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Serotypes and genotypes of *S. Pneumoniae* isolates from adult invasive disease in Spain: A 5-year prospective surveillance after pediatric PCV13 licensure. The ODIN study

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ABSTRACT

Serotypes/genotypes causing invasive pneumococcal disease (IPD) in adults are determined by vaccination strategies. The aim of this study was to assess the epidemiology of IPD in adults (≥18 years) after PCV13 introduction for children: serotypes, clonal complexes, antibiotic non-susceptibility and clinical presentations.

We performed a prospective, clinical surveillance of hospitalized culture-confirmed IPDs in adults in nine Spanish hospitals (August 2010–June 2015). A total of 1087 culture-confirmed IPD episodes were included, of which 772 (71.0%) had bacteremic pneumonia (401 complicated/371 uncomplicated pneumonia), 122 (11.2%) meningitis, 102 (9.4%) non-focal bacteremia, 34 (3.1%) peritonitis and 57 (5.3%) others. The most common serotypes were: 3 (12.7%), 19A (8.5%), 8 (7.7%), 7F (6.3%), 1 (4.2%), 6C (4.2%), 11A (4.2%), 22F (4.2%) and 14 (4.0%). Vaccine types (PCV13 + 6C) caused 49.8% of IPD episodes, with a significant decrease over the 5-year period, and significant decreases in serotypes 6C and 7F. The most common genotypes were: CC180 (8.4%), CC191 (6.0%), and CC53 (5.0%).

Vaccine types caused 53.9% (414/768) pneumonia episodes and 58.9% (235/399) complicated pneumonia, 53.4% IPD in adults <50 years (143/268), and 54.7% IPD in immunocompetent patients (337/616). Overall non-susceptibility was 25.9% to penicillin (1.1% for parenteral criteria), 24.9% to erythromycin and 2.7% to levofloxacin.

Conclusions: Although the percentage of vaccine-types causing IPDs in adults significantly decreased, it remained high. Associations of vaccine types with pneumonia (with complicated pneumonia for specific serotypes), and immunocompetent patients point to the burden of IPD caused by PCV13 serotypes.

1. Introduction

Streptococcus pneumoniae is a major human pathogen causing pneumonia, meningitis and other invasive diseases. The capsular polysaccharide is the main pneumococcal virulence factor that

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has been associated with differences in the ability of *S. pneumoniae* strains to colonize the nasopharynx and cause invasive disease, as well as variations in disease manifestation, population groups affected, antimicrobial resistance and geographical distribution [1]. Moreover, due to the plasticity of its genome, *S. pneumoniae* is able to respond to human interventions such as antibiotic treatment and vaccination for disease prevention. Serotype replacement can occur through expansion of existing serotypes/clones, emergence of new clones expressing non-vaccine serotypes or capsular switching [2]. This opens the question as to whether the genetic background (defined by lineages) has a role in clinical/epidemiological characteristics classically associated with capsules. Molecular typing, in addition to capsular serotyping, might provide valuable information on evolutionary changes and subsequent clinical consequences.

Soon after the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) and subsequent 13-valent PCV (PCV13) in childhood immunization programs, significant and progressive reductions were reported in the incidence of invasive pneumococcal disease (IPD) in children and adults [3–6], due, in the latter, to the herd effect [7–9]. Nevertheless, the higher incidence rates of IPD remain in older adults and children younger than 5.

Geographic serotype distribution is variable and partly conditioned by antimicrobial consumption and vaccine strategies. In Spain, PCV13 was introduced in June 2010 for immunization of healthy children but, in contrast to most European countries, it was not included into the universal immunization programm (NIP) and it was only available privately to parents until 2015–2016, except in two regions, Madrid (June 2010) and Galicia (January 2011) (Fig. 1).

The aim of this study was to perform an in-depth analysis of the serotype and genotype epidemiology of IPD in adults in Spain. To that end, we conducted a multicenter study to analyze the current serotype and genotype distributions, as well as the antibiotic susceptibility of invasive pneumococci. We also analyzed the relationship between serotypes and clones by clinical presentation of IPD.

2. Materials and methods

This is a prospective multicenter laboratory-based study performed in nine teaching hospitals of six Autonomous Regions in Spain (August 2010–June 2015). Adult patients (≥18 years) with IPD episodes were eligible to participate if they were hospitalized at the study site for at least 24 h. The study protocol was approved by the corresponding local ethics committee, and all patients provided written informed consent. Clinical follow-up was performed until discharge (or up to ≈90 days after if incomplete resolution at discharge) or death.

