Decreasing incidence and changes in serotype distribution of invasive pneumococcal disease in persons aged under 18 years since introduction of 10-valent and 13-valent conjugate vaccines in Portugal, July 2008 to June 2012

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The 10-valent pneumococcal conjugate vaccine (PCV10) became available in Portugal in mid-2009 and the 13-valent vaccine (PCV13) in early 2010. The incidence of invasive pneumococcal disease (IPD) in patients aged under 18 years decreased from 8.19 cases per 100,000 in 2008–09 to 4.52/100,000 in 2011–12. However, IPD incidence due to the serotypes included in the 7-valent conjugate vaccine (PCV7) in children aged under two years remained constant. This fall resulted from significant decreases in the number of cases due to: (i) the additional serotypes included in PCV10 and PCV13 (1, 5, 7F; from 37.6% to 20.6%), particularly serotype 1 in older children; and (ii) the additional serotypes included in PCV13 (3, 6A, 19A; from 31.6% to 16.2%), particularly serotype 19A in younger children. The decrease in serotype 19A before vaccination indicates that it was not triggered by PCV13 administration. The decrease of serotype 1 in all groups, concomitant with the introduction of PCV10, is also unlikely to have been triggered by vaccination, although PCVs may have intensified and supported these trends. PCV13 serotypes remain major causes of IPD, accounting for 63.2% of isolates recovered in Portugal in 2011–12, highlighting the potential role of enhanced vaccination in reducing paediatric IPD in Portugal.

Introduction

Introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) led to changes in the circulating serotypes of *Streptococcus pneumoniae* and also often to decreases in the incidence of invasive pneumococcal disease (IPD) worldwide [1-3]. Two new pneumococcal conjugate vaccine (PCV) formulations are now commercially available and are used for children [4]. A 10-valent formulation (PCV10) including, in addition to the PCV7 serotypes, serotypes 1, 5 and 7F and a 13-valent conjugate vaccine (PCV13), including all PCV10 serotypes plus serotypes 3, 6A and 19A. The few available early reports point to the effectiveness of these expanded valency vaccines against the serotypes included in their formulations [4-8].

In Portugal, PCVs are not included in the national immunisation plan. Had they been, they would have been offered free of charge. Nevertheless, since 2001, when the vaccine became available, there has been a steady increase in PCV7 uptake bought privately, without any reimbursement, reaching 75% of children aged 2 years or under in 2008 [9]. PCV10 became available for childhood vaccination in mid-2009 and PCV13 in early 2010. Soon after PCV13 became available, according to sales data, this vaccine was mostly used (data not shown).

In previous studies, we showed that significant changes in the serotypes causing IPD in children followed PCV7 availability in Portugal [9,10] and that there was evidence for a herd effect in the adult population [9,11,12]. Serotypes 1 and 7F, both included in PCV10 and PCV13, and serotype 19A, included in PCV13, emerged as major causes of paediatric (persons aged under 18 years) IPD in the post-PCV7 period in Portugal [10]. Given the limited information on the efficacy of PCV10 and PCV13, this study aimed at documenting the potential effects of vaccination on serotype distribution, antimicrobial resistance and incidence of paediatric IPD in Portugal from July (week 26) 2008 and June (week 25) 2012.

Table 1

Population under 18 years during the study period, Portugal, 2008–12

Calendar year	Number of persons per age group				Total
	0–11 months	12–23 months	2–4 years	5–17 years	
2008	103,746	101,339	318,186	1,454,385	**1,977,656**
2009	98,759	103,011	311,641	1,444,426	**1,957,837**
2010	100,492	96,995	302,067	1,429,777	**1,929,331**
2011	95,703	99,519	294,727	1,412,241	**1,902,190**
2012	92,651	97,591	295,215	1,405,356	**1,890,813**

Methods

Bacterial isolates

Since 2007, the Portuguese Group for the Study of Streptococcal Infections and the Portuguese Study Group of Invasive Pneumococcal Disease of the Paediatric Infectious Disease Society have monitored pneumococcal invasive infections in Portugal. During the study period, this involved the microbiology laboratories and paediatric departments of 61 hospitals throughout Portugal. The network includes centres covering the entire country, including all referral hospitals and most centres where microbiological diagnostic services are available. All centres included in the study reported during the entire period.

A case of IPD was defined as a person from whom an isolate of *S. pneumoniae* was recovered from a normally sterile body site (not including middle ear fluid) or from whom pneumococcal DNA was detected in cerebrospinal fluid (CSF) or pleural fluid. Isolates recovered up to 2008 were previously characterised [9,10,13]. Isolates recovered from patients aged under 18 years between July 2008 and June 2012 were included in the present study. Epidemiological years were defined as spanning from week 26 of one year to week 25 of the following year.

Only one isolate from each patient was considered. All strains were identified as *S. pneumoniae* by colony morphology and haemolysis on blood agar plates, optochin susceptibility and bile solubility. The *lytA* gene was used to identify pneumococci in CSF or pleural fluid.

