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Streptococcus pneumoniae serotype 19A in hospitalized children with invasive pneumococcal disease after the introduction of conjugated vaccines in Lima, Peru

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ABSTRACT

Background: The Pneumococcal conjugate vaccine (PCV) has decreased cases of invasive pneumococcal disease (IPD) worldwide. However, the impact of PCVs introduction may be affected by the serotype distribution in a specific context.
Methods: Cross-sectional multicenter passive surveillance study of IPD cases in pediatric patients hospitalized in Lima, Peru between 2016 and 2019 (after PCV13 introduction) to determine the serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae*. Serotyping was performed by a sequential multiplex PCR and confirmed by whole genome sequencing.
Results: Eighty-five *S. pneumoniae* isolates were recovered (4.07/100,000 among children < 60 months of age). Serotype 19A was the most common (49.4%). Children infected with serotype 19A in comparison with children infected with other serotypes were younger, had a lower rate of meningitis and higher rates of pneumonia, complicated pneumonia and antimicrobial resistance; 28.6% of patients with serotype 19A have received at least one dose of PCV13 vs. 62.8% of patients with other serotypes. Using MIC-breakpoints, 81.2% (56/69) of non-meningitis strains and 31.2% (5/16) of meningitis strains were susceptible to penicillin; 18.8% (3/16) of meningitis strains had intermediate resistance to ceftriaxone. Resistance to azithromycin was 78.8% (67/85). Serotype 19A frequency increased over time in the same study population, from 4.2% (4/96) in 2006–2008, to 8.6% (5/58) in 2009–2011, to 49.4% (42/85) in the current study (2016–2019) ($p < 0.001$).
Conclusions: After PCV13 introduction in Peru, serotype 19A remains the most prevalent; however, the vaccination coverage is still not optimal. Therefore, additonal surveillance studies are needed to determine the remaining IPD burden.

Introduction

The introduction of pneumococcal conjugated vaccines (PCVs) has decreased the rate of pneumococcal invasive disease (IPD) and deaths, non-bacteremic pneumonia and otitis media, as well as the

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rate of hospitalization for IPD and antimicrobial resistance [1]. In addition, PCV immunization has decreased the rate of pneumococcus colonization by vaccine serotypes in the nasopharynx, which has led to indirect protection to other populations and herd immunity [2]. However, the degree of this direct and indirect protection varies globally depending on the local baseline serotype distribution, type of vaccine used, schedule (3 +1, 3 +0, 2 +1), and the vaccine coverage in the target population [1].

After PCV7 introduction serotype 19A emerged as a predominant serotype in children and adults worldwide [3,4]. However, after PCV13 introduction, multiple studies have reported the decrease on serotype 19A and the important emergence of non-PCV13 serotypes [3,5–7]. In Latin America, in neighboring countries like Colombia, serotype 19A currently accounts for approximately one half of all IPD isolates in children [8]; whereas in Chile serotype 19A accounts for 12–23% of pediatric IPD cases [9].

On the other hand, PCV introduction has led to decrease on antimicrobial resistance; however, there are important regional differences. The antimicrobial resistance trends in Latin America are associated with the emergence of multidrug resistant clones of serotype 19A, with current resistance rates to penicillin (MIC ≥0.125 mg/mL) around 70% and to trimethoprim-sulfamethoxazole around 60% [10].

The introduction of PCVs has dramatically changed the prevalence of serotypes in IPD and nasopharyngeal colonization in children, a phenomena known as serotype replacement [11]. This has led to the development of new vaccine with more serotypes to cover the emerging ones [12]. Thus, it is important to monitor the serotype distribution over time after vaccine introduction to determine the impact of immunization. Understanding of the remaining IPD burden will allow to make recommendations for future vaccines.