IPD was defined as the isolation of *S. pneumoniae* in normally sterile fluids (blood, cerebrospinal fluid, pleural fluid, ascitic fluid and others) from a patient with clinical signs/symptoms of infection. The patient's age, immune status, and major clinical presentation (uncomplicated pneumonia, complicated pneumonia, meningitis, non-focal bacteremia, peritonitis and others) were

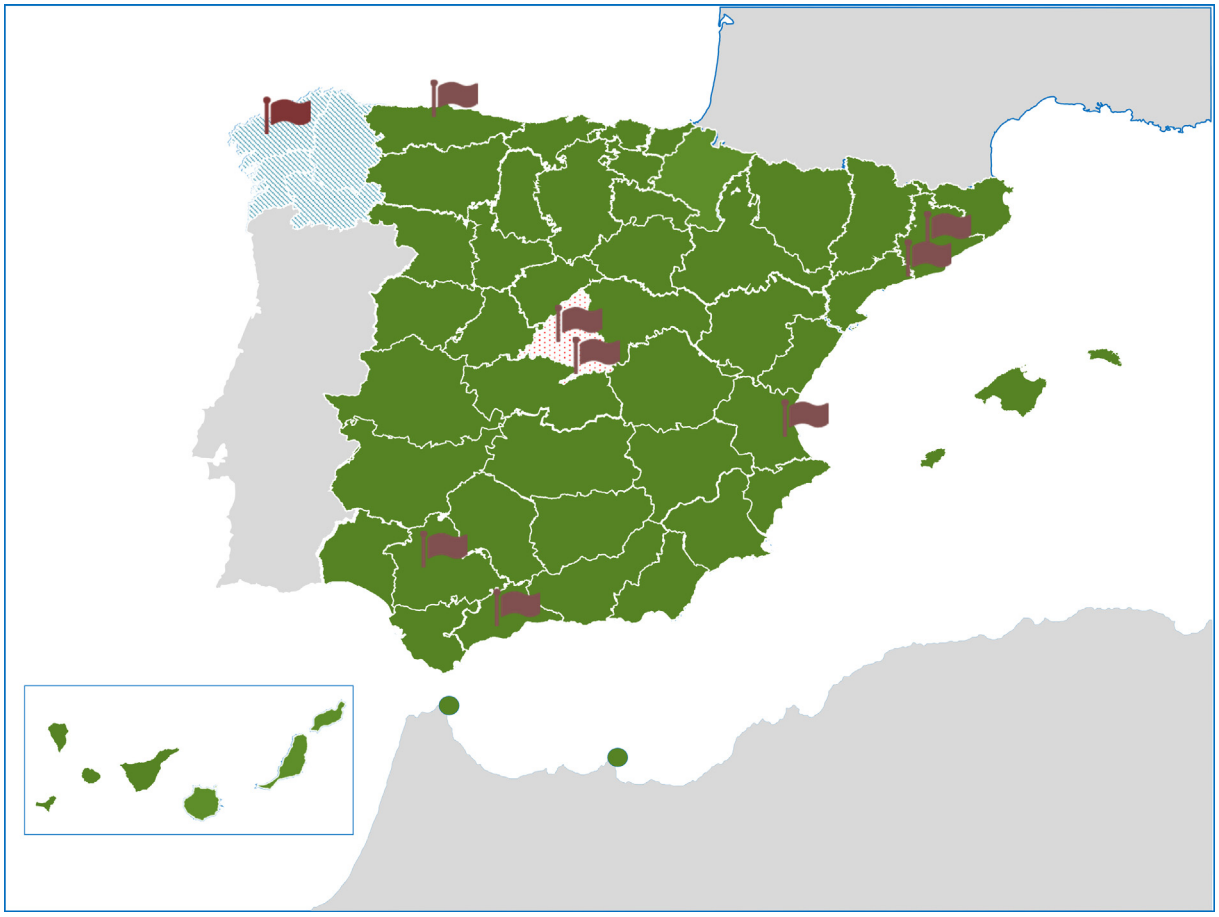

Fig. 1. Regional infant vaccination during the study period (2010–2015) and distribution of participating centers. Flags represents the location of different participant hospitals. Green indicates regions where PCV13 was not included in the official childhood immunization program during the study period, with an estimated vaccine uptake of 61% (3 + 1 schedule). Stripped or dotted regions where PCV13 was included in the immunization program (uptake around 95%; 2 + 1 schedule). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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recorded. Pneumonia with empyema, parapneumonic pleural effusion or multilobar infiltration was considered as complicated. As per the Advisory Committee on Immunization Practices (ACIP), patients were considered immunocompromised when cancer, HIV infection, chemotherapy, immunosuppressive treatment, chronic renal disease, nephrotic syndrome, or other causes of immunosuppression or immunodeficiency were present [10].

