

Original Paper

Cite this article: de Felipe B *et al* (2022). Molecular epidemiology of paediatric invasive pneumococcal disease in Andalusia, Spain. *Epidemiology and Infection* **150**, e163, 1–8. <https://doi.org/10.1017/S0950268822001376>

Received: 15 June 2022

Accepted: 5 August 2022



Key words:

Genotypes; invasive pneumococcal disease; pneumococcal conjugate vaccine; serotypes; *Streptococcus pneumoniae*

Author for correspondence:

Ignacio Obando,
E-mail: iobando@us.es

Molecular epidemiology of paediatric invasive pneumococcal disease in Andalusia, Spain

Beatriz de Felipe¹, Pablo Obando Pacheco², Begoña Carazo Gallego³, David López Martín⁴ , Juan Luis Santos Pérez⁵, Yolanda González Jiménez⁶, María José Muñoz Vilches⁷, Nerea Cardelo Autero⁴, Verónica González Galán⁸, Francisco José Morón¹, Juan Antonio Cordero Varela¹, María José Torres Sánchez¹, Antonio Medina Claros³, David Moreno Pérez⁹ and Ignacio Obando^{1,7} 

¹Biomedical Institute of Seville (IBiS), University of Seville, University Hospital Virgen del Rocío (HUVR)/CSIC, Seville, Spain; ²Pediatric Unit, Hospital Universitario Virgen de Valme, Seville, Spain; ³Pediatric Infectious Diseases and Immunodeficiencies Unit, Hospital Universitario Regional de Málaga, Málaga, Spain; ⁴Pediatric Unit, Hospital Costa del Sol, Marbella, Málaga, Spain; ⁵Pediatric Infectious Diseases and Immunodeficiencies Unit, Hospital Virgen de las Nieves, Granada, Spain; ⁶Pediatric Pneumology Unit, Hospital Universitario San Cecilio, Granada, Spain; ⁷Pediatric Infectious Diseases and Immunodeficiencies Unit, HUVR, Seville, Spain; ⁸Microbiology Department, HUVR, IBiS, Seville, Spain and ⁹Coordinador del Plan Andaluz de Vacunas de Andalucía (PVA), Pediatric Infectious Diseases and Immunodeficiencies Unit, Hospital Universitario Regional de Málaga, Málaga, Spain

Abstract

This study aimed to assess the impact of the introduction of pneumococcal conjugate vaccine 13 (PCV13) on the molecular epidemiology of invasive pneumococcal disease (IPD) in children from Andalusia. A population-based prospective surveillance study was conducted on IPD in children aged <14 years from Andalusia (2018–2020). Pneumococcal invasive isolates collected between 2006 and 2009 in the two largest tertiary hospitals in Andalusia were used as pre-PCV13 controls for comparison of serotype/genotype distribution. Overall IPD incidence rate was 3.55 cases per 100 000 in 2018; increased non-significantly to 4.20 cases per 100 000 in 2019 and declined in 2020 to 1.69 cases per 100 000 (incidence rate ratio 2020 vs. 2019: 0.40, 95% confidence interval (CI) 0.20–0.89, $P = 0.01$). Proportion of IPD cases due to PCV13 serotypes in 2018–2020 was 28% ($P = 0.0001$ for comparison with 2006–2009). Serotypes 24F (15%) and 11A (8.3%) were the most frequently identified non-PCV13 serotypes (NVT) in 2018–2020. Penicillin- and/or ampicillin-resistant clones mostly belonged to clonal complex 156 (serotype 14-ST156 and ST2944 and serotype 11A-ST6521). The proportion of IPD cases caused by PCV13 serotypes declined significantly after the initiation of the PCV13 vaccination programme in 2016. Certain NVT, such as serotypes 24F and 11A, warrant future monitoring in IPD owing to invasive potential and/or antibiotic resistance rates.

Introduction

Streptococcus pneumoniae is a commensal bacterium of the human respiratory tract. Under appropriate conditions, they become pathogenic and can cause high morbidity and mortality [1, 2]. This microorganism is a leading cause of mucosal diseases, such as otitis media, sinusitis and pneumonia. In addition, it is a prominent pathogen in invasive diseases, including bacteraemia, meningitis and sepsis.

Routine vaccination of infants and young children with pneumococcal conjugate vaccines (PCVs) has led to a prompt and sustained reduction in the incidence of invasive pneumococcal disease (IPD) rates. This reduction also extends to unvaccinated cohorts of older children and adults due to herd effect [3, 4]. Despite their success, PCVs have limitations: coverage is serotype dependent, and there is a potential increase in IPD caused by non-vaccine types (NVT) due to serotype replacement in their nasopharyngeal (NP) ecological niche [5].

Active surveillance in the epidemiology of IPD after PCVs introduction in different epidemiological settings is crucial to fully understand the effect of these vaccines on the incidence of IPD, the distribution of serotypes and whether there is some degree of evasion of vaccine immunity due to the increase in NVT in the IPD. Although epidemiological studies have suggested that capsular type is the primary determinant of invasiveness, a non-capsular genomic background may also influence the ability of pneumococci to cause invasive disease [6].

© The Author(s), 2022. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

Therefore, to assess the impact of PCV introduction in the community, genotyping invasive isolates would provide additional and more complete information to classical studies based only on information regarding serotype distribution. This comprehensive approach would allow the identification of emerging serotypes/clones that are not included in current PCVs and knowledge of their invasive potential and resistance rates.

Analysis of the impact of PCVs on the incidence of IPD in Spain has been difficult because variations in vaccination policies, evaluation periods and surveillance systems could account for differences in reported data between geographical areas [7, 8]. Andalusia is the most populous region of Spain. Although PCV7 was introduced in 2001, vaccination coverage was partial because PCVs were only available in the private market until the introduction of PCV13 in the paediatric immunisation programme in December 2016. In this study, we aimed to characterise the epidemiology and circulating serotypes/genotypes in IPD and carriage in the paediatric population of selected provinces of Andalusia to gain an in-depth understanding of the impact of PCV13 introduction in Andalusia at the population level.