In Peru, an upper-middle-income country, PCV7 was introduced in the National Immunization Program in 2009, PCV10 in 2011 and PCV13 in July 2015; however, from that time point PCV10 was still partially used by the National Immunization Program for several additional months and still it is being use in the private sector. Overall, PCV vaccination uptake in Peru was slow. The Peruvian group in pneumococcal research (Grupo Peruano de Investigación en Neumococo, GPIN) has been monitoring the *Streptococcus pneumoniae* serotype distribution and antimicrobial resistance since 2006. The first IPD study (IPD1) was conducted before PCV7 introduction in Peru (2006–2008) [13] and the second study (IPD2) right after PCV7 introduction (2009–2011) [14]. Currently, there is no information on *S. pneumoniae* after PCV13 introduction in Peru; therefore, we conducted this study (IPD3) with the following aims: (1) to determine the serotype distribution and antimicrobial resistance of *S. pneumoniae* in hospitalized children in Lima with IPD from 2016 to 2019, after the introduction of PCV13, (2) to describe and compare the clinical characteristics and antimicrobial resistance of serotype 19A vs. other serotypes, and (3) to describe the serotype evolution over time in children with IPD before, during and after PCV introduction in Peru.

Material and methods

Study design

Cross-sectional multicenter passive surveillance study of IPD in 7 national hospitals and 7 private clinics and laboratories in Lima, Peru between November 2016 and December 2019. These are the most important and representative public and private health care center in Lima. We collected *S. pneumoniae* positive cultures from normally sterile sites (blood, cerebrospinal fluid [CSF], pleural fluid, synovial fluid, or peritoneal fluid) in pediatric patients (< 18 years of age). All isolates recovered at the participating centers during the surveillance period were collected. Information on the demographic and clinical characteristics of patients with IPD was recovered from the hospital medical records.

For the comparison of serotypes before and after PVC introduction, we used the data from our two previous passive surveillance studies conducted with the same methodology and patient population: IPD1 conducted between 2006 and 2008 [13] and IPD2 conducted between 2009 and 2011) [14]. In order to compare the incidence data between studies, we used the same methodology as previously described [13,14]. We estimated that 75% of the total pediatric population of the Province of Lima is served by the study's participating hospitals and clinics. The mean annual IPD rate (number of cases/100,000 children) was calculated based on the rates in each year of the study among children < 2 years of age (1 month-24 months of age) and < 5 years of age (1–60 months of age). The population of each district within the Province of Lima was obtained from the national public databases (https://www.minsa.gob.pe/reunis/data/poblacion_estimada.asp).

Laboratory studies

The isolates were transported to the central study laboratory (Pediatric Infectious Diseases Laboratory at Universidad Peruana Cayetano Heredia) the same day of isolation and confirmed to be *S. pneumoniae* by standard microbiological tests based on the colony morphology, alpha hemolysis, Gram stain, bile solubility and optochin susceptibility. Antimicrobial susceptibility testing was performed in all isolates by Kirby Bauer method using antibiotic disks (Oxoid Ltd, Basingstoke, Hans, UK) for ten antibiotics: oxacillin, azithromycin, chloramphenicol, clindamycin, vancomycin, rifampin, trimethoprim-sulfamethoxazole (TMP/SMX), tetracycline, levofloxacin and linezolid. The minimal inhibitory concentration (MIC) was determined using E-test® (AB Biodisk, Solna, Sweden) for five antibiotics: penicillin, ceftriaxone, azithromycin, clindamycin, and chloramphenicol. Selection of antibiotics and antimicrobial resistance interpretation was carried out according to the Clinical and Laboratory Standards Institute guidelines (CLSI standard M100 29th ed. 2019) [15]. Serotyping was performed by a sequential multiplex PCR using previously described primers and a validated method from the literature [16]. Serotypes were confirmed by whole genome sequencing (WGS) at the reference laboratory (CDC Streptococcus Laboratory, USA). For all laboratory testing, *S. pneumoniae* ATCC® 49619 was used as a control strain.

Statistical analysis

Statistical analyses of characteristics between IPD serotypes were performed with Chi-square test ($\chi 2$), Fisher's exact test, T-test and Mann-Whitney U-test using the statistical package Stata/SE v.17.0.