Isolates were sent to the Spanish Reference Laboratory for Pneumococci and serotyped using the Quellung reaction/dot blot assay [11]. Only one isolate per episode was considered. Molecular typing was performed by pulsed-field gel electrophoresis (PFGE). Genomic DNA embedded in agarose plugs was restricted with SmaI (New England BioLabs), and fragments were separated by PFGE [12]. PFGE patterns were compared with representative international pneumococcal clones of the Pneumococcal Molecular Epidemiology Network [12]. Band patterns were visually compared. Major PFGE patterns were defined as those including >4 isolates. At least one representative strain of each major PFGE pattern was analyzed by multilocus sequence typing (MLST) [13]. Allele numbers and sequence types were assigned using the MLST website [13]. Major lineages were named as clonal complexes (CC), with the serotype as superscript (e.g., $CC306^{1}$; clonal complex 306 of serotype 1). Susceptibility was determined by agar dilution [12]. Isolates with intermediate- or high-level resistance to oral/-parenteral penicillin, cefotaxime (considering meningitis and non-meningitis breakpoints), erythromycin and levofloxacin determined using current Clinical and Laboratory Standards Institute (CLSI) breakpoints [14] were considered non-susceptible.

Vaccine types (VTs) were considered serotypes included in the PCV13 and serotype 6C based on previous data [15]. All other serotypes were considered non-vaccine types (NVTs).

Comparisons between proportions were performed using the χ^2 test, Fisher's exact test and the Likelihood ratio test, as necessary. For quantitative variables, since data did not show normality in the Kolmogorov-Smirnoff test, the Kruskal-Wallis and Mann-Whitney tests were used as necessary. Statistical analyses were performed using the statistical program SPSS v.19 (SPSS Inc, Chicago IL).

Temporal trends of VTs (individually and overall), NVTs (overall, and individually for the most prevalent) and CCs within serotypes were calculated by linear/non-linear regression using time as the independent variable. The model showing the highest ratio R^2/degrees of freedom was considered. Trends were considered significant when p-values were ≤ 0.05.

3. Results

3.1. Patients, episodes, serotypes and genotypes

Of the 1107 IPDs included during the 5-year study period, 20 were excluded because they not met the eligibility criteria (≥24 h of hospitalization); thus, 1087 episodes were evaluable (see Supplementary material Table S1 for details on recruitment by hospital and study period). Strains from four episodes were not available for further studies. Among evaluable cases, 269 (24.7%) occurred in 18–49-year-old patients, 273 (25.1%) in 50–64-year-old patients, 220 (20.2%) in 64–74-year-old patients, and 325 (29.9%) in patients older than 74. The clinical presentations were: pneumonia (772, 71.0%) (401 complicated and 371 uncomplicated), meningitis (122, 11.2%), non-focal bacteremia (102, 9.4%), peritonitis (34, 3.1%) and other IPDs (57, 5.2%).

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.10.098.

Among the 1084 available isolates, the most common serotypes were: serotype 3 (12.7%), 19A (8.5%), 8 (7.7%), 7F (6.3%), 1 (4.2%), 6C (4.2%), 11A (4.2%), 22F (4.2%) and 14 (4.0%), accounting for 56% of the total isolates. VT (PCV13 + 6C) accounted for 539 isolates (49.8%). Table 1 shows the serotype distribution by study period. The percentage of VTs decreased over time ($R^2 = 0.812$, $\beta = -0.901$, $p = 0.037$). Individually, serotypes 6C ($R^2 = 0.821$, $\beta = -0.906$, $p = 0.034$) and 7F ($R^2 = 0.937$, $\beta = -0.964$, $p = 0.007$)

Table 1
Annual distribution of serotypes causing IPDs in adults (≥18 years) in Spain (August 2010–June 2015).

	Isolates, *n* (%)	2010/2011	2011/2012	2012/2013	2013/2014	2014/2015
Serotypes	**1083**	**191**	**242**	**200**	**241**	**209**
1	46 (4.2)	12 (6.3)	8 (3.3)	13 (6.5)	12 (5.0)	1 (0.5)
3	137 (12.7)	22 (11.5)	27 (11.2)	27 (13.5)	28 (11.6)	33 (15.8)
4	27 (2.5)	7 (3.7)	5 (2.1)	6 (3.0)	4 (1.7)	5 (2.4)
5	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)
6A	8 (0.7)	3 (1.6)	2 (0.8)	1 (0.5)	2 (0.8)	0 (0.0)
6B	7 (0.6)	3 (1.6)	1 (0.4)	0 (0.0)	3 (1.2)	0 (0.0)
6C[a]	45 (4.2)	11 (5.8)	11 (4.5)	10 (5.0)	7 (2.9)	6 (2.9)
7F[a]	68 (6.3)	16 (8.4)	20 (8.3)	12 (6.0)	13 (5.4)	7 (3.3)
9V	19 (1.8)	2 (1.0)	7 (2.9)	2 (1.0)	5 (2.1)	3 (1.4)
14	43 (4.0)	11 (5.8)	9 (3.7)	11 (5.5)	8 (3.3)	4 (1.9)
18C	10 (0.9)	1 (0.5)	3 (1.2)	3 (1.5)	1 (0.4)	2 (1.0)
19A	92 (8.5)	18 (9.4)	21 (8.7)	20 (10.0)	20 (8.3)	13 (6.2)
19F	27 (2.5)	4 (2.1)	10 (4.1)	5 (2.5)	5 (2.1)	3 (1.4)
23F	9 (0.8)	2 (1.0)	3 (1.2)	2 (1.0)	1 (0.4)	1 (0.5)
All PCV13 + 6C[a]	**539 (49.8)**	**112 (58.6)**	**127 (52.5)**	**113 (56.5)**	**109 (45.2)**	**78 (37.3)**
8	83 (7.7)	12 (6.3)	17 (7.0)	9 (4.5)	17 (7.1)	28 (13.4)
11A[a]	46 (4.2)	0 (0.0)	10 (4.1)	10 (5.0)	15 (6.2)	11 (5.3)
22F	46 (4.2)	7 (3.7)	11 (4.5)	5 (2.5)	9 (3.7)	14 (6.7)
9N	38 (3.5)	6 (3.1)	4 (1.7)	4 (2.0)	10 (4.1)	14 (6.7)
12F	35 (3.2)	2 (1.0)	10 (4.1)	3 (1.5)	9 (3.7)	11 (5.3)
15A	32 (3.0)	6 (3.1)	9 (3.7)	9 (4.5)	6 (2.5)	2 (1.0)
24F	29 (2.7)	6 (3.1)	5 (2.1)	8 (4.0)	5 (2.1)	5 (2.4)
16F	28 (2.6)	7 (3.7)	4 (1.7)	6 (3.0)	5 (2.1)	6 (2.9)
10A	22 (2.0)	4 (2.1)	4 (1.7)	0 (0.0)	8 (3.3)	6 (2.9)
All Non-PCV13[a]	**544 (50.2)**	**79 (41.4)**	**115 (47.5)**	**87 (43.5)**	**132 (54.8)**	**131 (62.7)**