Patients and methods

Study setting and design

This was a population-based prospective active clinical and molecular surveillance study of laboratory-confirmed cases of IPD in children aged <14 years from January 2018 to December 2020. PCV13 uptake ranged from 97.2% in 2018 to 98.2% in 2020.

The study was conducted in 28 hospitals with paediatric departments located in three of the four most populated Andalusian provinces: Seville, Malaga and Granada. The total population <14 years under surveillance on 1 January 2018 was 684 694 (with 83 587, 114 181 and 486 926 persons aged <2, 2–4 and 5–13 years, respectively) according to the public data from the Statistical National Institute. IPD cases were defined as *S. pneumoniae* cultured from normal sterile fluid samples. The study protocol was approved by the local research ethics committee.

Data collection

Basic demographic data (age, sex and PCV vaccination status), clinical presentation (meningitis, pneumonia or primary bacteraemia), underlying comorbidities and outcomes were retrieved from standardised case report forms completed by the attending physicians who managed the cases. The collected information was added anonymously to the dataset specifically designed for this study.

Microbiological studies, serotyping and genotyping

All pneumococcal invasive isolates were cultured and identified in different participating health centres using standard laboratory protocols. All presumptive pneumococcal strains were sent to the Microbiology Department at the University Hospital Virgen del Rocío (HUVR) for antimicrobial susceptibility, serotyping and genotyping studies. Genomic DNA from *S. pneumoniae* isolates was fully sequenced, and serotypes were deduced bioinformatically based on the CPS locus [9]. In addition, some participating centres sent pneumococcal isolates to the Spanish National Reference Laboratory to complete serotype detection

by the Quellung reaction, as they had been routinely performed prior to the study period. The PCV13 group (VT) included the serotypes included in the PCV13 vaccine. All other serotypes were considered non-PCV13 serotypes (NVT).

Genotyping was performed using multilocus sequence typing (MLST) as reported elsewhere [10]. A clonal complex (CC) was defined as sequence type (ST) sharing five out of seven allelic variants.

The susceptibility to antimicrobials was tested using an *e* test. Interpretative minimum inhibitory concentration (MIC) breakpoints for antibiotics were defined according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing. Meningitis breakpoints for β -lactam antibiotics were used in meningitis cases and non-meningitis breakpoints in all other cases. Intermediate and fully resistant strains were combined into the non-susceptible category.

Pre-PCV13 historical controls

To compare the serotype distribution and antibiotic resistance rates between post-PCV13 and pre-PCV13, we used a historical collection of 75 isolates collected from paediatric patients with IPD in two major Andalusian tertiary paediatric hospitals (HUVR, Seville and Hospital Regional Universitario, Malaga) during the 2006–2009 period, partially included in a previous study [11].

Invasive disease potential

The invasive potential of the serotypes identified in IPD cases was estimated by the odds ratio (OR) in relation to their prevalence in IPD and NP carriage. Invasive OR was calculated by referring to all other serotypes as follows: $OR = (ad)/(bc)$, where *a* is the number of invasive A serotypes, *b* is the number of carriage A serotypes, *c* is the number of invasive non-A serotypes and *d* is the number of carriage non-A serotypes [12]. Serotypes with $OR > 1$ were considered to have an increased possibility of IPD. OR was calculated by adding a single carrier for the specific serotypes identified in IPD, but not in NP carriage.

A total of 76 colonising isolates were recovered from an NP carriage study conducted during the same period (2018–2020) in 394 healthy children aged 6 months to 5 years attending primary healthcare centres in Seville during a well-being visit. NP sampling was performed as previously reported, and written informed consent was obtained from the parent/guardian of the study children [13]. Pneumococci identification, serotyping and genotyping were performed at HUVR, as previously described.

Statistical analyses

Statistical analyses were performed using SPSS 26 (SPSS Inc., Chicago, IL, USA) and Epidat 4.2 (Conselleria de Sanidade, Xunta de Galicia, Spain). Incidence rates (IRs) were calculated as the number of cases per 100 000 inhabitants, using population data from the Spanish census population estimates for the corresponding year. Incidence rate ratios (IRRs), with their respective 95% confidence intervals (CIs), were calculated by comparing incidence in different years. Categorical variables were analysed using the χ^2 test and Fisher's exact test, as appropriate.

Results

Evolution of the IPD

A total of 63 IPD cases were identified during the study period. Thirty-one (49%) cases were diagnosed in children <2 years old, 21 (33%) cases in children 2–4 years old and 11 (17%) cases in children 5–13 years old. Pneumonia (with or without associated pleural empyema) was the most frequent clinical condition ($n=28$, 44%), followed by primary bacteraemia ($n=22$, 35%), meningitis ($n=12$, 19%) and primary peritonitis ($n=1$, 2%), respectively. A total of 40 (64%) and 4 (6%) patients had been immunised with PCV13 and PCV7, respectively. Nine (14%) children had pre-existing risk factors for IPD, including preterm, congenital heart disease and chronic pulmonary disease ($n=2$ each) and haematopoietic stem cell transplantation, congenital CMV infection and primary immunodeficiency ($n=1$ each). There were no deaths, and two (3%) patients with meningitis developed neurological sequelae.

