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Clin. Vaccine Immunol. 2013, 20(10):1524. DOI: 10.1128/CVI.00239-13.
Published Ahead of Print 7 August 2013.

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Expansion of Serotype Coverage in the Universal Pediatric Vaccination Calendar: Short-Term Effects on Age- and Serotype-Dependent Incidence of Invasive Pneumococcal Clinical Presentations in Madrid, Spain

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In Madrid, Spain, the 13-valent pneumococcal conjugate vaccine (PCV13) replaced PCV7 in the pediatric universal vaccination calendar in June 2010. A prospective clinical surveillance that included all children hospitalized with culture- and/or PCR-confirmed invasive pneumococcal disease (IPD) was performed in all Madrid hospitals. The incidence rates (IRs) (defined as the number of cases/100,000 inhabitants aged <15 years) in the PCV7 (May 2007 to April 2010) versus PCV13 (May 2011 to April 2012) periods were compared. There were 499 cases in the PCV7 period and 79 cases in the PCV13 period. Globally, the IR significantly decreased from 17.09 (PCV7 period) to 7.70 (PCV13 period), with significant decreases (PCV7 versus PCV13 periods) in all age groups for bacteremic pneumonia (5.51 versus 1.56), parapneumonic pneumococcal empyema (PPE) (5.72 versus 3.12), and meningitis (2.16 versus 0.97). In the PCV13 period, significant reductions (the IR in the PCV7 period versus the IR in the PCV13 period) were found in IPDs caused by PCV13 serotypes (13.49 versus 4.38), and specifically by serotypes 1 (globally [4.79 versus 2.53], for bacteremic pneumonia [2.23 versus 0.97], and for PPE [2.26 versus 1.17]), serotype 5 (globally [1.88 versus 0.00], for bacteremic pneumonia [0.89 versus 0.00], and for PPE [0.55 versus 0.00]), and serotype 19A (globally [3.77 versus 0.49], for bacteremic pneumonia [0.72 versus 0.00], for PPE [0.89 versus 0.00], and for meningitis [0.62 versus 0.00]). IPDs caused by non-PCV13 serotypes did not increase (IR, 3.60 in the PCV7 period versus 3.31 in the PCV13 period), regardless of age or presentation. No IPDs caused by the PCV13 serotypes were found in children who received 3 doses of PCV13. The number of hospitalization days and sanitary costs were significantly lower in the PCV13 period. The switch from PCV7 to PCV13 in the universal pediatric vaccination calendar provided sanitary and economical benefits without a replacement by non-PCV13 serotypes.

In the Madrid autonomous community (approximately 6 million inhabitants), the 7-valent pneumococcal conjugate vaccine (PCV7) was included in the systematic childhood vaccination calendar in November 2006 for the universal vaccination of children aged <24 months, with doses at 2, 4, 6, and 18 months of age. In June 2010, the 13-valent vaccine (PCV13) replaced the PCV7, with an immunization schedule of 2, 4, and 15 months and a catch-up program for children aged 18 to 24 months.

The HERACLES study (1–4), a yearly clinical surveillance of all pediatric laboratory-confirmed invasive pneumococcal disease (IPD) requiring hospitalization in the autonomous community of Madrid, Spain, started in May 2007, 6 months after the introduction of PCV7 in the pediatric calendar for universal vaccination. Over the 6 years of this ongoing surveillance, successive yearly analyses have shown that the incidence rate of total IPDs was not reduced in the PCV7 era, despite the significant decreasing trend of IPDs caused by PCV7 serotypes, due to the increase in IPDs by non-PCV7 serotypes (mainly serotypes 1, 7F, and 19A) (4). In the period immediately after the switch from PCV7 to PCV13 (2010 to 2011), significant reductions in the incidence rate of global IPD, mainly achieved by the reduction of cases caused by serotypes 1 and 19A, were evidenced (4).

It has been postulated that a time lag after universal vaccination is necessary before disease rates stabilize and greater decreases in IRs are shown compared to the baseline (5, 6). Mathematical

modeling has suggested a reduction in the overall incidence rate of IPDs following the introduction of PCV13, with uncertainty about serotype replacement (7). Early after the implementation of PCV7, there was worldwide evidence of a rapid serotype replacement, at different degrees, which reduced the overall effectiveness of PCV7 (6, 8). Thus, monitoring IPD following PCV13 introduction is important, since the extent to which replacement occurs after PCV13 universal vaccination is yet to be determined (9).

Considering, as a baseline, the incidence rates from the PCV7 universal vaccination era, the aim of this study was to compare the incidence rates for all hospitalized IPDs during the PCV13 universal vaccination period (2011 to 2012) versus during the PCV7 universal vaccination period (2007 to 2010), with the analysis measured by clinical presentation, serotype, and age groups.