Non-PCV13 serotypes (serotypes not included in PCV13 + serotype 6C) individually shown are those accounting for ≥2% of total isolates; [a]significant time trends (see text). Period 2010/2011: 01/08/2010–1/06/2011; period 2011/2012: 02/06/2011–1/06/2012; period 2012/2013: 02/06/2012–01/06/2013; period 2013/2014: 02/06/2013–01/06/2014; period 2014/2015:02/06/2014–01/06/2015.

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decreased over time. NVTs significantly increased over the study period; individually, the percentage of serotype 11A ($R^2 = 0.974$, $\beta = 3.543$, $p = 0.027$) increased over time.

The percentage of VT isolates was higher in the area with low PCV13 pediatric uptake (Barcelona) than in the area with high coverage (Madrid) (53.9% *vs.* 40.8%, $p = 0.001$), with differences for serotype 14 (4.8% *vs.* 1.8%, $p = 0.048$). Among NVTs, serotypes 8 and 16F were more common in the region with high coverage (16.9% *vs.* 3.9%, $p < 0.001$, and 4.9% *vs.* 0.8%, $p = 0.002$, respectively), while serotype 24F was more common in the region with low uptake (5.3% *vs.* 0.7%, $p = 0.001$).

Fig. 2 shows the major pneumococcal genotypes (CCs with ≥10 isolates) by serotype. In general, there was an association between CCs and serotype, with the exception of $CC386^{6C,6B}$, $CC156^{14,11A,9V,19A,24F}$, $CC230^{19A,24F,19F}$ and $CC63^{8,15A,23B,19F}$, which were associated with more than one serotype. Serotypes 3, 8 and 11A had two major CCs each. Although serotype 19A isolates were genetically diverse, two CCs ($CC230^{19A}$ and $CC320^{19A}$) accounted for 54.3% of 19A isolates. The changes in the CCs over the study period are shown in Supplementary material Table S2.

Relationship of serotypes/genotypes with age, clinical presentation, and immune status