The annual IR of IPD in children <14 years was 3.55/100 000 cases in 2018, increased non-significantly in 2019 to 4.20/100 000 cases (2019 vs. 2018; IRR: 1.18, 95% CI 0.69–2.04). IR in 2020 decreased significantly by 60% in 2020 to 1.69/100 000 cases, compared to 2019 (2020 vs. 2019; IRR: 0.40, 95% CI 0.20–0.81, $P=0.01$) (Table 1 and Fig. 1). The temporal evolution of annual IRs stratified by clinical conditions and age groups are shown in Table 1 and Figures 1 and 2.

Serotype distribution

Twenty serotypes were identified among the 60 isolates available for serotyping/genotyping during the post-PCV13 period. Five serotypes were VT, and the remaining 15 serotypes were NVT. VT cases included four cases of vaccine failure in children who had been fully immunised with PCV13: pleural empyema and/or bacteraemic pneumonia due to serotypes 3 ($n=3$) and 14 ($n=1$), respectively. Additionally, two patients with a single dose of PCV13, aged 2- and 3-month-old patients, developed IPD due to serotypes 18C and 19A. The proportion of VT significantly decreased in 2018–2020 compared to historical controls in the pre-PCV13 period (28% vs. 88%; $P=0.0001$).

NVT most frequently identified during the 2018–2020 period were according to their rank in IPD: 24F ($n=9$, 15%), 11A ($n=5$, 8.3%), 22F ($n=4$, 6.7%), 8, 12F and 33F ($n=3$, 5% each). The proportion of the first three NVT increased between the pre- and post-PCV13 periods ($P=0.01$ for serotype 24F and $P=0.02$ and 0.04 for serotypes 11A and 22F, respectively) (Fig. 1). On the other hand, the most prevalent VT in the contemporary period were: 3 ($n=6$, 10%), 1 ($n=4$, 6.7%) and 14 and 19A ($n=3$, 5% each), with a significant decrease in the proportion of serotype 1 isolates in relation to the historical period ($P=0.0002$). Finally, serotypes 5 and 7F, which circulated significantly in the pre-CNV13 period, were not identified during 2018–2020 ($P=0.03$, 2018–2020 vs. 2006–2009).

Table 1. IR of IPD and IRR in children <14 years globally and stratified by age groups and clinical conditions

Age group clinical condition	2018 incidence (per 100 000)	2019 incidence (per 100 000)	IRR 2019 vs. 2018 (95% CI)	2020 incidence (per 100 000)	IRR 2019 vs. 2018 (95% CI)
<14 years					
Global	3.55	4.20	1.18 (0.69–2.04)	1.69	0.40 (0.20–0.81)*
Pneumonia	1.63	1.80	1.11 (0.49–2.51)	0.77	0.43 (0.15–1.21)
Primary bacteraemia	1.33	1.50	1.13 (0.47–2.77)	0.46	0.41 (0.13–1.30)
Meningitis	0.59	0.90	1.52 (0.36–7.33)	0.30	0.34 (0.07–1.69)
<2 years					
Global	16.46	14.49	0.88 (0.39–1.96)	9.61	0.66 (0.26–1.71)
Pneumonia	6.33	5.27	0.83 (0.17–3.87)	4.12	0.78 (0.11–4.62)
Primary bacteraemia	7.60	5.27	0.69 (0.20–2.46)	4.12	0.78 (0.11–4.62)
Meningitis	2.53	3.95	1.56 (0.18–18.68)	1.37	0.35 (0.07–4.33)
2–4 years					
Global	5.97	8.38	1.39 (0.56–3.47)	1.59	0.19 (0.04–0.84)†
Pneumonia	2.23	3.05	1.36 (0.23–9.31)	1.59	0.52 (0.05–3.64)
Primary bacteraemia	2.23	3.81	1.70 (0.33–10.98)	0	–
Meningitis	1.49	1.52	1.02 (0.07–14.11)	0	–
5–13 years					
Global	0.65	1.31	2.03 (0.43–12.53)	0.44	0.35 (0.07–1.71)
Pneumonia	0.65	0.87	1.34 (0.23–9.17)	0	–
Primary bacteraemia	0	0.22	–	0	–
Meningitis	0	0.22	–	0.22	1.01 (0.01–79.62)

IRR, incidence rate ratio.

* P value = 0.01, † P value = 0.02.