Incidences were calculated based on the entire Portuguese population of the relevant age groups using data available from the Instituto Nacional de Estatística [14] (Table 1), using the population data of the first calendar year of each epidemiological year. The calculation assumes that all IPD cases were treated at the 61 hospitals in our network. Four age groups were considered: infants aged less than 12 months, children aged 12–23 months, children aged from two to four years and children and adolescents aged from five years to less than 18 years.

Serotyping and antimicrobial susceptibility testing

Serotyping was performed by the standard capsular reaction test using the chessboard system [15] and specific sera (Statens Serum Institut, Copenhagen, Denmark). Serotypes were classified into vaccine serotypes, i.e. those included in PCV7 (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F), the additional three found in PCV10 (addPCV10: 1, 5, 7F), the additional three found

Table 2

Cases of invasive pneumococcal disease and available *Streptococcus pneumoniae* isolates from patients aged under 18 years, Portugal, July 2008–June 2012[a]

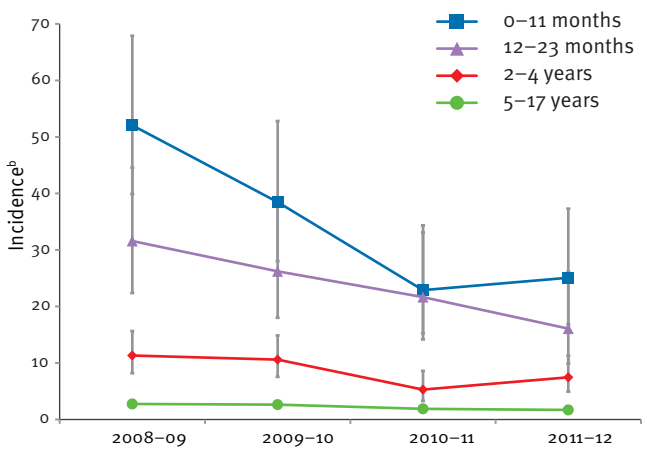
Epidemiological years[a]	Number of cases[b]/available isolates, by age group				Total
	0–11 months	12–23 months	2–4 years	5–17 years	
2008–09	54/49	32/26	36/25	40/33	**162/133**
2009–10	38/35	27/23	33/21	38/35	**136/114**
2010–11	23/20	21/19	16/13	27/25	**87/77**
2011–12	24/17	16/16	22/19	24/16	**86/68**
Total	**139/121**	**96/84**	**107/78**	**129/109**	**471/392**

[a] From week 26 of one year to week 25 of the following year.

[b] Case numbers comprise patients from whom pneumococci were isolated from a normally sterile site or pneumococcal DNA was detected in cerebrospinal fluid or pleural fluid.

Figure 1

Incidence of invasive pneumococcal disease in persons aged under 18 years, Portugal, July 2008–June 2012[a]



The bars represent 95% confidence intervals for the incidence estimates.

[a] Epidemiological years: from week 26 of one year to week 25 of the following year.

[b] Number of cases per 100,000 in specified age group.

in PCV13 (addPCV13: 3, 6A, 19A) and non-vaccine serotypes (NVT).

Etest strips (AB Biodisk, Solna, Sweden) were used to determine the minimal inhibitory concentrations (MICs) for penicillin, cefotaxime, ceftriaxone and levofloxacin. We opted to use the Clinical and Laboratory Standards Institute (CLSI) guidelines because these were the standards in use in Portugal during the study period. In 2008, the CLSI changed the recommended breakpoints to those currently used to interpret MIC values [16]. Unless otherwise stated, we have used the CLSI-recommended breakpoints before 2008 [17] as epidemiological breakpoints that allow comparison with previous studies.

Isolates were further characterised by determining their susceptibility to erythromycin, clindamycin, vancomycin, linezolid, tetracycline, trimethoprim-sulfamethoxazole and chloramphenicol by the Kirby-Bauer disk diffusion technique, according to the CLSI recommendations and interpretative criteria [16]. Macrolide resistance phenotypes were identified using a double disc test with erythromycin and clindamycin. Simultaneous resistance to erythromycin and clindamycin defines de MLS_B phenotype (resistance to macrolides, lincosamides and streptogramin B) while non-susceptibility only to erythromycin defines the M phenotype.

Statistical analysis

Simpson's index of diversity (SID) and respective 95% confidence intervals (CIs) was used to measure the serotype diversity [18]. The SID measures the probability that two isolates sampled at random will not share the same serotype. The Cochran–Armitage test (a modification of the Pearson chi-squared test to incorporate a suspected ordering in the effects of the categories of the second variable) was used for trends with the false discovery rate (FDR) correction for multiple testing [19]. A p value of less than 0.05 was considered significant for all tests.

Results

Isolate collection

Between July 2008 and June 2012, a total of 471 cases of IPD were reported in Portugal. Their distribution by age and epidemiological year is shown (Table 2) and the annual incidence of IPD by age group is presented (Figure 1). Although starting from very disparate values, IPD incidence decreased significantly in all age groups when comparing 2008–09 with 2011–12 (0–11 months, p<0.001; 12–23 months and 2–4 years, p=0.002; and 5–17 years, p=0.003; all robust after FDR) (Table 3).