Received 19 June 2013 Returned for modification 15 July 2013

Accepted 30 July 2013

Published ahead of print 7 August 2013

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doi:10.1128/CVI.00239-13

TABLE 1 Estimated person-year data on children in Madrid for the different study age groups by study period, according to the Spanish Instituto Nacional de Estadística

Age group (mo)	No. of cases in:	
	2007 to 2010 ^a	2011 to 2012
<12	224,692	73,521
≥12 to <24	221,154	75,200
≥24 to <60	651,757	222,127
≥60	1,822,743	655,687
Total	2,920,346	1,026,535

^a See reference 17. The person-years shown are summated values covering the first 3 years of the HERACLES study: 1st year, May 2007 to April 2008; 2nd year, May 2008 to April 2009; and 3rd year, May 2009 to April 2010.

MATERIALS AND METHODS

A prospective active clinical surveillance study on laboratory-confirmed IPD was carried out in all hospitals with pediatric departments located in the autonomous community of Madrid (Spain), beginning in May 2007. IPD was defined as the presence of *Streptococcus pneumoniae* in normal sterile fluids, and only children aged <15 years with laboratory-confirmed IPD by culture and/or PCR were considered. The analysis was performed by considering data from two periods: (i) a 3-year period (May 2007 to April 2010) of PCV7 universal vaccination (PCV7 period) and (ii) a 1-year period (May 2011 to April 2012) starting 1 year after the switch from PCV7 to PCV13 in May 2010 to ensure the completion of catch-up vaccine administration (PCV13 period).

Basic demographic data (age, gender, and vaccination status) and clinical presentation (bacteremic pneumonia, parapneumonic pneumococcal empyema [PPE], primary bacteremia, meningitis, and others) were recorded. Local research ethics committees approved the study protocol. Written informed consent was obtained from parents or guardians before inclusion of child participants in the study.

Samples were sent to the clinical microbiology laboratory at each center for microbiological culture and/or PCR detection. All pneumococcal isolates were sent to a single reference laboratory (Microbiology Department of the University Clinic Hospital in Madrid) for serotyping by Quellung reaction. Pleural and cerebrospinal fluids not yielding positive culture were also sent to the reference laboratory for PCR detection of the pneumolysin (*ply*) and autolysin (*lyt*) genes (10, 11). Pneumococci that were confirmed by PCR were serotyped by real-time PCR assay using the LightCycler SYBR green format followed by melting-curve analysis, as previously described (12), and detected serotypes 1, 3, 4, 5, 6, 7F, 14, 19A, and 19F. The molecular typing of 19A isolates was carried out by multi-locus sequence typing (MLST) as previously described (13), and sequence types (STs) were allocated using the online MLST database (<http://www.mlst.net>).

Susceptibilities to penicillin, cefotaxime, and erythromycin were determined by microdilution according to CLSI recommendations (14). Current CLSI breakpoints (15) were considered for susceptibility interpretation. Isolates with intermediate- or high-level resistance were defined as nonsusceptible.

The incidence rate (IR) was calculated as the number of cases per 100,000 inhabitants using estimated person-years data on children in Madrid (for each study period, by age group, and for total children <15 years of age) from the Spanish Instituto Nacional de Estadística (see <http://www.ine.es/jaxiBD/tabla.do?per=03&type=db&divi=EPA&idtab=261nogo>), shown in Table 1.

The sanitary costs for the different clinical presentations were obtained from the Spanish Ministry of Health statistical website (<http://msssi.gob.es/estadEstudios/estadisticas/cmbd.htm>), which provides the costs per hospitalized case based on codes from the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) (16) in 2010 (more recent data are now available). The costs were directly

TABLE 2 Numbers (incidence rates) of overall IPDs by study period and of the different clinical presentations by age group individually

Symptoms by age group	No. (IR) of cases in:		P
	2007 to 2010	2011 to 2012	
Total IPDs	499 (17.09)	79 (7.70)	0.0000
<12 mo	107 (47.62)	20 (27.20)	0.0260
≥12 to <24 mo	89 (40.24)	11 (14.63)	0.0014
≥24 to <60 mo	189 (29.00)	28 (12.61)	0.0000
≥60 mo	114 (6.25)	20 (3.05)	0.0034
Bacteremic pneumonia	161 (5.51)	16 (1.56)	0.0000
<12 mo	13 (5.79)	3 (4.08)	0.8351
≥12 to <24 mo	20 (9.04)	2 (2.66)	0.1124
≥24 to <60 mo	80 (12.27)	6 (2.70)	0.0001
≥60 mo	48 (2.63)	5 (0.76)	0.0080
PPE	167 (5.72)	32 (3.12)	0.0019
<12 mo	4 (1.78)	1 (1.36)	1.0000
≥12 to <24 mo	30 (13.57)	2 (2.66)	0.0224
≥24 to <60 mo	90 (13.81)	19 (8.55)	0.0710
≥60 mo	43 (2.36)	10 (1.53)	0.2728
Primary bacteremia	60 (2.05)	11 (1.07)	0.0595
<12 mo	35 (15.58)	8 (10.88)	0.4572
≥12 to <24 mo	13 (5.88)	3 (3.99)	0.7832
≥24 to <60 mo	7 (1.07)	0 (0.00)	0.7135
≥60 mo	5 (0.27)	0 (0.00)	0.9995
Meningitis	63 (2.16)	10 (0.97)	0.0236
<12 mo	36 (16.02)	4 (5.44)	0.0492
≥12 to <24 mo	10 (4.52)	2 (2.66)	0.7583
≥24 to <60 mo	5 (0.77)	1 (0.45)	1.0000
≥60 mo	12 (0.66)	3 (0.46)	0.8199

