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Invasive *Streptococcus pneumoniae* isolates from pediatric population in Argentina for the period 2006–2019. Temporal progression of serotypes distribution and antibiotic resistance

Jonathan Zintgraff [a,*], Paula Gagetti [b], Daniela Napoli [a], Nahuel Sanchez Eluchans [a], Lucia Irazu [c], Maria Moscoloni [a], Argentina Spn Working Group [1] Mabel Regueira [a], Claudia S. Lara [a], Alejandra Corso [b]

[a] Servicio de Bacteriología Clínica, Departamento de Bacteriología. Instituto Nacional de Enfermedades Infecciosas (INEI)-ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina
[b] Servicio Antimicrobianos, Departamento de Bacteriología. Instituto Nacional de Enfermedades Infecciosas (INEI)-ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina
[c] Instituto Nacional de Enfermedades Infecciosas (INEI)-ANLIS "Dr. Carlos G. Malbrán", Buenos Aires, Argentina

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ABSTRACT

Streptococcus pneumoniae is a major cause of severe invasive disease associated with high mortality and morbidity worldwide. A total of 2908 pneumococcal isolates were analyzed between 2006 and 2019. Gold standard pneumococcal serotyping (the Neufeld-Quellung reaction) was performed to identify the serotypes associated with infection in children < 5 years in Argentina and agar dilution method was carried out to determine their profiles to 14 antimicrobial agents.

In 2012, the 13-valent pneumococcal conjugate vaccine (PCV13) was included in the National Immunization Program. In this work we have analyzed the local epidemiology of invasive pneumococcal diseases before and after the introduction of this vaccine in order to understand the epidemiological relevance and impact of PCV13.

During the periods compared in the present study there was a significant increase in the proportion of non-PCV13 serotypes, serogroup 24 (246.7%) and 12F (85.7%), and a significant decrease in PCV13 serotypes, including serotypes 14 (91.2%), 5 (95.6%) and 1 (84.6%) among others. Another observation was that serotypes 3 (7.4%) and 19A (4.9%) still remain among the most frequent serotypes despite being part of the PCV13 formulation. Regarding antimicrobial resistance, in the present study we observed an increase in erythromycin resistance during the period of study mainly associated to serotype 14 in the pre-PCV13 period and to serogroup 24 in the post-PCV13 period, which also was the major NVT serotype associated with antimicrobial resistance and MDR. Serotypes 14, 24A/B/F and 19A were in the first three places among isolates resistant to all the antibiotics tested.

Our data highlight the importance of continuous surveillance to assess the impact of pneumococcal vaccines and the use of antibiotics in the dynamic of pneumococcal serotypes.

1. Introduction

Streptococcus pneumoniae causes invasive pneumococcal disease (IPD) which is an outstanding cause of morbidity and mortality all over the world despite the availability of pneumococcal conjugate vaccines [1]. It can produce IPD, including bacteremia and meningitis, and non-invasive pneumococcal disease such as pneumonia, otitis media, sinusitis, among others [2]. Pneumonia is considered an IPD when this pathogen is obtained from pleural fluid or blood. *S. pneumoniae* is a major human pathogen producing structurally diverse capsular polysaccharides, which is the primary virulence factor of this pathogen and also crucial for immune evasion.

One hundred capsular serotypes have been identified [3]. Three vaccines are currently available for the prevention of disease caused by *S. pneumoniae*: 10-valent pneumococcal conjugate vaccine (1, 4, 5,6B, 7F, 9V, 14,18C, 19F and 23F), 13-valent pneumococcal conjugate vaccine (PCV10 plus 3, 6A, and 19A) and 23-valent pneumococcal polysaccharide vaccine (PPSV23: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F). At the moment of this publication, three additional investigational PCVs are today undergoing clinical studies: PCV15

* Corresponding author.
E-mail address: jzintgraff@anlis.gob.ar (J. Zintgraff).
[1] Members of the Argentina Spn Working Group are listed in Acknowledgments.

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(PCV13 + 22F and 33F), PCV20 (PCV15 + 8, 10A, 11A, 12F, 15BC) and PCV24 (PCV20 + 2, 9N, 17F, 20) [4–7]. Recently, in 2020, the Serum Institute of India, the largest vaccine manufacturer in the world by doses, announced the launch of India's first indigenously developed pneumococcal vaccine – called PNEUMOSIL®. As a PCV, PNEUMOSIL® is similar to the pediatric pneumococcal vaccine already on the market and it targets serotypes 1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F and 23F [8,9].

Different hypotheses have been postulated to understand the changes in serotype and antimicrobial resistance over the years. Many studies have shown that the introduction of PCVs has resulted in changes in epidemiology of pneumococcal diseases and can drive an increase in the frequency of preexisting resistant variants of non-vaccine serotypes due to the removal of competition from vaccine serotypes. On the other hand, widespread use of antibiotics has led to the rise of resistance. [10–16].

In Argentina the 7-valent pneumococcal conjugate vaccine (nowadays, discontinued) was available since 2000 but only in the private health sector. In 2012, PCV13 was included in the National Immunization Program in a 2 + 1 schedule (2, 4, 12 months) and catch up for children between 12 and 24 months of age during the first year with a two-dose scheme to achieve an impactful reduction of invasive pneumococcal diseases in the country in the shortest possible time [17].

To establish some national background for data of IPD, we may add that a previous study which evaluated impact of PCV13 on Consolidated Pneumonia (CP) and Pneumococcal Pneumonia (PP) burden [18], had shown a significant decrease in children (mainly in the group 12–23 months of age), a significant decrease in vaccine serotypes, as well as an increase in non-vaccine serotypes.

Surveillance of antimicrobial resistance allows us to monitor changes associated with specific serotypes, as is the case of penicillin non-susceptible pneumococci (PNSP), which in Argentina has been historically associated with serotypes 14, 19A, 9V, 6A/B and 23F [19,20].

In this study, we describe the epidemiology of IPD in children < 5 years old (0–59 months of age) over the years 2006–2019, through the analysis of the site of infection and antibiotic resistance stratified by cases caused by PCV13 versus non-PCV13 serotypes isolates. Because IPD occurs most commonly in children, especially those under 2 years of age, two groups of age were evaluated: <2 years old (0–23 months of age) versus 2–4 years old (24–59 months of age).

2. Materials and methods

A total of 2908 *S. pneumoniae* isolates (one pneumococcal isolate per IPD case) from sterile fluids (including cerebrospinal fluid, pleural fluid, blood, joint fluid, peritoneal fluid) in children younger than 5 years, were collected from 169 hospitals belonging to 23 provinces and Buenos Aires city, between January 2006 and December 2019 as part of the *S. pneumoniae* Surveillance Program (SIREVA II-OPS/WHO). Thirty hospitals were incorporated into the surveillance network in 2010. *S. pneumoniae* isolates and epidemiologic data were submitted to the National Reference Laboratory, INEI-ANLIS "Dr Carlos G. Malbrán"- Clinical Bacteriology and Antimicrobial Agent Divisions- for serotyping and susceptibility testing.

A UK external quality assurance Program NEQAS (UK National external quality assessment Schemes) monitors and ensures proficiency in developing the quality of results.

Five periods were defined: Early pre-PCV13 (2006–2008), late pre-PCV13 (2009–2011), transitional period (2012–2013), early post-PCV13 (2014–2016) and late post-PCV13 (2017–2019).

To evaluate changes in serotype distribution and to know the situation at the time of incorporation of the vaccine, late pre-PCV13 versus late post-PCV13 were compared, whereas in order to evaluate the changes in the susceptibility profiles to antimicrobial agents, we compared early pre-PCV13 and late post-PCV13.

Changes in serotype distribution before and after PCV13 introduction according to the main clinical diagnosis (invasive pneumonia, meningitis, sepsis/bacteremia) were calculated using the following formula:

$$\text{percentage change} = \left(\frac{\text{number of serotypes post} - \text{PCV period} - \text{number of serotypes pre} - \text{PCV period}}{\text{number of serotypes pre} - \text{PCV period}}\right) \times 100$$

A positive change indicated an increase in the proportion of the serotype, whereas a negative change indicated a reduction.

