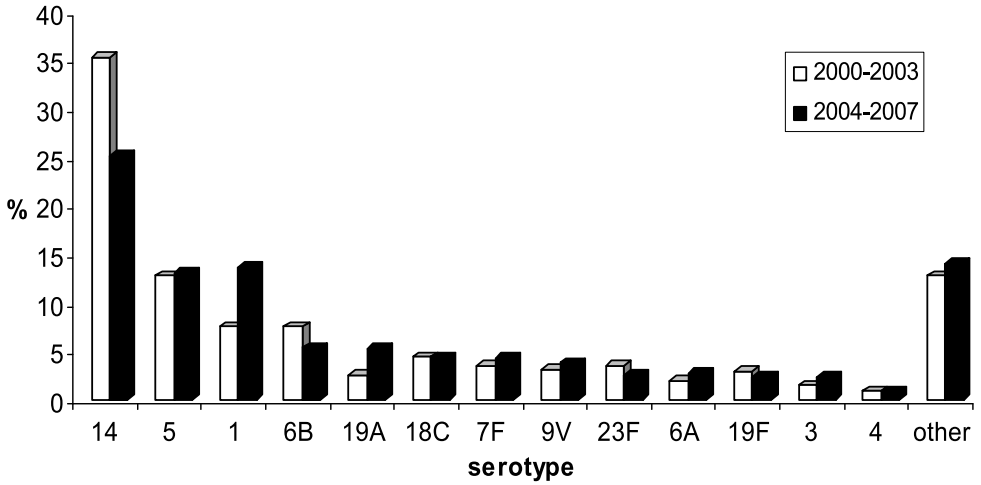


Fig. 1. Distribution of main serotypes. Comparison of the periods 2000–2003 and 2004–2007. Note: there were statistically significant variations only in serotypes 14, 1 and 19A.

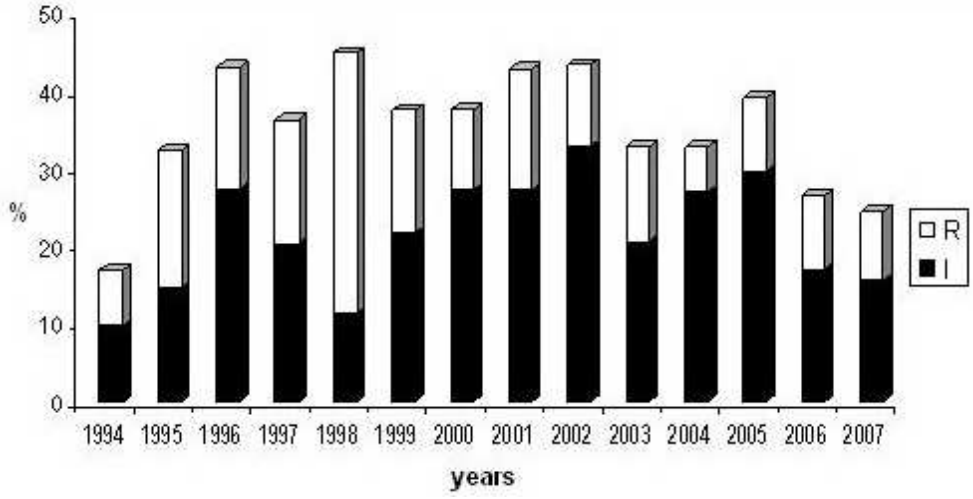


Fig. 2. Annual distribution of *S. pneumoniae* according to susceptibility to penicillin, period 1994–2007 ($n = 2196$).

## 4. Discussion

The results reported by the SIREVA network showed a large burden of IPD, especially in the $< 2$ years old group [5,6].

Active surveillance studies to evaluate the incidence of IPD and their incidence in pneumonia have been carried out recently in several Latin American countries, some of them sponsored by Pan American Health Organization.

In Argentina, Ruvinsky et al. [9] have developed a population-based surveillance study to determine the incidence of bacterial pneumonia, based on evaluation of chest X-ray and a protocol proposed by the WHO, (period 2002–2004). The mean annual incidence was 1100/100,000 in children $< 6$ years of age and 2000 cases/100,000 in children $< 2$ years of age [9]. Using the same methodology, Tregnaghi et al. [10] found a mean incidence of 2422 cases/100,000 children/year in children $< 2$ years old in the city of Cordoba. The mean global incidence of IPD was 206.8 cases/100,000 children per year, which is substantially higher than that reported in Chile by Lagos et al. [11]. The incidence of IPD expressed as bacteremia in hospitalized and ambulatory children $< 3$ years old, was 64.6/100,000/year.

Hortal et al. [12] reported the incidence of consolidated pneumonia in Uruguay between June 2001 to May 2004. The annual incidence rate ranged

from 1692/100,000/year to 1839/100,000/year in the $<$ 1 year old group; and from 1757/100,000/year to 2017/100,000/year for the 12–23 months old group. Studies from Chile and Brazil that were also based on the WHO protocol showed lower incidence rates: in Santiago the rate was 928/100,000/year and in Goiania 758/100,000/year in patients 0–23 months of age [11].