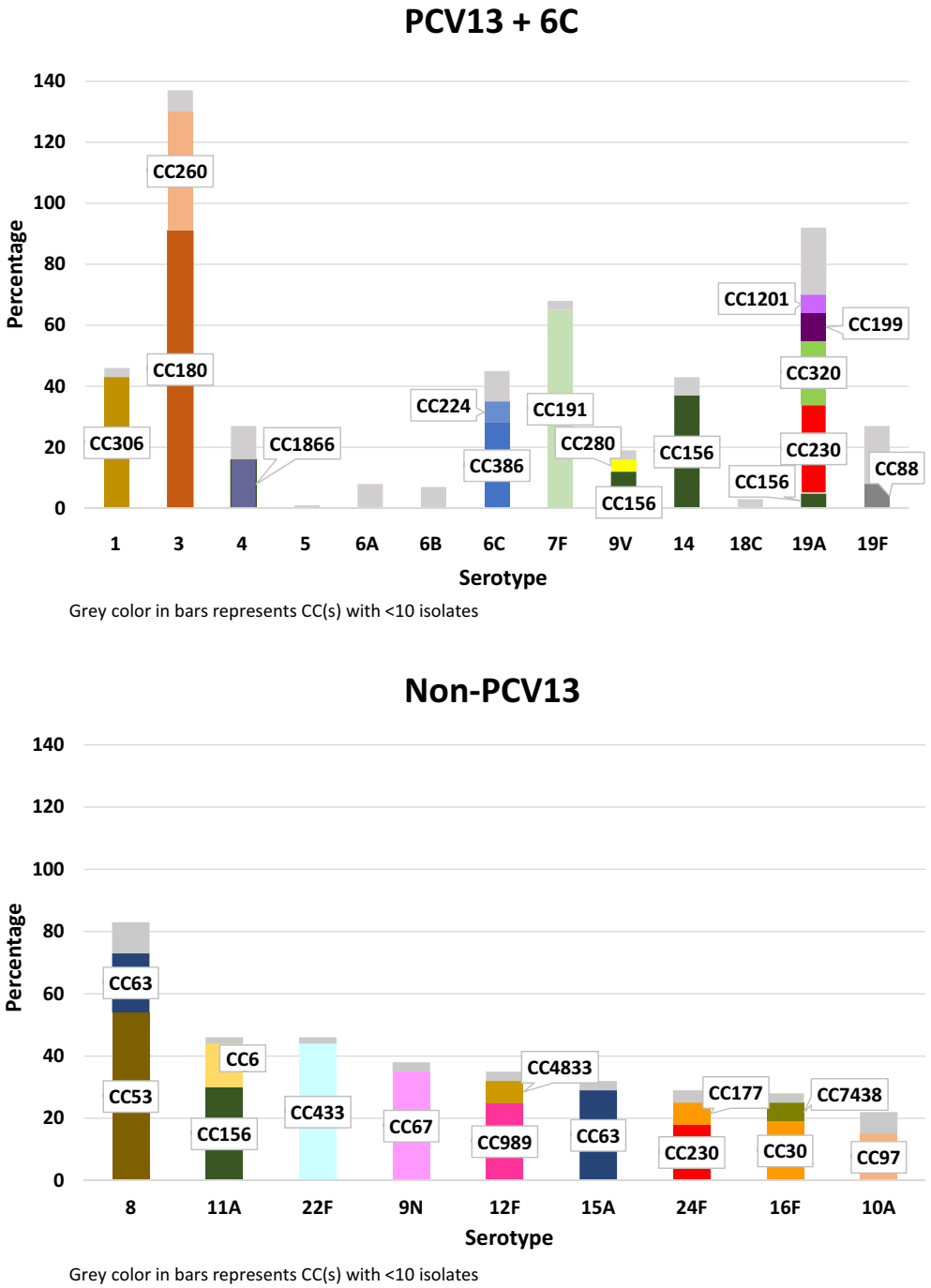

Fig. 2. Clonal composition of major *S. pneumoniae* serotypes isolated from adults with IPD in Spain (2010–2015). Only serotypes with ≥10 isolates are shown. Gray color in bars represents CC(s) with <10 isolates.

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The most common serotypes among pneumonia isolates were: serotypes 3 (15.1%), 8 (9.6%), 19A (9.5%) and 7F (7.7%). VTs caused 53.9% (414 out of 768) of all pneumonia episodes, and 58.9% (235 out of 399) of complicated pneumonia episodes (Supplementary material Table S3). Serotype 1 was most common among complicated (7.0%) compared to uncomplicated pneumonia (3.0%, $p = 0.01$). There were no differences regarding clonal composition of isolates causing complicated or uncomplicated pneumonia, except among serotype 3 CCs. There was an association between CC180[3] and complicated pneumonia (50 of 381 complicated pneumonia *vs.* 24 of 359 non-complicated pneumonia, $p = 0.003$).

Among pneumonia isolates, 0.9% had penicillin minimum inhibitory concentrations (MICs) ≥ 4 μg/mL and 2.5% had cefotaxime MICs ≥ 2 μg/mL (non-meningeal breakpoints).

There were 122 meningitis episodes; serotype 3 (9.8%), and serotypes 11A and 19A (7.4% each) were the most common (Supplementary Table S3). VT accounted for 43.4% of meningitis isolates. Using meningeal breakpoints, penicillin (≥ 0.12 μg/mL) and cefotaxime (≥ 1 μg/mL) resistance rates were 30.3% and 12.3%, respectively.

Finally, serotypes 15A (10.8%), 6C (9.8%), 11A (6.9%) and serotypes 3 and 9N (5.9% each) were the most common among non-focal bacteremia isolates, with serotypes 11A (14.7%), 6C (8.8%) and serotypes 7F, 14, 15B, 17F, 19A, 19F and 24F (5.9% each) the most common among peritonitis episodes. VTs accounted for 35.3% and 38.2% of non-focal bacteremia and peritonitis isolates, respectively.

By age group, the most common serotypes in young adults (<50 years) were serotypes 8 (10.4%), 3 (10.1%), 7F (8.6%), 19A (6.7%), 1 and 14 (5.6% each), and 6C (5.2%); VTs accounted for 53.4% of isolates. In patients ≥65 years, the most common serotypes were 3 (13.5%), 8 (5.5%), 19A (7.9%) and 22F (5.4%), while VT-IPD represented 46.1% of all IPD (Supplementary Table S4).