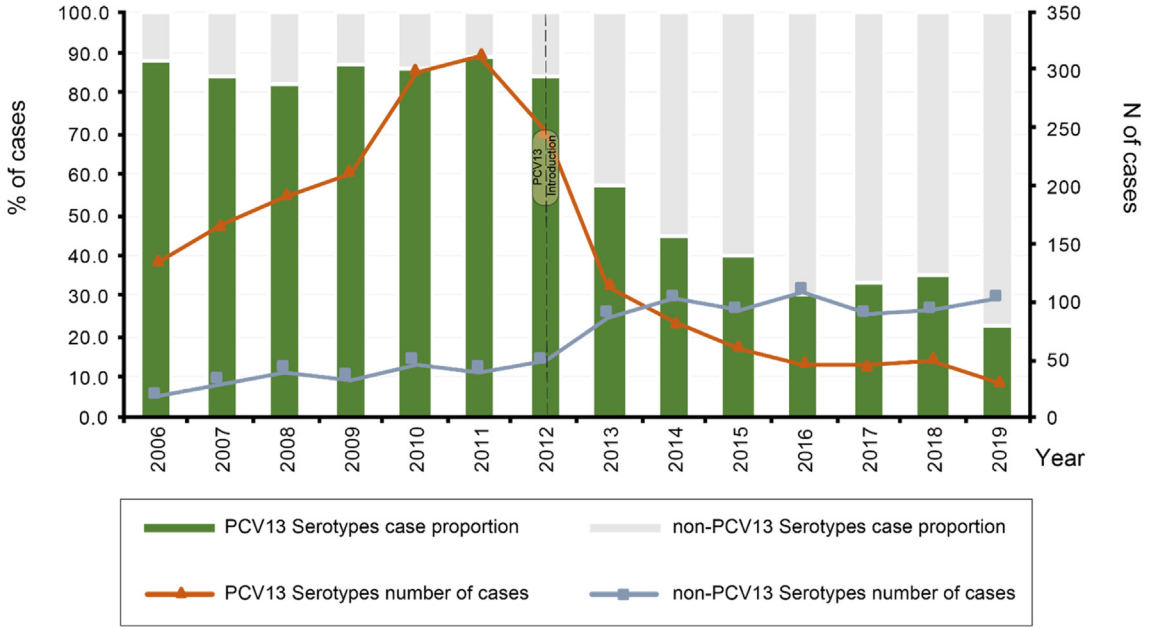

Fig. 1. Changes in proportion and number of cases of invasive pneumococcal disease by PCV13 serotypes and non-PCV13 serotypes in children < 5 yo for the period 2006–2019 (n = 2908).

Table 1
PCV13 and non-PCV13 Serotypes (top ten most prevalent) distribution in children < 5 yo.

Serotype		Year																							
		2006		2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018		2019	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
PCV13 SEROTYPES	1	21	13.82	25	12.82	19	8.23	41	16.80	48	13.95	41	11.92	34	11.60	32	16.00	23	12.64	21	13.91	7	4.52	7	5.26	10	7.04	3	2.27
	3	4	2.63	4	2.05	5	2.16	9	3.69	14	4.07	10	2.91	12	4.00	16	8.00	8	4.40	10	6.62	10	6.45	7	5.26	11	7.75	12	9.09
	4	3	1.97	1	0.51	3	1.30	0	0	4	1.16	4	1.16	3	1.02	1	0.50	0	4.40	1	0.66	0	0	0	0	0	0	0	0
	5	13	8.55	25	12.82	42	18.18	29	11.89	22	6.40	63	18.31	48	16.38	14	7.00	1	0.55	1	0.66	0	0	2	1.50	2	1.41	1	0.76
	6A	2	1.32	10	5.13	10	4.33	4	1.64	25	7.27	18	5.23	14	4.78	3	1.50	5	2.75	4	2.65	2	1.29	0	0	0	0	0	0
	6B	9	5.92	8	4.10	5	2.16	11	4.51	18	5.23	16	4.65	9	3.07	6	3.00	4	2.20	1	0.66	1	0.65	0	0	1	0.70	0	0
	7F	5	3.29	9	4.62	19	8.23	12	4.29	21	6.10	21	6.10	22	7.51	11	5.50	7	3.85	10	6.62	6	3.87	6	4.51	1	0.70	2	1.52
	9V	9	5.92	5	2.56	5	2.16	9	3.69	9	2.62	10	2.91	7	2.39	4	2.00	6	3.30	5	3.31	1	0.65	4	3.01	5	3.52	0	0
	14	41	26.97	48	24.92	57	24.36	65	26.64	83	24.13	79	22.97	51	17.41	16	8.00	10	5.49	4	2.65	7	4.52	5	3.76	8	5.63	7	5.30
	18C	10	6.58	8	4.10	9	3.90	7	2.87	13	3.78	11	3.20	14	4.78	0	0	4	2.20	0	0	1	0.65	1	0.75	1	0.70	1	0.76
	19A	9	5.92	12	6.15	9	3.90	12	4.92	21	6.10	20	5.81	20	6.83	8	4.00	10	5.49	3	1.99	8	5.16	8	6.02	9	6.34	3	2.27
	19F	5	3.29	5	2.56	4	1.73	7	2.87	9	2.62	9	2.62	5	1.71	1	0.50	2	1.10	0	1.99	3	1.94	2	1.50	2	1.41	1	0.76
	23F	3	1.97	5	2.56	4	1.73	6	2.46	12	3.49	10	2.91	8	2.73	2	1.00	1	0.55	0	0	1	0.65	2	1.50	0	0	0	0
NON-PCV13 SEROTYPES	12F	5	3.28	7	3.58	7	3.03	7	2.86	11	3.18	3	0.85	10	3.38	7	3.50	10	5.49	14	9.27	18	11.61	13	9.77	18	12.67	8	6.06
	24F	0	0	0	0	0	0	0	0	1	0.28	6	1.70	1	0.33	21	10.50	15	8.24	14	9.27	12	7.74	5	3.75	15	10.56	9	6.81
	23B	1	0.65	0	0	4	1.73	3	1.22	1	0.28	0	0	5	1.69	1	0.50	7	3.84	3	1.98	9	5.80	7	5.26	2	1.40	8	6.06
	22F	0	0	3	1.53	3	1.29	0	0	3	0.86	0	0	5	1.69	5	2.50	1	0.54	0	0	5	3.22	1	0.75	8	5.63	3	2.27
	15B	1	0.65	0	0	2	0.86	3	1.22	2	0.57	1	0.28	3	1.01	1	0.50	6	3.29	2	1.32	6	3.87	3	2.25	3	2.11	3	2.27
	23A	1	0.65	0	0	0	0	1	0.40	1	0.28	2	0.56	2	0.67	1	0.50	4	2.19	2	1.32	7	4.51	3	2.25	1	0.70	6	4.54
	11A	0	0	0	0	1	0.43	2	0.81	2	0.57	1	0.28	3	1.01	2	1.00	5	2.74	3	1.98	3	1.93	3	2.25	3	2.11	0	0
	9N	0	0	0	0	1	0.43	0	0	3	0.86	2	0.56	2	0.67	1	0.50	7	3.84	3	1.98	3	1.93	1	0.75	1	0.70	3	2.27

2.1. Isolates identification and conventional serotyping

All isolates were identified on the basis of optochin sensitivity (Oxoid, Basingstokes, UK), and bile (sodium deoxycholate) solubility test [21]. Gold standard of pneumococcal serotyping (the Neufeld-Quellung reaction) was performed using pool, group, type and factor specific commercial antisera produced by the Statens Serum Institute (Copenhagen, Denmark). Capsular types were assigned in accordance with the Danish nomenclature system [22–24]**.** Isolates were classified as non-typeable (NT) only if the Quellung reaction and subsequent sequential multiplex PCR [25] failed to classify them into one of the known serotypes.

2.2. Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed by agar dilution method to penicillin, amoxicillin, cefotaxime, meropenem, ceftaroline (only for isolates from 2014), ceftobiprole (from 2017), erythromycin, tetracycline, doxycycline (from 2013), chloramphenicol, cotrimoxazole, levofloxacin, rifampicin and vancomycin, according to CLSI guidelines [26,27]. Penicillin resistance was defined as MIC of ≥ 0.12 μg/ml according to meningitis break-point. For cefotaxime, intermediate and resistant categories were defined as MIC of 1, and $\geq$ 2 μg/ml, respectively. Ceftobiprole was interpreted according to EUCAST break-points: S $\leq$ 0,5; R $\geq$ 1 ug/ml. *S. pneumoniae* ATCC 49,619 and *Staphylococcus aureus* ATCC 29,213 were used as quality control strains. Macrolide resistance phenotypes were identified using a double disc test with erythromycin (15 μg) and clindamycin (2 μg) following the recommendation of the guidelines mentioned above. Isolates with intermediate or full resistance were defined as non-susceptible (NS) and multidrug resistant (MDR) was defined as non-susceptible to three or more classes of antimicrobial agents [28].