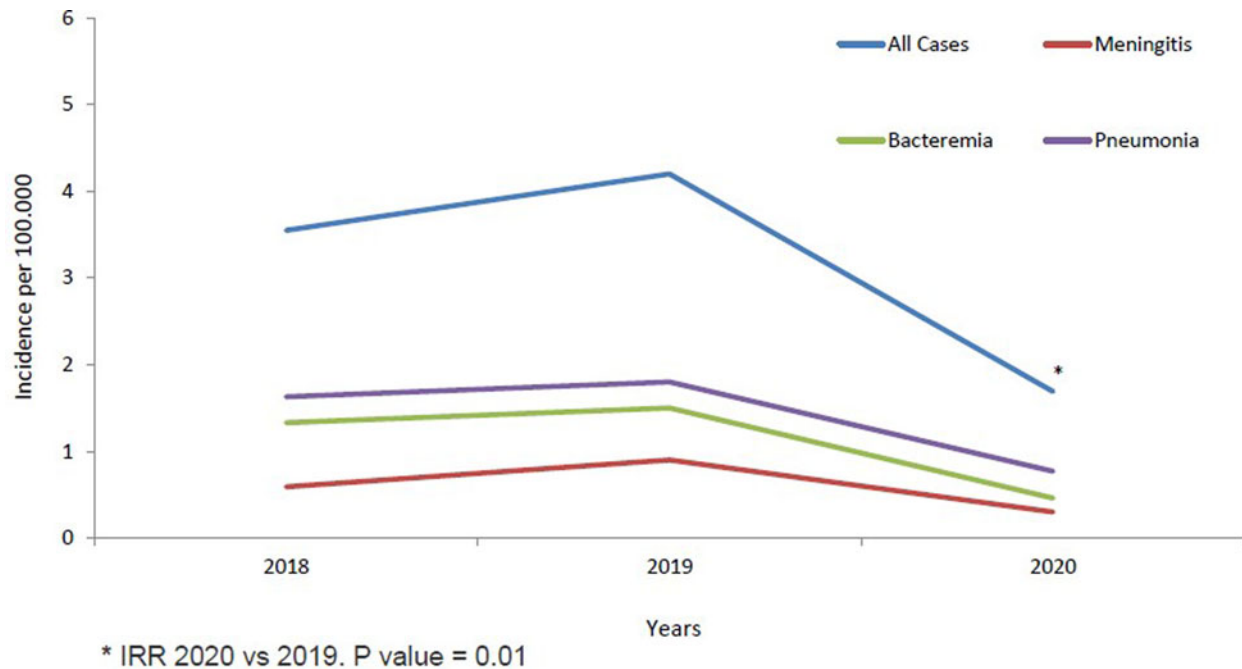


Fig. 1. IPD incidence in children <14 years from 2018 to 2020 stratified by clinical conditions.

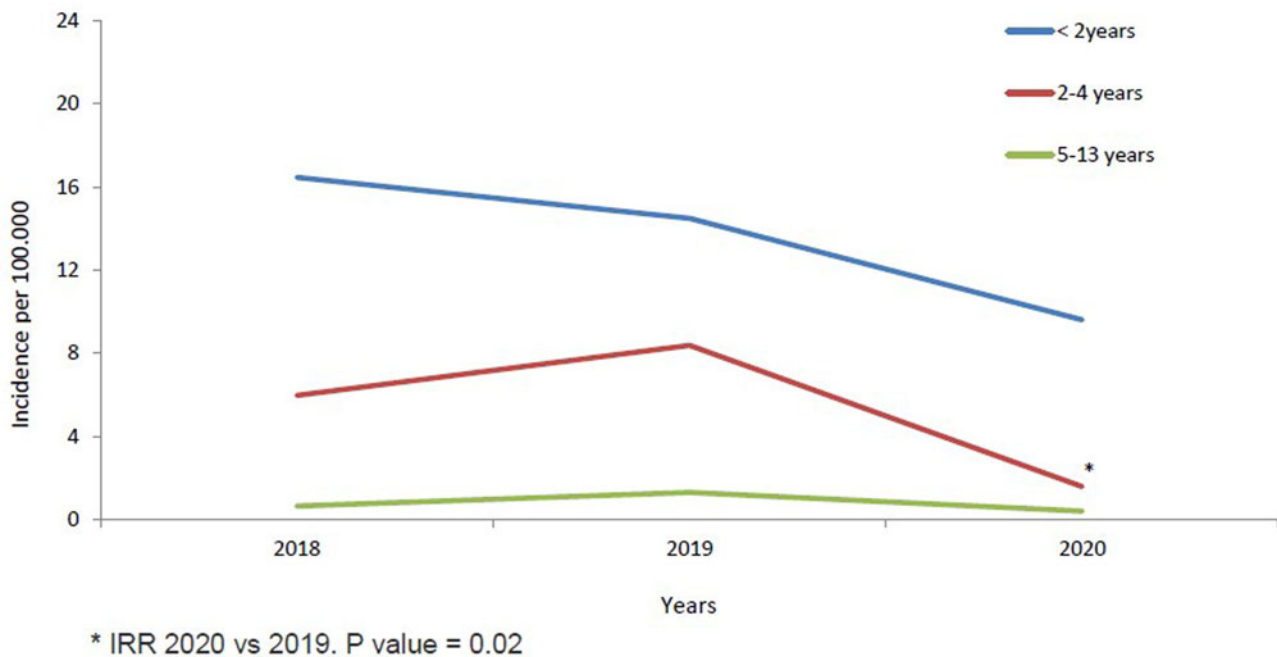


Fig. 2. IPD incidence in children <14 years from 2018 to 2020 stratified by age groups.

Genotype distribution

Twenty-six different STs were identified by molecular typing using MLST in the post-PCV13 period, being CC230 ($n=9$, 15%) and CC156 ($n=8$, 13.3%) the most common clones in the post-PCV13 (Table 2). CC230, expressed as serotype 24F, was associated with pneumonia, primary bacteraemia and meningitis and comprised of two clones, ST230 and the single-locus variant (SLV) ST4677. ST230 circulated during both study

periods, and ST4667 was only detected in the post-PCV13 period (Fig. 3).

CC156 was associated with serotypes 14 (ST156 and SLV ST2944) and 11A (double locus variant ST6521) in the 2018–2020 period and with serotypes 14 (ST156) and 9V (SLV ST838) in the 2006–2009 period. CC156 was associated with pneumonia and bacteraemia in serotype 14, and exclusively with primary bacteraemia in serotype 11A.

Table 2. CCs and STs with ≥ 2 isolates for the study periods 2018–2020 or 2006–2009 and their associated serotypes

CC/ST	Serotype	2018–2020	2006–2009
		No. of cases (%) <i>N</i> = 60	No. of cases (%) <i>N</i> = 75
CC230		9 (15)	2 (2.7)
ST230	24F	4 (6.7)	2 (2.7)
ST4677	24F	5 (8.3)	0
CC156		8 (13.3)	4 (5.3)
ST156	14	2 (3.3)	4 (5.3)
ST2944	14	1 (1.7)	0
ST6521	11A	5 (8.3)	0
ST180	3	5 (8.3)	4 (5.3)
ST306	1	4 (6.7)	15 (20)
ST433	22F	4 (6.7)	0
ST1110	8	3 (5)	0
ST2372		3 (5)	0
	19A	1 (1.7)	0
	23B	2 (3.3)	0
ST717	33F	3 (5)	0
ST260	3	1 (1.7)	3 (4)
ST393	38	2 (3.3)	1 (1.3)
ST66	9N	2 (3.3)	0
ST1551	10A	2 (3.3)	0
ST1078	15A	2 (3.3)	0
ST1262	15B/C	2 (3.3)	0
ST2690	35B	2 (3.3)	0
ST392	19A	1 (1.7)	2 (2.7)
ST1201	19A	1 (1.7)	2 (2.7)
ST228	1	0	9 (12)
CC289			9 (12)
ST289	5	0	3 (4)
ST1221	5	0	6 (8)
ST191	7F	0	9 (12)
ST1876	6C	0	2 (2.7)
ST276	19A	0	2 (2.7)

ST in bold were detected only during the 2018–2020 period.