Regarding immune status, 616 IPD episodes occurred in immunocompetent patients and 467 in immunocompromised patients (Supplementary Table S5). Forty-four different serotypes were found among immunocompetent patients, five of which accounted for more than 51% of episodes; the number of serotypes found among immunocompromised patients was similar (40), eight of which accounted for 50% of episodes. Serotypes 1 (5.8% *vs.* 2.1%, $p = 0.003$), 3 (16.0% *vs.* 8.1%, $p < 0.001$), 7F (7.8% *vs.* 4.3%, $p = 0.018$) and 8 (9.3% *vs.* 5.6%, $p = 0.024$) were more frequent among immunocompetent patients, whereas serotypes 10A (3.6% *vs.* 0.8%, $p = 0.002$), 11A (5.8% *vs.* 3.1%, $p = 0.043$) and 15A (5.1% *vs.* 1.3%, $p \leq 0.001$) were more frequent among immunocompromised patients. In these two groups, VTs caused 54.7% (337 out of 616) of IPD in immunocompetent patients and 43.3% (202 out of 467) in immunocompromised patients.

3.2. Antimicrobial non-susceptibility

Table 2 shows non-susceptibility data. Overall, non-susceptibility (MIC ≥ 0.12 mg/L) was 25.9% [1.1% for parenteral MIC ≥ 4 mg/L)] to penicillin, 24.9% to erythromycin and 2.7% to levofloxacin. No differences in non-susceptibility were found between regions with high and low coverage, except for levofloxacin non-susceptibility, with a significantly higher percentage in the region with high coverage (6.7% *vs.* 1.1%, $p < 0.001$), associated with a higher percentage of serotype 8.

Table 3 shows non-susceptibility rates for serotypes/CCs. Only 12 isolates were non-susceptible to parenteral penicillin (all MIC = 4 mg/L): seven serotype 19A (all CC320), two 14 (both CC156), one 9V (CC156), one 6B and one 23F. According to non-meningitis breakpoints for cefotaxime, 29 (2.7%) isolates (28 MIC = 2 mg/L, one MIC = 4 mg/L) were non-susceptible: 16 serotype 19A (eleven CC320, three CC230, one CC156), nine serotype 14 (eight CC156), two 9V (CC156), one 6B and one 23F. Of the 270 isolates resistant to erythromycin, 239 (88.5%) exhibited MIC ≥ 128 mg/L. Serotypes with levofloxacin non-susceptibility >10% were 9V (21.1%), 8 (19.3%), 23F (11.1%) and 9N (10.5%). Eighteen out of 29 (62.1%) isolates non-susceptible to levofloxacin belonged to CC63, of which 15 were serotype 8 and three were serotype 15A. Non-susceptibility by serotype within CCs was examined for three CCs expressing more than one capsule: CC63[8,15A,23B,19F], CC156[14,11A,9V,19A,24F] and CC230[19A,24F,19F]. Within CC63, all isolates belonging to serotype 8 were susceptible to penicillin while all serotype 15A were non-susceptible; levofloxacin non-susceptibility was 78.9% and 10.3% among isolates of serotypes 8 and 15A, respectively ($p < 0.001$). Within CC156, erythromycin non-susceptibility was 100% (19A), 25% (9V) and ≤8% (14 and 11A), while non-susceptibility to levofloxacin was 33.3% (9V), 2.7% (14) and 0% (11A and 19A).

4. Discussion

This multicenter study presents an overview of serotype and genotype distribution causing adult IPD in Spain, a country with regional differences in PCV pediatric uptake. Approximately half of IPDs in adults were caused by VTs, although the percentage decreased over the 5-year study period, reflecting herd protection by pediatric vaccination. Among NVTs, significant increases over time were found only for serotype 11A (appearing in period 2011/2012). Regarding serotype 8 CC63[8] decreased and CC53[8] increased. A recent Canadian study described genetic shifts within serotypes among isolates collected from children ≤5 years old because of the impact of PCV13, and suggested selective

Table 2
Antimicrobial non-susceptibility rates, range, MIC_{50} and MIC_{90} of invasive pneumococci collected from adults (≥18 years) in Spain (2010–2015) regarding clinical presentation.

