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Original Article

Resilience and emergence of pneumococcal serotypes and lineages in adults post-PCV13 in Spain: A multicentre study

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ABSTRACT

Background: *Streptococcus pneumoniae* causes invasive pneumococcal disease (IPD) in adults. The introduction of pneumococcal conjugate vaccines (PCVs) has reduced vaccine serotypes but has also led to the rise of non-vaccine serotypes. The aim of this study was to analyse pneumococcal lineages and their association with recent changes in IPD among adults in Spain.
Methods: Data from adult IPD cases (≥18 years) were collected from six Spanish hospitals in 2019–2021. Strains were serotyped, tested for antibiotic susceptibility and subjected to whole genome sequencing (WGS). Findings were compared with data from previous periods (2008–2016).
Results: A total of 655 IPD episodes were examined. Pneumonia was the main focus (515/655), and 366 episodes occurred in adults over 64 years. Although IPD incidence decreased during COVID-19 pandemic, the burden of disease caused by PCV13 serotypes was significant. Notably, serotype 3 persisted (GPSC12-ST180 and GPSC83-ST260), and a new serotype 4 lineage emerged (GPSC162-ST13022). Among non-PCV13 serotypes, serotype 8 expanded (GPSC3-ST53) and a new serotype 12F lineage emerged (GPSC55-ST8060). Most serotypes presented a dominant Global Pneumococcal Sequencing Cluster (GPSC) like GPSC16-ST67 of 9N or GPSC19-ST433 of 22F. Nevertheless, some GPSCs were associated with several serotypes, the most numerous were GPSC3 (serotypes 8, 11A, and 33F) and GPSC6 (serotypes 11A and 14). The overall penicillin non-susceptibility rate was 17.0 %, 14.6 % resistance for meningitis and 1.6 % for pneumonia (15.1 % susceptible at increased exposure [SIE]). Serotypes 11A and 14 (GPSC6-ST156/6521) and 19A (GPSC1-ST320) had penicillin MICs above 1 mg/L. Acquired resistance genes associated with macrolide and/or tetracycline resistance were present in 19.4 % of isolates, particularly among serotypes 6C (GPSC47-ST386/4310) and 19A (GPSC1-ST320).
Conclusions: The burden of PCV13 serotypes in adult IPD remains significant, and serotype 3 is the primary contributor. However, the rise of stable lineages associated with non-PCV13 serotypes, particularly 8, 9N, and 22F highlights a shifting epidemiology. The persistence of multidrug-resistant lineages, such as GPSC6-ST156 and GPSC1-ST320, emphasizes the need for continued surveillance. Vaccination of high-risk adults with current and broader coverage PCVs would help to control the burden of pneumonia and IPD among adults.

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Introduction

Streptococcus pneumoniae is a life-threatening pathogen that is commonly found in the nasopharynx of children under 5 years old, who are considered the main pneumococcal carriers [1]. Among adults, invasive pneumococcal diseases (IPD) are frequently associated with the elderly as well as with immunocompromised individuals or those with comorbidities such as diabetes, chronic renal, pulmonary or heart diseases among others. In these patients, IPD could be more severe, including bacteraemic pneumonia or meningitis [2]. Pneumococcal vaccines are based on the development of antibodies against the polysaccharide capsule, which is the main pneumococcal virulence factor [3]. Although more than 105 different serotypes have been described, current vaccines target only a few of them.

The introduction of pneumococcal conjugate vaccines (PCVs) into paediatric vaccination schedules has led to a decrease in vaccine serotypes among adults (herd protection), as well as an increase in non-vaccine serotypes [4,5]. In Spain, PCV7 was introduced in 2001, followed by the introduction of PCV13 in 2011 and the universal children vaccination was stablished in 2016. Thereafter, the overall benefits of paediatric vaccination have not been sufficient to control the burden of adult IPD especially in older people as incidence continue to increase [5]. The recent introduction of broader vaccines, such as PCV15, PCV20, and PCV21, and their increasing use in adults could change the epidemiology of pneumococcal serotypes in the near future.

Pneumococcal recombination allows bacteria to switch capsules and evade the effects of vaccines [6,7]. Thus, highly invasive lineages can evade the effect of vaccines by expressing non-vaccine serotypes, as occurred with the spread of the serotype 19A-CC320 lineage after the PCV7 introduction [8], or with the serotype 11A variant of Spain^{9V}-ST156 (GPSC6) detected in Europe [9]. Therefore, it is important to understand the impact of vaccination on the lineage composition of pneumococcal serotypes to explain their dynamics. For instance, highly homogenous genetic serotypes that are targeted by PCVs, such as 1, 6B, or 7F, rapidly disappeared as a cause of IPD in both children and adults [10,11]. However, the decrease in disease caused by more diverse serotypes, such as 14, 19A, or 19F, has been slow [12,13]. Additionally, other serotypes, such as serotype 3, have persisted over the years with a limited herd effect, which is likely due to the unique characteristics of this serotype [11,14,15]. In our area, rates of this serotype remained stable from 1.58/100,000 inhabitants (1994–2001) to 1.61/100,000 inhabitants (2006–2010) and to 1.28/100,000 inhabitants (2016–2020) [16]. However, a clonal shift was observed from the GPSC83-CC260 to the globally disseminated GPSC12-CC180 [16].