For the majority of IPD cases (n=430), pneumococci were isolated from a normally sterile site. Only 41 cases involved solely the identification of pneumococcal DNA in CSF or pleural fluid: for these cases, no capsular serotype information is available. Of the 430 IPD cases from whom *S. pneumoniae* bacteria were recovered, 392 isolates were available for further characterisation. The remaining 38 isolates were lost before reaching the central laboratory for characterisation. Available isolates were recovered from blood (n=335, 85.5%), CSF (n=39, 9.9%), pleural fluid (n=15, 3.8%) and peritoneal fluid (n=3, 0.8%).

Serotype distribution

We detected 39 different capsular types as well as non-typable isolates among the 392 available isolates (Figure 2). The most frequent, which accounted for 57% (n=225) of isolates from all analysed IPD cases in the study period, were serotypes 1 (n=74, 18.9%), 19A (n=72, 18.4%), 7F (n=43, 11.0%), and 14 (n=36, 9.2%). The proportion of IPD cases caused by PCV7 serotypes remained relatively constant, at around 21%. However, the fraction of IPD cases that could have been potentially prevented by PCV10 and PCV13 decreased significantly during the study period, from 59.4% to 47.1% (p=0.008) and from 91.0% to 63.0% (p=0.024), respectively. This was accompanied by an increase in serotype diversity when comparing 2008–09 (SID=0.846, CI95%: 0.810 to 0.882) with 2011–12 (SID=0.957, 95% CI: 0.939–0.975).

In order to estimate the incidence of IPD due to individual serotypes, the cases for which no isolate was available (n=79) were assumed to have the same serotype distribution as that found among isolates from

Table 3

Incidence of invasive pneumococcal infections caused by *Streptococcus pneumoniae* serotypes included in conjugate vaccine formulations by age group, Portugal, July 2008–June 2012[a]

Age group	Serotype group	Incidence[b] (95% confidence intervals)			
		2008–09	2009–10	2010–11	2011–12
0–17 years	PCV7	1.75 (1.26–2.44)	1.41 (0.97–2.04)	0.82 (0.51–1.34)	1.10 (0.72–1.69)
	PCV10	4.98 (4.09–6.07)	3.66 (2.90–4.61)	2.01 (1.47–2.75)	2.17 (1.60–2.94)
	PCV13	7.50 (6.38–8.81)	5.27 (4.34–6.39)	3.10 (2.41–3.99)	2.87 (2.21–3.74)
	NVT	0.69 (0.41–1.17)	1.68 (1.20–2.36)	1.41 (0.97–2.05)	1.65 (1.16–2.33)
	All serotypes	8.19 (7.06–9.55)	6.95 (5.92–8.22)	4.51 (3.68–5.56)	4.52 (3.66–5.58)
5–17 years	PCV7	0.33 (0.14–0.79)	0.38 (0.17–0.85)	0 (0–0.27)	0 (0–0.27)
	PCV10	2.17 (1.53–3.07)	1.65 (1.11–2.46)	0.83 (0.47–1.46)	1.17 (0.72–1.88)
	PCV13	2.67 (1.95–3.65)	2.10 (1.48–3.00)	1.51 (0.99–2.30)	1.17 (0.72–1.88)
	NVT	0.08 (0.02–0.41)	0.53 (0.26–1.06)	0.38 (0.17–0.86)	0.53 (0.26–1.07)
	All serotypes	2.75 (2.02–3.74)	2.63 (1.92–3.61)	1.89 (1.30–2.75)	1.7 (1.14–2.53)
2–4 years	PCV7	1.36 (0.55–3.38)	2.02 (0.94–4.33)	0.41 (0.08–2.00)	2.36 (1.14–4.88)
	PCV10	9.5 (6.67–13.55)	8.07 (5.47–11.9)	3.67 (2.05–6.55)	3.14 (1.67–5.92)
	PCV13	11.31 (8.17–15.66)	9.58 (6.71–13.69)	4.07 (2.35–7.07)	5.11 (3.10–8.42)
	NVT	0 (0–1.21)	1.01 (0.35–2.90)	1.22 (0.46–3.26)	2.36 (1.14–4.88)
	All serotypes	11.31 (8.17–15.66)	10.59 (7.54–14.87)	5.30 (3.26–8.60)	7.46 (4.93–11.3)
12–23 months	PCV7	12.15 (7.00–21.08)	7.98 (4.08–15.61)	6.84 (3.25–14.38)	7.03 (3.41–14.52)
	PCV10	14.57 (8.80–24.14)	11.40 (6.48–20.04)	6.84 (3.25–14.38)	7.03 (3.41–14.52)
	PCV13	27.93 (19.37–40.29)	20.51 (13.44–31.32)	13.67 (8.03–23.27)	9.04 (4.76–17.19)
	NVT	3.64 (1.37–9.71)	5.70 (2.59–12.53)	7.98 (4.00–15.92)	7.03 (3.40–14.52)
	All serotypes	31.58 (22.37–44.57)	26.21 (18.02–38.13)	21.65 (14.16–33.1)	16.08 (9.9–26.12)
0–11 months	PCV7	12.75 (7.48–21.71)	7.70 (3.83–15.45)	8.01 (4.07–15.78)	7.38 (3.58–15.18)
	PCV10	21.24 (14.04–32.16)	10.99 (6.12–19.76)	9.15 (4.85–17.28)	8.85 (4.57–17.15)
	PCV13	43.55 (32.57–58.23)	21.99 (14.48–33.38)	12.59 (7.30–21.69)	14.75 (8.81–24.71)
	NVT	8.50 (4.44–16.26)	16.49 (10.19 – 26.68)	10.30 (5.65 – 18.77)	10.32 (5.59–19.08)
	All serotypes	52.05 (39.9–67.90)	38.48 (28.04 – 52.81)	22.89 (15.25 – 34.34)	25.08 (16.85–37.31)

NVT: non-vaccine serotypes; PCV: pneumococcal conjugate vaccine.

