Pneumococcal serotypes in children and relationship with clinical presentation and antimicrobial susceptibility in the PCV13 era

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Abstract

Background: The aim of this study was to analyse the serotypes causing invasive pneumococcal disease (IPD) according to the clinical presentation, and antimicrobial susceptibility in children aged ≤17 years before the introduction of the 13-valent pneumococcal conjugate vaccine (PCV13) in the official paediatric schedule. Methods: We conducted a prospective study in children ≤17 years with IPD attended in three Catalan hospitals between January 2012 and June 2016. IPD cases were diagnosed by culture or RT-PCR. Demographic, microbiological, and clinical data were analysed. Associations were assessed using the odds ratio (OR) and 95% confidence intervals (CI). Results: Of the 253 cases included, 34.4% were aged < 2 years, 38.7% 2-4 years and 26.9% 5-17 years. Over 64% of cases were PCV13 serotypes. Cases with PCV13 serotypes were associated with overall pneumonia (OR:7.47[4.0-13.96]) and complicated pneumonia (OR:7.2[4.04-12.75]), overall and in each age group (p<0.05). Serotypes 3 and 1 were associated with complicated pneumonia (p<0.05). Non-PCV13 serotypes were associated with meningitis (OR:7.32[2.33-22. 99]) and occult bacteremia (OR:3.6[1.56-8.76]). Serotype 19A was more frequent in children aged <2 years and serotype 3 and 1 in those aged 2-4 years and >4 years, respectively. Forty-four cases (36.1%) were non-susceptible to penicillin and 16.4% were also non-susceptible to cefotaxime. There were no significant differences between PCV13 and non-PCV13 cases with non-susceptible penicillin strains (36.1% and 36.0%, respectively), while PCV13 cases showed more frequently non-susceptible cefotaxime strains (23.6%; p=0.010). Serotypes 19A and 14 were associated with non-susceptibility to both penicillin and cefotaxime strains (p=0.003 and p<0.001, respectively) and serotype 19A with resistant to penicillin (p=0.002) Conclusions: PCV13 serotypes were the most frequent serotypes in children aged ≤17years, mainly serotype 3, 1 and 19A. Non-PCV13 serotypes were associated with meningitis and occult bacteraemia while PCV13 serotypes with pneumonia. PCV13 and non-PCV13 cases presented high frequency of non-susceptibility to penicillin. Serotypes 19A, and 14 were associated with non-susceptibility to both penicillin and cefotaxime, and serotype19A with penicillin resistance.

Background

Streptococcus pneumoniae infections are associated with substantial morbidity and mortality worldwide. The World Health Organization estimated that approximately 335,000 (240,000–460,000) deaths were due to *S. pneumoniae* in children aged <5 years in 2015 [1].

The epidemiology of invasive pneumococcal disease (IPD) has changed after the introduction of pneumococcal conjugate vaccines [2].In countries where the 7-valent pneumococcal conjugate vaccine (PCV7) (with serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) was introduced, the global IPD incidence decreased but the non-PCV7 serotypes emerged [2–5]. Later, higher-valent vaccines [10-valent pneumococcal conjugate vaccine (PCV10), that includes PCV7 serotypes plus serotypes 1, 5 and 7F, and 13-valent pneumococcal conjugate vaccine (PCV13) that includes PCV10 serotypes plus 3, 6A and 19A] [6] were introduced and a significant decline in some of additional serotypes was observed [7].

In Catalonia, Spain, PCV7 was available in 2001, but only children with risk factors were vaccinated for free [8,9]. An estimated vaccine coverage of around 50% was reached [9].Between 2005 and 2007, a change in IPD serotypes was found in children aged <2 years, compared with the pre-vaccine period, with a decrease in PCV7 serotypes and an increase in non-PCV7 serotypes [10]. PCV13 replaced PCV7 in 2010 and finally, in July 2016, was included in the paediatric official calendar [11]. PCV13 could cover up to 70% of IPD cases in our reference area [12].

Studies worldwide have found that the changing distribution of pneumococcal serotypes has led to changes in the rates of *S. pneumoniae* resistance to antibiotics [13,14].

The aim of this study was to analyse the distribution of *S. pneumoniae* serotypes according to the clinical presentation of IPD and antimicrobial susceptibility in children aged <18 years in a community with intermediate PCV13 coverage before the introduction of this vaccine in the official paediatric schedule.

Methods

2.1 Study design

We conducted a prospective observational study between January 2012 and June 2016 in children aged <18 years with IPD attended in three paediatric hospitals: Hospital Vall d'Hebron of Barcelona, Hospital Sant Joan de Déu of Esplugues, Barcelona and Hospital de Nens of Barcelona. The estimated reference population aged <18 years of these hospitals was 442,761, representing 31.9% of this age group in Catalonia.

IPD was diagnosed through the isolation of *S. pneumoniae* by culture or detection of bacterial DNA by real-time polymerase chain reaction (RT-PCR) in any normally sterile site [15].

2.2 Data collected

Epidemiological and clinical characteristics, including age, gender, underlying medical condition, clinical presentation, intensive care unit (ICU) admission, clinical outcome and PCV13 vaccination status were collected. The method of diagnosis, serotype and antimicrobial susceptibility were also analysed.