In a previous multicenter study in Spain, we analysed the impact of the paediatric introduction of PCV13 on the epidemiology of adult IPD. We demonstrated a continuous decrease in IPD due to PCV13 serotypes throughout the periods (from 7.7 in 2008-2009 to 3.5 in 2012-2013 to 2.3 per 100,000 inhabitants in 2015-2016; $p < 0.01$). This decline was offset by a progressive increase in IPD due to non-PCV13 serotypes, especially in the late-PCV13 period (2015–2016; IRR 1.31; 95 % CI 1.14–1.51), resulting in stabilization of the overall incidence [11]. These trends led to an early decrease in adult IPD cases (2012–2013), followed by a plateau (2015–2016) [5,11]. The present study continues this monitoring with an analysis of the epidemiology of adult IPD in Spain, including the universal-PCV13 period (2019–2021), which reached vaccination rates over 97 % among children (pestadistico.inteligenciadegestion.sanidad.gob.es/publicoSNS/I/sivamin/informe-de-evolucion-de-coberturas-de-vacunacion-por-vacuna). Furthermore, in-depth analysis was done using whole genome sequencing (WGS) to identify persistent lineages associated with PCV13 serotypes and those related to non-PCV13 serotypes.

Methods

Study design

A laboratory-based multicentre study was conducted by collecting data on IPD in adults (≥18 years old) from six Spanish hospitals, whose details have been published previously [5,11]. These hospitals were located in three different regions of Spain: Catalonia, in the northeast of the country (Hospital Universitari de Bellvitge [HUB], Hospital Germans Trias i Pujol [HUGTiP], Consorci Corporació Sanitària Parc Taulí [CCSPT] and Hospital Vall d'Hebron [VH]); the Basque Country, in northern Spain (Hospital Universitario de Donostia [HUD]); and Madrid, in the central region of Spain (Hospital General Universitario Gregorio Marañón [HGUGM]) [5,11]. For comparisons, four periods were analysed: 2008–2009 (pre-PCV13), 2012–2013 (early-PCV13), 2015–2016 (late-PCV13), and 2019–2021 (universal-PCV13) (Supplementary Fig. S1). IPD was defined by the isolation of *S. pneumoniae* from a normally sterile site in a patient with signs and symptoms of infection. Serotypes were grouped according to the serotypes included in the PCVs to facilitate the analyses (Supplementary Fig. S2).

Serotyping, antibiotic susceptibility testing, and statistical analysis

Pneumococcal isolates were identified by standard microbiological procedures [11] and serotyped at the Spanish Pneumococcal Reference Laboratory [17]. Antimicrobial susceptibility was assessed by microdilution according to guidelines and criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org/clinical_breakpoints/). Incidence was calculated as the number of episodes per 100,000 inhabitants based on the population of the three Spanish regions involved, and serotypes were classified as indicated in Supplementary Fig. S2. A *Chi*-square test was used to assess the association between the categorical variables, with statistical significance set at $\alpha < 0.05$ (two-tailed). The test was performed using the chisq.test function in R (version 4.3.2).

Whole genome sequencing

A QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used to extract pneumococcal genomic DNA, which was then quantified using dsDNA Qubit quantification assays (Promega, Wisconsin, USA). Genomic libraries were prepared using a Nextera XT kit and paired-end sequencing (2 ×300 bp) on a MiSeq platform (Illumina, San Diego, USA). Reads were deposited in the European Nucleotide Archive (ENA) (https://www.ebi.ac.uk/ena/browser/home), and metadata are outlined in Supplementary Table S1. The molecular characterization and analysis results are shown in Supplementary Table S2.

Ethics statement

This work was conducted in accordance with the Declaration of Helsinki of the World Medical Association and approved by the Clinical Research Ethics Committee of Hospital Universitari de Bellvitge (PR153/18 and PR283/21). The requirement for written informed consent was waived as this study was retrospective and

Table 1
Serotype distribution of pneumococci causing IPD in adults in the universal-PCV13 period (2019–2021) by focus of infection.

		No of Episodes	Incidence (a)	PCV13 (b)		Additional PCV20 (b)		PCV21-V116 (b)		Other serotypes (b)	
				Frequency (%)	Most frequent serotypes (%) (c)	Frequency (%)	Most frequent serotypes (%) (c)	Frequency (%)	Most frequent serotypes (%) (c)	Frequency (%)	Most frequent serotypes (%) (c)
Manifestation of infection	Pneumonia	515 (78.6 %)	4.3	23.3	**3** (9.5); **14** (3.5); **19A** (3.3)	40.2	**8** (21.9); **22F*** (5.6); **12F** (4.1); **33F*** (2.3)	73.6	**8** (21.9); **3** (9.5); **22F*** (5.6); **12F** (4.1); **9N** (3.9); **19A** (3.3)	11.5	**6C** (3.5)
	Meningitis	55 (8.4 %)	0.5	21.8	**3, 23F** (5.5); **19A, 19F** (3.6)	25.5	**8** (12.7); **11A**, **22F*** (3.6)	61.8	**8** (12.7)**; 9N** (10.9); **3, 23B** (5.5); **11A**, **19A**, **22F**, **31** (3.6)	16.4	**6C, 35F** (5.5)
	Bacteraemia without focus	39 (6.0 %)	0.3	12.8	**3** (5.1)	33.3	**8** (12.8); **12F**, **22F*** (7.7); **33F*** (2.6)	74.4	**8** (12.8); **12F**, **15A**, **22F, 23B** (7.7); **3, 16F**, **23A**, **31** (5.1)	7.7	-
	Other	24 (3.6 %)	0.2	8.3	**3** (8.3)	45.8	**8** (20.8); **10A, 22F*** (8.3)**; 11A, 15B** (4.2)	75.0	**8** (20.8); **3, 10A, 16F, 22F** (8.3); **9N**, **11A**, **15B**, **17F**, **23B** (4.2)	12.5	**6C, 13, 38** (4.2)
	Peritonitis	22 (3.4 %)	0.2	18.2	**3** (13.6); **14** (4.5)	18.2	**11A, 12F, 15B, 33F*** (4.5)	50.0	**3** (13.6); **11A**, **12F**, **15B**, **16F, 20, 23A, 23B, 33F** (4.5)	36.4	**6C** (18.2); **35F** (9.1)**; 21**, **38** (4.5)
Age group	Overall	655 (100 %)	5.5	21.8	**3** (9.0); **14, 19A** (3.1)	38.2	**8** (19.8); **22F*** (5.5); **12F** (4.0); **11A** (3.1); **33F*** (2.1)	72.1	**8** (19.8); **3** (9.0); **22F** (5.5); **9N** (4.1); **12F** (4.0); **11A**, **19A** (3.1)	12.5	**6C** (4.1)
	18–64	289 (44.1 %)	2.4	23.5	**3** (8.7); **4** (3.5)	45.3	**8** (27.7); **12F** (5.5); **22F*** (5.2); **33F*** (1.7)	69.9	**8** (27.7); **3** (8.7); **12F** (5.5); **22F** (5.2); **9N** (3.5)	10.4	**6C** (3.8)
	> 64	366 (55.9 %)	3.1	20.5	**3** (9.3); **14, 19A** (3.8)	32.5	**8** (13.7); **22F*** (5.7); **11A** (3.8); **33F*** (2.5)	73.5	**8** (13.7); **3** (9.3); **22F** (5.7); **9N** (4.6); **11A**, **19A** (3.8); **23B** (3.6); **15A** (3.3); **16F, 23A** (3.0)	14.2	**6C** (4.4)