[a] Epidemiological years: from week 26 of one year to week 25 of the following year.
[b] Number of cases per 100,000 in specified age group.

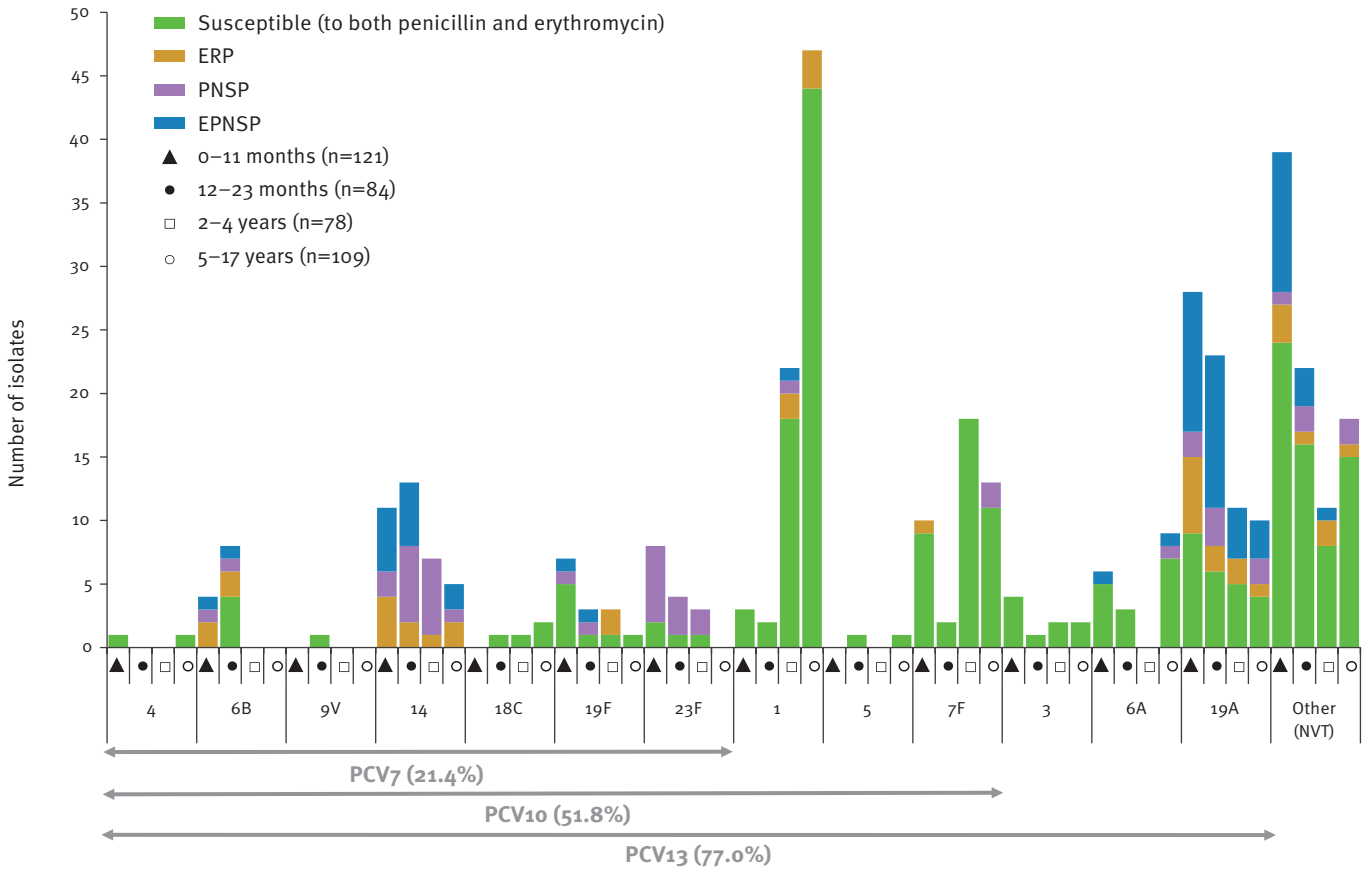
the same epidemiological year and age group. The overall incidence of IPD in all age groups due to PCV7, addPCV10 and addPCV13 serotypes decreased during the study period (p=0.026, p<0.001 and p<0.001 respectively, robust after FDR) while that of cases with NVT increased (p=0.027, also robust after FDR) (Figure 3A, Table 3). While IPD incidence due to the serotypes included in the conjugate vaccines decreased, these were still a significant cause of IPD in all age groups, accounting for 62.3% (n=43) of the isolates in 2011–12. When considering individual serotypes (for which at least five isolates were detected), the incidence of IPD due to serotypes 1 (p<0.001), 19A (p<0.001), 7F (p=0.024) and 14 (p=0.015) decreased and increases in serotype 10A (p=0.001) were noted. However, only the decrease in serotypes 1 and 19A incidence and the increase in 10A incidence were supported after adjustment by FDR.