obtained, when available, or adapted from related processes. For pneumococcal meningitis (code 320.1), the cost was 11,524.3 euros. The cost for pneumococcal pneumonia (code 481), 4,330.75 euros, was considered for bacteremic pneumonia, the cost for empyema (code 510), 6,743.42 euros, was considered for pneumococcal parapneumonic empyema, and the cost for bacteremia (code 790.7), 4,994.38 euros, was considered for primary bacteremia. The costs globally and per clinical presentation were extrapolated to 100,000 inhabitants using person-years data from each study period (Table 1).

The total days of hospitalization were calculated globally and per clinical presentation and extrapolated to 100,000 inhabitants using person-years data in each study period (Table 1).

Comparisons between the two study periods were performed with Epidat version 3.1.

RESULTS

A total of 499 IPDs were identified in the 3-year PCV7 period and 79 IPDs in the 1-year PCV13 period. The median (interquartile range) ages (in months) of children suffering with IPD were 32.0 (13.0 to 56.0) in the PCV7 period (56.1% males) and 40.0 (11.0 to 62.0) in the PCV13 period (48.1% males). Table 2 shows, by study period, the number (IR) of total IPDs, bacteremic pneumonia, PPE, primary bacteremia, and meningitis. Other presentations (such as septic arthritis, peritonitis, cerebral abscess, periorbital cellulitis, otomastoiditis, and periotic abscess) accounted for 48 cases in the PCV7 period and 10 cases in the PCV13 period.

PPE diagnoses were based exclusively on PCR (negative cultures) in 102 out of 167 (61.1%) cases in 2007 to 2010 and in 22 out

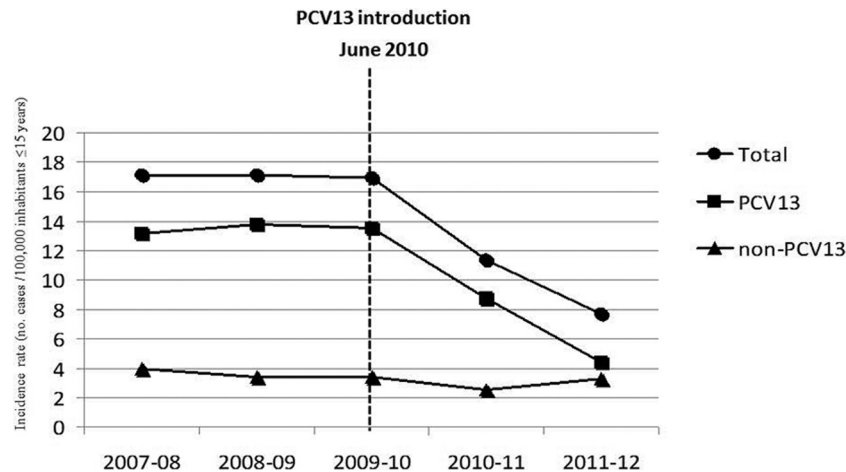


FIG 1 Yearly evolution of IRs of total IPDs, IPDs by PCV13 serotypes, and IPDs by non-PCV13 serotypes over the five HERACLES study time periods.

of 32 (68.8%) in 2010 to 2011. In the case of meningitis, diagnoses were based exclusively on PCR (negative cultures) in 3 out of 63 (4.8%) cases in 2007 to 2010 and in 1 out of 10 (10.0%) in 2010 to 2011.

Figure 1 shows the yearly evolution of the IR of total IPDs, of IPDs by PCV13 serotypes, and of IPDs by non-PCV13 serotypes over the five HERACLES study periods.

IR by clinical presentation. In the PCV7 period, the IR of total IPDs was 17.09. This IR significantly ($P < 0.0001$) decreased to 7.70 in the PCV13 period, with significant ($P < 0.05$) decreases for all age groups (Table 2). The decrease in overall IPDs was due to significant decreases in the IRs of bacteremic pneumonia (globally and in children aged >24 months) ($P < 0.01$), PPE (globally and in children aged ≥ 12 to <24 months) ($P < 0.05$), and meningitis (globally and in children aged <12 months) ($P < 0.05$). The decrease in the IR of primary bacteremia did not reach significance ($P = 0.0595$).