Abreviations: PCV13, 13-valent pneumococcal conjugate vaccine; PCV20, 20-valent pneumococcal conjugate vaccine; PCV21-V116, 21-valent pneumococcal conjugate vaccine
The numbers in boldface are serotypes
(a) Incidence (number of episodes/100,000 inhabitants)
(b) PCV13, serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F; Addiotional PCV20, serotypes 8, 10A, 11A, 12F, 15B, 22F* and 33F* (*additional serotypes included also in the PCV15); PCV21-V116, serotypes 3, 6A, 7F, 8, 9N, 10A, 11A, 12F, 15A, 15B, 16F, 17F, 19A, 20, 22F, 23A, 23B, 24F, 31, 33F and 35B; Other serotypes, serotypes not included in PCV described above.
(c) Serotypes with a frequency higher than 3.0 % are showed, except for serotype 22F and 33F (PCV15)

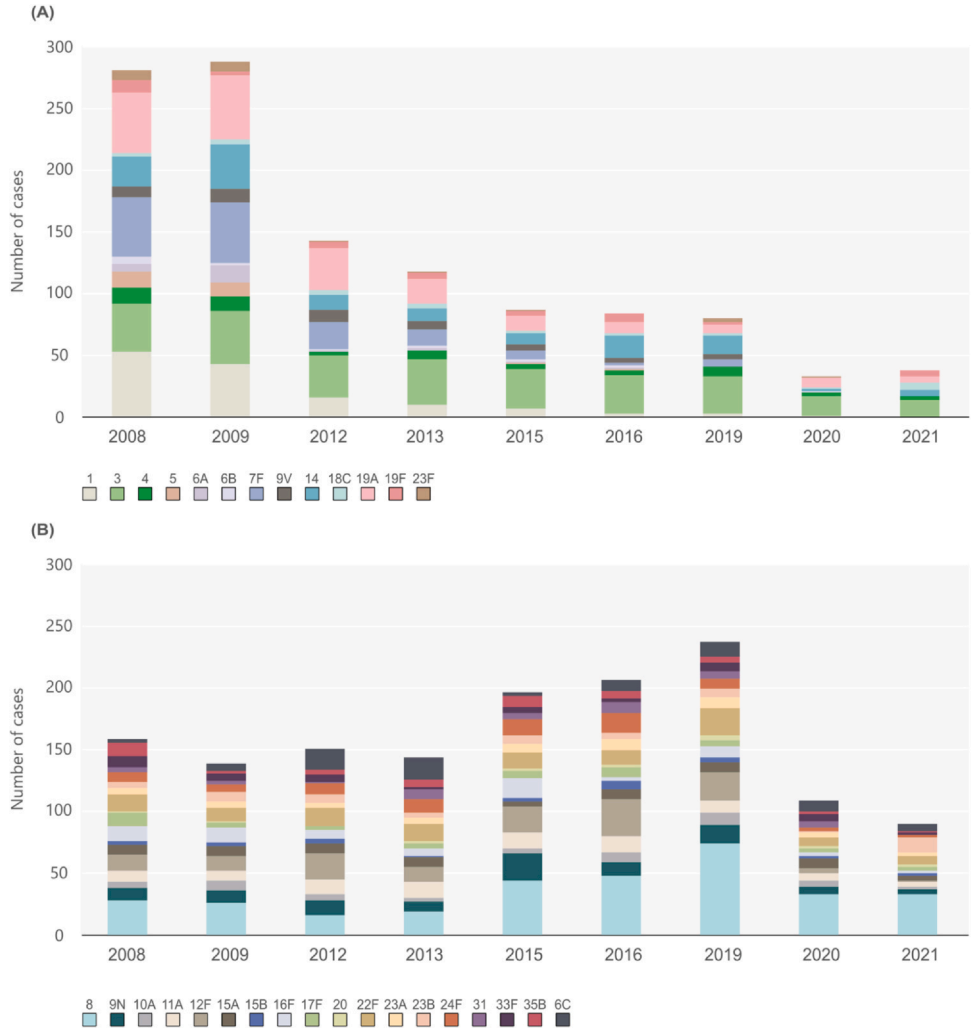

Fig. 1. Periodic distribution of serotypes that cause invasive pneumococcal disease. (A) Serotypes included in the PCV13 vaccine and (B) non-PCV13 serotypes.

observational. All the data were anonymized, and patient confidentiality was always protected in accordance with current laws in Spain (LOPD 3/2018 and RD 1720/2007).