Results

A total of 85 *S. pneumoniae* isolates were recovered from pediatric patients with IPD from November 2016 to December 2019. During the study period the province of Lima had a mean population of 288,189 children < 24 months of age and 865,708 children < 60 months of age. The estimated annual IPD incidence rate in children < 24 months was 6.05/100,000 in 2017, 5.09/100,000 in 2018 and 4.14/100,000 in 2019, with an average of 5.09/100,000. Among children < 60 months of age the incidence rates was 5.38/100,000 in 2017, 3.69/100,000 in 2018 and 3.13/100,000 in 2019, with an average of 4.07/100,000 (Table 1).

Serotype 19A was the most common, representing 49.4% of all isolates. The average annual incidence of serotype 19A was 1.54 per 100,000 children < 24 months and 2.09 per 100,000 children < 60 months (Table 1). Children infected with serotype 19A compared to

Table 1
S. pneumoniae serotypes and IPD incidence in pediatric patients in Lima, Peru (2016–2019) (N = 85).

Serotypes	Total pediatric cases n (%)	Cases in children < 24 months	Incidence per 100 000 children < 24 months	Cases in children < 60 months	Incidence per 100 000 children < 60 months
19A	42 (49.4)	10	1.54	34	2.09
24F	18 (21.2)	12	1.85	15	0.92
38	2 (2.4)	2	0.31	2	0.12
35B	2 (2.4)	0	0	1	0.06
23B	2 (2.4)	1	0.15	1	0.06
16F	2 (2.4)	1	0.15	1	0.06
6C	2 (2.4)	0	0	2	0.12
33F	1 (1.2)	1	0.15	1	0.06
24B	1 (1.2)	1	0.15	1	0.06
23A	1 (1.2)	0	0	1	0.06
15C	1 (1.2)	0	0	0	0
15B	1 (1.2)	0	0	0	0
14	1 (1.2)	0	0	1	0.06
10A	1 (1.2)	1	0.15	1	0.06
7F	1 (1.2)	1	0.15	1	0.06
7C	1 (1.2)	0	0	0	0
6A	1 (1.2)	1	0.15	1	0.06
3	1 (1.2)	1	0.15	1	0.06
15B/15C[a]	2 (2.4)	1	0.15	1	0.06
24serogroup[a]	1 (1.2)	0	0	1	0.06
NT[a]	1 (1.2)	0	0	0	0
Total	85	33	5.09	66	4.07

[a] Pending WGS confirmation.

those infected with other serotypes were younger (26.2% vs. 55.8% were < 2 years), had a lower rate of meningitis (4.8% vs. 32.6%) and higher rates of pneumonia (80.9% vs. 34.9%) and complicated pneumonia at discharge (61.1% vs. 24.3%). Case fatalty rate was the same (Table 2).

Regarding the immunization characteristics of the children with IPD, 87.2% had received at least one dose of PCV of any type. We were not able to define the type of vaccine used in 21 children (24.7%). 45.9% of the immunized children received PCV13; 28.6% of patients with serotype 19A received at least one dose of PCV13 vs. 62.8% of patients with other serotypes. Only 7 of 12 children infected by serotype 19A and immunized with PCV13 were fully immunized (Table 3).

The pneumococcal strains had high antimicrobial resistance rates to common antibiotics. Serotype 19A strains had significantly higher resistance rates than other serotypes for penicillin (97.6% vs. 79.1%), azithromycin (95.2% vs. 58.1%), TMP/SMX (92.9% vs. 58.1%), tetracycline (88.1% vs. 51.2%) and clindamycin (85.7% vs. 53.5%) using disk diffusion (Table 1S). All strains were susceptible to chloramphenicol, levofloxacin, linezolid, rifampin and vancomycin using disk diffusion. Using MIC breakpoints, 81.2% (56/69) of non-meningitis strains were susceptible to penicillin, 31.2% (5/16) of meningitis strains were susceptible to penicillin, and 18.8% (3/16) of meningitis strains were intermediate resistant to ceftriaxone. Resistance to azithromycin by MIC was 78.8% (67/85) (Table 4). The penicillin and ceftriaxone MIC distribution are presented in the supplementary files (Fig. 1S).