The median age (in months) of children suffering from PPE was significantly higher in the PCV13 period than in the PCV7 period (47.0 versus 42.0; $P = 0.032$). In the case of meningitis, the difference in mean age (17.5 months in the PCV13 period versus 8.0 months in the PCV7 period) did not reach statistical significance ($P = 0.125$), and no differences were found for bacteremic pneumonia (45.0 versus 43.0 months, respectively; $P = 0.690$) or primary bacteremia (7.0 versus 8.0 months, respectively; $P = 0.508$).

IR by serotype. The reduction in the IR of total IPDs was due to significant ($P < 0.01$) reductions in IPDs caused by PCV13 serotypes in all age groups (Table 3). The reduction found in the IR of IPDs caused by PCV13 serotypes was due to significant reductions in IPDs caused by serotype 1 (globally and in the group of children aged ≥ 24 to <60 months) ($P < 0.01$), serotype 5 (globally and in children aged ≥ 24 to <60 months) ($P < 0.05$), and serotype 19A (globally and in children aged <60 months) ($P < 0.05$). IPDs caused by non-PCV13 serotypes did not increase in the PCV13 period either globally or in any of the age groups. The overall 105 cases caused by non-PCV13 serotypes in the PCV7 period were caused by 25 different serotypes (none with >4 isolates/year), while in the PCV13 period, the 34 cases were caused by 15 different serotypes (serotype 22F [4 cases], 11A and 15B [3 cases each], 12F and 25A [2 cases each], and 20 cases of other serotypes).

The median age (in months) of children suffering from IPDs caused by serotypes included in PCV13 was significantly higher in the PCV13 period than in the PCV7 period (48.0 versus 34.5; $P = 0.037$), mainly due to significant differences for serotype 1 (65.0 versus 52.0; $P = 0.016$), without statistical significance for serotypes 3 (37.5 versus 25.0; $P = 0.436$), 7F (40.0 versus 22.0; $P = 0.285$), or 19A (14.0 versus 15.0; $P = 0.560$).

IR by clinical presentation and serotype. Table 4 shows the IRs of IPD clinical presentations caused by PCV7, by additional serotypes in PCV13 (individually), by PCV13 (globally), and by non-PCV13 serotypes for the different age groups in the study. Reductions ($P < 0.05$) in the IRs of meningitis, PPE, and bacteremic pneumonia were due to significant ($P < 0.01$) reductions in the IRs of these clinical presentations caused by PCV13 serotypes. Specifically, significant reductions were found for meningitis caused by serotype 19A ($P = 0.05$), for PPE caused by serotypes 1 and 19A ($P < 0.05$), and for bacteremic pneumonia caused by serotypes 1, 5, and 19A ($P < 0.05$). Although the reduction in the IR of primary bacteremia did not reach statistical significance ($P = 0.0595$), the IR of primary bacteremia caused by PCV13 serotypes significantly ($P = 0.0061$) decreased in the PCV13 period.

The median age (in months) of children suffering from PPE caused by serotypes included in the PCV13 was significantly higher in the PCV13 period than in the PCV7 period (52.0 versus 44.0; $P = 0.028$), mainly due to significant differences for serotype 1 (74.0 versus 49.5; $P = 0.002$), without statistical significance for other serotypes.

IPDs in the PCV13 period and relationship to vaccination status. In the PCV13 period, 33 out of the 79 children with IPDs had been vaccinated with at least one dose of PCV13. Of them, 12 suffered from an IPD by a serotype included in PCV13: one child that had received only one dose (had primary bacteremia caused by serotype 7F), 7 children that had received two doses (3 had primary bacteremia caused by 19A-ST320, one had bacteremic pneumonia caused by serotype 1, one had PPE caused by serotype 3, one had meningitis caused by serotype 1, and one had otomastoiditis caused by 19F), 3 children that had received one catch-up dose (one had bacteremic pneumonia caused by serotype 3, one had PPE caused by serotype 3, and one had a brain abscess related to otomastoiditis caused by serotype 19A-ST320), and 1 child that

TABLE 3 Numbers (incidence rates) of IPDs caused by different serotypes, by age group and study period