Results

IPD decreased in the COVID-19 pandemic period with marked PCV13 herd protection and maintenance of serotype 3

A total of 655 IPD episodes were recorded between 2019 and 2021, and 55.9 % of them occurred in adults over 64 years old. Pneumonia was the main focus (78.6 %), followed by meningitis (8.4 %) (Table 1). Overall, the rate of disease due to PCV13 serotypes was still high (21.8 %) in all clinical presentations, such as pneumonia (23.3 %) and meningitis (21.8 %), and serotype 3 was the most frequent (9.0 %) (Table 1). Serotype 4 was stable in this period, especially among young adults. Conversely, serotype 8 was the most frequent in all manifestations except peritonitis and accounted for nearly a fifth of all IPD episodes (19.8 %) (Table 1).

Serotype 22F accounted for more than 5 % of most IPD cases, and serotype 9N was present in 10.9 % of meningitis episodes. Notably, there was a wide dispersion of serotypes among IPD cases with an abdominal focus (Table 1). According to age group, serotype 8 was the leading cause of disease in all age groups examined, although it was more frequent among young adults (18–64 years old) (27.7 %) than older adults (> 65 years old) (13.7 %; $p < 0.001$) (Table 1), as was serotype 12F (5.5 % vs. 2.7 %). Serotypes 3, 9N, 11A, 14, 19A, 22F, and 33F were more frequent among the elderly, although these differences did not reach the statistically significance (Supplementary Fig. S3).

Compared to previous results, the incidence of IPD decreased from 12.2 in 2009 to 7.5 in 2013 ($p < 0.001$) [5,11] followed by an increase to 9.2 in 2019 ($p = 0.009$) (Supplementary Fig. S4). During the SARS-CoV-2 pandemic, a decrease occurred from 4.0 in 2020 to 3.2 in 2021 ($p = 0.09$). This dynamic was most marked in the elderly group with an incidence of 21.2 in 2019, which decreased to 8.9 in 2020 ($p < 0.001$) and to 6.4 in 2021 ($p = 0.146$). In young adults, the IPD incidence decreased from 4.9 in 2019 to 2.2 in 2020 ($p < 0.001$) and 1.6 ($p = 0.409$) in 2021.

The distribution of serotypes by period is shown in Fig. 1. Since the introduction of PCV13 to children's vaccination schedules in 2010, some serotypes such as 1, 7F, and 23F drastically decreased. The prevalence of these three serotypes changed from 10.0 % (95/949), 10.2 % (97/949), and 1.7 % (16/949) (2008–2009) to 0.6 % (4/655), 0.9 % (6/655), and 0.6 % (4/655), respectively (2019–2021) (Fig. 1A). However, other PCV13 serotypes like serotype 3, 19A, and 19F persisted over time with frequencies of 8.9 % (85/949), 10.6 % (101/949), and 1.4 % (13/949) in 2008–2009 and 9.2 % (60/655), 3.1 %