2.3. Statistical analysis

Statistical analyses were performed by using GraphPad Prism 9.0 (GraphPad Software, Inc., San Diego, California, USA). Fisher's exact test or X^2 test was used to determine the statistically significant differences between serotypes and periods; and association between clinical diagnosis and serotypes, as appropriate. Differences between groups and association between serotypes were considered to be significant at a p value of < 0.05.

Finally, we theorized the coverage of current PCV13 and the other three additional investigational PCVs.

3. Results

In the period between 2006 and 2019, 2908 isolates were studied, of which 1518 were admitted during all pre-PCV13 period (2006–2011) and 1390 after the PCV13 introduction in Argentina (2012–2019).

Trends in vaccine serotypes (VT), non-vaccine serotypes (NVT) and age group variation were described based on the number of isolates per year, and the results are summarized in Fig. 1. As the figure shows, the proportion of IPD caused by PCV13 serotypes, significantly decreased from 88.2% in 2006 to 22.8% in 2019 (p < 0.001). On the other hand, a statistically significant increase in non-PCV13 serotypes was observed from 11.8% to 77.2% during the studied period (p < 0.001).

The ratio of cases submitted by each group (<2 yo vs 2–4 yo) was 65.7:34.3 for the first six years of study, but changed in the last two years, approaching 54.5:45.5.

Regarding clinical diagnosis, the distribution in order of prevalence was: invasive pneumonia (49.8%; n = 1448) followed by meningitis (21.6%; n = 628) and sepsis/bacteremia (13.6%; n = 395). Other diagnoses (including septic arthritis and peritonitis, among others) were 15%.

Considering the entire period studied, the most prevalent PCV13 serotypes in invasive pneumonia were 14 (n = 277; 19.1%; $p < 0.001$, 1 (n = 259; 17.9; $p < 0.001$, 5 (n = 164; 11.3%; $p < 0.001$, 3 (n = 91; 6.3%; $p < 0.001$, 19A (n = 23; 6.6%) and 7F (n = 83; 5.7%); in meningitis cases were 14 (n = 95; 15.3%), 5 (n = 54; 8.6%), 18C (n = 50, 7.9%; $p < 0.001$), 7F (n = 40; 6.4%) and 1 (n = 36; 5.7%; $p < 0.001$) and in sepsis/bacteremia serotypes were 14 (n = 44; 11.7%; $p < 0.05$), 19A (n = 23; 6.6%) and 6A (n = 21; 5.3%).

Among the non-PCV13 serotypes, 12F and 24F were associated (both $p < 0.001$) with invasive pneumonia, meningitis (n = 35; 2.4% and n = 36; 2.5%); and only 24F (n = 14; 3.9%; $p < 0.001$ was associated with sepsis/bacteremia.

As mentioned before, in Argentina the PCV13 was included in the National Immunization Schedule in 2012. Since then, the distribution of serotypes has changed considerably as can be observed in Table 1 and Figs. 2 and 3. On the other hand, a total of 73 different serotypes were found (Supplementary S1) showing the diversity of the serotypes circulating.

Throughout the entire period of study, serotype 1 was associated with the group of 2–4 yo (n = 248; 22.6%; $p < 0.001$). By contrast, serotypes 14 (n = 325; 17.9%; $p < 0.001$), 19A (n = 107; 5.9%; $p < 0.05$), 23F (n = 42; 2.3%; $p < 0.05$), 12F (n = 106; 5.8%; $p < 0.001$) and serogroup 24 (n = 115; 6.3%; $p < 0.05$) were associated with the < 2 yo group (Fig. 5).

A statistically significant decrease in serotypes 14 ($p < 0.001$), 1 ($p < 0.001$), 5 ($p < 0.001$), 6A ($p < 0.001$), 6B ($p < 0.001$), 7F ($p < 0.05$), 18C ($p < 0.05$) and 23F ($p < 0.05$) was observed comparing the late pre-PCV13 (2009–2011) and the late post-PCV13 (2017–2019) periods. A significant increase in serotypes 12F ($p < 0.001$), 23B ($p < 0.001$), 22F ($p < 0.001$), 23A ($p < 0.001$), 16F ($p < 0.001$), 3 ($p < 0.05$), 15A ($p < 0.001$) and Serogroup 24 ($p < 0.001$) was also observed. Serotype 19A also showed a decrease, however, it was not statistically significant (Fig. 4).

Regarding non-typeable isolates, during the entire period of study we detected 44 NT (1.5%) *Streptococcus pneumoniae.*

An individual analysis was performed to serogroup 24, in order to evaluate if there was a statistically significant difference between the serotypes 24A, 24B, and 24F. Five isolates belonging to the Pre-vaccine era (2009–2011) were excluded, because they could not be serotyped. There was not a statistically significant difference ($p = 0.57$) between the distribution of serotypes included in the serogroup 24 comparing late pre-PCV13 period against the late post-PCV13 period.

The analysis of serotype distribution according to clinical diagnosis, is shown in Fig. 5.

Only serotype 3 showed a positive change in pneumonia. The remaining PCV13 serotypes showed a negative value, which indi-

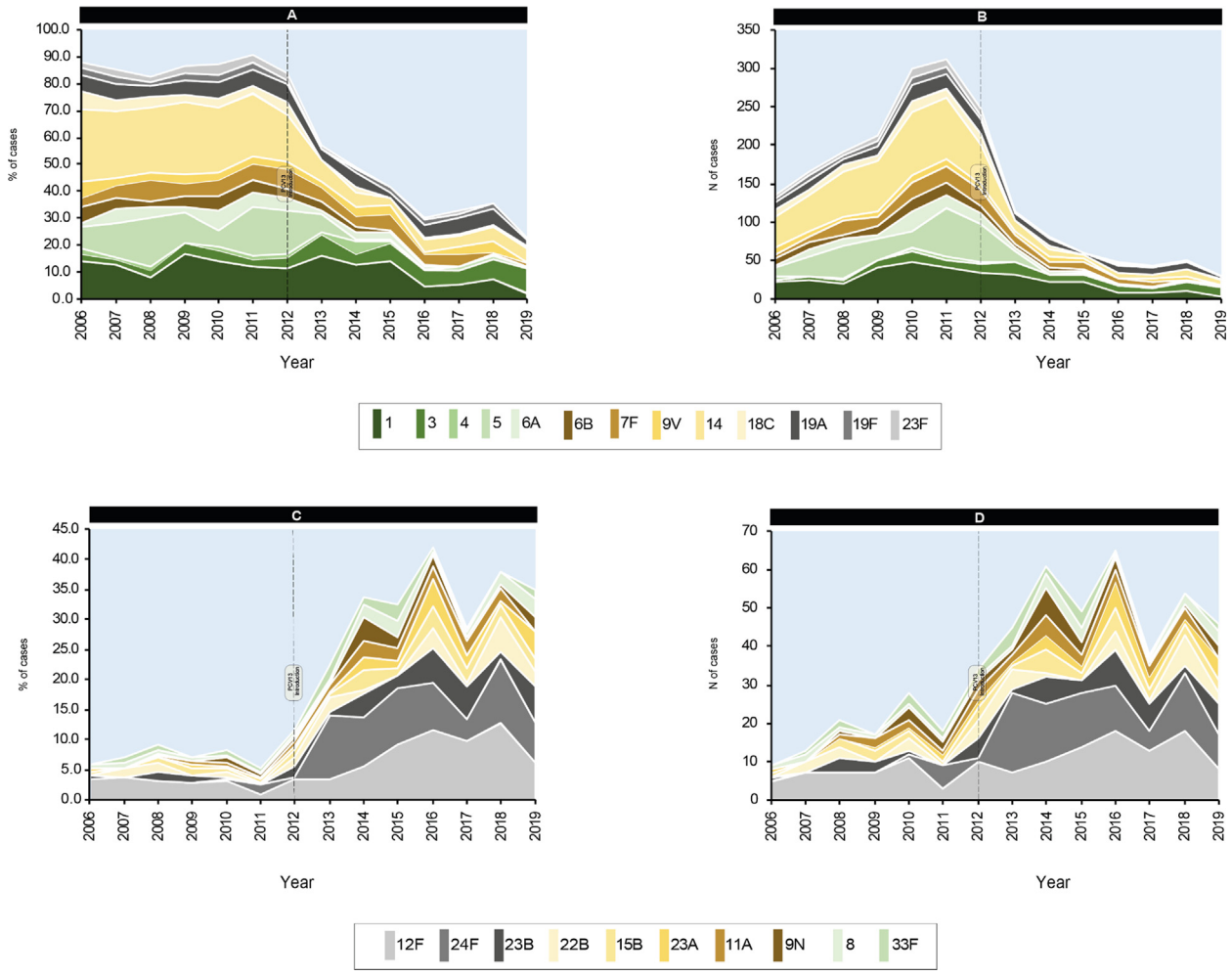

Fig. 2. Serotype distribution between 2006 and 2019. **A/B**- Proportion and Number of cases according to the PCV13 Serotypes in children < 5 yo. **C/D**- Proportion and Number of cases by non-PCV13 Serotypes (top ten most prevalent) in children < 5.