In contrast to the continual decreases in the number of isolates of serotype 1 and 19A during the study period, serotype 7F decreased only in 2010–11 after the introduction of PCV10 (Table 4). These resulted in decreases in the proportion of isolates of addPCV10 from 37.6% in 2008–09 to 20.6% in 2011–12 and of addPCV13 from 31.6% in 2008–09 to 16.2% in 2011–12.

The overall changes in IPD incidence are the result of very different dynamics in the various age groups (Figure 3 and Table 3). In the youngest age group (0–11 months), IPD incidence due to the additional serotypes included in the expanded valency conjugate vaccines decreased (addPCV10, p=0.006; addPCV13, p<0.001, both robust after FDR) while the incidence due to PCV7 serotypes and NVTs did not change significantly. A similar situation occurred in those aged 12–23 months, but here only the addPCV13 (p=0.004) was robust after FDR.

Figure 2

Streptococcus pneumoniae isolates expressing serotypes present in conjugate vaccines causing invasive pneumococcal infections, Portugal, July 2008–June 2012 (n=392)



EPNSP: isolates presenting both erythromycin resistance and penicillin non-susceptibility; ERSP: erythromycin-resistant isolates; NVT: non-vaccine serotypes; PNSP: penicillin non-susceptible isolates.

The number of isolates expressing each serotype in each of the age groups considered is shown. The serotypes included in each of the conjugate vaccines are indicated by the arrows and the percentage of the total number of isolates expressing the serotypes included in each of the vaccines is indicated. A total of 27 NVT were detected, representing 90 isolates as follows: 10A, 15A and 23B (n=7 each); 22F (n=6); 8, 15B, 15C, 21 and 24F (n=5 each); 25A (n=4); 9N, 11A, 20, 24A, 33A and non-typable (n=3 each); 6C, 16F, 29, 33F and 35F (n=2 each); 7C, 12B, 18B, 28A, 34 and 45 (n=1 each).

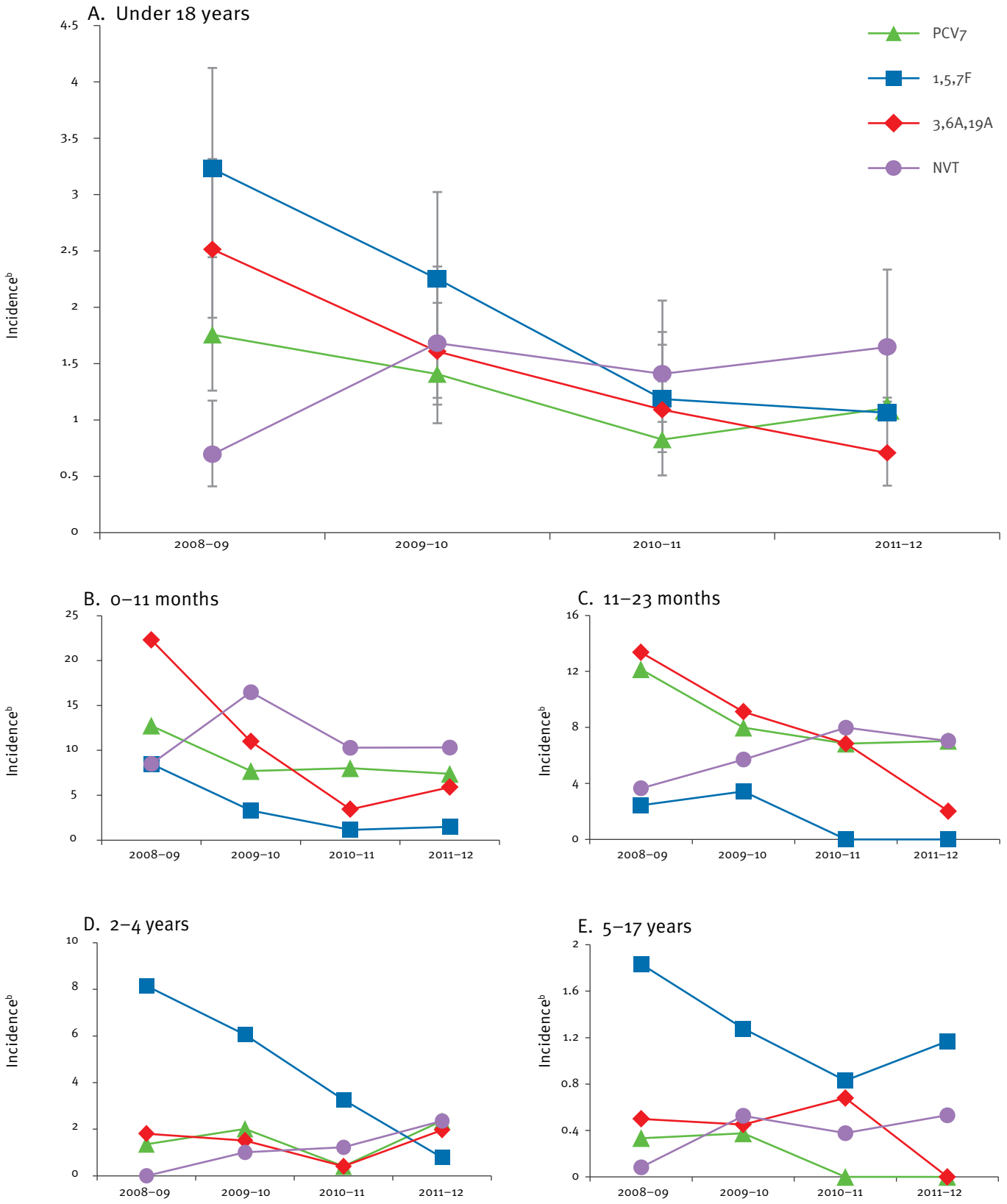
In patients aged 2–4 years, there was no significant change in IPD incidence due to addPCV13, but there was a substantial decrease in IPD incidence due to the addPCV10 serotypes ($p<0.001$) accompanied by an increase in IPD incidence due to NVTs ($p=0.007$), both supported after adjustment by FDR. In the older age group (5–17 years), in which no individuals were vaccinated with PCV10 or PCV13, the decreases in IPD incidence due to addPCV10 and addPCV13 serotypes were not statistically significant: the only significant change was the decrease in IPD incidence due to PCV7 serotypes ($p=0.006$, robust after FDR).