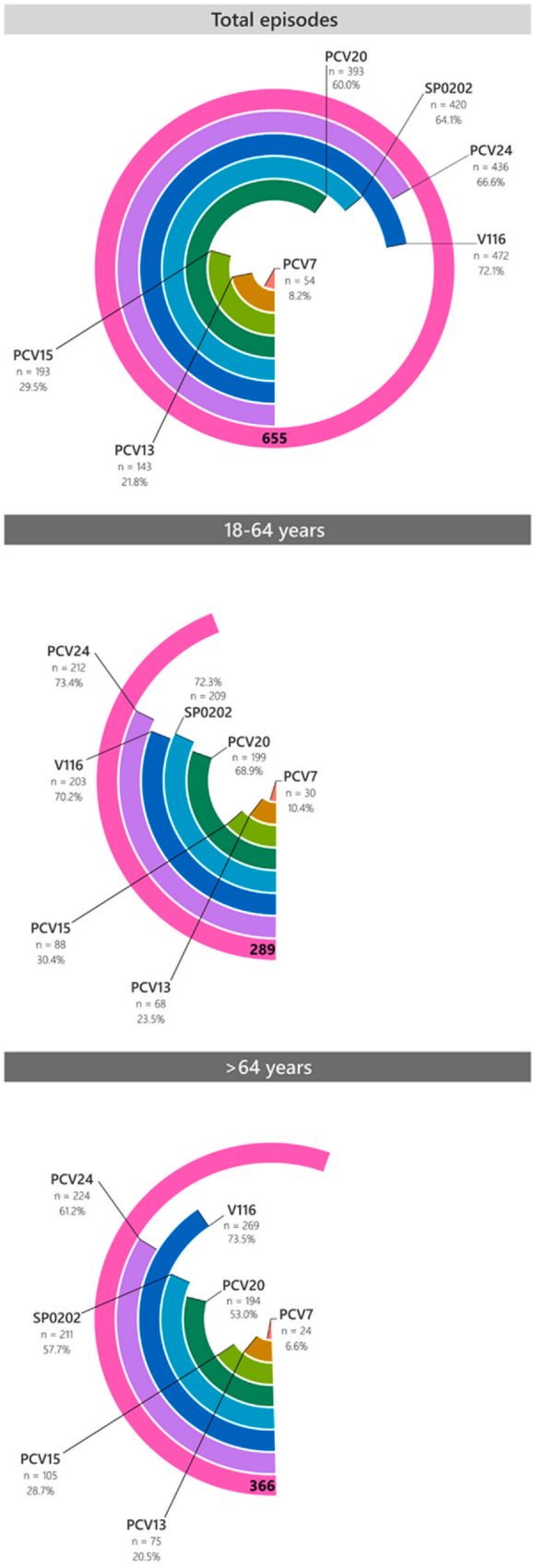

Fig. 2. Coverage of different pneumococcal vaccines during the study period. The total episodes are represented in pink. Each PCV shows the number of IPD cases caused by vaccine serotypes and the percentage of coverage. Red: PCV7; orange: PCV13; green: PCV15; dark green: PCV20; blue: PCV21-SP0202 (Sanofi); dark blue: PCV21-V116 (MSD); purple: PCV24.

(20/655), and 1.1 % (7/655) in 2019–2021, respectively (Fig. 1A). Non-PCV13 serotypes like 8, 9N, 10A, and 22F increased from 5.7 % (54/949), 2.1 % (20/949), 1.4 % (13/949), and 2.6 % (25/949) (2008–2009) to 21.4 % (140/655), 3.8 % (25/655), 2.6 % (17/655), and 5.5 % (36/655) (2019–2021), respectively (Fig. 1B).

New PCVs have recently been introduced or are under investigation for the prevention of adult pneumococcal disease, and their potential coverage is shown in Fig. 2. Among the currently licensed vaccines, PCV21-V116 had the highest coverage (72.1 %), while PCV20 (60.0 %) and PCV15 (29.5 %) had lower rates. PCVs that are being researched in different clinical trials, such as PCV21-SP0202 and PCV24, had slightly higher coverage than PCV20. For example, the percentage of invasive pneumonia covered by PCV20 and PCV21-V116 was 63.5 % and 73.6 %, respectively (Table 1).

The spread and emergence of lineages explain the serotype dynamics in the post-pandemic period

We analysed the genetic lineages by serotype through WGS (Fig. 3). Most serotypes are composed of multiple lineages or global pneumococcal sequencing clusters (GPSCs), although a dominant lineage is present. For instance, four GPSCs were identified among serotype 8 isolates, and GPSC3 was dominant (78.5 %). Likewise, the GPSC19 was dominant (86.7 %) among serotype 22F isolates. However, serotypes 9N, 24F, and 33F had unique GPSCs.

The analysis results of GPSCs that encompass more than one serotype are shown in Supplementary Fig. S5. The most numerous was GPSC3, which was shared by serotypes 8, 11A, and 33F. GPSC6 equally represented by serotypes 11A (ST6521) and 14 (ST156); ST6521 was a *double locus variant* (DLV) of ST156 (Supplementary Table S1). This recombination with the acquisition of the capsular operon of 11A allow this lineage to evade the vaccines, as formerly it was 9 V and 14. Another example is GPSC18, which includes ST1201 and was shared by serotypes 7F and 19A. Several GPSCs became more common in this period, such as GPSC3, which explained the increase of serotype 8. A new lineage, GPSC162-ST13022, was detected in the case of serotype 4, while serotype 12F showed an increase related to GPSC55-ST8060.

Antibiotic non-susceptibility in IPD demonstrates conserved resistance mechanisms over time

Decreased susceptibility to penicillin (minimum inhibitory concentration (MIC) ≥ 0.12 mg/L; PEN-NS) was observed in 17 % of the isolates. The clinical impact of this epidemiological breakpoint was only significant in cases of meningitis, in which the resistance rate was 14.6 %. For pneumonia, the rate of penicillin resistance was 1.6 %, whereas 15.1 % of episodes were susceptible at increased exposure (SIE) using non-meningeal breakpoints (`Fig. 4A).

Most of the isolates with PEN-NS (MIC range 0.12–0.50 mg/L) expressed serotypes 6C and 24F (not included in PCV13) (Fig. 4B) and were related to GPSC47-ST386/ST4310 and GPSC10-ST4677 (Fig. 3). Other serotypes associated with higher MICs for PEN-NS (range 1–4 mg/L) were 11A and 14 (both related to GPSC6-ST156/ST6521). Cefotaxime showed enhanced activity in vitro, and the isolates were classified as SIE in 6.6 % of pneumonia episodes and 5.4 % of meningitis episodes. Serotypes 11A, 14 (GPSC6-ST156/ST6521), and 19A (GPSC1-ST320) were associated with this decreased susceptibility (Figs. 3 and 4B).

Acquired resistance was detected in 19.4 % of isolates and was mainly related to the acquisition of macrolide (*erm*(B) and *mef*(E)) or tetracycline (*tet*(M)) resistance genes. Macrolide resistance (18.5 %) was associated with serotypes 6C, 14, 19A, 24F, and 33F, which harboured *erm*(B) embedded in Tn*6002* (Supplementary Table S3). Tetracycline resistance (19.4 %) was associated with the same serotypes due to the presence of *tet*(M) in Tn*6002*.