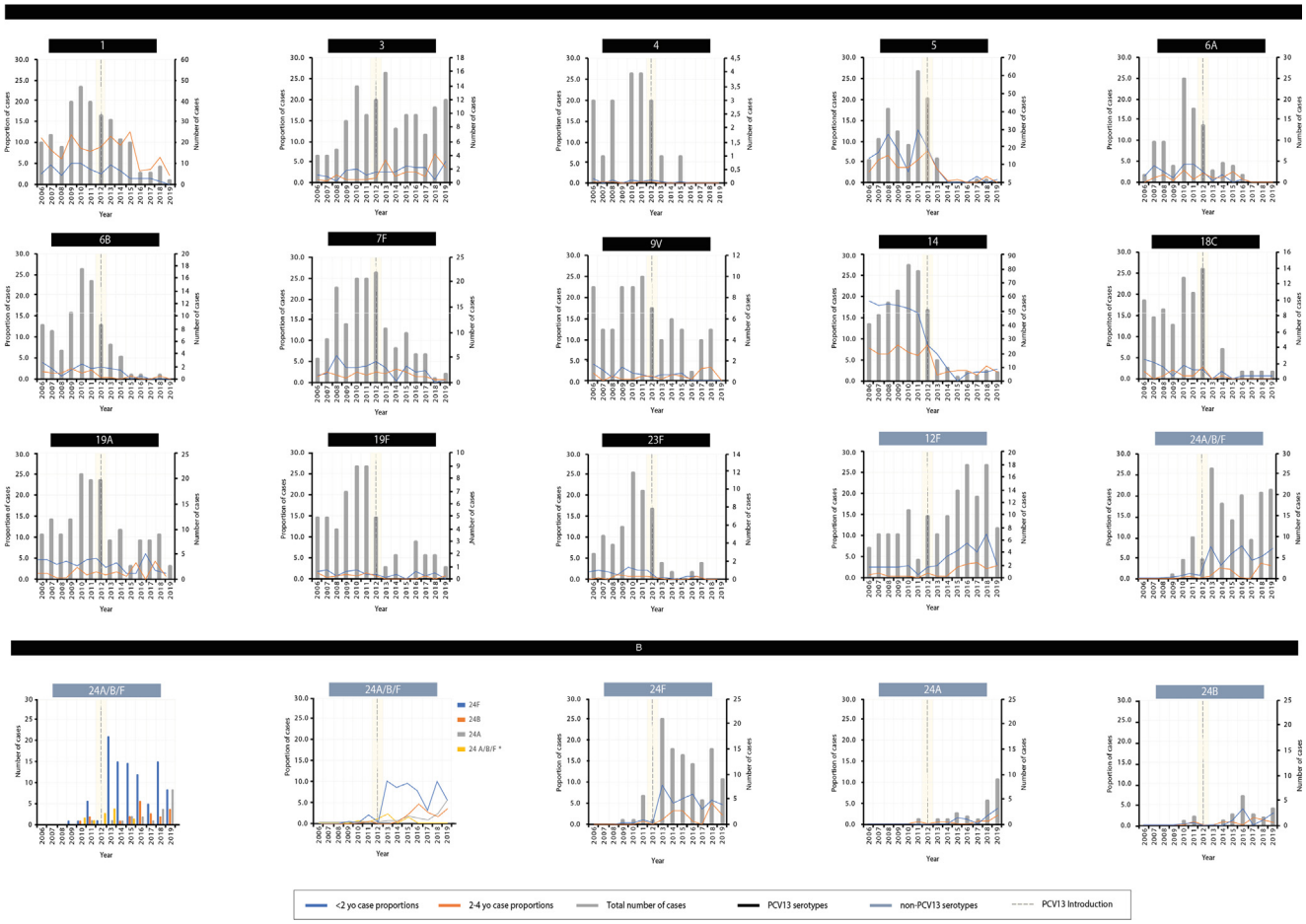

Fig. 3. A- Serotype distribution of PCV13 isolates and the most prevalent non-PCV13 serotypes (12F and serogroup 24) according to age group between 2006 and 2019. **B-** Individual analysis of serogroup 24. *Twelve isolates could not be serotyped as 24F, 24A or 24B, but they are included in Case proportions 24 A/B/F and Total Number of Cases 24 A/B/F.

cates a reduction of these serotypes in the main clinical diagnosis. In contrast, some non-PCV13 serotypes showed positive values, which indicates an increase of these serotypes, in the main clinical diagnosis.

The serotype coverage (theoretical vaccination coverage) of current and future vaccine formulations for children under 59 months with IPD is displayed in Fig. 6. The serotypes covered by PCV13 and the other three investigational PCVs are shown in this figure providing a preliminary view of the theoretical vaccination coverage and the potential impact of new three vaccine products.

As mentioned before, the coverage of PCV13 serotypes has firmly declined over the study period. When we extended the analysis to the additional serotypes included in PCV15, we noticed a 3.9% (n = 407) gain in theoretical vaccination coverage compared to PCV13 over the last three years. Regarding the additional serotypes in PCV20 compared to PCV15 we found that another extra 17.5% theoretical vaccination coverage would be gained (n = 210).

On the other hand, the theoretical vaccination coverage of PCV24 (n = 221) increased 2.4% in relation to PCV20. The difference between the coverage proportion of PCV20 and PCV24 in the period 2017–2019 proved to be statistically significant ($p < 0.05$).

Antimicrobial susceptibility tests were performed to 2798 isolates. A total of 929 (33.2%) were PNSP. Cefotaxime-NS in 153 (5.5%), meropenem-NS in 187 (6.7%), amoxicillin-NS in 20 (0.7%) and ceftobiprole-NS in 3 (0.8%). A total of 1213 (43.4%) isolates were cotrimoxazole-NS, 745 (26.6%) were erythromycin-NS, 550 were (19.7%) resistant to tetracycline and 2 (0.1%) to chloramphenicol. All the isolates were susceptible to ceftaroline, levofloxacin, rifampicin and vancomycin (Fig. 7).

Regarding antimicrobial NS, erythromycin and tetracycline showed a statistically significant increase in the late post-PCV13 period (2017–2019) compared to early pre-PCV13 period (Fig. 7, Table 2). Multidrug resistance was 16.1% overall, and showed a statistically significant increase ($p < 0.05$) from 8.0% in early pre-PCV13 to 21.1% in late post-PCV13 period.

Among erythromycin NS isolates of the whole collection, MLS_B phenotype represented 48.0% and M phenotype 52.0%. However, the prevalence of the MLS_B phenotype increased during the period of study, from 26.1% in early pre-PCV13 to 83.8% in late post-PCV13 period ($p = 0.06$). Most of the isolates with MLS_B phenotype belonged to serogroup 24 (35.7%), followed by serotypes 14 (14.6%) and 19 A (9.7%).

Serotypes 14, 24A/B/F, 19A, 6A, 6B, 23B, 9V, 16F, 19F and 5 represented 84.3% of the PNSP overall. In early pre-PCV13 period serotype 14 represented 55.3% of the PNSP, followed by serotype 19A (12.0%), 6B (8.0%) and others. On the other hand, in late post-PCV13 period serogroup 24 represented 35.2% of the PNSP, followed by serotype 19A (14.4%), 23B (12.0%) and others.