A subject was considered vaccinated if they had received the last dose of PCV13 ≥15 days before symptom onset.

2.3 Serotype identification and antimicrobial susceptibility

Strains isolated by culture were serotyped using the Quellung reaction or dot-blot at the National Centre of Microbiology (Majadahonda, Spain) [16].When the diagnosis was made only by RT-PCR, serotypes were identified at the Molecular Microbiology Department, Hospital Sant Joan de Déu in accordance with

previously-validated methods [15].The procedure allows the identification of 40 serotypes by a RT-PCR threshold cycle ≤30.

Serotypes were categorized into PCV13 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, 7F, 3, 6A and 19A) and non-PCV13 serotypes (all other serotypes). Serotypes identified by RT-PCR as group level (6A/6C, 9V/A, 19FBC, and 7FA) were defined as PCV13 serotypes 6A, 9V, 19F and 7F, respectively.

Susceptibility to penicillin and cefotaxime were determined by the microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) criteria. Current EUCAST breakpoints were used to interpret susceptibility [17]. A minimum inhibitory concentration (MIC) study was carried out at the National Microbiology Center. The penicillin breakpoints used were: MIC >0.06 mg/L, non-susceptible, and MIC >2 mg/L, resistant. For cefotaxime breakpoint was: MIC >0.5 mg/L, non-susceptible.

2.4 Statistical analysis

Proportions were compared using the χ^2 test or Fisher's exact test, as appropriate. All statistical tests were two tailed and statistical significance was established as $p<0.05$. If the number of cases was zero, this was corrected by adding 0.5, and p-values were calculated according to Sheskin's method [18]

Associations between variables were assessed using the odds ratio (OR) and 95% confidence intervals (CI).

The analyses were conducted using the Statistical Package for Social Sciences (SPSS 19.0 for Windows) and EPIDAT (program for the epidemiological analysis of tabulated data; version 3.1).

2.5 Data confidentiality and ethical aspects

Informed consent was obtained from all individual 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 Ethics Committee of Hospital Sant Joan de Déu approved the study.

Results

3.1 Baseline characteristics

During the study period, 263 cases of IPD were diagnosed in children aged <18 years. Serotyping was not possible in 10 cases (3.8%), which were excluded. Of the 253 cases included, 151 (59.7%) were male; 87 (34.4%) were in children aged <2 years, 98 (38. 7%) in those aged 2–4 years, and 68 (26.9%) in those aged 5–17 years (Table 1).

There was a seasonal variation, with 72% of cases detected during the cool months (October to March; $p<0.001$).

Eighty-three patients (32.8%) had received ≥1 dose of PCV13, while 129 (51.0%) had received no dose of any conjugate vaccine.

Fifteen cases (6.0%) had a chronic disease, including congenital immunodeficiency (2 cases), congenital heart disease (3 cases), chronic pulmonary disease (1 case), chronic kidney disease (1 case), and cerebrospinal fluid leak (1 case), or had received immunosuppressive therapy in the previous 6 months (7 cases).

The most frequent clinical presentation was pneumonia (187 cases; 73.9%), of which 150 cases (80. 2%) were complicated pneumonia. It was followed by occult bacteraemia (25 cases; 9.9%) and meningitis (18 cases; 7.1%). The presentation in the remaining 23 cases (9.1%) was septic shock (7 cases; 2.8%), mastoiditis (7 cases; 2.8%), osteoarticular infection (6 cases; 2.3%), orbital cellulitis (2 cases; 0.8%) and pancreatitis (1 case; 0.4%). Figure 1 shows the distribution of clinical manifestations by age group. Complicated pneumonia was the most frequent clinical presentation in all age groups.

Forty–nine cases (19.4%) were admitted to the ICU: 7 of 7 (100%) with septic shock, 15 of 18 (83.3%) with meningitis, 23 of 150 (15.3%) with complicated pneumonia, 1 of 7 (14.3%) with mastoiditis, 2 of 37 (5.4%) with non-complicated pneumonia and 1 of 25 (4%) with occult bacteraemia (in this case ICU admission was caused by convulsions). Of the 49 ICU cases, 55.1% were caused by PCV13 serotypes, and 14.3% had an underlying disease.

Two patients died: an 18-month-old child with serotype–35F meningitis who had congenital immunodeficiency, and a 23-month-old child with septic shock caused by a non-vaccine serotype which was not identified.

3.2 Serotypes and clinical presentation

One hundred and sixty-three (64.4%) cases were due to PCV13 serotypes (Figure 2); It was 41.5% in patients who had received ≥ 1 dose of PCV13 (34/82 cases) and 75.2% in patients who had not received any conjugated vaccine (97/129; $p<0.001$).

The most frequent serotypes were: 3 (20.9 %), 1 (19.0%), 19A (6.7%) and 14 (5.9%) all included in PCV13.

Overall and complicated pneumonia were mainly caused by PCV13 serotypes (143/187 cases, 76.4% and 123/150 cases, 82.0%, respectively). PCV13 serotypes caused overall pneumonia in 87.7% of cases and non-PCV13 serotypes in 48.9% (OR: 7.47 [4.0–13.96]). Similarly, PCV13 serotypes led to complicated pneumonia in 75.5% of cases and non-PCV13 serotypes in 30% (OR: 7.2 [4.04–12.75]) (Table 2).