Antimicrobial susceptibility

Resistance to the tested antimicrobials is summarised in Figure 2 and Table 5. Overall, 110/392 isolates (28.1%) were non-susceptible to penicillin (PNSP): 82 (20.9%) expressed low-level resistance and 28 (7.1%) high-level resistance [17]. Considering the current CLSI breakpoints for parenteral penicillin [16], 16/39 isolates from CSF would have been considered resistant and three isolates (0.8%) from 353 non-meningitis cases would have been considered non-susceptible, all intermediately resistant. Resistance to erythromycin (ERP) was found in 103/392 isolates (26.0%), of which 78 isolates (75.7%) expressed the MLS_B phenotype and 25 (24.3%) the M phenotype. All isolates were susceptible to levofloxacin, vancomycin and linezolid. The simultaneous expression of erythromycin resistance and penicillin non-susceptibility (EPNSP) was found in 64/392 (16.3%) of the isolates. Within the study period, there was a modest decrease in ERP (when comparing 36/133 (27.1%) in 2008–09 with 16/68 (23.5%) in 2011–12) and a more noticeable decrease in PNSP (when comparing 45/133 (33.8%) in 2008–09 with 14/68 (20.6%) in 2011–12).

Figure 3

Incidence of invasive pneumococcal disease due to vaccine serotypes in patients aged under 18 years, Portugal, July 2008–June 2012[a]



NVT: non-vaccine serotypes; PCV7: 7-valent pneumococcal conjugate vaccine.

[a] Epidemiological years: from week 26 of one year to week 25 of the following year.

[b] Number of cases per 100,000 in specified age group.

Table 4

Serotypes of the isolates responsible for invasive pneumococcal disease in patients aged under 18 years, Portugal, July 2008–June 2012[a] (n=392)

Serotype	Number of isolates			
	2008–09	2009–10	2010–11	2011–12
1	33	20	14	7
3	2	3	1	3
4	1	0	0	1
5	2	0	0	0
6A	5	5	5	3
6B	5	2	2	3
7F	15	15	6	7
9V	0	0	1	0
14	13	12	6	5
18C	0	3	0	1
19A	35	19	13	5
19F	5	2	3	4
23F	5	4	2	4
NVT	12	29	24	25
Total	**133**	**114**	**77**	**68**

NVT: non-vaccine type.

[a] Epidemiological years: from week 26 of one year to week 25 of the following year.

Table 5

Antimicrobial resistance of *Streptococcus pneumoniae* isolates responsible for invasive pneumococcal disease in patients aged under 18 years, Portugal, July 2008–June 2012[a] (n=392)

Antibiotic	Number of resistant isolates (%)[b]			
	0–11 months (n=121)	12–23 months (n=84)	2–4 years (n=78)	[5–17 years (n=109)
PEN[c]	43 (35.5)	38 (45.2)	15 (19.2)	14 (12.8)
MIC_{90}	0.75	1.5	0.75	0.064
MIC_{50}	0.023	0.032	0.016	0.016
CTX	4 (3.3)	7 (8.3)	1 (1.3)	2 (1.8)
MIC_{90}	0.75	0.75	0.5	0.125
MIC_{50}	0.023	0.032	0.023	0.023
CRO	6 (5.0)	4 (4.8)	0 (0.0)	2 (1.8)
MIC_{90}	0.75	1	0.5	0.125
MIC_{50}	0.032	0.032	0.032	0.023
ERY	46 (38.0)	29 (34.5)	15 (19.2)	13 (11.9)
CLI	34 (28.1)	23 (27.4)	13 (16.7)	8 (7.3)
CHL	6 (5.0)	4 (4.8)	0 (0.0)	4 (3.7)
SXT	27 (22.3)	21 (25.0)	16 (20.5)	15 (13.8)
TET	29 (24.0)	21 (25.0)	11 (14.1)	13 (11.9)