VTs were the major contributors to penicillin NS in early pre-PCV13 period, representing 88.7% of the PNSP. In contrast, in late post-PCV13 period, 76.0% of the PNSP were NVTs. A similar scenario was observed in the MDR isolates, in which the NVTs showed a statistically significant increase from 24.4% in early pre-PCV13 to 75.6% in late post-PCV13 period. Serotypes 24 A/B/F are the major

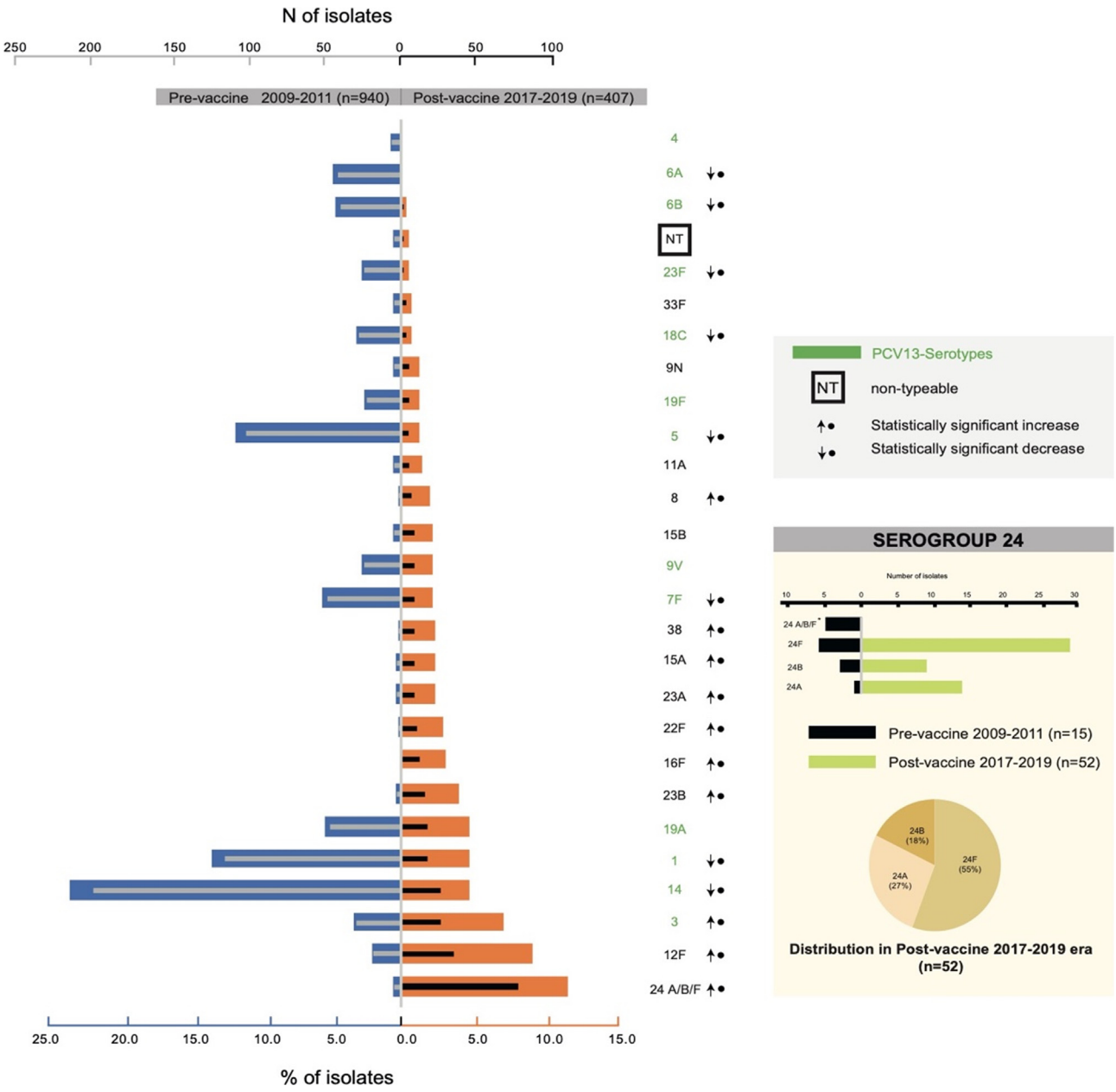

Fig. 4. Pneumococcal serotype distribution before (2009–2011) and after PCV13 (2017–2019) introduction. (Top 30 most prevalent serotypes in Post-vaccine era, including NT). *Five isolates could not be serotyped as 24F, 24A or 24B.

NVT serotypes associated with these antimicrobial NS and MDR in the late post-PCV13 period, accounting for 46.3% (44/95) of penicillin NS, 53.7% (44/82) of erythromycin NS, 55.8% (43/77) tetracycline NS, 30.4% (42/138) cotrimoxazole NS and 72.9% (43/59) MDR. Serotype 19A isolates showed a proportion around 5% during all the period of study, but penicillin NS increased from 62.1% in early pre-PCV period to 94.7% in late post-PCV13 period. Similarly, considering the same periods of study, 19A serotype isolates showed a statistically significant increase of MDR from 3.4% to 84.2% ($p < 0.05$).

Fig. 8 shows the association between the most prevalent serotypes and antimicrobial agents NS in the whole period of study (the other serotypes group is plotted in supplementary data as S2). Serotypes 14 and 24A/B/F were in the first three places among isolates non-susceptible to all antimicrobial agents.

4. Discussion and conclusions

S. pneumoniae is an important cause of invasive diseases, in particular in children and elderly people. Data generated from the laboratory-based surveillance SIREVA has provided valuable and important information regarding trends of pneumococcal serotype changes in Latin America [29,30]. The introduction of PCVs has changed the epidemiology of pneumococcal diseases, and, as a result, IPD rates have been significantly reduced in both children and the elderly population through direct and indirect (herd immunity) protection [31–34]. However, the proportion of pneumococcal diseases caused by non-PCV13 serotypes has risen, due to the increase in existing serotypes and/or the emergence of new serotypes, some of which are associated with antimicrobial resistance [35–37].

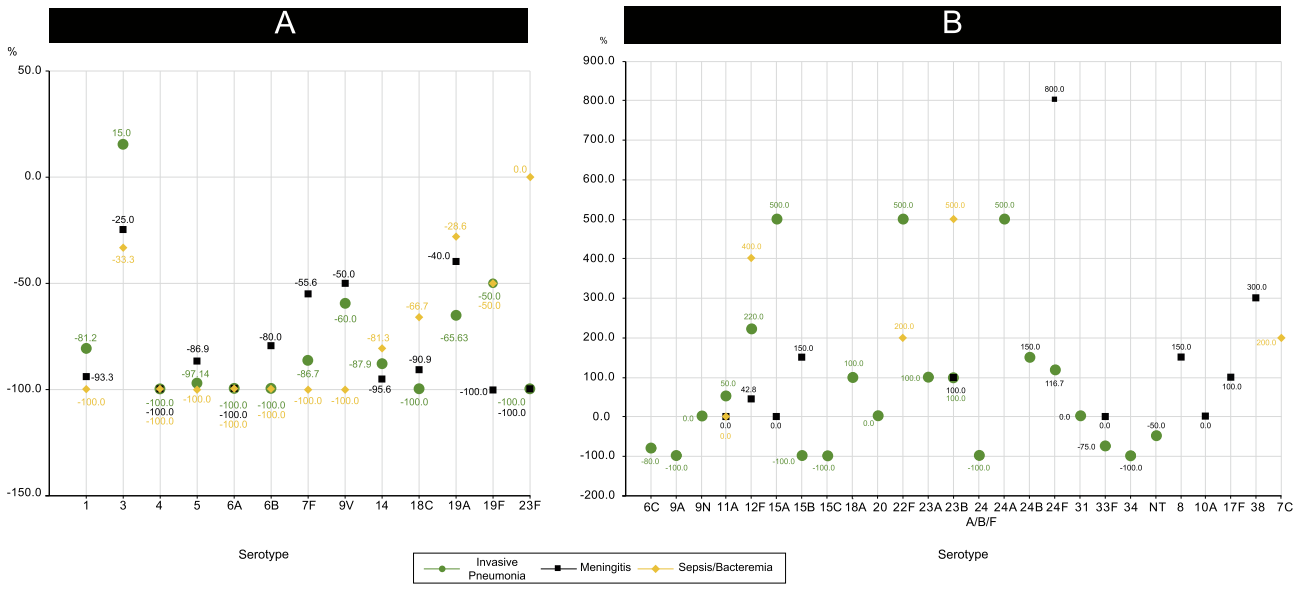

Fig. 5. Serotype distribution according to the main clinical diagnosis. Late pre-PCV13 (2009–2011) versus Late post-PCV13 (2017–2019) periods were compared. **A-** Change of PCV13 serotypes. **B-** Change of non-PCV13 serotypes.