Over the 13 years of surveillance (2006–2019), serotype 19A frequency has significantly increased over time in Lima, as well as serotypes not included in PCV13. Serotype 19A frequency varied from 4.2% (4/96) in our first IPD study (2006–2008), to 8.6% (5/58) in the second study (2009–2011), to 49.4% (42/85) in the current study (2016–2019) ($p < 0.001$). The average annual incidene of serotype 19A has increased from 0.69 per 100,000 children < 60 months in 2006–2008, to 0.67 in 2009–2011, to 2.09 in 2016–2019. On the other hand, serotypes included in PCV7 have decreased over time, with no PCV7 serotypes recovered in last 2 years (Fig. 1). Serotypes included in PCV7 decreased from 68.8% in the period pre-PCV7 introduction to 1.2% post-PCV13 introduction; serotypes included in PCV10 decreased from 75.0% to 2.4%, and serotypes included in PCV13 decreased from 84.4% to 54.8%, repectively (Fig. 2S).

Table 2
Demographic and clinical characteristics of pediatric patients with IPD by serotype (N = 85)†.

Characteristics	All	Serotype	
		19A	Other serotypes
	N = 85 n (%)	N = 42 n (%)	N = 43 n (%)
Male sex	42 (49.4)	17 (40.5)	25 (58.1)
Age group*			
Infants (< 2years)	35 (41.2)	11 (26.2)	24 (55.8)
Pre-school children (2–6 years)	38 (44.7)	27 (64.3)	11 (25.9)
School children (≥ 6 years)	12 (14.1)	4 (9.5)	8 (18.6)
Comorbidities[a]	40 (52.0)	19 (51.4)	21 (52.5)
Culture site**			
Blood	50 (58.8)	17 (40.5)	33 (76.7)
Pleural fluid	24 (28.2)	23 (54.8)	1 (2.3)
CSF	9 (10.6)	1 (2.4)	8 (18.6)
Other[b]	2 (2.4)	1 (2.4)	1 (2.3)
Primary clinical diagnosis**			
Pneumonia	49 (57.7)	34 (80.9)	15 (34.9)
Meningitis	16 (18.8)	2 (4.8)	14 (32.6)
Bacteremia	16 (18.8)	4 (9.5)	12 (27.9)
Other[c]	4 (4.7)	2 (4.8)	2 (4.6)
Complications			
Complicated pneumonia	19 (39.6)	15 (45.5)	4 (26.7)
Sepsis	27 (34.6)	14 (36.8)	13 (32.5)
Septic shock	11 (14.1)	6 (15.8)	5 (12.5)
Discharge status*			
Live without sequela	45 (63.4)	17 (48.6)	28 (77.8)
Live with sequela	20 (28.2)	15 (42.9)	5 (13.9)
Dead	6 (8.4)	3 (8.5)	3 (8.3)
Discharge diagnosis**			
Complicated pneumonia	31 (42.5)	22 (61.1)	9 (24.3)
Pneumonia	16 (21.9)	9 (25.0)	7 (18.9)
Meningitis	14 (19.2)	2 (5.6)	12 (32.5)
Bacteremia	9 (12.3)	3 (8.3)	6 (16.2)
Other[d]	3 (4.1)	0	3 (8.1)
Hospital stay in days, median (IQR)	14 (8–26)	15.5 (11–27.5)	12 (7–22)

† Some variables may add up less than 85 due to missing data. IQR: interquartile range, CSF: cerebrospinal fluid

[a] The most common comorbidities were malnutrition in 11 patients, congenital malformations in 9, asthma in 9, anemia in 7, congenital heart disease in 7, prematurity in 6 and nephrotic syndrome in 6. Patients could have had more than one comorbidity.

[b] Peritoneum fluid, skin abscess.

[c] Bacterial peritonitis, pre sternal abscess.

[d] Peritonitis, pyelonephritis

* $p < 0.05$,

** $p \leq 0.001$, for the comparison of serotype 19A and other serotypes using Chi-squared test

Discussion

This study found that after sixteen months since introduction of PCV13 in Peru, serotype 19 A remains as the most prevalent among children with IPD, despite being part of the PCV13 formulation. Serotye 19A is associated with high rates of pneumonia, complicated pneumonia and antimicrobial resistance.