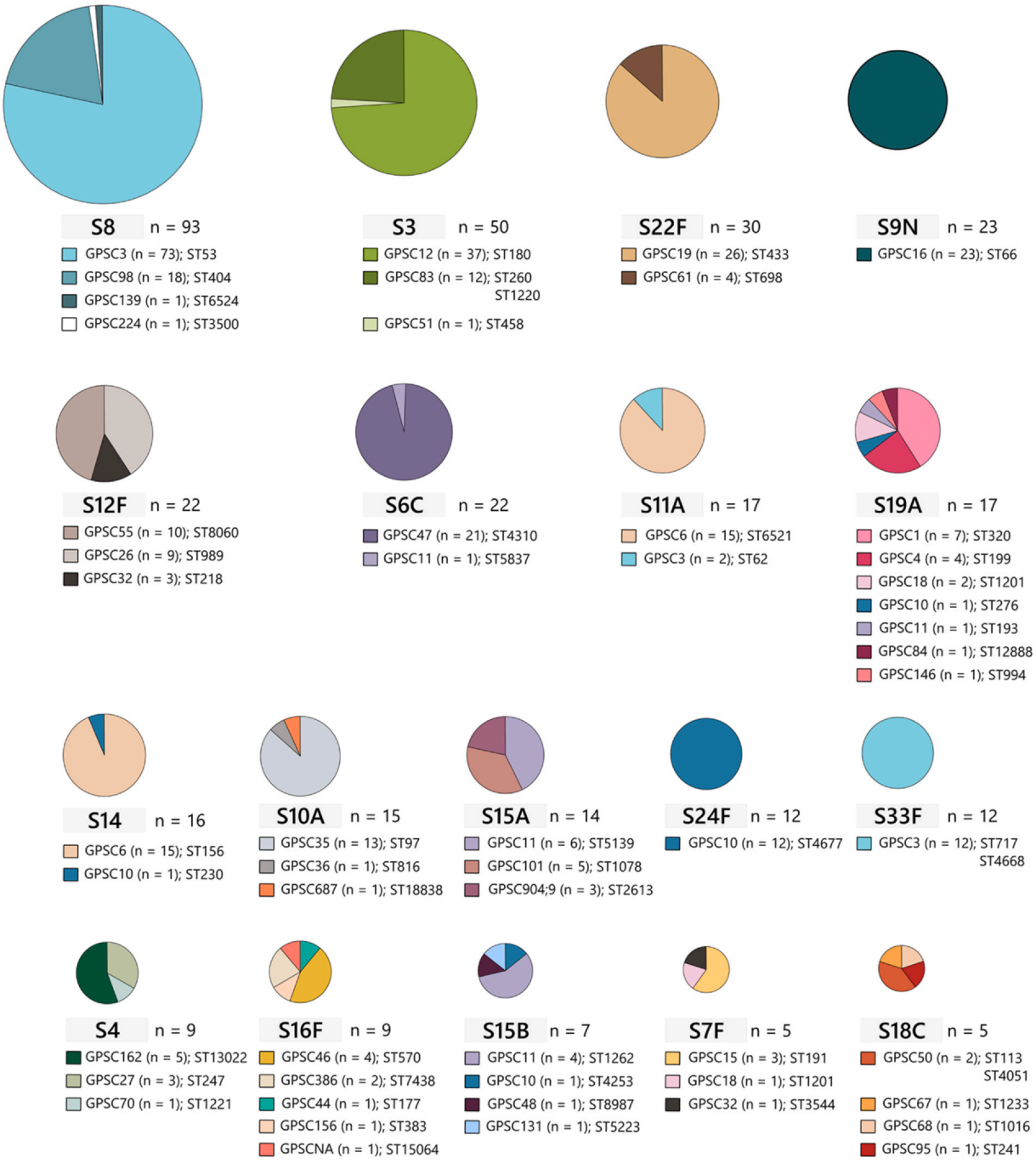

Fig. 3. Serotypes (S) and associated Global Pneumococcal Sequencing Clusters (GPSCs) during the period of 2019–2021. Each circle represents a different serotype, and the size of the circle is proportional to the number of cases. Within each serotype, the various GPSCs are depicted in different colours, and the dominant sequence type (ST) is indicated. The legend indicates the total number of occurrences for each serotype and for GPSC (NA refers to GPSC not assigned).

Other isolates such as serotype 12F were only tetracycline resistant due to the presence of Tn*916,* which harbours *tet*(M) (Supplementary Table S3). Most of these elements were embedded in larger structures of Tn*5252* or Tn*5353*. Finally, mutations in chromosomal genes that lead to AA changes were responsible for non-susceptibility to co-trimoxazole (14.5 %; I100L in FolA) and levofloxacin (5.8 %; single ParC (n = 5) or double ParC/ParE plus GyrA (n = 2) AA changes at QRDR) (Supplementary Figure S3). Resistance to co-trimoxazole was mainly observed in serotypes 11A, 14, 15A, 19A, and 23B, while resistance to levofloxacin was predominantly found in serotypes 4, 8, 9N, 12F, 15A, and 19A (Fig. 4B).

Discussion

The dynamics of IPD and pneumococcal lineages showed sudden alterations that probably resulted from non-pharmaceutical interventions (NPI) against COVID-19, as observed globally [18]. Despite an overall decrease in the incidence of IPD, certain serotypes have shown resilience and continue to challenge disease management. This work examines the evolving dynamics of IPD and pneumococcal lineages several years after the introduction of PCVs.