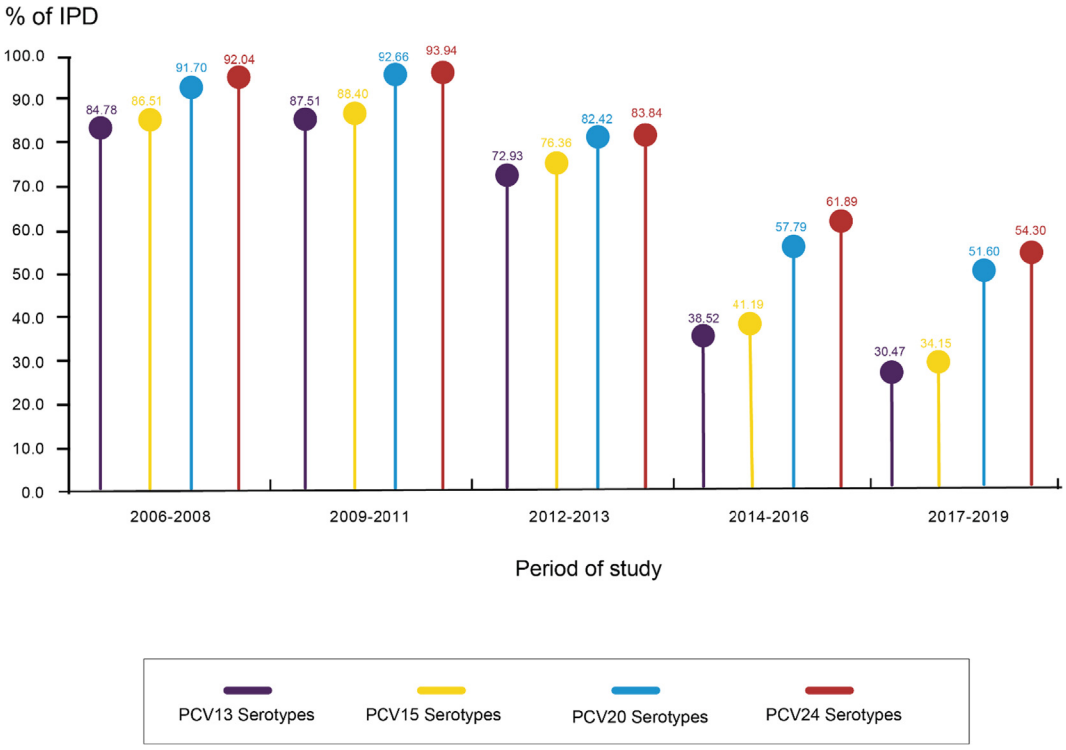

Fig. 6. Proportion of IPD cases due to serotypes included in current and upcoming vaccines during the entire period studied.

In this study, we observed a clear decrease in PCV13 serotypes 14, 5 and 1 which were highly prevalent before the incorporation of the PCV13. We also found the persistence of serotype 3, resembling the findings of earlier studies [2]. Although it is known that in several countries serotype 19A experimented an important decrease after the introduction of PCV13 [38], in our case it still remains between the ten most frequent serotypes. Serotype 19A was rare (≤3%) in PCV13-using countries but represented almost a quarter of the cases ≤ 5 years in PCV10-using countries [25]. In Argentina, serotype 19A is still responsible for 4.91% of the total cases. But this result, or even a higher prevalence, has been found in others countries using PCV13 [39]. PCV13 immunization coverage among 1-year-olds in Argentina was: 22% in 2012, 85% in 2013, 89% in 2014, 82% in 2015, 83% in 2016, 78% in 2017, 88% in 2018 and 80% in 2019 [40]. Although it is necessary the improvement of the vaccines coverages it is likely that other factors are playing a role in the current presentation of this serotype, since years with higher coverage (2016, 2018) and higher prevalence (in the post vaccine period) were observed.

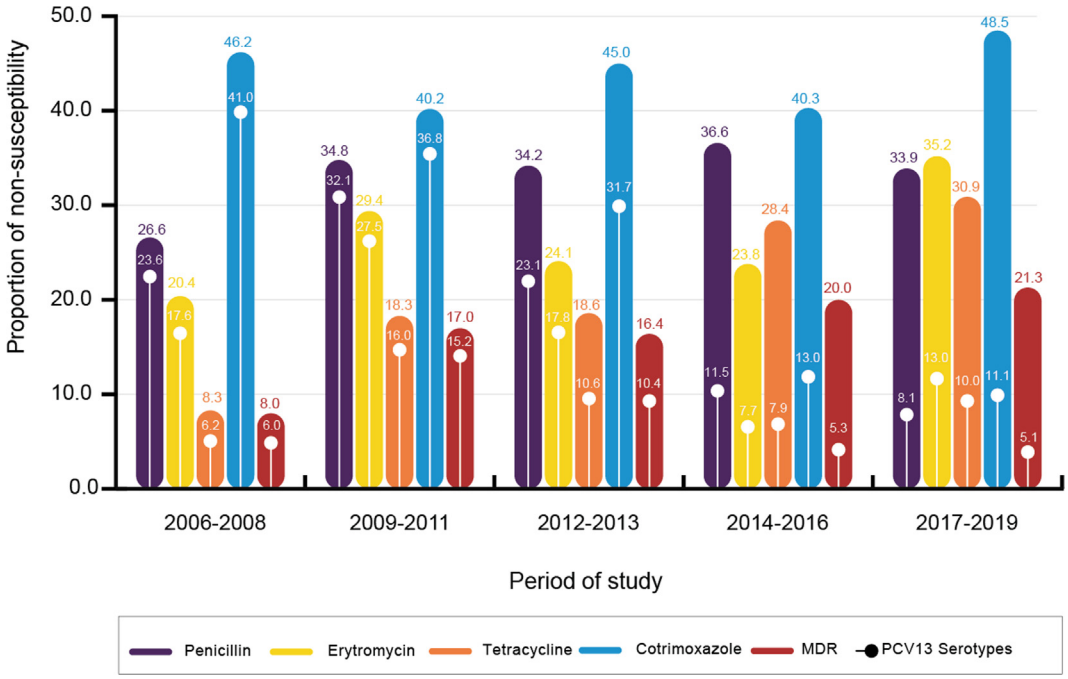

Fig. 7. Proportion of antimicrobial non-susceptibility and PCV13 serotypes according to different periods of study (n = 2798).

Table 2
Antimicrobial resistance among 2798 *S. pneumoniae* isolated between 2006 and 2019 from children under 5 years old in Argentina. Comparison between periods of study.

Antimicrobial Agent	Total				2006–2008			2009–2011			2012–2013			2014–2016			2017–2019		
	(n)	(S)	(NS)	(%NS)	(n)	(NS)	(%NS)	(n)	(NS)	(%NS)	(n)	(SN)	(%NS)	(n)	(NS)	(%NS)	(n)	(NS)	(%NS
Penicillin	2798	1877	929	33.2	563	150	26.6	923	321	34.8	489	167	34.2	454	166	36.6	369	125	33.9
Amoxicillin	2798	2778	20	0/7	563	5	0.9	923	4	0.4	489	1	0.2	454	1	0.2	369	9	2.4
Cefotaxime	2798	2645	153	5.5	563	44	7.8	923	52	5.6	489	22	4.5	454	15	3.3	369	20	5.4
Meropenem	2798	2611	187	6.7	563	51	9.1	923	77	8.3	489	22	4.5	454	15	3.3	369	22	6.0
Ceftaroline	637	637	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	268	0	0	369	0	0
Ceftobiprole	363	360	3	0,8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	363	3	0.8
Erythromycin	2798	2053	742	26.5	563	115	20.4	923	271	29.4	489	118	24.1	454	108	23.8	369	130	35.2
Tetracycline	2798	2248	550	19.7	563	47	8.3	923	169	18.3	489	91	18.6	454	129	28.4	369	114	30.9
Doxycycline	1017	715	302	29.7	NA	NA	NA	NA	NA	NA	NA	NA	NA	454	129	28.4	369	114	30.9
Chloramphenicol	2798	2793	5	0.2	563	4	0.7	923	1	0.1	489	0	0	454	0	0	369	0	0
Cotrimoxazole	2798	1585	1213	43.4	563	260	46.2	923	371	40.2	489	220	45.0	454	183	40.3	369	179	48.5
Levofloxacin	2798	2798	0	0	563	0	0	923	0	0	489	0	0	454	0	0	369	0	0
Rifampicin	2798	2798	0	0	563	0	0	923	0	0	489	0	0	454	0	0	369	0	0
Vancomycin	2798	2798	0	0	563	0	0	923	0	0	489	0	0	454	0	0	369	0	0
MDR	2798	2347	451	16.1	563	45	8.0	923	157	17.0	489	80	16.4	454	91	20.0	369	78	21.1

S: susceptible; NS: non-susceptible; NA: not available; MDR: multidrug resistant.