The incidence of IPD among children < 24 months of age in Lima has decreased significantly from 18.45/100,000 in our first study [13] to 5.05/100,000 in the second study [14], and no significant change between our second and third study, 5.09/100,000. Overall, we found a significant decrease after PCV introduction in infants, as described in many countries [1]. Among children < 60 months of age there has been a non-significant decrease of IPD incidence from 7.7/

Table 3
Immunization characteristics of pediatric patients with IPD by serotype (N = 85).

Vaccine	All N = 85 n (%)	19A serotype N = 42 n (%)	Other serotypes N = 43 n (%)
Vaccinated with any PCV	68 (87.2)	31 (81.6)	37 (92.5)
PCV type*			
PCV-7	5 (5.9)	1 (2.4)	4 (9.3)
PCV-10	20 (23.5)	15 (35.7)	5 (11.6)
PCV-13	39 (45.9)	12 (28.6)	27 (62.8)
No data	21 (24.7)	14 (33.3)	7 (16.3)
PCV10 doses (N = 20)			
One dose	1 (5.0)	1 (6.7)	0
Two doses	4 (20.0)	3 (20.0)	1 (20.0)
Three doses	13 (65.0)	10 (66.7)	3 (60.0)
No data	2 (10.0)	1 (6.7)	1 (20.0)
PCV13 doses (N = 39)			
One dose	11 (28.2)	2 (16.7)	9 (33.3)
Two doses	14 (35.9)	3 (25.0)	11 (40.7)
Three doses	14 (35.9)	7 (58.3)	7 (25.9)

PCV, Pneumococcal conjugated vaccine
* $p < 0.05$, for the comparison of serotype 19A and other serotypes using Chi-squared test

100,000 (IPD1) to 6.07/100,000 (IPD2) and 4.07/100,000 in the current study.

After PCV7 introduction serotype 19A emerged as a predominant serotype in children and adults worldwide [3,4]. However, after PCV13 introduction, multiple studies have reported the decrease on serotype 19A and the important emergence of non-PCV13 serotypes [3,5–7]. Nevertheless, in order to achieve this, it is fundamental to have good vaccination coverage and several years of immunization with the vaccine, in order to see serotype replacement and herb immunity. We conducted our study only 16 months after starting PCV13 vaccination in Peru. In addition, the PCV13 vaccination coverage in Peru during the initial vaccination program has been slow with rates of 87.0% in 2016 and 78.6% in 2017 for the third PCV dose at one year of age (in Peru the 2 +1 schedule is used) [17]. Moreover, during the first year of PCV13 introduction, both vaccines, PCV10 and PCV13, were used in the country. In our study, less than half of the enrolled children were vaccinated with PCV13.

In Latin America, before the widespread PCV implementation, serotype 19A was responsible for a small number of pneumococcal disease cases, with an estimated prevalence rate of 3.8% between 1990 and 2010 [18]. A systematic review in the region found a 19.9% reduction in the total number of IPD isolates comparing the periods 2006–2009 and 2010–2015, most likely associated with PCVs introduction; however, the proportion of serotype 19A isolates doubled in relation to the overall number of pneumococcal isolates [19]. The estimated prevalence of serotype 19A during 2010–2015 was 11.1% in children younger than 5 years in Latin America [19].