A significant decline was observed in the proportion of serotype 1 ST306 that was the most common clone in the pre-PCV13 period (from 20% to 6.3%, $P = 0.04$). Other highly prevalent clones in this period, such as serotype 5 CC289, serotype 7F ST191 and serotype 1 ST228, disappeared completely in the post-PCV13 period.

Invasive potential

There were important differences in the ORs of the different serotypes identified in the IPD (Fig. 4). Serotype 24F had

significantly high invasive potential (OR 13.2, 95% 1.7–107.7), and serotype 3 was close to significance (OR 8.3, 95% CI 0.97–71.2). Serotypes 1, 14, 19A and 18C (VT) and serotypes 12F and 33F (NVT) had non-statistically significant highly invasive disease; however, ORs were calculated by adding a single carrier because these serotypes were not identified in NP carriage. The remaining serotypes had either non-statistically significant high invasive potential (serotypes 8, 22F, 9N, 15A and 38) or low invasive potential (serotypes 23B, 35B, 11A, 35F, 10A, 23A and 15B/C).

Antimicrobial non-susceptibility

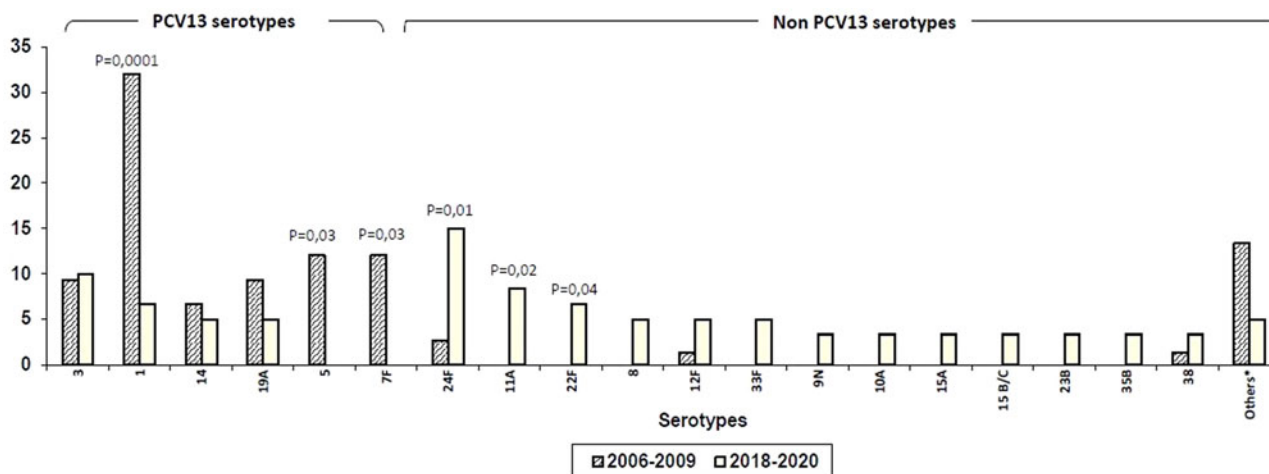
A total of 18 (28.6%) strains were penicillin non-susceptible (PNS) and five (7.9%) strains were penicillin-resistant (PR). Non-susceptibility and resistance rates to other β -lactam antibiotics commonly used in the treatment of IPD were as follows: ampicillin (12.7% and 12.7%), cefotaxime (7.9% and 0%) and ceftriaxone (3.2% and 0%). Fifteen (23.8%) strains were also erythromycin-resistant (ER).

Table 3 shows the distribution of the serotypes with reduced susceptibility to β -lactam antibiotics and erythromycin. Resistance to penicillin and/or ampicillin was detected mostly in strains belonging to CC156 (serotypes 11A and 14). Furthermore, there were two cases of meningitis due to serotype 24F that with meningitis breakpoint were also PR. This serotype was universally PNS (MIC 0.5–1 mg/l) and ER.

Discussion

The present study is the first report of the evolution of IPD among Andalusian children after the introduction of the PCV13 universal childhood vaccination, and it was conducted in parallel with an NP carriage study and clonal analysis of the invasive isolates. The annual IR of IPD in <14 years peaked in 2019 at 4.20/100 000 cases and decreased by 60% in 2020. The temporal evolution of the IR during the study period is difficult to interpret because of the disruptive effects of the COVID-19 pandemic. In this regard, in a comprehensive study with data from 26 countries and territories, the incidence of reported *S. pneumoniae* infections decreased by 68% at 4 weeks and 82% at 8 weeks following the week when significant changes in population movements were recorded [14]. Furthermore, marked reductions in bacteraemic pneumococcal pneumonia and non-pneumonia IPD were reported in Israel during the COVID-19 pandemic (IRR January 2020 through February 2021 vs. mean monthly rates of 0.19 and 0.42, respectively, in 2016–2019) [15]. The decline in pneumococcus-associated disease was temporally associated with the full suppression of respiratory syncytial virus (RSV), influenza virus and human metapneumovirus (hMPV) circulation. To better understand the impact of the COVID-19 pandemic, PCV13 and viral circulation on IPD epidemiology, it is advisable to continue monitoring the evolution of IPD in the coming years.