Finally, an increase in non-PCV13 serotypes with persistently high prevalence of serotype 12F and serogroup 24 was found. Emergence of serotype 12F after introduction of PCV13 was observed in Israel [41], Japan [42] and during an outbreak in Canada that began just after the second wave of the H1N1 pandemic in the autumn of 2009 [43]. A similar scenario was observed in Uruguay after the introduction of PCV13 [44].

Sequence types (ST), clonal complexes (CC) and Global Pneumococcal Sequence Clusters (GPSCs) are useful tools to provide further context for the distribution of serotypes and antibiotic resistance across pneumococcal lineages, which can be helpful to assess the impact of PCVs introduction.

Serotype 14, which represented 55.3% of the PNSP in early pre-PCV period, was historically the most frequent serotype associated to penicillin NS in Argentina. More than 80% of the PNSP in the nineties from children with IPD belonged to the widespread international Spain$^{9V/14}$-3 clone sequence type (ST) 156 expressing serotype 14 and NS to cotrimoxazole [45]. Therefore, the reduction in penicillin and cotrimoxazole NS and the prevalence of serotype 14 observed during 2006–2007 could be associated with the decrease of this clone [19].

In Argentina, erythromycin NS emerged in 1995 and increased over time mainly associated to the England^{14}-9 (ST9), Poland^{6B}-20 (ST315) and Spain$^{9V/14}$-3 (ST156) clones [46]. In the present study we observed an increase in erythromycin NS during the period of study mainly associated to serotype 14 in early pre-PCV13 period and to serogroup 24 in the late post-PCV13 period. Furthermore, most of the serogroup 24 isolates present the MLS_B phenotype. Although the proportion of serotype 19A was similar along the time, a higher prevalence of PNSP and MDR was observed. In the present study serotype 19A isolates showed 80% of penicillin NS and 30% of MDR, both higher than those found in a study carried out with isolates recovered between 1994 and 2014. The previous study showed 66% of penicillin NS and 11% of MDR in serotype 19A isolates, 54.5% of them were ST1131, a single-locus variant of ST172, followed by 11% of ST8121 [47]. Five major clonal

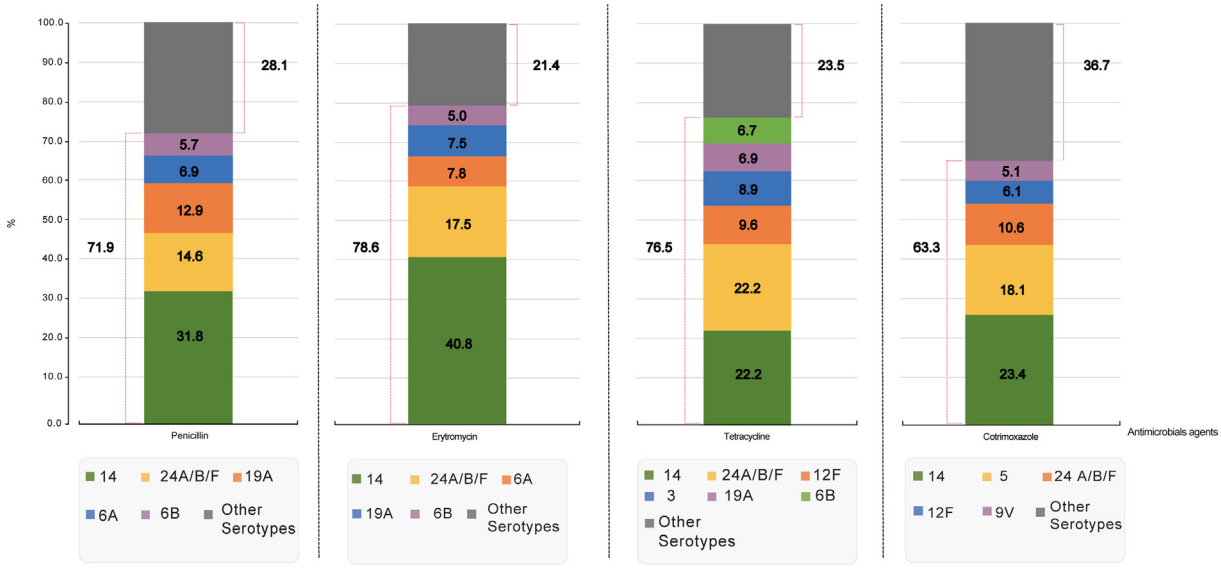

Fig. 8. Association between serotypes and antimicrobial agents non-susceptibility.

complexes (CC) are related to serotype 19A, specifically CC81, CC193, CC199, CC276 and CC320 [48], however, only two of them (CC199 and CC320) were detected in isolates from our country in a very low proportion (data not shown).

There are many theories about the increase of serotype 19A isolates in the world, mainly associated to vaccine induced serotype replacement, antibiotic pressure, introduction of new clones and/or the increase of previously circulating clones. The increase of *S. pneumoniae* serotype 19A in our country was mainly due to the dissemination of ST1131 and ST8121. Our findings suggest that the increase in serotype 19A was in part associated with the dissemination of preexisting clones circulating before the introduction of PCV13 into the National Immunization Program [47].

Serogroup 24 was the major NVT serotype associated with antimicrobial NS and MDR in the late post-PCV13 period of the present study. In Argentina serogroup 24 ranking first in < 2 years old from 2013 and showed a high proportion of MDR [49]. Increases in serogroup 24 after PCV13 introduction was also observed in other countries and it was reported to be one of the dominant NVT serotypes causing IPD in Portugal and Germany [50,51]. Globally, serotype 24F was mainly associated to three lineages: ST162, CC230 and CC72, and in Argentina it was mainly associated with ST230, Denmark^{14}-32 PMEN clone [49].

In Argentina, serotype 12F has not been associated with antimicrobial NS so far. According to GPSC (Global Pneumococcal Sequence Clusters) database serotype 12F isolates are globally susceptible to antibiotics [39] except the isolates from Israel originated by the expansion of the ST3774 clone [41]. A recent study carried out in Canada reported outbreaks of 12F strains resistant to macrolides belonging to ST218, commonly susceptible to antibiotics, which acquired determinants of resistance to macrolides by horizontal gene transfer [52]. Considering the PCVs in development, the average theoretical coverage for the last 3 years was: PCV13 30.3%, PCV15 33.9%, PCV20 51.4% and 54.1% for PCV24.

The decision to incorporate a new pneumococcal vaccine into the national immunization program is multifactorial. In this context of analysis, a vaccine that contains as many serotypes as possible and especially those which correlate with the local epidemiology of the circulating serotypes, is clearly a crucial factor to take into consideration to achieve its incorporation into this program. The difference between the coverage proportion of PCV20 and PCV24 turned out to be statistically significant, however, we express our concern regarding the non-incorporation of serotype 24F in any of the new vaccines, which, as reflected in this work, is one of the most prevalent serotypes in Argentina as well as in other countries [35,53–55].

It is equally important to achieve a pneumococcal vaccine that covers a broad range of serotypes and protects against the nasopharyngeal colonization and the invasive pneumococcal diseases. Several pneumococcal proteins are being studied as vaccine candidates [56].

Continued surveillance of pneumococcal epidemiology is strongly suggested, in order to render information on the emergence of multi-drug- resistant strains available.

One weakness of our study is the lack of availability of IPD incidence rates as well as scarce epidemiological-clinical data.

On the other hand, given the large number of hospitals that participated in the surveillance, we consider that the changes here described are representative of the baseline population in Argentina.