Serotype 3 caused overall pneumonia in 92.5% of cases, especially complicated pneumonia (83.0%), and was associated with both clinical presentations (OR:5.5 [1.9–15.92] and, OR:4.3 [2.01–9.35], respectively). Serotype 1 was also associated with overall pneumonia (OR: 21.82 [2.95–161.63]) and complicated pneumonia (OR: 5.16 [2.21–12.04]).

Meningitis was mainly caused by non-PCV13 serotypes (14/18, 77.8%). Although there was no predominant serotype, non-PCV13 serotypes caused meningitis in 15.6% of cases and PCV13 serotypes in 2.5% (OR: 7.32 [2.33–22.99]).

Occult bacteraemia was also caused mainly by non-PCV13 serotypes (16/25, 64%). Non-PCV13 serotypes caused occult bacteraemia in 17.8 % of cases and PCV13 serotypes in 5.5% (OR: 3.6 [1.56–8.76]). Some non-PCV13 serotypes were associated with occult bacteraemia namely 12F (OR: 19.73 [1.72–226.12]) and 10A (OR: 10.22 [1.94–53.74]). Also serotype 18C (PCV13 serotype), showed a slight association (OR: 48.61 [2.26–1043.02]).

Other clinical presentations were caused mainly by non-PCV13 serotypes (16/23; 69.6%). Non-PCV13 serotypes caused these presentations in 17.8% of cases and PCV13 in 4.3% (OR: 4.81 [1.90–12.22]).

3.3 Serotypes and clinical presentation according to age group

PCV13 serotypes were most frequent in the 5–17 years age group (77.9%; OR: 2.41 [1.27–4.59]) and non-PCV13 in <5 years, mainly in the <2 years age group (55.2% OR: 3.63 [2.10–6.29]. Serotype 19A was more frequent in the <2 years age group (58.8%; OR: 2.95 [1.08–8.05]), serotype 3 in the 2–4 years age group (58.5%; OR: 2.79 [1.50–5.20]) and serotype 1 in the 5–17 years age group (60.4%; OR: 6.50 [3.31 –12.77]) (Figure 2).

Table 3 and figure 3 show the serotype distribution by age groups and the association to clinical presentation.

In children aged <2 years, PCV13 serotypes were associated with overall pneumonia (OR: 5.29 [2.08–13.41]) and complicated pneumonia (OR: 4.84 [1.91–12.22]). Serotype 3 caused overall pneumonia in 91.7% of cases (OR: 12.57 [1.54–102.33]) and complicated pneumonia in 83.3% (OR: 10.62 [2.16–52.3]). Serotype 19A was associated with complicated pneumonia (OR: 4.32 [1.03–18.07]) in 70% of cases.

In the 2–4 years age group, PCV13 serotypes were associated with overall pneumonia (OR: 5.42 [1.70–17.23]) and complicated pneumonia (OR: 6.92 [2.61–18.36]), while non-PCV13 serotypes were associated with meningitis (OR: 27.38 [1.42–527.7]). Serotype 3 was associated with complicated pneumonia (OR: 6.7 [1.85–24.24]) and serotype 9V with occult bacteraemia (OR: 102.7 [4.27–2472.3]).

In the 5–17 years age group, PCV13 serotypes were associated with overall pneumonia (OR: 8.17 [1.91–34.86]) and complicated pneumonia (OR: 6.05 [1.81–20.18]), whereas non-PCV13 serotypes were associated with other clinical forms [OR: 9.3 [1.51–57.12]]. Serotype 1 was associated with complicated pneumonia (OR: 10.43 [2.17–50.12])

3.4 Serotypes and method of diagnosis

The diagnosis was made by culture in 62 cases (24.5%), culture and RT-PCR in 69 (27.3%) and by RT-PCR in 122 (48.2%).

PCV13 and non-PCV13 serotypes showed no significant difference in the percentage of cases diagnosed exclusively by PCR (52.1% versus 41.1%).

There were significant differences between serotypes in the method of diagnosis: 86.8% of cases due to serotype 3 were diagnosed only by PCR, versus 38% in the other serotypes ($p<0.001$). By contrast, other serotypes, such as serotype 14 and 24F were diagnosed less frequently by PCR (13.3%, 2/15 and 0%, 0/6) than the other serotypes ($p = 0.005$ and $p = 0.030$, respectively).

3.5 Non-susceptible antibiotics by serotypes

Antimicrobial susceptibility was studied in 122/131 (93.1%) strains isolated. Forty-four cases (36.1%) were non-susceptible to penicillin, and 4 cases (3.3%) were penicillin resistant. Twenty cases (16.4%) were also non-susceptible to cefotaxime (Table 4).

There were no significant differences in the percentage of non-susceptible penicillin strains between PCV13 serotypes (36.1%) and non-PCV13 serotypes (36.0%). PCV13 serotypes were associated with strains not susceptible to cefotaxime ($p = 0.010$) and with isolates not susceptible to both penicillin and cefotaxime ($p = 0.010$).

Serotype 19A showed a penicillin MIC >0.06 mg/L in 90.9% of cases studied, ($p<0.001$) and MIC>2 mg/L in 27.3% ($p = 0.002$). In addition, 54.5% of serotype 19A strains were non-susceptible to cefotaxime ($p = 0.003$) and also non-susceptible to penicillin ($p = 0.003$).