The trends in serotype 19A prevalence over time vary by country and region. For example, in Colombia, serotype 19A emerged as the predominant IPD serotype after PCV10 introduction in children [20]. Among pneumococcal pneumonia cases comparing the pre-PCV10 (2008–2011) and post-PCV10 (2014–2019) periods in Colombia, serotype 19A increased from 3% to 30.2%, associated with higher rates of complicated pneumonia, intensive care unit admission and higher rates of antimicrobial resistance [8]. Similar to our study, in Colombia it is estimated that serotype 19A currently accounts for one half of all IPD isolates in the pediatric population [8]. In Chile, PCV10 was introduced in the national immunization program in 2011. Serotype 19A increased from < 5% before 2010 to 12–23% in 2014–2015, associated with high rates of antimicrobial resistance [9]. In Brazil, PCV10 has been introduced in the national immunization program in 2010, with important reduction in IPD; however, serotype replacement has been also an issue. During the late post-vaccine years, since 2014, serotype 19A has been the leading serotype, responsible for 28–45% of all IPD in children under 5 years [21]. On the other hand, in Argentina the rate of serotype 19A from IPD cases in children increased from 3% in 1993 to 6% in 2011 in the pre-vaccination period [22]. After PCV13 introduction in 2012, the rate of serotype 19A decreased, but not significantly. Currently (2017–2019), serotype 19A is responsible for approximately 5% of all IPD cases [23]. The prevalence of serotype 19A in Argentina seems to be much lower than the current prevalence in Colombia, Chile, Brazil and Peru.

There are several hypotheses to try to explain the increase of serotype 19A isolates in the world: 1) vaccine-induced serotype replacement, 2) introduction of new clones, 3) increase in previously circulating clones, and 4) antibiotic pressure [23]. Thus, to have a better understanding of the phenomena, it is necessary to study the pneumococcal clones and sequence types (ST). In order to control the spread of this strain, it is fundamental to improve vaccination coverage with PCV13 or other conjugated vaccines containing serotype 19A, and improve vaccination of other eligible population, such as the elderly.

Currently, new vaccine formulations to cover additional serotypes have been developed; however, these vaccines are still not available in Latin America. Based on the current IPD serotype distribution in Peru, the addition of new serotypes in PCV15 (PCV13 + 22 F and 33 F) and PCV20 (PCV15 + 8, 10 A, 11 A, 12 F and 15B) [12] have a modest increase in serotype coverage, from 54.8% with PCV13, to 56.0% with PCV15 and 59.8% with PCV20 (Fig. 2S). However, this report is based on a relatively small number of strains.

In relation to the clinical characteristics, we found an overall high rate of complicated pneumonia at discharge (42.5%). In the United States the rate of complicated pneumonia in children, specifically empyema, increased after PCV7 introduction and decreased significantly after PCV13 [24]. Similarly, in Colombia, the rate of complicated pneumonia increased from 13.4% to 31.8% after PCV10 introduction [8]. This could be explained because historically serotypes 1, 19A, 3, 14 and 7F were the most commonly associated with para-pneumonic empyema [25]; all serotypes were not part of PCV7, except 14. However, in our study serotype 19A still is responsible of half of all IPD cases and is associated with higher rates of complicated pneumonia (61.1%). In addition, as previously described in other studies, we found that infections with serotype 19A occurred

Table 4
Antibiotic susceptibility of *S. pneumoniae* strains by minimum inhibitory concentration (MIC) (N = 85).

Antibiotic	Breakpoint	N	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Penicillin	Non-MEC (R, MIC ≥8.0)	69	3 (4.3)	10 (14.5)	56 (81.2)
	MEC (R, MIC ≥0.12)	16	11 (68.8)	-	5 (31.2)
Ceftriaxone	Non-MEC (R, MIC ≥4.0)	69	1 (1.5)	7 (10.1)	61 (88.4)
	MEC (R, MIC ≥2.0)	16	0 (0.0)	3 (18.8)	13 (81.2)
Azithromycin		85	67 (78.8)	6 (7.1)	12 (14.1)
Clindamycin		85	58 (68.2)	2 (2.4)	25 (29.4)
Chloramphenicol		85	18 (21.2)	-	67 (78.8)