Serotype cause of IPDs by age group	No. (IR) of cases in:		<i>P</i>
	2007 to 2010	2011 to 2012	
PCV7 serotypes	21 (0.72)	2 (0.19)	0.0791
<12 mo	4 (1.78)	2 (2.72)	0.9157
≥12 to <24 mo	3 (1.36)	0 (0.00)	1.0000
≥24 to <60 mo	4 (0.61)	0 (0.00)	1.0000
≥60 mo	10 (0.55)	0 (0.00)	0.3375
Serotypes added in PCV13			
Serotype 1	140 (4.79)	26 (2.53)	0.0032
<12 mo	6 (2.67)	1 (1.36)	0.9072
≥12 to <24 mo	9 (4.07)	1 (1.33)	0.4714
≥24 to <60 mo	71 (10.89)	9 (4.05)	0.0054
≥60 mo	54 (2.96)	15 (2.29)	0.4522
Serotype 3	24 (0.89)	6 (0.58)	0.5877
<12 mo	5 (2.23)	1 (1.36)	1.0000
≥12 to <24 mo	5 (2.26)	1 (1.33)	1.0000
≥24 to <60 mo	12 (1.84)	4 (1.80)	1.0000
≥60 mo	2 (0.11)	0 (0.00)	1.0000
Serotype 5	55 (1.88)	0 (0.00)	0.0001
<12 mo	11 (4.90)	0 (0.00)	0.3298
≥12 to <24 mo	5 (2.26)	0 (0.00)	1.0000
≥24 to <60 mo	24 (3.68)	0 (0.00)	0.0125
≥60 mo	15 (0.82)	0 (0.00)	0.0990
Serotype 6A	5 (0.17)	0 (0.00)	1.0000
<12 mo	2 (0.89)	0 (0.00)	1.0000
≥12 to <24 mo	2 (0.90)	0 (0.00)	1.0000
≥24 to <60 mo	0 (0.00)	0 (0.00)	0.8875
≥60 mo	1 (0.05)	0 (0.00)	0.9182
Serotype 7F	39 (1.44)	6 (0.58)	0.0770
<12 mo	12 (5.34)	1 (1.36)	0.1121
≥12 to <24 mo	8 (3.62)	0 (0.00)	0.5828
≥24 to <60 mo	14 (2.15)	5 (2.25)	1.0000
≥60 mo	5 (0.27)	0 (0.00)	0.9995
Serotype 19A	110 (3.77)	5 (0.49)	0.0000
<12 mo	37 (16.47)	2 (2.72)	0.0082
≥12 to <24 mo	37 (16.73)	2 (2.66)	0.0065
≥24 to <60 mo	26 (3.99)	1 (0.45)	0.0178
≥60 mo	10 (0.55)	0 (0.00)	0.3375
PCV13 serotypes	394 (13.49)	45 (4.38)	0.0000
<12 mo	85 (37.83)	8 (10.88)	0.0005
≥12 to <24 mo	61 (27.58)	3 (3.99)	0.0003
≥24 to <60 mo	151 (23.17)	19 (8.55)	0.0000
≥60 mo	97 (5.32)	15 (2.29)	0.0025
Non-PCV13 serotypes	105 (3.60)	34 (3.31)	0.7494
<12 mo	30 (13.35)	13 (17.68)	0.5017
≥12 to <24 mo	20 (9.04)	7 (9.31)	0.8765
≥24 to <60 mo	38 (5.83)	9 (4.05)	0.4124
≥60 mo	17 (0.93)	5 (0.76)	0.9077

had received the third PCV13 dose 2 days before hospital admission for an otomastoiditis and peritotic abscess caused by serotype 19A-ST320. No further cases of IPDs caused by the PCV13 serotypes were found in children that had been completely vaccinated with PCV13 (3 doses).

Susceptibility. In the PCV13 period, all 19A isolates ($n = 5$) were nonsusceptible to cefotaxime and oral penicillin (3 of them resistant to parenteral penicillin) and erythromycin, while all iso-

TABLE 4 Numbers (incidence rates) of different clinical presentations caused by different serotypes globally and by study period