PCV13 vaccination has provided evident herd protection in children over the years, but this study has shown that the remaining

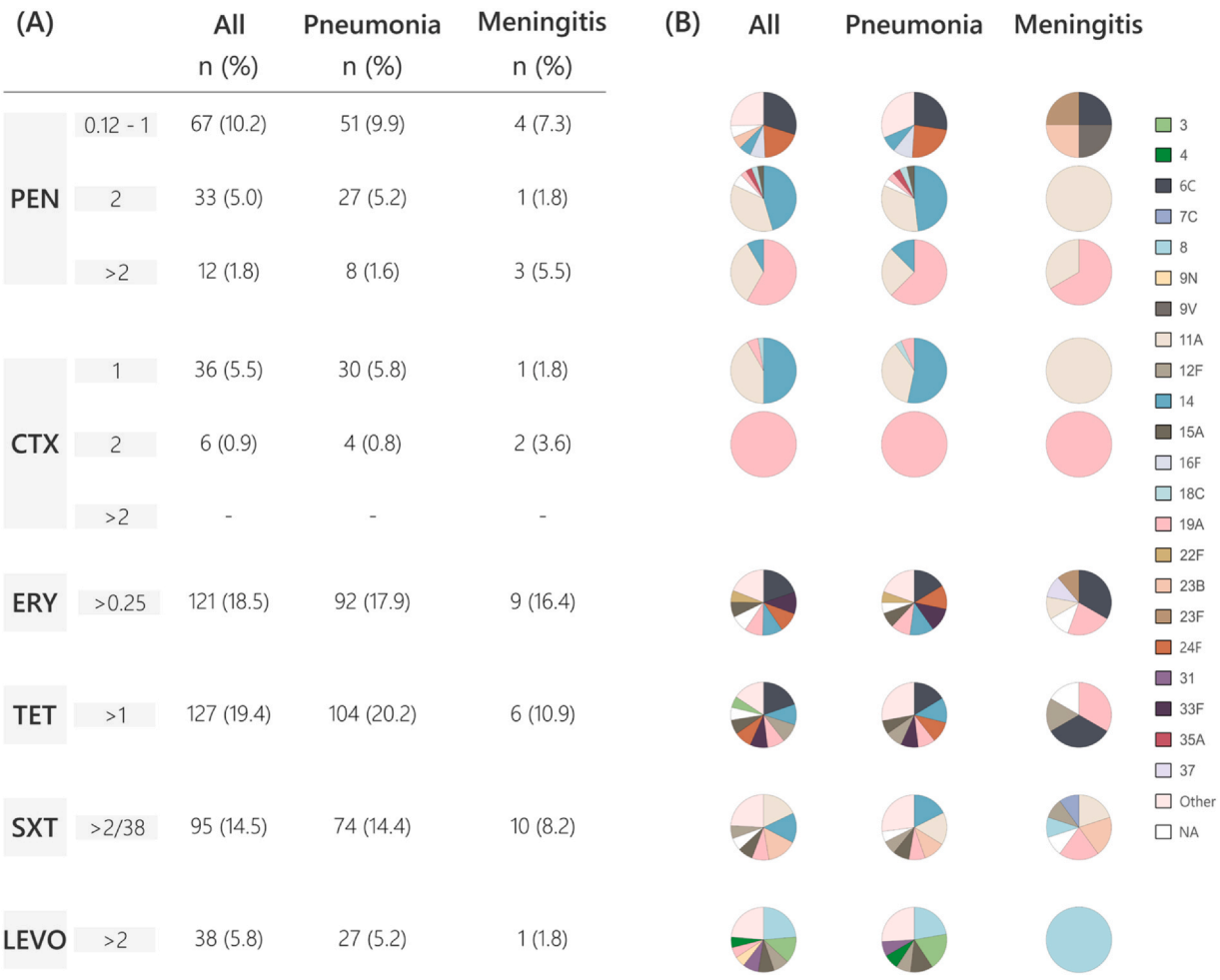

Fig. 4. Overall, pneumonia, and meningitis antimicrobial non-susceptibility rates and serotypes. (A) The number of isolates (n) and the percentage (%) of non-susceptibility rates for penicillin (PEN), cefotaxime (CTX), erythromycin (ERY), tetracycline (TET), co-trimoxazole (SXT), and levofloxacin (LEV). EUCAST meningeal breakpoints: PEN, S≤ 0.06 and R > 0.06; CTX, S≤ 0.5 and R > 0.5. Non-meningeal breakpoints: PEN, S≤ 0.06 and R > 2; CTX, S≤ 0.5 and R > 2. (B) The most prevalent serotypes within the non-susceptibility range. Only serotypes with a frequency higher than 5 % are represented (NA refers to serotype not available).

disease due to PCV13 serotypes is still significant and may justify direct vaccination for adults. Worldwide, serotype 3 continues to be a leading cause of IPD [10,13,14]. Although the main reason remains unclear, potential factors could include the global expansion of GPSC12-CC180 or the role of capsule shedding in disease pathogenesis [15,16]. Additionally, the re-emergence of serotype 4 is a cause for concern. This rise has been recently linked to outbreaks such as one that occurred in Canada related to GPSC27-CC244 [19] and one detected in Finland among shipyard workers associated with GPSC162-ST801 [20]. In the present study, the maintenance of serotype 4 was linked to GSPC162-ST13022 with notable prevalence in the age group of 18–64 years, as recently reported in the UK [15]. One hypothesis is that young and unvaccinated adults act as reservoirs and facilitate its persistence. The detection of this new lineage in Europe, a region with a well-stablished vaccination programme, is a cause for concern.