The proportion of IPD cases due to VT decreased by 68% between 2018–2020 and 2006–2009 and this was due to the eradication of serotypes 5 and 7F, which were widely represented in the pre-VCN13 period. In addition, there was a marked reduction (83%) in the circulation of serotype 1, the most prevalent serotype in the historical period [7]. Similar findings were reported in Madrid in a Heracles study, except for a more pronounced decline in serotype 19A IPD rates. This was the most frequently detected serotype in Madrid during the pre-PCV13 period, related to the

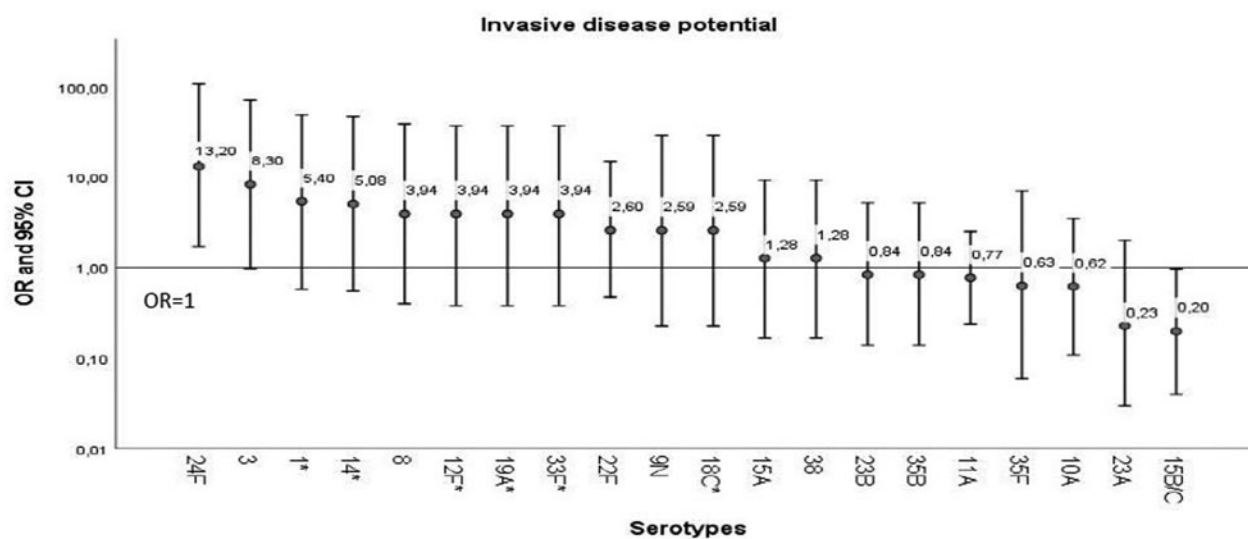


*Others included the following serotypes (n) in time periods:

2006-2009: 6A (2), 6C (3), 18C (1), 19F (1), 23F (1), 29 (1), 38 (1)

2018-2020: 18C (1), 23A (1), 35F (1)

Fig. 3. Distribution of serotypes causing IPD in the pre-PCV13 period (2006–2009) and post-PCV13 period (2018–2020).



* OR were calculated by adding one single carrier because these serotypes were not identified in NP carriage

Fig. 4. Invasive disease potential (ORs and 95% CIs) of the specific serotypes identified in IPD over the period 2018 until 2020. OR displayed on a logarithmic scale, error bars indicate 95% CIs.

expansion of the multi-resistant clone ST320, a phenomenon not detected in our geographical area. On the other hand, serotype 3, the most common VT identified in the study period, caused three of the four breakthrough IPD in children fully vaccinated with PCV13. Serotype 3 is currently one of the leading causes of breakthrough IPD in paediatric patients, which might likely reflect the reduced vaccine effectiveness of PCV13 against serotype 3 compared to other serotypes [16–18]. Serotype 3 declined by 32% between 2009 and 2019 in Spanish children. Nevertheless, it remains an important cause of paediatric IPD, as in certain European countries [19–21].

Sixteen NVT cases were identified, with 24A, 11A and 22F being the most prevalent. They showed differences in invasive potential, antibiotic resistance and coverage by the new generation of PCV vaccines licensed by the Food and Drug Administration for adults aged ≥ 18 years (15-valent PCV (PCV15) and 20-valent PCV (PCV20)). According to the distribution of serotypes in 2018–2020, the estimated coverage would have been 41.7% for PCV15 and 68.8% for PCV20, respectively.

The wide distribution of NVT in IPD is a predictable finding previously reported in a systematic review and meta-analysis based on epidemiological surveillance data for IPD in 20

Table 3. Distribution of serotypes associated with reduced susceptibility to β -lactams antibiotics and erythromycin

Serotype	Proportion (%) of strains with reduced susceptibility (% resistant) to				
	PEN	AMP	CTX	CRO	ERY
11A	100 (0)	100 (100)	40 (0)	0 (0)	0
14	100 (100)	100 (100)	100 (0)	66.7 (0)	0
15A	0	0	0	0	100 (100)
23A	0	0	0	0	100 (100)
23B	100 (0)	0	0	0	0
24F	100 (22.2 ^a)	0	0	0	100 (100)
33F	0	0	0	0	100 (100)
Total	28.6 (7.9)	12.7 (12.7)	7.9 (0)	3.2 (0)	23.8 (23.8)

PEN, penicillin; AMP, ampicillin; CTX, cefotaxime; CRO, ceftriaxone; ERY, erythromycin.