In conclusion, this work could be an aid to monitor the dynamic changes in serotypes and antimicrobial resistance. Besides, it may become a guidance for the development of new generation of vaccines for our country.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Argentina Spn Working Group:

Claudia Hernández, Alejandra Blanco, Vanessa Reitjman: **Hospital de Pediatria "Prof.Dr. Juan P. Garrahan"**, Carlos Vay: **Htal de Clinicas "Jose de San Martin"**, Claudia Alfonso: **Hospital General de Agudos "Donación Francisco Santojanni"**, Claudia Garbaz: **Hospital General de Agudos "Dr. Ignacio Pirovano"**, Claudia Etcheves: **Sanatorio Franchin**, Flavia Amalfa**: Hospital General de Agudos" Dr. Parmenio Piñero"**, Debora Stepanik**: Sanatorio Trinidad Palermo**, **Hospital Italiano,** Liliana Fernandez Caniggia**: Hospital Alemán**, Marisa Turco, Adriana Procopio, Miryam Vazquez**: Hospital de Niños "Dr. Ricardo Gutiérrez",** Mariela Soledad

Zarate**: Sanatorio Güemes,** Marta Giovanakis**: Hospital Británico**, Marta Flaibani**: Hospital General de Agudos " Dr. Carlos.G. Durand",** Nora Gómez**: Hospital General de Agudos Dr. Cosme Argerich**", Rosana Pereda**: Hospital de Niños" Pedro de Elizalde",** Liliana Guelfand**: Hospital General de Agudos " Dr. Juan A. Fernández**", Silvana Manganello**: Hospital General de Agudos " Dalmacio Vélez Sarsfield**", Iliana Martinez**: Hospital Churruca - Visca**, Ana Togneri**: HIGA Evita**, Andrea Pacha**: HIGA San Juan De Dios**, Maria L Benvenutti, Mabel Rizzo: **HIGA "Dr. Jose Penna**", Silvia Beatriz Fernandez**: HIGA "Dr. Pedro Fiorito**", Maria Cervelli: **HIGA "Dr. Diego Paroissien**", Mónica Machain**: HIGA "Dr. Abraham F. Piñeyro**", Andrea Fascente**: HIGA "Luisa C. de Gandulfo**", Laura Paniccia**: Hospital Municipal de Agudos "Dr. Leónidas Lucero**", María Adelaida Rosetti**: HIGA" Presidente Perón** ", Hebe Gullo, Maria Susana Commisso**: HIGA "Vicente Lopez y Planes**"; Nory Cerda, Carolina Vaccino**: Hospital Zonal General de Agudos "Dr. Carlos Bocalandro**", Victoria Monzani, Laura Morvay**: Hospital Materno Infantil " Dr. Victorio Tetamanti**", Micaela Sogga**: Hospital Municipal "Dr. Bernardo Houssay**, Viviana Vilches**: Hospital Universitario Austral**, Ana Laura Mariñazky**: Hospital "Dr. Arturo Oñativia"**, Appendino Andrea, Laura Biglieri**: Hospital Zonal Especializado de Agudos y Crónicos "Dr. A. Cetrángolo"**, Sandra Bognanni**: Hospital Zonal "Gdor. Domingo Mercante"**, Cecilia Vescina**: Hospital de Niños "Sor Maria Ludovica"**; Johanna Pérez: **Hospital Municipal de Pediatría "Dr. Federico Abete"**, Liliana Meccia**: Hospital del Niño de San Justo**: Marisa Almuzara**: HIGA "Eva Perón"**, Alejandra Sale**: Hospital Municipal "Dr. Enrique Sturiz"**, Mónica Sparo**: Hospital Municipal " Ramón Santamarina"**, Sofía Murzicato**: Hospital Municipal " Dr. Emilio Zerboni"**, Gabriela Galán**: Hospital Municipal "Dr. Federico Falcón"**, Roxana Depardo**: Hospital Municipal "Ostaciana V. Lavignolle"**, Maricel Garrone: **HIGA Simplemente Evita**, Adriana Fernández Laussi, Graciela Priore**: Hospital Nacional "Prof. Dr. Alejandro Posadas**", Mónica Vallejo**: Hospital Privado de Comunidad**, Cecilia Barrachia**: Hospital Municipal "Dr. Pedro Orellana"**, Adriana Melo**: Hospital Zonal General de Agudos "Virgen del Carmen"**, Victoria Ascua**: Hospital Interzonal General de Agudos "Dr. Enrique Erril"**, Patricia Lopez**: Hospital Zonal Especializado Materno Infantil "Argentina Diego"**, Daniela Carrizo**: Laboratorio Central de Salud Pública**, Patricia Valdez, Mariela Silvia Farfan**: Hospital Interzonal de Niños "Eva Perón"**, Viviana David**: Hospital Interzonal "San Juan Bautista"**, Leyla Guadalupe Gómez Capara, Mónica Graciela Sucin, Viviana Isabel Saito**; Hospital Pediatrico "Dr. Avelino Castelán"**, Laura Pícoli, Mariana Carol Rey, Isabel Ana Marques**: Hospital "Dr Julio C. Perrando"**, Norma Ester Cech**: Hospital 4 de Junio " Dr. Ramón Carrillo"**, Susana Ortiz: **Hospital Regional " Dr. Victor M. Sanguinetti**", Mario Flores**: Lab.de la Dirección de Patología Prevalente y Epidemiologia,** Teresa M Strella**: Htal Zonal de Trelew**, Omar Daher**: Htal. Zonal de Esquel**, Ana Littvik**: Hospital "Dr. Guillermo Rawson"**; Claudia Amareto De Costabella**: Hospital Regional "Dr. Louis Pasteur"**, Lidia Wolff De Jakob: **Clínica Privada Vélez Sarsfield**, Lilia Norma Camisassa**: Hospital Regional "Domingo Funes",** Liliana González**: Hospital Infantil Municipal**, Patricia Montanaro**: Hospital de Niños "Santísima Trinidad"**, Marina Botiglieri**: Clínica Universitaria "Reina Fabiola"**, Paulo Cortéz**: Hospital Pediátrico Del Niño Jesús**, Ana María Pato: **Hospital "Ángela Llano"**, Juan Pellegrini**: Hospital Pediátrico "Juan Pablo II"**, Lorena del Barco, María Eugenia de Torres, María Silvia Díaz**: Hospital Materno Infantil "San Roque",** María Ofelia Moulins, Luis Otaegui, Norma Yoya**: Hospital "Delicia C. Masvernat"**, Nancy Comello, Silvana Vivaldo: **Hospital de La Madre y El Niño**, Nancy Noemí Pereyra**; Hospital Central**, Marcelo Tóffoli, Gabriela Granados**: Hospital de Niños "Dr. Héctor Quintana"**, Beatriz Resina**: Laboratorio Central de Salud Pública**, Adriana Pereyra**: Hospital "Gobernador Centeno"**, Gladys Almada**: Hospital "Dr. Lucio Molas",** Sonia Flores, Mónica Romanazi**: Hospital Regional "Dr. Enrique Vera Barros"**, Ada Zanusso, Adriana Edith Acosta**: Hospital" Dr. Teodoro J. Schestakow"**, Alfredo Matile, Beatriz Garcia**: Hospital Pediátrico "Dr. Humberto Notti"**, Lorena Leguizamón**: Hospital Provincial de Pediatría "Dr. Fernando Barreyro"**, Viviana Villalba**: Hospital Escuela de Agudos " Dr. Ramón Madariaga"**, Cristina Pérez**: Hospital Provincial "Dr. Castro Rendón"**, Fernanda Bulgueroni**. Hospital " Dr. Horacio Heller"**, Néstor Blazquez, María Laura Álvarez**: Hospital Zonal Bariloche "Dr. Ramon Carrillo",** Cristina Carranza, Mariela Roncallo**: Hospital Area Cipolletti**, Gonzalo Crombas, Daniela Durani**: Hospital "Francisco López Lima"**, Graciela Stafforini, Maria Gabriela Rivolier: **Hospital "Artémides Zatti",** Cristina Bono, Eloisa Aguirre**: Hospital "Presidente Perón"**, Ana Berejnoi**: Hospital Público Materno Infantil**, Silvia Amador**: Hospital "San Vicente de Paul"**, Norma Sponton**: Hospital del Milagro**, Hugo Castro**: Hospital "Marcial Quiroga"**, Marisa López**: Hospital "Dr. Guillermo Rawson"**, Ema María Fernández**: Policlínico Regional "Juan Domingo Perón** ", Hugo Rigo**: Hospital Privado**, Josefina Villegas**: Hospital Zonal de Caleta Olivia "Pedro Tardivo"**, Vilma Krause, Alejandra Vargas, Mariel Borda**: Hospital Regional de Río Gallegos**, Andrea Badano, Adriana Ernst**: Hospital de Niños "Dr. Victor J. Vilela"**, Andrea Nepote, Maria Gilli**: Laboratorio Central de Salud Publica,** Emilce Mendez, Alicia Nagel**: Hospital" Dr. José M. Cullen"**, Maria Inés Zamboni**: CEMAR,** Noemi Borda**: Hospital Español**, Stella Virgolini, Maria Rosa Baroni**: Hospital de Niños "Dr. Orlando Alassia"**, Marciana Cragnolino**: Htal. Regional "Dr. Ramón Carrillo"**, Maria Elisa Pavón**: Hospital de Niños (CePSI) "Eva Perón"**, Manuel Boutureira**: Hospital Regional**, Marcela Vargas, Alejandra Guerra**: Hospital Regional**, Ana Maria Villagra De Trejo, José Assa**: Hospital del Niño Jesús María**, Fernández de Gandur: **Hospital de Clínicas "Presidente Dr. Nicolás Avellaneda"**, Norma Cudmani: **Laboratorio de Salud Pública.**

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.12.008.

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