All serotype 14 strains (100%) showed a penicillin MIC >0.06 mg/L ($p<0.001$), and 81.8% of cases were non-susceptible to both cefotaxime ($p<0.001$) and penicillin ($p<0.001$).

Serotype 11A showed non-susceptibility to cefotaxime in 60% of strains compared with 14.5% for other serotypes ($p = 0.031$). These strains also were non-susceptible to penicillin ($p = 0.031$).

Other serotypes showed significant differences in antimicrobial susceptibility, namely 24F which showed penicillin MIC >0.06 mg/L in 83.3% ($p = 0.023$), and 23B in 100% ($p = 0.045$).

Discussion

PCV13 serotypes caused 64.4% of IPD in children aged <18 years during the study period, and the most frequent serotypes were 3, 1, 19A and 14. Other studies in Catalonia have shown a higher frequency of PCV13 serotypes despite the decrease in the incidence in all age groups in this period [19].However, other authors have found a higher percentage of non-PCV13 serotypes. Makwana et al observed that 77.5% of cases of IPD in children aged <5 years in England and Wales between 2010 and 2016 were caused by non-PCV13 serotypes [20]. A study conducted in Madrid (Spain) between 2012 and 2015, found that this percentage was 68.2% in children aged <15 years [21], a figure a little higher than the 55.2% described by Janoir et al [14] in France in 2011–2012 in the same age group. The differences between these results might be explained, at least in part, by a lower PCV13 coverage in Catalan children than in other areas because, during the study period, PCV13 was not included in the Catalan immunization schedule.

In relation with the distribution of the main serotypes, Ceyhan et al [22] found that, in Turkish children in the same age group than us, serotype 19F was the most frequent, followed by 14, 3 and 6B (in equal amounts), while Camilli et al [23], in Italian children aged <5 years in 2012–2014, found that serotypes 1, 19A and 14 were among the four most frequent serotypes, together with 24F. The diagnostic techniques used in these studies may be one of the main explanatory factors for these differences [24]. In our study, serotype 3 was the most frequent and the most frequently diagnosed only by PCR (87% of cases due to serotype 3). When PCR is not available for the diagnosis, another distribution of serotypes in the same age groups may be found, due to under detection.

Serotype 19A was associated with age <2 years, serotype 3 with the 2–4 years age group and serotype 1 with the 5–17 years age group. Other studies have found similar results, with 100% of cases due to serotype 19A in children aged <2 years, 63.6% of cases due to serotype 3 in the 2–4 years age group, and 80% of cases due to serotype 1 in children aged >4 years [21].

PCV13 serotypes were associated with overall pneumonia and complicated pneumonia in children <18 years, mainly serotype 19A in children aged <2 years, serotype 3 in those aged <5 years and serotype 1 in the 5–17 years age group. In a Polish study conducted between 2011 and 2013 in all age groups, it was observed that PCV13 serotypes caused 78.4% of cases of pneumonia, and serotype 3 and serotype 1 were associated to pneumonia as our study [25].

In our study, non-PCV13 serotypes were associated with occult bacteraemia, especially 10A and 12F, as well as meningitis, which showed this association only to in the 2–4 years age group. Similarly, other authors have reported that 90% of meningitis cases and 64% of occult bacteraemia cases during 2011–2012 were produced by non-PCV13 serotypes [26].

The percentage of strains not susceptible to penicillin (36.1%) or to cefotaxime (16.4%) was higher than that described by other authors [23,25]. Serotypes 19A and 14 were associated with strains not susceptible to penicillin, nor to cefotaxime, whereas serotype 23B and 24F were not susceptible to penicillin. Other authors have also described non-susceptibility to penicillin in serotypes 19A and 14 [23,25], in serotype 24F [14,23] or in serotype 23B [27]. Skoczyńska et al [25] found that not susceptibility to penicillin besides was associated with the vaccine-serotypes 19F, 6B and 9V, which in our study could not be demonstrated, perhaps due to the small number of strains studied. They found association of not susceptibility to cefotaxime in serotype 19A and 14 as in our study. The non-susceptibility to antibiotics of non-PCV13 serotypes previously commented is a worrisome fact and should be monitored.

Among the strengths of the study is the high percentage of cases serotyped, since serotyping was not possible in only 3.8% of cases. In addition, the same diagnostic techniques (culture and PCR) were used uniformly in all cases, improving both the diagnostic sensitivity and the range of serotypes identified, which permitted a more precise estimate of the burden of IPD.

There were some limitations. First, the population studied corresponds to three paediatric hospitals, although these hospitals serve 32% of the population aged <18 years requiring hospitalization in Catalonia. In the subgroup analyses, the small number of cases observed in some situations may explain that the confidence intervals of the associations were very wide. Finally, antibiotic sensitivity could only be studied in 48% of cases serotyped, since a large part of the cases were diagnosed by PCR.

Conclusions

PCV13 serotypes were the most frequently found IPD serotypes in children aged <18 years, especially serotype 19A in children aged <2 years, serotype 3 in the 2–4 years age group and serotype 1 in the 5–17 years age group.

Non-PCV13 serotypes were the main cause of meningitis, occult bacteraemia and other clinical presentations, while PCV13 serotypes were mainly responsible for pneumonia.