Presentation and serotype	No. (IR) of cases in:		<i>P</i>
	2007 to 2010	2011 to 2012	
Total IPDs	499 (17.09)	79 (7.70)	0.0000
PCV7	21 (0.72)	2 (0.19)	0.0791
1	140 (4.79)	26 (2.53)	0.0032
3	24 (0.89)	6 (0.58)	0.5877
5	55 (1.88)	0 (0.00)	0.0001
6A	5 (0.17)	0 (0.00)	1.0000
7F	39 (1.44)	6 (0.58)	0.0770
19A	110 (3.77)	5 (0.49)	0.0000
PCV13	394 (13.49)	45 (4.38)	0.0000
Non-PCV13	105 (3.60)	34 (3.31)	0.7494
Bacteremic pneumonia	161 (5.51)	16 (1.56)	0.0000
PCV7	7 (0.24)	0 (0.00)	0.6849
1	65 (2.23)	10 (0.97)	0.0178
3	4 (0.14)	1 (0.10)	1.0000
5	26 (0.89)	0 (0.00)	0.0154
6A	0 (0.00)	0 (0.00)	0.9051
7F	16 (0.55)	1 (0.10)	0.0833
19A	21 (0.72)	0 (0.00)	0.0231
PCV13	139 (4.76)	12 (1.17)	0.0000
Non-PCV13	22 (0.75)	4 (0.39)	0.3118
PPE	167 (5.72)	32 (3.12)	0.0019
PCV7	7 (0.24)	0 (0.00)	0.6849
1	66 (2.26)	12 (1.17)	0.0444
3	15 (0.51)	5 (0.49)	1.0000
5	16 (0.55)	0 (0.00)	0.0833
6A	0 (0.00)	0 (0.00)	0.9051
7F	9 (0.31)	4 (0.39)	0.8991
19A	26 (0.89)	0 (0.00)	0.0154
PCV13	139 (4.76)	21 (2.05)	0.0003
Non-PCV13	28 (0.96)	11 (1.07)	0.8964
Primary bacteremia	60 (2.05)	11 (1.07)	0.0595
PCV7	3 (0.10)	0 (0.00)	1.0000
1	3 (0.10)	0 (0.00)	1.0000
3	2 (0.07)	0 (0.00)	1.0000
5	6 (0.21)	0 (0.00)	0.8403
6A	1 (0.03)	0 (0.00)	0.9051
7F	9 (0.31)	1 (0.10)	0.4441
19A	22 (0.75)	3 (0.29)	0.1574
PCV13	46 (1.58)	4 (0.39)	0.0061
Non-PCV13	14 (0.48)	7 (0.68)	0.5860
Meningitis	63 (2.16)	10 (0.97)	0.0236
PCV7	2 (0.07)	0 (0.00)	1.0000
1	2 (0.07)	1 (0.10)	1.0000
3	1 (0.03)	0 (0.00)	0.9051
5	4 (0.14)	0 (0.00)	1.0000
6A	3 (0.10)	0 (0.00)	1.0000
7F	4 (0.14)	0 (0.00)	1.0000
19A	18 (0.62)	0 (0.00)	0.0500
PCV13	34 (1.16)	1 (0.10)	0.0034
Non-PCV13	29 (0.99)	9 (0.88)	0.8873

lates of serotypes 1 ($n = 26$), 3 ($n = 6$), and 7F ($n = 6$) were susceptible to oral penicillin.

Associated costs. Table 5 shows the total numbers of days of hospitalization and sanitary costs per 100,000 inhabitants aged <15 years by clinical presentation in the two study periods. The

TABLE 5 Total numbers of days of hospitalization per 100,000 inhabitants aged <15 years and sanitary costs by clinical presentation in the two study periods

Clinical presentation	2007 to 2010		2011 to 2012	
	No. of days of hospitalization	Sanitary costs (€) ^d	No. of days of hospitalization	Sanitary costs (€) ^d
Bacteremic pneumonia	51.36	23,875.62	10.62 ^a	6,750.09 ^a
PPE	105.43	38,562.25	61.08 ^a	21,021.15 ^a
Primary bacteremia	16.23	10,261.21	8.77 ^a	5,351.81 ^a
Meningitis	43.21	24,861.13	27.86 ^a	11,226.41 ^a
Others ^b	19.52		15.78 ^c	
Total IPDs	235.76	97,560.21	124.11 ^a	44,349.46 ^a

^a $P \geq 0.0001$ versus 2007–2010 period.^b Other presentations (such as septic arthritis, peritonitis, cerebral abscess, periorbital cellulitis, otomastoiditis and periotic abscess) accounted for 48 cases in the PCV7 period and 10 cases in the PCV13 period.^c $P = 0.0188$ versus that for 2007–2010 period.^d For calculations of sanitary costs, only bacteremic pneumonia, PPE, primary bacteremia, and meningitis were considered.

number of days of hospitalization per 100,000 inhabitants aged <15 years and the sanitary costs per 100,000 inhabitants aged <15 years were significantly lower in the PCV13 period for IPDs globally and for the four major presentations individually. The reductions in the days of hospitalization per 100,000 inhabitants aged <15 years from the PCV7 to the PCV13 period were 47.4% globally, 79.3% for bacteremic pneumonia, 42.1% for PPE, 46.0% for primary bacteremia, 35.5% for meningitis, and 19.2% for other presentations. The estimated approximate reductions in sanitary costs per 100,000 inhabitants aged <15 years in the PCV13 period were 53,000 euros globally, 17,000 euros for bacteremic pneumonia, 17,500 euros for PPE, 4,900 euros for primary bacteremia, and 13,600 euros for meningitis.