CHL: chloramphenicol; CLI: clindamycin; CRO: ceftriaxone; CTX: cefotaxime; ERY: erythromycin; LEV: levofloxacin; MIC: minimal inhibitory concentration; PEN: penicillin; SXT: trimethoprim-sulfamethoxazole; TET: tetracycline.

[a] Epidemiological years: from week 26 of one year to week 25 of the following year.

[b] Unless otherwise specified.

[c] Number of isolates and percentage of penicillin non-susceptible isolates is indicated.

Together, serotypes 19A and 14 contributed greatly to ERP (62/103, 60.2%) and PNSP (64/110, 58.2%) (Figure 2). Serotypes included in PCV10 represented 33/46 (71.7%), 21/39 (53.8%) and 17/64 (26.6%) of PNSP, ERP and EPNSP, respectively, while serotypes included in PCV13 constituted 41/46 (89.1%), 32/39 (82.1%) and 49/64 (76.6%), respectively.

Discussion

The most important finding of our study is the decrease in incidence of IPD in all age groups analysed. Moreover, the changes in distribution of the serotypes of pneumococci causing IPD in Portugal that accompanied the introduction of PCV7 [9,10] have continued with the introduction of PCV10 and PCV13. Overall, IPD incidence due to the serotypes included in the PCVs decreased (Table 3 and Figure 3A): this affected particularly the additional serotypes included in PCV10 and PCV13 and to a lesser extent the serotypes included in PCV7. The incidence of IPD means that the actual number of isolates per serotype in each epidemiological year is small (Table 4). This is even more marked when stratifying by age group and in the last years of the study, due to the decrease in IPD incidence.

The reasons behind the persistence of PCV7 serotypes as causes of IPD may be multifactorial [12,20]. Chief among those could be the slower uptake and lower vaccination coverage in Portugal when compared with countries where PCV7 was introduced in the national immunisation plan [1,2]. As with serotype 19A (see below), the high proportion of resistant isolates expressing PCV7 serotypes, particularly to penicillin and macrolides, could be an important factor in their persistence. Serotype 14, which is known to represent more virulent clones [21], remains the most important PCV7 serotype in IPD. In contrast to our data, a recent study in Portugal found the virtual elimination of PCV7 serotypes from nasopharyngeal carriage, with the exception of serotype 19F, a serotype already associated with carriage before the introduction of PCVs [22].

The serotype dynamics underpinning the changes in IPD incidence were different in the various age groups. In the youngest children (under 2 years), including those vaccinated with either PCV10 or PCV13, there were decreases in IPD caused by the serotypes included in the PCVs (Figure 3, B and C). While a reduction in

incidence due to addPCV10 could be expected in 2009–10, since the PCV10 vaccine was available from the outset of the epidemiological year, the reduction in incidence due to addPCV13 occurred in spite of PCV13 becoming available only in early 2010. IPD incidence due to PCV7 serotypes decreased slightly, but not significantly: these serotypes remain important causes of IPD in spite of more than a decade of PCV7 use.

The 2–4 years age group started including children potentially vaccinated with at least one dose of PCV10 or PCV13 in 2010–11. In this age group, there was a strong decrease in the IPD incidence due to addPCV10 serotypes already in 2009–11 (Figure 3D), whereas no significant change was seen in incidence due to addPCV13. In the older age group, (5–17 years), the decreases in IPD incidence caused by addPCV10 occurring between 2008–09 and 2010–12 and those due to addPCV13 serotypes in 2011–12 were not statistically significant. Taken together, our data suggest that there may be different reasons for the decreases in the incidence due to the various serotypes included in both PCV10 and PCV13 and those included only in PCV13.

Limited data available from observational studies in different geographical regions have documented decreases in IPD incidence due to PCV13 serotypes following PCV13 introduction and of serotypes 1 and 19A in particular [6,7]. A field study showed clear effectiveness of PCV13 against serotype 19A IPD [4], while another study demonstrated reduced nasopharyngeal acquisition of both serotypes 1 and 19A [8].

Decreases in the proportion of isolates expressing serotypes 1 and 5, included in both PCV10 and PCV13, and of serotype 6A, included only in PCV13, were noted in the adult (≥18 years) population in Portugal between 2009 and 2011 [12]. In contrast, the proportion of isolates expressing serotype 19A, also included only in PCV13, did not change significantly as a cause of IPD in the adult population in the country [12]. The herd effect that is known to occur due to PCV7, and now also expected for PCV10 and PCV13, is predicted to be delayed relative to the effects on the vaccinated children [2,9,23]. It is therefore unlikely that the changes seen in adults, and now in older unvaccinated paediatric age groups, can be attributed to PCV use. These data emphasise the importance of unexplained temporal trends in the distribution of pneumococcal serotypes [24] as potential confounders in observational studies such as ours. Similar to our observations, a vaccine trial in Alaska saw decreases in the incidence of PCV13 serotype IPD even before a direct effect of vaccination would be expected [20].