PCV13 and non-PCV13 cases presented high frequency of non-susceptibility to penicillin. Non-susceptibility to both penicillin and cefotaxime was associated with serotypes 19A and 14, and serotype 19A was associated with resistance to penicillin.

The non-susceptibility to antibiotics of non-PCV13 serotypes is a worrisome fact and should be monitored to apply the appropriate disease prevention strategies.

List Of Abbreviations

IPD: Invasive pneumococcal disease; PCV7: 7-valent pneumococcal conjugate vaccine; PCV13: 13-valent pneumococcal conjugate vaccine; NonPCV13: serotypes not included in 13-valent pneumococcal conjugate vaccine; RT-PCR: real-time polymerase chain reaction; ICU: intensive care unit; CI: confidence intervals.

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all individual participants included in the study. Written informed consent was obtained from a parent o guardian for participants under 16 years old. 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 Ethics Committee of Hospital Sant Joan de Déu approved the study.

Consent for publication

Not applicable

Availability of data and material

The dataset supporting the conclusions of this article is included within the article.

Competing interests

JJ Garcia-Garcia has received honoraria for speaking at symposia (Pfizer and GSK), and Financial support for attending symposia (Pfizer). All outside the submitted work.

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Authors' contributions

CI made substantial contributions to the data collection process and to the conception, design, analysis, and interpretation of data. CI and PC drafted and revised the manuscript and gave the final approval of the version to be published, and CI is the corresponding author. SH, JJGG, CE, FMLL, ADC, JMO, ASR, MFS, SGP, GC, AMP, SU, MC, CMA, LS, and AD made contributions to the interpretation of the data, provided comments on the draft and have read and approved the final version.

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Not Applicable

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Tables

Table 1. Demographic characteristics and clinical presentation in children ≤17 years with IPD.

	N (%) (253 cases)
Age group	
<2 years	87 (34.4)
2-4 years	98 (38.7)
5-17 years	68 (26.9)
Sex	
Male	151 (59.7)
Seasonality	
October-March	182 (72.0)
Diagnosis	
Culture only	62 (24.5)
PCR only	122 (48.2)
Culture + PCR	69 (27.3)
Clinical presentation	
Overall pneumonia	187 (73.9)
Complicated pneumonia	150 (80.2)
Occult bacteraemia	25 (9.9)
Meningitis	18 (7.1)
Other forms[a]	23 (9.1)
ICU admission	
Yes	49 (19.4)
Underlying medical condition	
Yes	15 (6.0)
Case fatality rate	2 (0.8)
Yes	
Serotype groups	
PCV13	163 (64.4)
Non-PCV13	90 (35.6)
*Pneumococcal vaccination**	
PCV7 (≥1 dose)	33 (13.1)
PCV10 (≥1 dose)	7 (2.8)
PCV13 (≥1 dose)	83 (32.9)
Non-vaccinated	129 (51.2)
Antimicrobial susceptibility (n=122 strains)	
Penicillin non-susceptible**	44 (36.1)
Cefotaxime non-susceptible	20 (16.4)

*1 missing case **4 cases were penicillin resistant.

[a] Other forms: septic shock (7 cases), mastoiditis (7 cases), osteoarticular infection (6 cases), orbital cellulitis (2 cases), pancreatitis (1 case).

Abbreviations: IPD, invasive pneumococcal disease; CFR, case fatality rate; ICU, intensive care unit; PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine.

Table 2. Distribution of *Streptococcus pneumoniae* serotypes causing IPD in children aged ≤17 years by clinical presentation.