	All isolates ($n = 1083$)				Meningitis ($n = 122$)				Pneumonia ($n = 768$)			
	Range	MIC_{50}	MIC_{90}	%	Range	MIC_{50}	MIC_{90}	%	Range	MIC_{50}	MIC_{90}	%
Penicillin	**≤0.015–4**	**≤0.015**	**1**		**≤0.015–4**	**≤0.015**	**1**	**30.3**	**≤0.015–4**	**≤0.015**	**1**	**0.9**
≥0.12	–	–	–	25.9	–	–	–	**30.3**	–	–	–	22.9
2	–	–	–	6.7	–	–	–	4.9	–	–	–	6.4
4	–	–	–	1.1	–	–	–	2.5	–	–	–	**0.9**
Cefotaxime	**≤0.015–4**	**≤0.015**	**1**		**≤0.015–4**	**≤0.015**	**1**	**12.3**	**≤0.015–2**	**≤0.015**	**1**	**2.5**
1	–	–	–	12.4	–	–	–	9.8	–	–	–	9.6
≥2	–	–	–	2.7	–	–	–	2.5	–	–	–	2.5
Erythromycin	**≤0.12–≥128**	**≤0.12**	**≥128**	**24.9**	**≤0.12–≥128**	**≤0.12**	**≥128**	**29.5**	**≤0.12–≥128**	**≤0.12**	**≥128**	**21.0**
Levofloxacin	**≤1–≥64**	**≤1**	**≤1**	**2.7**	**≤1–2**[a]	**≤1**	**≤1**	**0.0**	**≤1–≥64**	**≤1**	**≤1**	**2.9**

Rates of non-susceptibility regarding specific breakpoints for each group are shown in bold. Breakpoints for non-susceptibility [oral/meningitis penicillin: ≥0.12 mg/L; parenteral penicillin: ≥4 mg/L; cefotaxime (meningitis): ≥1 mg/L; cefotaxime (non-meningitis): ≥2 mg/L; erythromycin: ≥0.5 mg/L; levofloxacin: ≥4 mg/L].

[a] Two isolates with MIC = 2 mg/L.

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Table 3
Antimicrobial non-susceptibility rates for serotypes and related CCs of *S. pneumoniae* strains collected from adults (≥18 years) with IPD in Spain (2010–2015).

Serotype	Related CCs[a]	Penicillin	Cefotaxime	Erythromycin	Levofloxacin
6C (*n* = 45)		44.4/–	–/–	66.7	–
	CC386 (*n* = 28)	50.0/–	–/–	100	–
8 (*n* = 83)		–/–	–/–	22.9	19.3
	CC53 (*n* = 54)	–/–	–/–	–	1.9
	CC63 (*n* = 19)	–/–	–/–	100	78.9
9 N (*n* = 38)		5.3/–	–/–	2.6	–
	CC67 (*n* = 35)	2.9/–	–/–	2.9	–
9 V (*n* = 19)		47.4/5.3	36.8/10.5	15.8	21.1
	CC156 (*n* = 12)	75.0/8.3	58.3/16.7	25.0	33.3
10A (*n* = 22)		–/–	–/–	31.8	–
	CC97 (*n* = 15)	–/–	–/–	13.3	–
11A (*n* = 46)		69.6/–	50.0/–	28.3	–
	CC156 (*n* = 30)	100/–	76.7/–	6.7	–
	CC62 (*n* = 14)	–/–	–/–	71.4	–
14 (*n* = 43)		95.3/4.7	83.7/20.9	11.6	2.3
	CC156 (*n* = 37)	100/5.4	89.2/21.6	8.1	2.7
15A (*n* = 32)		90.6/–	9.4/–	90.6	9.4
	CC63 (*n* = 29)	100/–	10.3/–	100	10.3
15B (*n* = 20)		–/–	–/–	25.0	–
16F (*n* = 28)		28.6/–	–/–	17.9	–
	CC30 (*n* = 19)	5.3/–	–/–	26.3	–
19A (*n* = 92)		68.5/7.6	50.0/17.4	60.9	1.1
	CC230 (*n* = 29)	100/–	51.7/10.3	65.5	3.4
	CC320 (*n* = 21)	100/33.3	100/52.4	95.2	–
19F (*n* = 27)		59.3/–	33.3/–	70.4	3.7
23A (*n* = 20)		10.0/–	–/–	60.0	–
	CC42 (*n* = 18)	–/–	–/–	55.6	–
23B (*n* = 21)		66.7/–	4.8/–	9.5	–
	CC2372 (*n* = 15)	80.0/–	–/–	6.7	–
24F (*n* = 29)		69.0/–	–/–	69.0	–
	CC230 (*n* = 18)	100/–	–/–	100	–
35B (*n* = 18)		50.0/–	22.2/–	5.6	–

Absence of non-susceptible isolates is shown by (–).
[a] Only CC accounting for ≥10 isolates are show. Breakpoints for non-susceptibility [oral/meningitis penicillin: ≥ 0.12 mg/L; parenteral penicillin: ≥ 4 mg/L; cefotaxime (meningitis): ≥ 1 mg/L; cefotaxime (non-meningitis): ≥ 2 mg/L; erythromycin: ≥ 0.5 mg/L; levofloxacin: ≥ 4 mg/L].