The higher frequency of *S. pneumoniae* isolation during the cold months suggests that viral infection is a risk factor for pneumococcal disease [13]. We found a greater frequency of meningitis in children $<$ 1 year old, which was the group with the highest risk of death (10%–15%), and neurological sequelae (30%) [14].

Serotype 14 represented more than 30% of isolates in the period 1994–1999 [15]. However, a reduction of 10% was observed after 2003.

A significant increase in serotype 1 isolation was noted during the later years of this study, as seen in Uruguay [16]. Serotype 1 is of particular interest because of its ability to provoke outbreaks in crowded and closed institutions [17] and cause severe episodes of pneumonia and empyema in children. This serotype is prevalent in some European countries, Israel, India, Africa, the southern cone of Latin America and Colombia. In contrast this serotype has low incidence of recovery in the US [18–20]. Serotype 5 was prevalent in Argentina and other countries in southern Latin America, but not in Brazil [20].

The increase incidence of isolation of serotype 19A in recent years has paralleled the worrisome increase in resistance to penicillin and erythromycin. Similar data was reported in other countries such as Korea, France, Spain and Israel that occurred before the introduction of PCV7 into their national vaccination schedules [21].

The increase of serotype 6A, though still not significant, highlights the importance of the continuous surveillance of the new serotype 6C necessary.

Age also influenced serotype distribution with a higher frequency of serotype 14 and 6B in children $<$ 2 years old and a lower prevalence of serotype 1 and 5 than in children $\geqslant$ 2 years old. This could be due to the differences in PCV7 coverage age groups. Similar data has been reported from other countries including Brazil [19].

In a previous study, we determined that more than 80% of the penicillin non-susceptible isolates showed characteristics of the widespread international Spain$^{9V/14}$-3 (ST156) clone, expressing serotype 14 and resistance to trimethoprim-sulfamethoxazole [22]. Therefore, the reduction in penicillin and trimethoprim-sulfamethoxazole resistance and the prevalence of serotype 14 observed during 2006–2007 could be associated, at least in part, with the decrease of this clone.

New MIC breakpoints for penicillin for non-meningitis isolates (susceptible penicillin MIC $\leqslant$ 2 $\mu$g/mL; resistant $\geqslant$ 8 $\mu$g/mL) were recommended by the CLSI in 2008 [23], implying that penicillin or ampicillin continues to be the drug of choice for the initial empirical treatment of pneumonia in children.

In accordance with the guidelines of the Argentine Pediatric Society, children $\geqslant$ 4 years old with pneumonia usually receive ampicillin or penicillin. Similar therapy is used in other countries of the region. Cefotaxime or ceftriaxone are utized for meningitis and bacteremias in infants [24].

In Argentina, erythromycin resistance emerged in 1995 and increased from 0% in 1994 to 13.8% in 2007 [25]. A similar change has also been reported in other countries and is possibly associated with increased macrolide usage, mainly azithromycin [21]. Recently, we determined that the England$^{14}$-9 (ST9), Poland$^{6B}$-20 (ST315) and Spain$^{9V/14}$-3 (ST156) clones were responsible for the emergence of pneumococcal macrolide resistance in the pediatric population of Argentina [25]. Resistance to other antimicrobial agents showed no significant changes during that period studied.

Variations in the distribution of *S. pneumoniae* serotypes, along with geographic and temporal stability of circulating serotypes, have been described [26] and account for the variations in the coverage of the PCV7 in different countries. Significant reduction in pneumococcal disease in vaccinated as well as unvaccinated (herd immunity) subjects have been documented in countries that have introduced the PCV7 into their vaccination schedules [27]. The coverage of PCV7 in children $<$ 2 years old in Argentina is inferior to the one observed in the United States. This is due to the absence in this formulation of serotypes 1 and 5, both highly prevalent in Argentina. Thus, the inclusion of the PCV7 in the national vaccination schedule of Argentina has been controversial due to it's deficient serotype coverage and high cost. Consensus exists among pediatricians on its administration to children $<$ 2 years old with risk conditions, as suggested by the American Academy of Pediatrics, and this approach has already been implemented in the public health immunization program in Argentina and other Latin American countries. Currently, the PCV7 is not included in the national schedule for immunization in Argentina, and its use depends on the decision of the attending pediatrician and the family.

The new conjugate vaccines that include other serotypes, particularly 1 and 5, greatly improve the serotype coverage in Argentina where these serotypes represented almost 25% of isolates studied in 2007. The incorporation of these vaccines into the Argentine childhood immunization schedule could prevent a significant amount of IPD, particularly in children up to 2 years old, which is the population most at risk.

In conclusion, this study demonstrates a reduction in serotype 14, penicillin non-susceptibility and trimethoprim-sulfamethoxazole resistance as well as an increase of serotypes 1, 19A and erythromycin resistance in recent years. These results emphasize the need for the introduction of a conjugate pneumococcal vaccine and continued surveillance for changes in serotype distribution and antimicrobial resistance. Data on the incidence of IPD are necessary to estimate the efficacy of a national vaccination program.

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