Although marked fluctuations in the incidence of serotype 1 IPD have been known to occur, no such changes have been documented in IPD due to serotype 19A, one of the most important serotypes that emerged in the post-PCV7 era and a serotype that remains a stable cause of IPD in adults in Portugal [10,12,25]. Another important difference between isolates expressing these two serotypes is their antimicrobial resistance. While serotype 1 isolates are mostly susceptible to the antimicrobials most frequently used to treat pneumococcal infections, serotype 19A isolates are frequently resistant to penicillin and the macrolides [10,24,25], a characteristic that has been maintained throughout the study period (Figure 2).

Our data argue that factors other than vaccination, such as unrelated temporal trends, triggered the decrease in the incidence of the two serotypes that had become the leading causes of paediatric IPD in the post-PCV7 years: serotypes 1 and 19A (Table 4) [10]. The reason for these changes remains unknown and may be different for these two serotypes. However, even if the observed serotype changes were not triggered by vaccination, PCV use might have reinforced them.

Although the decreases in incidence of PCV13 serotypes were partly offset by an increase in incidence of NVT IPD, the overall result was a substantial reduction in the incidence of paediatric IPD. An increase in NVT IPD incidence was noted in most age groups, although only in the 2–4 year-olds was this increase statistically significant. The decrease in the incidence of cases due to PCV13 serotypes resulted in multiple serotypes becoming more prominent causes of IPD, leading to increased serotype diversity of IPD cases. Serotype 10A was the only NVT serotype rising significantly in an overall analysis. However, this serotype was not among the most frequent NVT serotypes detected in a carriage study [22] nor was it found to be particularly invasive [21], raising the possibility that this will not be a sustained increase.

The decrease in overall PNSP during the study period reflected primarily the decrease of serotype 19A. When comparing with previous data [10], resistance to penicillin and cefotaxime remained unchanged in all age groups and there was a modest decline in ceftriaxone resistance. On the other hand, resistance to erythromycin and clindamycin rose in the youngest children (aged <2 years) but remained approximately constant in the older age groups. Serotype 19A and the PCV7 serotypes, particularly serotype 14, remained the most important serotypes in terms of resistance, suggesting a potential influence of PCVs in the dynamics of antimicrobial resistance.

Since the criterion for identification of an IPD case is the isolation of pneumococci from a normally sterile body site or the identification of pneumococcal DNA in cerebrospinal fluid (CSF) or pleural fluid, and this is almost exclusively done in hospital laboratories, we believe that few cases of laboratory-confirmed IPD would have been diagnosed outside of our network. The active nature of the surveillance and the involvement of a large number of hospitals covering the entire country offer further reassurance that our surveillance system identified most cases. We cannot guarantee that

the serotype distribution of the cases where isolates were unavailable followed the serotype distribution of available isolates, but we consider that the approach adopted minimises potential bias and describes the actual situation. However, the small proportion of cases with unknown serotype information ensures that our extrapolation does not affect the results.

In spite of substantial decreases in PCV13 IPD incidence in all age groups (Table 3), these serotypes remain the most important causes of IPD, being responsible for 63.2% of IPD cases in patients under 18 years in 2011–12 (Table 4). The persistence of these serotypes, including the PCV7 serotypes that have been subject to vaccine pressure for more than a decade, suggests that this could be due to the relatively modest vaccination coverage in Portugal. Coverage peaked around 2008 at 75% but declined to 63% in 2012 (data not shown), a lower coverage than in countries where PCVs are in the national immunisation plan and highlighting the potential benefits of increasing vaccination coverage. Although the study period is probably too close to vaccine introduction to expect a herd effect in older unvaccinated children, the relatively lower vaccination coverage may also compromise the extent of this effect. This may reduce the overall benefits of vaccination, including the potential protection of infants younger than 90 days [26] who are not currently protected by direct vaccination. Continued surveillance will monitor the extent of PCV13 success by evaluating the capacity of PCV13 serotypes to persist in spite of vaccination and in documenting the changes in antimicrobial resistance that accompany the changing serotypes and the emergence of any NVT replacement serotypes.

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Conflict of interest

JMC research grants administered through his university and honoraria for serving on speakers bureaus of Pfizer, Gilead and Novartis. MR honoraria for serving on the speakers bureau of Pfizer and for consulting for GlaxoSmithKline.

Authors' contributions

JMC, MJB and MR developed the design for the study; MJB, JMC and the Portuguese Group for the Study of Streptococcal Infections and the Portuguese Study Group of Invasive Pneumococcal Disease of the Paediatric Infectious Disease Society coordinated the collection of the surveillance data; SIA, ANH and JPL characterised the isolates; JMC, SIA and MR analysed the data; JMC and MR drafted the manuscript; all co-authors reviewed and contributed to the final version of the manuscript.

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