advantages derived from genetic diversity, frequent recombination and drug resistance potential related to specific clones [16]. Based on this, not only the decline in VTs, but also the clonal changes in our study could be due to the herd effect after PCV13 introduction for children (only in those cases in which there was an association with serotypes included in the vaccine). However, explaining changes as derived from PCV13 uptake in children should be considered with caution, since PCV7/13 had not been universally administered for pediatric vaccination in all Spanish geographical areas, and isolates came from adults. In this sense, the analysis of isolates from a region with high vaccine uptake (PCV7/13 included in the pediatric immunization program; estimated uptake >80%) versus one region with low uptake [PCV7/13 not included by the period analyzed; estimated uptake = 55% [17] was performed to explore possible herd effects. Based on our findings, indirect effects can be inferred, since adult VT-IPDs were significantly less frequent in the area with high pediatric coverage. It has been reported that there is a decline in carriage of PCV13 serotypes in ≥50% of non-immunized children only in communities where pediatric PCV13 coverage is ≥75%, [18], which indirectly reduces the burden of VT-IPD. Finally, the genetic diversity or homogeneity of some serotypes and their variations could be related to changes in the clonal composition. For example, since nearly all serotype 7F strains belonged to CC191, the decrease in this serotype is associated with a decrease in the CC191. In contrast, the decrease in serotype 14 could not be related with a genotype, since CC156 isolates could express different capsular types including non-PCV13 serotypes.

In this study we have shown differences in the serotype composition in terms of clinical presentation, age and immune status, as has been previously described. Thus, VTs accounted for more than a half of pneumonia episodes. Since pneumonia is a major threat in the adult population, a PCV13 vaccination strategies could reduce the burden of this disease. In addition, some VTs (serotypes 1, 3, 19A and 7F) were associated with complicated pneumonia as recently reported in Spain [19,20]. Notably, among pneumonia episodes due to serotype 3 those caused by CC180[3] were more frequent among complicated pneumonia suggesting that other factors beside capsule are also important in the pathogenesis of infection. Moreover, with respect to immune status non-invasive serotypes (11A, 9 N, 15A, 10A) were more frequent among immunosuppressed patients, suggesting that the impaired immune system could facilitate the invasiveness of these serotypes [21]. Furthermore, invasive serotypes were more common among immunocompetent patients (1, 3, 7F, 8) highlighting the major role of the capsular polysaccharide in the invasiveness.

Previous studies have suggested that adults with high-risk conditions may not benefit from indirect protection as much as immunocompetent patients [10,22]. Nevertheless, VTs caused 43.3% IPD in immunocompromised patients that could take advantage of PCV13 vaccination until herd protection from pediatric PCV13 is fully established [23].However, considering our data from the area, where the proportion of VT-IPDs remains high despite >80% pediatric coverage, a better strategy to reduce the burden of adult IPD would be to offer a specific PCV13 vaccination program not only to high-risk adults (as currently done in Spain), but to all persons aged ≥60 years old. Hence, ACIP recommends that PCV13 is routinely administered to all adults aged ≥65 years [24]. Specific vaccination programs in adults have been suggested, since serotypes causing IPDs in adults are rarely found in the nasopharynx of children [25].

In line with other studies performed in Spain [11,26], our results shows that penicillin/erythromycin non-susceptibility is linked to serotypes 15A, 19A, 6B, 19F and 24F, while levofloxacin

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non-susceptibility was mainly linked to serotype 8 [27–29]. The relationship between serotype 8 and levofloxacin-resistance was previously described associated with the spread of a recombinant clone 8-ST63 (related to clone Sweden15A-ST63) initially confined in Madrid area and associated with HIV patients [27,29], that expanded to other regions [27]. Although the main reservoir of pneumococci are children, isolates of serotype 8 are rarely found, neither as colonizers or agents of invasive disease [30]. Notably, since fluoroquinolones are not used in children, the quinolone-resistance in CC63[8] represents a problem strictly in adults. Importantly, in our study we observed a decrease of CC63 within serotype 8, with a significant increase in CC53[8] (susceptible to penicillin, erythromycin and levofloxacin). Finally, the continuing presence of CCs strongly linked to high beta-lactam resistance, such as CC320 (serotype 19A) and CC156 (serotypes 14, 9V and 11A), is also a concern. However, the percentage of serotype 14 was higher among regions with lower vaccination coverage in children (data not shown) [17]. This reinforces the need for high vaccination coverage in children to protect the main population affected, and through the herd effect to protect subjects not targeted by vaccination strategies.

The results of the present surveillance study showed that, although the percentage of VTs as a cause of IPDs in adults in Spain is decreasing, it remains high, even in areas where PCV7/13 is included in the pediatric immunization program. Moreover, this study shows differences in the serotype distribution as regards clinical presentation, age or immune status highlighting the role of capsular type in the pathogenesis of pneumococcal infections. The main limitation of this study is that it is not a population-based study which hampered to know the real impact of children vaccination among the burden of adult IPD. However, our study analyzes a large pneumococcal series causing IPD in adults allowing us to analyze the serotype composition and vaccine coverage of IPD in different populations targeted by the current 13-valent conjugate vaccine.

Potential conflicts of interest

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