MEC, meningitis isolates; Non-MEC, Non-meningitis isolates; R, resistant

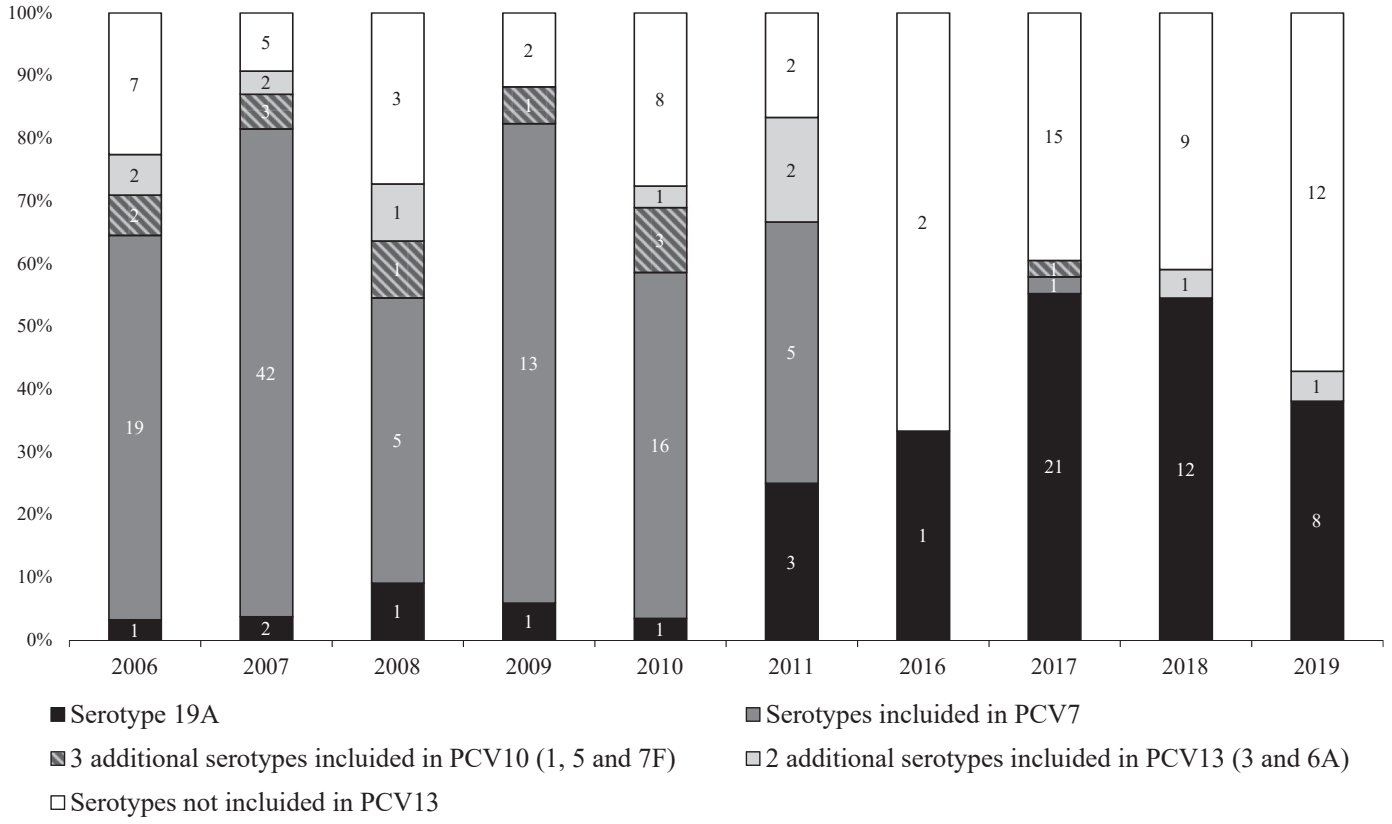

Fig. 1. *S. pneumoniae* serotype distribution of IPD strains from children hospitalized in Lima, Peru from 2006 to 2019.

in younger children and strains were more resistant to antibiotics [4,26].

Globally, PCV introduction has led to decrease on antimicrobial resistance. However, there are important regional differences based on the type of vaccine used and the circulation of specific serotypes, clones and STs. In Lima the rate of penicillin non-susceptibility among meningeal strains changed from 46.2% in 2006–2008 [13] to 68.8% in 2016–2019 (current study). In Brazil, during the first 3 years after PCV10 introduction there was a reduction of penicillin resistance; however, in the following 6 years there has been a gradual increase of penicillin resistance, associated with non-PCV10 serotypes [27]. The rate of penicillin non-susceptibility (MIC > 0.12 ug/mL) among children < 5 years of age with meningitis in Brazil changed from 47.5% in 2007–2009 to 53.9% in 2017–2019 [27]. While in Argentina, this rate varied from 26.6% in 2006–2008 to 33.9% in 2017–2019 [23].