Besides serotypes 3 and 4, the burden of remaining disease due to PCV13 serotypes in adults was low and associated with heterogeneous serotypes, and no major lineage was identified. However, there was persistence of some beta-lactam or multidrug-resistant lineages, such as GPSC6-ST156 in serotype 14 or GPSC1-ST320 in serotype 19A. These are major contributors to the decreased susceptibility to penicillin and third-generation cephalosporins in Spain [10] and deserve continued surveillance. The rise of non-PCV13 serotypes has been observed in both national and global surveillance [17,18]. Serotypes 6C, 8, 9N, 12F, 15A, 22F, and 23B have been reported as some of the most frequent serotypes causing IPD during the COVID-19 pandemic [17,21–23]. In the present study, serotypes 6C, 8, 22F, and 23B have also been prevalent, and most were characterised by a single or dominant GPSC.

The increase in serotype 8 was mostly associated with GPSC3-ST53, which is linked to the globally disseminated PMEN clone (Netherlands^{8}-ST53) [21,24] and partially to the maintenance of GPSC98-ST404. We also detected the emergence of a new lineage, GPSC55-ST8060, among serotype 12F isolates. This lineage has been previously detected in Israel, as indicated by the GPSC data [25], although it was susceptible to antibiotics (with the exception of cotrimoxazole). Serotype 12F (GPSC26-ST989) was associated with resistance to tetracycline (*tet*(M)), chloramphenicol (*cat*), and co-trimoxazole (FolA AA changes), as reported in the literature [21,25]. Another concern is the persistence of GPSC10 related to serotype 24F, a multidrug-resistant lineage associated with meningitis [26].

The occurrence of different GPSCs shared among various serotypes underscores the dynamic nature of pneumococcal epidemiology and the pathogen's adaptive capacity [27]. We identified eight different GPSCs associated with more than one serotype, which had varying levels of dominance. Among these, the most notable was GPSC3, which included isolates of serotype 8 (ST53), 11A (ST62), and 33F (ST717 and

ST4668). This association was also detected in a Canadian study, although GPSC3 was largely related to serotype 33F, followed by 11A [28].

The second most important lineage was GPSC6, which included serotypes 14 (ST156) and 11A (ST6521), which are both associated with the globally disseminated PMEN3 clone. Serotype 11A isolates were escape vaccines through recombination of this successful clone, which has been detected in Europe and has typically expressed capsular types 9 V and 14 [9]. Interestingly, GPSC6-ST3811, a *single locus variant* from this successful lineage (PMEN3, ST156), was detected in Canada and was associated with serotype 15A [21].

The persistence of certain serotypes and the emergence of antibiotic-resistant lineages highlight the need for continued vaccine development and optimisation. During the COVID-19 pandemic, IPD cases practically disappeared as pneumococci stopped circulating. As COVID-19 NPI measures relaxed, IPD cases returned to pre-pandemic levels [15,17]. Although more than 90 % of isolates were susceptible to beta-lactams, the high rate of SIE highlights the need to use high doses of penicillin in the empirical therapy for pneumonia.

Among the resistant pneumococcal strains, serotypes 11A, 14, and 19A had the highest MICs, as reported in the literature [29–31]. Fortunately, the vaccine development pipeline for the prevention of pneumococcal diseases is expanding. Vaccines such as PCV20 (approved for adults and children in Spain, 2022 and 2024, respectively), PCV21 (V116, licensed for adults by the Food and Drug Administration [FDA]), and PCV24 (under investigation) offer high putative coverage and suggest potential benefits in the current scenario of pneumococcal disease [5,11]. However, as seen with PCV13, a serotype replacement phenomenon could occur, as could the persistence of successful lineages through recombination or the resilience of serotype 3.

This study has some limitations. Although it was a multicentre study, it does not represent the entire country. Additionally, the analysis focused on adult IPD, but children have been the main target of vaccines. However, one of the strengths of the study is that it builds on two previous studies on the same population, which allows us to observe the evolution of IPD over time in the same regions. Furthermore, we included extensive molecular analysis through WGS, which provided a detailed description of GPSCs in adult IPD.

Conclusion

The burden of IPD remains significant in adults despite universal paediatric vaccination. Notably, among PCV13 cases, serotype 3 remains prevalent, and we detected the emergence of a new serotype 4 lineage (GPSC162). Non-PCV13 serotypes, such as 8, 9N, and 23B, have shown increasing trends linked to the expansion of previously described lineages. This shift underscores the need for continued vaccine development to enhance coverage and address emerging antibiotic resistance. The persistence of specific serotypes and their association with multidrug-resistant GPSCs emphasise the dynamic nature and adaptive capacity of pneumococci, which warrant ongoing surveillance.

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CRediT authorship contribution statement

SCS, AGD, JMM, EC, MDQ, AC, NL, DB, MA, MB, LSE, JY, SM, JC, CA.

CA, AGD conceptualised, designed, and supervised the study. JMM, EC, MDQ, AC, NL, DB, MA, MB, JY, JC contributed to collect IPD cases and clinical characteristics. SCS, AGD, LSE, SM designed and performed the whole genome sequencing approaches. SCS, AGD, JC, DB, CA conducted the final analysis of data. SCS, AGD, CA verified the data of the study and wrote the original draft of the manuscript and had full access to all the data in the study. All authors participated in manuscript review and editing and had final responsibility for the decision to submit for publication.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sara Calvo-Silveria declares that the Project was partially funded by PFIS predoctoral grant number FI22/00279.

José Yuste declares grants or contracts paid to his institution from MSD, Pfizer and Meiji. Support for attending meetings and/or travel from MSD and Pfizer. Participation on a Data Safety Monitoring Board or Advisory Board from GSK, MSD and Pfizer.

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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.2024.102619.

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