^aMeningitis breakpoint.

countries where PCV10/VCN13 had been introduced [22]. In this review, the prevalence of NVT (22F, 12F, 33F, 24F, 15C, 15 B, 23 B, 10A, 38 and 15A) did not differ significantly from those identified in the present study. These serotypes, as well as other emerging serotypes in recent years, such as serotypes 8, 9N and 11A, should be of special vigilance in the future [19, 20, 23–25].

Serotype 24F, the most frequent serotype in IPD cases during the study period, circulated as clonal types belonging to CC230 (ST230 and the single locus variant ST4677) and was not detected in the pre-PCV13 period. Data from Spanish and French national surveillance systems identified serotype 24F as the most frequent cause of paediatric IPD in the most recent years, accounting for 11.3% (2019) of IPD cases in Spanish children <17 years and 24.4% (2015–2017) in French children <2 years [19, 25]. Interestingly, this serotype was also the most frequent NVT in these age groups and countries prior to PCV13 introduction (2.9% (2009) in Spain and 5.7% (2006–2010) in France).

Serotype 24F is not covered by PCV15 and PCV20 and has a high invasive potential, as shown in the present study as well as in two recent and much larger studies after PCV13 implementation in France and Belgium [26, 27]. Moreover, this serotype is associated with PNS and ER. Based on these findings, it is likely that vaccine and antibiotic pressure had a contributory role in the observed increases in NVT in several European countries and Canada since 2015 [19, 20, 23, 25, 28].

Serotype 11A was the second most prevalent NVT in IPD in Andalusia and NP carriage. In contrast, it ranked 15th in Spain for IPD in children aged <5 years. This serotype, covered by PCV20 but not PCV15, had a low invasive potential and was associated with ST6521, an ampicillin/amoxicillin-resistant CC156 related clone not detected in the pre-PCV13 period [24]. This variant has arisen due to genomic recombination between fragments of two main clones (11A-ST62 and NT-ST344) and the successful global clone Spain^{9V}-ST156 associated with serotypes 9V and 14 [29]. ST6521 has also a greater biofilm production capacity, which may have contributed to the recent expansion of serotype 11A in acute exacerbations of chronic obstructive pulmonary disease and acute otitis media in Spain [30, 31].

The present study has several limitations. First, the study included IPD cases from three Andalusian provinces and therefore may not be fully representative of the entire region. Second, the limited study period did not allow us to analyse

longer-term changes in IPD. Third, the evolution of IPD IRs in 2020 is difficult to interpret because of the potential impact of the COVID-19 pandemic on IPD epidemiology. Fourth, historical controls were collected from two hospitals that represented approximately 40% of IPD cases in the PCV13 period. Finally, the limited number of IPD and NP carriage isolates could undermine the ability to detect a statistically significant high invasive potential.

In conclusion, the proportion of IPD cases due to PCV13 serotypes declined significantly in 2018–2020 after the initiation of the PCV13 universal vaccination programme in late 2016. However, certain NVT, such as serotypes 24 and 11A, warrant future monitoring in IPD due to invasive potential and/or antibiotic resistance rates.

Acknowledgements. We are grateful to Raquel Merino Ingelmo, Elena Vázquez and Inmaculada López Hernández for providing the pneumococcal isolates for this study. The authors thank Manuel José Rodríguez Ortega for reviewing the manuscript and providing technical assistance with bioinformatics analysis.

Author contributions. B. F.: methodology, project administration, supervising. P. O. P.: resources, methodology, writing review. B. C. G.: resources, writing review. D. L. M.: resources. J. L. S. P.: resources. Y. G. J.: resources. M. J. M. V.: resources. N. C. A.: resources. V. G. G.: methodology, microbiological analysis. F. J. M.: genomic analysis. J. A. C. V.: bioinformatic analysis. M. J. T. S.: microbiological analysis. A. M. C.: resources. D. M. P.: conceptualisation, methodology, writing review. I. O.: conceptualisation, methodology, analysis, funding acquisition, writing original draft and editing.

Financial support. This work was supported by Pfizer (grant 53233485).

Conflict of interest. B. C. G. reports travel grants from Pfizer. A. M. C. reports a travel grant from the GSK. J. L. S. P. reports travel grants and personal fees from Pfizer. I. O. reports congress registration fees and a grant from Pfizer, while others declare no potential conflicts of interest.

Ethical standards. Informed consent was obtained from a parent or guardian for participants included in the study. The study complies with the principles of the Declaration of Helsinki and the legal structure with respect to international human rights and biomedicine and protection of personal data laws. The Ethic Committee of Hospital Universitario Virgen del Rocío approved the study (CI 2017/76).

Data availability statement. The data that support the findings of this study are openly available in Open Science Framework at <https://osf.io/profile/>, reference number DOI:10.17605/OSF.IO/T4FRH.