Serotype	Total		Meningitis			Overall pneumonia			Complicated pneumonia			Occult bacteraemia			Others		
	n	%	n	%	p value*	n	%	p value*	n	%	p value*	n	%	p value*	n	%	p value*
PCV13																	
1	48	19.0	0	0.0	-	47	97.9	<0.001	41	85.4	<0.001	0	0.0	-	1	2.1	ns
3	53	20.9	1	1.9	ns	49	92.5	0.001	44	83.0	<0.001	0	0.0	-	3	5.7	ns
4	2	0.8	0	0.0	-	1	50.0	ns	0	0.0	-	1	50.0	ns	0	0.0	-
6A[a]	3	1.2	0	0.0	-	3	100.0	ns	3	100.0	ns	0	0.0	-	0	0.0	-
6B	2	0.8	0	0.0	-	2	100.0	ns	1	50.0	ns	0	0.0	-	0	0.0	-
7F[a]	9	3.6	0	0.0	-	9	100.0	ns	9	100.0	ns	0	0.0	-	0	0.0	-
9V[a]	6	2.4	0	0.0	-	4	66.7	ns	4	66.7	ns	2	33.3	ns	0	0.0	-
14	15	5.9	1	6.7	ns	12	80.0	ns	9	60.0	ns	1	6.7	ns	1	6.7	ns
18C	2	0.8	0	0.0	-	0	0.0	-	0	0.0	-	2	100.0	0.009	0	0.0	-
19A	17	6.7	1	5.9	ns	15	88.2	ns	12	70.6	ns	0	0.0	-	1	5.9	ns
19F[a]	5	2.0	1	20.0	ns	1	20.0	ns	0	0.0	-	2	40.0	ns	1	20.0	ns
23F	1	0.4	0	0.0	-	0	0.0	-	0	0.0	-	1	100.0	ns	0	0.0	-
Non-PCV13																	
6C	1	0.4	0	0.0	-	1	100.0	ns	0	0.0	-	0	0.0	-	0	0.0	-
8	2	0.8	0	0.0	-	1	50.0		1	50.0	ns	0	0.0	-	1	50.0	ns
10A	6	2.4	1	16.7	ns	1	16.7	ns	0	0.0	-	3	50.0	0.014	1	16.7	ns
11A	5	2.0	0	0.0	-	3	60.0	ns	1	20.0	ns	0	0.0	-	2	40.0	ns
12F	3	1.2	0	0.0	-	1	33.3	ns	1	33.3	ns	2	66.7	0.026	0	0.0	-
13	1	0.4	1	100.0	ns	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
15A	3	1.2	1	33.3	ns	0	0.0	ns	0	0.0	-	1	33.3	ns	1	33.3	ns
15B	2	0.8	1	50.0	ns	0	0.0	-	0	0.0	-	1	50.0	ns	0	0.0	-
15C	1	0.4	0	0.0	-	0	0.0	-	0	0.0	-	1	100.0	ns	0	0.0	-
16F	2	0.8	1	50.0	ns	0	0.0	-	0	0.0	-	1	50.0	ns	0	0.0	-
22F	2	0.8	1	50.0	ns	0	0.0	-	0	0.0	-	0	0.0	-	1	50.0	ns
23A	1	0.4	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-	1	100.0	ns
23B	3	1.2	0	0.0	-	1	33.3	ns	1	33.3	ns	1	33.3	ns	1	33.3	ns
24	2	0.8	0	0.0	-	1	50.0	ns	0	0.0	-	1	50.0	ns	0	0.0	-
24A	2	0.8	1	50.0	ns	1	50.0	ns	0	0.0	-	0	0.0	-	0	0.0	-
24F	6	2.4	1	16.7	ns	4	66.7	ns	2	33.3	ns	1	16.7	ns	0	0.0	
25F	1	0.4	0	0.0	-	0	0.0	-	0	0.0	-	1	100.0	ns	0	0.0	-
27	2	0.8	0	0.0	-	0	0.0	-	0	0.0	-	1	50.0	ns	1	50.0	ns
31	2	0.8	0	0.0	-	0	0.0	-	0	0.0	-	1	50.0	ns	1	50.0	ns
33F	2	0.8	0	0.0	-	1	50.0	ns	0	0.0	-	0	0.0	-	1	50.0	ns
35B	1	0.4	0	0.0	-	0	0.0	-	0	0.0	-	1	100.0	ns	0	0.0	-
35F	1	0.4	1	100.0	ns	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
38	2	0.8	0	0.0	-	1	50.0	ns	1	50.0	ns	0	0.0	-	1	50.0	ns
ONV	37	14.6	5	13.5	-	28	75.7	-	20	54.1	-	0	0.0	-	4	10.8	-
Total	253	100.0	18	7.1	-	187	73.9	-	150	59.3	-	25	9.9	-	23	9.1	-

[*] The p value indicates differences in the relationship between a serotype and a clinical form with respect to the other serotypes. "ns" indicates p value≥0.05.

[a] 7F: Four cases were identified as group level (7FA); 9V: two cases were identified as group level (9V/A); 19F: one case was identified as group level (19FBC); 6A: two cases were identified as group level (6A/6C).

Abbreviations: IPD, invasive pneumococcal disease; ONV, Other non-vaccine serotypes.

Table 3. Distribution of *Streptococcus pneumoniae* serotype groups causing IPD in children ≤17 years by clinical presentation and age group.

Age groups	Serotype group	Total		Clinical presentation														
				Meningitis			Overall pneumonia			Complicated pneumonia			Occult bacteraemia			Other forms		
0-17 years		n	%	n	%	p value*	n	%	p value*	n	%	p value*	n	%	p value*	n	%	p value*
	PCV13	163	64.4	4	2.5		143	87.7	<0.001	123	75.5	<0.001	9	5.5		7	4.3	
	Non-PCV13	90	35.6	14	15.6	<0.001	44	48.9		27	30.0		16	17.8	0.002	16	17.8	<0.001
	Total	253	100.0	18	7.1	-	187	73.9	-	150	59.3	-	25	9.9	-	23	9.1	-
<2 years																		
	PCV13	39	44.8	3	7.7	-	29	74.4	<0.001	23	59.0	0.001	4	10.3	-	3	7.7	-
	Non-PCV13	48	55.2	10	20.8	ns	17	35.4	-	11	22.9	-	12	25	ns	9	18.8	ns
	Total	87	100.0	13	14.9		46	52.9		34	39.1		16	18.4		12	13.8	
2-4 years																		
	PCV13	71	72.4	0	0	-	65	91.5	0.004	57	80.3	<0.001	4	5.6	-	2	2.8	-
	Non-PCV13	27	27.6	4	14.8	0.005	18	66.7	-	10	37.0	-	2	7.4	ns	3	11.1	ns
	Total	98	100.0	4	4.1		83	84.7		67	68.4		6	6.1		5	5.1	
5-17 years																		
	PCV13	53	77.9	1	1.9	ns	49	92.5	0.006	43	81.1	0.002	1	1.9	-	2	3.8	-
	Non-PCV13	15	22.1	0	0	-	9	60.0	-	6	40.0	-	2	13.3	ns	4	26.7	0.018
	Total	68	100.0	1	1.5		58	85.3		49	72.1		3	4.4		6	8.8	

* The p value indicates differences in the relationship between a serotype group and a clinical form with respect to the other serotypes.