DISCUSSION

The incidence data regarding IPDs vary widely depending on vaccination policy, geographical areas, and differences in the study methodologies and surveillance systems (17, 18). In Spain, where PCV7 was introduced in 2001 in the private market, the differences between geographical areas are evident according to the published data. The incidence rates of IPDs in children aged <2 years were reduced in the Basque Country and Navarre from 93.5 (1998 to 2001) to 56.3 (2003), with a vaccine uptake of 28% and 45%, respectively, with significant reductions in pneumococcal meningitis and pneumococcal bacteremia, and also in children aged <5 years (19). In Barcelona, the incidence rates of IPDs in children decreased from 96.9 in 1999 to 90.6 in 2004, with one-third of children having received PCV7, and with significant reductions in meningitis and bacteremia but increases in empyema (20). Interestingly, another study in Barcelona showed a significant increase in the incidence of IPDs in children aged <5 years, from 76.2/100,000 inhabitants in 2007 to 109.9/100,000 inhabitants in 2009, due to the increase in cases caused by non-PCV7 serotypes (21). Similar incidence rates (~61.55/100,000 inhabitants) were reported in the autonomous community of Madrid in the period of 1998 to 2006 (prior to PCV7 inclusion in the systematic vaccination calendar for childhood immunizations) (22). These values contrast with the IRs of IPDs in the PCV7 period of the present study: 17.09 globally (<15 years) and 47.62 in children

aged <12 months. The decrease reported for the period 2007 to 2011 (4) has continued, leading to IRs of 7.70 (global IPDs) and 27.2 (children aged <12 months) in the present yearly analysis (2011 to 2012) of the HERACLES study. The decrease in the PCV13 period was significant for all pediatric age groups and for bacteremic pneumonia, PPE, and meningitis, with IRs of 1.56, 3.12, and 0.97, respectively, in 2011 to 2012.

IPD incidence is age dependent. The results of the present study suggest that due to PCV13 vaccination of the youngest children, the mean age of children suffering from IPDs has increased. Nevertheless, probably due to the limited number of cases, statistical significance was only found in the case of PPE and, among them, for serotype 1 cases. There has been a significant reduction in PPE cases among children aged ≥ 12 to <24 months, a population group that had been vaccinated with PCV13 the previous year, but not among older children that were not in the target population for vaccination. Previous articles from the HERACLES study had shown that serotype 1 was linked to respiratory presentations of IPD and children aged >36 months (1). In the present analysis, respiratory presentations (bacteremic pneumonia and PPE) were the most prevalent IPDs in both periods (65.72% in the PCV7 period and 60.76% in the PCV13 period). However, the similar distributions of bacteremic pneumonia and PPE in the PCV7 period changed in the PCV13 period, with bacteremic pneumonia comprising 20.25% of total IPDs and PPE 40.51% of total IPDs. In any case, the IRs of IPD caused by serotypes 1 and 19A have markedly decreased, without any cases of bacteremic pneumonia, PPE, and meningitis caused by serotype 19A in the PCV13 period. Serotype 19A was linked to non-respiratory presentations and children aged <12 months in the PCV7 era (1). The five serotype 19A cases in the PCV13 period of the present analysis were primary bacteremia (3 cases) and 2 IPDs linked to otic foci. All isolates belonged to ST320. The marked reduction in serotype 19A cases is important from the point of view of antibiotic resistance, since in our country, ST320 is a worrisome clone showing multidrug resistance (2, 23, 24).

The significant reduction in the IR of serotype 5, without any cases in 2011 to 2012, could not be attributed only to vaccine pressure, since although this serotype was the third most prevalent (after serotypes 1 and 19A) in the PCV7 period, it has been linked to community outbreaks (25), such as that which occurred in the autonomous community of Madrid in the PCV7 period.

In a previous study using real-time PCR for identification, serotype 3 was reported to be a common serotype causing IPD in children in Spain (26). In the present analysis, cases caused by serotype 3 represented <8% in both periods, without a significant reduction in IR in the PCV13 period. No variations over 3 decades were described for this serotype, despite its high antimicrobial susceptibility as shown in Spain in a previous study (27). The inclusion of this serotype in PCV13 will probably have epidemiological effects once a time lag period passes after universal PCV13 vaccination, since none of the six cases of IPD caused by serotype 3 occurred in children who had received the 3-dose schedule of PCV13.

In the present study, no PCV13 vaccine failures were detected with use of the complete vaccination schedule, since the child with otomastoiditis by serotype 19A had received the third PCV13 dose only 2 days before hospital admission.

Of high importance is that, according to data in the present study, the IR of IPDs caused by non-PCV13 serotypes did not vary

after the switch from PCV7 to PCV13. Thus, the replacement issue detected early after the PCV7 introduction affecting both carriage and disease (9), which created uncertainty about replacement effects after PCV13 introduction, was not detected in the present study. No increase in cases caused by non-PCV13 serotypes was found in the PCV13 period for the four major clinical presentations studied, with the IRs of IPD by non-PCV13 serotypes being ≤ 1 . In addition, the 34 non-PCV13 cases found in 2011 to 2012 were caused by 15 different serotypes, none of them with >4 isolates, suggesting no specific serotype to be a potential candidate for future serotype replacement.