Resistance to macrolides has also increased from 24.8% in 2006–2008 [13] to 78.8% in 2016–2019. This trend is similar to what has been described among nasopharyngeal carriers in children < 2 years of age in Peru; from 33.5% during 2006–2008 to 50.0% during 2018–2019 [28], and similar to what has been found in other countries in the region [23,27,29].

In general, the antimicrobial resistance trends in Latin America are associated with the emergence of multidrug resistant clones of serotype 19A. As part of the SIREVA II (Sistema Regional de Vacunas, Vaccine Regional System) network of the Pan American Health Organization (PAHO), during 2010–2015, 185 serotype 19A isolates were analyzed, finding high rates of antimicrobial resistance to penicillin (MIC ≥0.125 mg/mL) (68.6%), tetracycline (63.7%), trimethoprim-sulfamethoxazole (63.2%), and erythromycin (43.2%) [10]. The most frequent STs found were ST320 (32.4%), ST199 (14.1%) and ST172 (10.8%) [20]. In Chile, during 2015, in children < 5 years almost 100% of 19A meningitis strains were penicillin resistant and 48.0% belong to the clonal complex 320 [9]. Despite the increasing trend in penicillin resistance of *S. pneumoniae*, penicillin or amoxicillin at high dose continue to be the drug of choice for treatment of ambulatory pneumonia cases; however, we need to be cautious in complicated pneumonia cases. On the other hand, although we only had few meningeal strains, around 19% were intermediate resistant to ceftriaxone; thus, the empiric antibiotic treatment of meningitis in Peruvian children should be ceftriaxone plus vancomycin, until culture and susceptibility results.

This study has several limitations. First, although we collected data and strains from the main public and private hospitals and clinical laboratories in Lima, we have not included all health care facilities of the city of Lima. Second, our study was conducted only in Lima, and although it accounts for one third of Peru´s population, additional nationwide surveillance studies are needed to better represent the entire country. Third, the number of samples included in this study is too small to make significant conclusions on the trends over time in serotype distribution and antimicrobial resistance in Peru. Nevertheless, the number of strains included in this study and in the previous ones from our research group (IPD1 and IPD2) [13,14] are larger than the number of strains collected as part of the national surveillance system in Peru, and as part of previous SIREVA surveillances, as reported for other countries in the region [18–20,30]. Fourth, some clinical information was missing, since we reviewed data only from the medical charts without an interview with the patients or parents. This was especially relevant for the immunization status of the children; we were not able to document the type of PCV received in one fourth of the subjects. Fifth, for MIC value between 0.5 and 2 mg/L obtained by E-test, we have not performed an alternative method (broth microdilution), as recommended by EUCAST, since E-test seems to underestimate MIC-values around the resistance breakpoints [31]. Finally, the current report does not include data on circulating clones and STs in order to understand the

predominance of serotype 19A and the current antimicrobial resistance profile in Lima.

Despite these limitations, the current study is an important contribution to the current understanding of pneumococcal serotype distribution and antimicrobial resistance in Peruvian children. This information is relevant to monitor vaccine impact and to guide empiric management of pneumococcal infections. Additional surveillance studies are needed to determine the remaining IPD burden in the late post-PCV13 years in Peru.

Data sharing

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Contributors

TO designed the study, obtained funding and supervised all activities. OA, IR, EC, MC, FC, AS, RH were in charge of IRB approval in each hospital, patient enrollment and data acquisition. ALM, FCK and AM were the study coordinators, in charge of strain collection, data acquisition and data entry. BG and EM performed the laboratory analysis. BG analyzed and interpreted the results. TO interpreted the results and wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

Ethical approval statement

This study was approved (Ethics code: SIDISI 67445) by the Institutional Review Board of Universidad Peruana Cayetano Heredia (Lima, Peru) and by each participating hospital ethics committee.

Role of the funding source

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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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2023.10.047.

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