"ns" indicates p value≥0.05.

Table 4: Distribution of *Streptococcus pneumoniae* serotypes causing IPD in children ≤17 years by non-susceptibility to antibiotics.

Serotype	Total cases	Analyzed cases		Penicillin >0.06 [a]			Penicillin>2[b]			Cefotaxime>0.5[c]			Penicillin >0.06 + Cefotaxime>0.5[d]		
	n	n	%	n	%	p value*	n	%	p value*	n	%	p value*	n	%	p value *
PCV13															
1	48	27	56.3	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
3	53	6	11.3	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
4	2	2	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
6A[e]	3	1	33.3	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
6B	2	1	50.0	1	100	ns	0	0.0	-	0	0.0	-	0	0.0	-
7F[e]	9	1	11.1	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
9V[e]	6	5	83.3	1	20.0	ns	0	0.0	-	1	20.0	ns	1	20.0	ns
14	15	11	73.3	11	100.0	<0.001	0	0.0	-	9	81.8	<0.001	9	81.8	<0.001
18C	2	2	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
19A	17	11	64.7	10	90.9	<0.001	3	27.3	0.002	6	54.5	0.003	6	54.5	0.003
19F[e]	5	4	80.0	3	75.0	ns	1	25.0	ns	1	25.0	ns	1	25.0	ns
23F	1	1	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
Non-PCV13															
6C	1	1	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
8	2	2	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
10A	6	5	83.3	0	0.0	-	0	0.0	-	0	0.0		0	0.0	
24F	6	6	100.0	5	83.3	0.023	0	0.0	-	0	0.0	-	0	0.0	-
11A	5	5	100.0	3	60.0	ns	0	0.0	-	3	60.0	0.031	3	60.0	0.031
12F	3	3	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
13	1	1	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
15A	3	3	100.0	1	33.3	ns	0	0.0	-	0	0.0	-	0	0.0	-
15B	2	2	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
15C	1	1	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
16F	2	2	100.0	1	50.0	ns	0	0.0	-	0	0.0	-	0	0.0	-
22F	2	2	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
23B	3	3	100.0	3	100.0	0.045	0	0.0	-	0	0.0	-	0	0.0	-
24	2	2	100.0	2	100.0	ns	0	0.0	-	0	0.0	-	0	0.0	-
24A	2	2	100.0	2	100.0	ns	0	0.0	-	0	0.0	-	0	0.0	-
27	2	2	100.0	1	50.0	ns	0	0.0	-	0	0.0	-	0	0.0	-
31	2	2	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
33F	2	2	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
35B	1	1	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
35F	1	1	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
38	2	2	100.0	0	0.0	-	0	0.0	-	0	0.0	-	0	0.0	-
PCV13	163	72	44.2	26	36.1	ns	4	5.6	ns	17	23.6	0.010	17	23.6	0.010
Non-PCV13	90	50	55.6	18	36.0		0	0.0		3	6.0		3	6.0	
Total	253	122	48.2	44	36.1	--	4	3.3	--	20	16.4	--	20	16.4	--

[(a)] Number and percentage of isolates of a given serotype, with penicillin MIC >0.06mg/L; [(b)] With penicillin MIC >2.0mg/L; [(c)] With cefotaxime MIC >0.5mg/L; [(d)] Not susceptible to both penicillin and cefotaxime.

[(e)] 7F: Four cases were identified as group level (7FA); 9V: two cases were identified as group level (9V/A); 19F: one case was identified as group level (19FBC); 6A: two cases were identified as group level (6A/6C).

[*] The p value indicates differences in the relationship between a serotype and non-susceptibility to an antibiotic with respect to the other serotypes. “ns” indicates p value≥0.05.

Abbreviations: IPD, invasive pneumococcal disease; ONV, Other non-vaccine serotypes.