References

1. Subramanian K, Henriques-Normark B and Normark S (2019) Emerging concepts in the pathogenesis of the *Streptococcus pneumoniae*: from nasopharyngeal colonizer to intracellular pathogen. *Cellular Microbiology* **21**, e13077.
2. Wahl B *et al.* (2018) Burden of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000–15. *Lancet Global Health* **6**, e744–e757.
3. Pilishvili T *et al.* (2010) Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *Journal Infectious Diseases* **201**, 32–41.
4. Waight PA *et al.* (2015) Effect of the 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease in England and Wales 4 years after its introduction: an observational cohort study. *Lancet Infectious Diseases* **15**, 535–543.
5. Hanquet G *et al.* (2022) Serotype replacement after introduction of 10-valent and 13-valent pneumococcal conjugate vaccines in 10 countries, Europe. *Emerging Infectious Diseases* **28**, 137–138.
6. Browall S *et al.* (2014) Intracolon variations among *Streptococcus pneumoniae* isolates influence the likelihood of invasive disease in children. *Journal Infectious Diseases* **209**, 377–388.
7. Picazo JJ *et al.* (2019) Impact of 13-valent pneumococcal conjugate vaccination on invasive pneumococcal disease in children under 15 years old in Madrid, Spain, 2007 to 2016: the HERACLES clinical surveillance study. *Vaccine* **37**, 2200–2207.
8. Redin A *et al.* (2021) Serotypes and clonal composition of *Streptococcus pneumoniae* isolates causing IPD in children and adults in Catalonia before 2013 to 2015 and after 2017 to 2019 systematic introduction of PCV13. *Microbiology Spectrum* **22**, e0115021.
9. Epping L *et al.* (2018) SeroBA: rapid high-throughput serotyping of *Streptococcus pneumoniae* from whole genome sequence data. *Microbial Genomics* **4**, e000186.
10. Enright MC and Spratt BG (1998) A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. *Microbiology (Reading, England)* **144**, 3049–3060.
11. Obando I *et al.* (2008) Pediatric parapneumonic empyema, Spain. *Emerging Infectious Diseases* **14**, 1390–1397.
12. Brueggemann AB *et al.* (2003) Clonal relationships between invasive and carriage and serotype- and clone-specific differences in invasive disease potential. *Journal Infectious Diseases* **187**, 1424–1432.
13. Cilveti R *et al.* (2017) Epidemiology of otitis media with spontaneous perforation of the tympanic membrane in young children and association with bacterial nasopharyngeal carriage, recurrences and pneumococcal vaccination in Catalonia, Spain – the prospective HERMES study. *PLoS One* **12**, e0170316.
14. Brueggemann AB *et al.* (2021) Changes in the incidence of invasive disease due to *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* during the COVID-19 pandemic in 26 countries and territories in the Invasive Respiratory Infection Surveillance Initiative: a prospective analysis of surveillance data. *Lancet Digital Health* **3**, e360–e370.
15. Danino D *et al.* (2022) Decline in pneumococcal disease in young children during the COVID-19 pandemic in Israel associated with suppression of seasonal respiratory viruses, despite persistent pneumococcal carriage: a prospective cohort study. *Clinical Infectious Diseases* **75**, e1154–e1164.
16. Andrews NJ *et al.* (2014) Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. *Lancet Infectious Diseases* **14**, 839–846.
17. Sings HL *et al.* (2019) Effectiveness of 13-valent pneumococcal conjugate vaccine against invasive disease caused by serotype 3 in children: a systematic review and meta-analysis of observational studies. *Clinical Infectious Diseases* **68**, 2135–2143.
18. Ricketson LJ *et al.* (2022) Vaccine effectiveness of the 7-valent and 13-valent pneumococcal conjugate vaccines in Canada: an IMPACT study. *Vaccine* **40**, 2733–2740.
19. De Miguel S *et al.* (2021) Nationwide trends of invasive pneumococcal disease in Spain from 2009 through 2019 in children and adults during the pneumococcal conjugate vaccine era. *Clinical Infectious Diseases* **73**, e3778–e3787.
20. Ladhani SN *et al.* (2018) Rapid increase in non-vaccine serotypes causing invasive pneumococcal disease in England and Wales, 2000–17: a prospective national observational cohort study. *Lancet Infectious Diseases* **18**, 441–451.
21. Silva-Costa C *et al.* (2021) Pediatric invasive pneumococcal disease three years after PCV13 introduction in the national immunization plan – the continued importance of serotype 3. *Microorganisms* **9**, 1428.
22. Balsells E *et al.* (2017) Serotype distribution of *Streptococcus pneumoniae* causing invasive disease in children in the post-PCV era: a systematic review and meta-analysis. *PLoS One* **12**, e0177113.
23. Dion SB *et al.* (2021) Invasive pneumococcal disease in Canada 2010–2017: the role of current and next-generation higher-valent pneumococcal conjugate vaccines. *Vaccine* **39**, 3007–3017.
24. Aguinagalde L *et al.* (2015) Emergence of amoxicillin-resistant variants of Spain^{9V}-ST156 pneumococci expressing serotype 11A correlates with their ability to evade the host immune response. *PLoS One* **10**, e0137565.
25. Ouldali N *et al.* (2021) Invasive pneumococcal disease incidence in children and adults in France during the pneumococcal conjugate vaccine era: an interrupted time-series analysis of data from a 17-year national prospective surveillance study. *Lancet Infectious Diseases* **21**, 137–147.
26. Cohen R *et al.* (2021) Invasive disease potential of pneumococcal serotypes in children after PCV13 implementation. *Clinical Infectious Diseases* **72**, 1453–1456.
27. Desmet S *et al.* (2021) In-depth analysis of pneumococcal serotypes in Belgian children (2015–2018): diversity, invasive disease potential, and antimicrobial susceptibility in carriage and disease. *Vaccine* **39**, 372–379.
28. Weinberger R *et al.* (2018) Invasive pneumococcal disease in children under 16 years of age: incomplete rebound in incidence after the maximum effect of PCV13 in 2012/13 in Germany. *Vaccine* **36**, 572–577.
29. González-Díaz A *et al.* (2020) Two multi-fragment recombination events resulted in the β -lactam-resistant serotype 11A-ST6521 related to Spain^{9V}-ST156 pneumococcal clone spreading in south-western Europe, 2008 to 2016. *Euro Surveillance* **25**, 1900457.
30. Shoji H *et al.* (2018) Overview of pneumococcal serotypes and genotypes causing diseases in patients with chronic obstructive pulmonary disease in a Spanish hospital between 2013 and 2016. *Infection and Drug Resistance* **11**, 1387–1400.
31. Morales M *et al.* (2018) Changes in the serotype distribution of *Streptococcus pneumoniae* causing otitis media after PCV13 introduction in Spain. *PLoS One* **13**, e0209048.