Figures

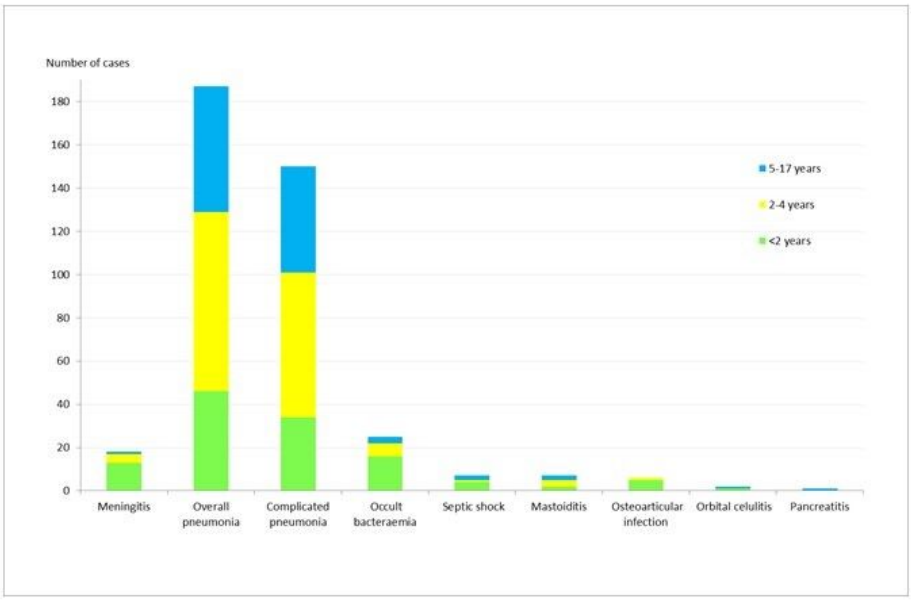

Figure 1

Distribution of IPD clinical presentation in children ≤17 years by age group. IPD: invasive pneumococcal disease

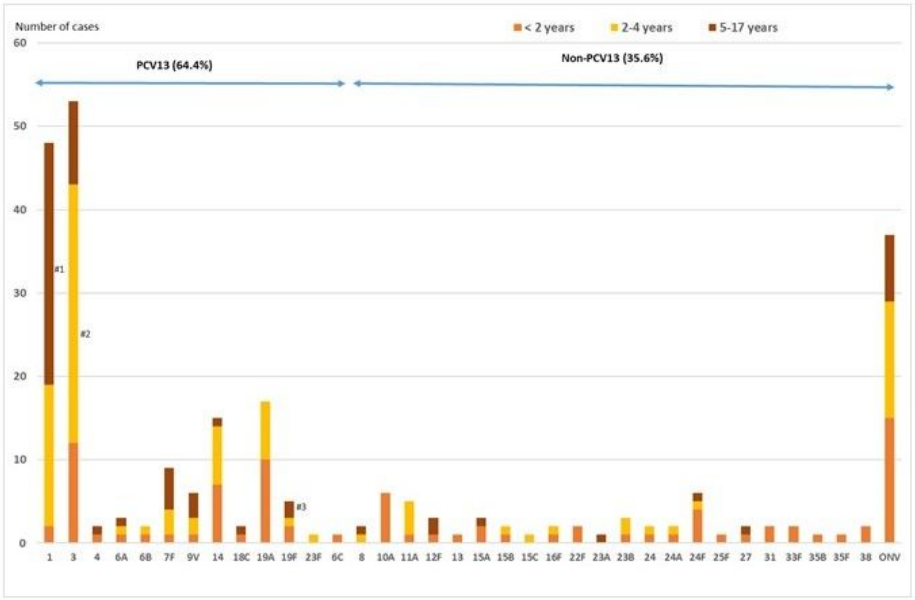

Figure 2

Distribution of Streptococcus pneumoniae serotypes causing IPD in children ≤17 years by age groups. IPD: invasive pneumococcal disease. ONV: Other non-vaccine serotypes. #1: p<0.001; #2: p=0.001; #3: p=0.028

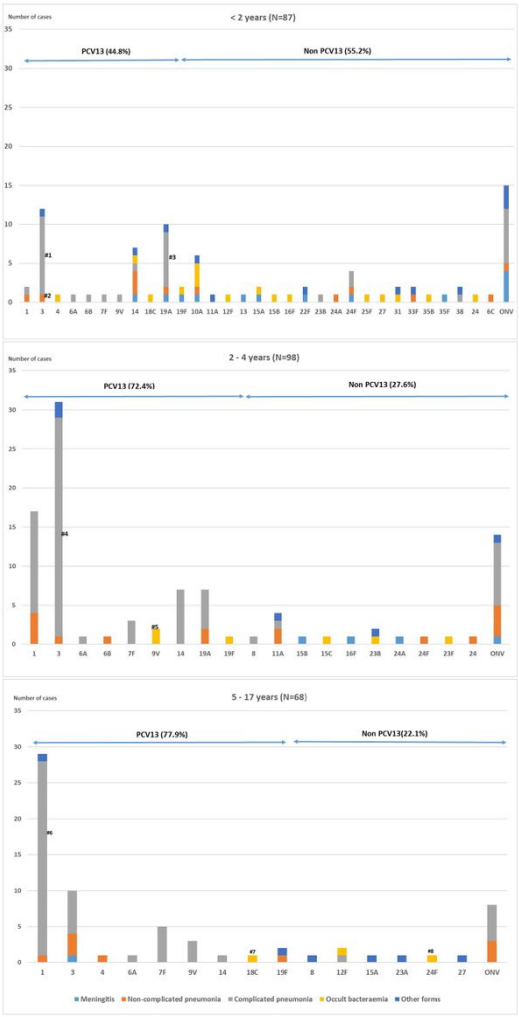

Figure 3

Distribution of Streptococcus pneumoniae serotypes in children ≤17 years by clinical presentation. • <2 age group. #1: p<0.001; #2: p=0.004 (Non-complicated and complicated pneumonia); #3:p= 0.033. • 2-4 age group. #4: p=0.002; #5:p=0.003 • 5-17 age group. #6:p<0.001; #7: p=0.004; #8: p=0.004 The p value indicates differences in the relationship between a serotype and a clinical form with respect to the other serotypes. IPD: invasive pneumococcal disease. ONV: Other non-vaccine serotypes.

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