The incidence rates of global IPD (IR, ~ 17.0) and of IPD by PCV13 serotypes (IR, ~ 13.5) remained unchanged from 2007 to 2010 (4). In contrast, there was a significant reduction in disease demonstrated in the present study after the switch from PCV7 to PCV13 for universal vaccination. This substantial reduction was linked to significant reductions in the estimated sanitary costs per 100,000 inhabitants aged <15 years and in days of hospitalization per 100,000 inhabitants for IPDs globally and for the four major presentations individually. Previous mathematical modeling of the long-term effects of replacing PCV7 with PCV13 showed the cost-effectiveness of such a switch in England and Wales (7), as well as of the implementation of PCV13 universal vaccination by the public health system in Valencia, Spain (28).

The results of the present analysis provide useful evidence on the benefits of replacing PCV7 with PCV13 universal vaccination. This is important since, at least with PCV7, stopping universal vaccination is predicted to cause a rebound in the number of IPD cases to the pre-PCV7 level (7). In the present study, no replacement by non-PCV13 serotypes was found in serotypes causing IPDs in the PCV13 period. However, if a possible replacement effect occurs, it is unlikely that this might lead to scenarios where PCV13 universal vaccination becomes less beneficial than the option of discontinuing conjugate vaccination altogether (7).

ACKNOWLEDGMENTS

This work was supported in part by an unrestricted grant from Pfizer S.L.U., Madrid, Spain.

J.P. and J.R.-C. have received travel fees from Pfizer for attending and/or speaking at symposiums and congresses. C.M. is an employee of Pfizer S.L.U., Madrid, Spain. The other authors have no conflicts of interest or funding to disclose.

Other members of the HERACLES study group include A. Delgado-Iribarren and M. Bueno (Hospital Universitario Fundación de Alcorcón), A. Alhambra and M. T. García (Hospital Sanchinarro), A. Rivas-Castillo and P. Fernandez (Hospital San Rafael), A. Gutiérrez (Hospital La Paz), B. Hernández (Hospital Niño Jesús), M. Zafra and J. Jaqueti (Hospital de Uenlabrada), E. Ríos, E. Culebras, F. González, and I. Rodríguez-Avial (Hospital Clínico San Carlos), C. Calvo and I. Wilhelmi (Hospital Severo Ochoa), C. Serrano and E. García-Peñuela (Hospital de la Zarzuela), E. Bouza and E. Cercenado (Hospital Gregorio Marañón and CIBER of Respiratory Diseases [CIBERES]), E. Gomez (Hospital Ramón y Cajal), F. Sanz, E. Giangaspro, and I. Sánchez (Hospital 12 de Octubre), I. Gadea and M. N. Domínguez (Fundación Jiménez Díaz), I. Romero and A. Alhambra (Hospital de Torreldones), J. L. Gómez-Garcés and M. A. Roa (Hospital de Móstoles), J. T. Ramos and J. Cacho (Hospital de Getafe), J. C. Sanz (LRSP), M. L. García-Picazo and S. Gallego (Hospital de El Escorial), M. J. Cilleruelo and M. I. Sánchez (Hospital Puerta de Hierro), P. Gomez and M. Penín (Hospital Príncipe de Asturias), S. Salso and M. Corcino (Hospital de Montepíncipe), A. Rodríguez (Hospital del Sur este), J. Clemente (Hospital del Henares), J. L. Ruibal (Hospital Infanta Cristina), F. J. Sanz and J. Zapardiel (Hospital Infanta Elena), B. Agundez

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REFERENCES

- Picazo J, Ruiz-Contreras J, Casado-Flores J, Giangaspro E, Del Castillo F, Hernández-Sampelayo T, Otheo E, Balboa F, Ríos E, Méndez C, Heracles Study Group. 2011. Relationship between serotypes, age, and clinical presentation of invasive pneumococcal disease in Madrid, Spain, after introduction of the 7-valent pneumococcal conjugate vaccine into the vaccination calendar. *Clin. Vaccine Immunol.* 18:89–94.
- Picazo J, Ruiz-Contreras J, Hernandez B, Sanz F, Gutierrez A, Cercenado E, Meseguer MA, Delgado-Iribarren A, Rodriguez-Avial I, Méndez C. 2011. Clonal and clinical profile of *Streptococcus pneumoniae* serotype 19A causing pediatric invasive infections: a 2-year (2007–2009) laboratory-based surveillance in Madrid. *Vaccine* 29:1770–1776.
- Picazo J, Ruiz-Contreras J, Casado-Flores J, Negreira S, Del Castillo F, Hernández-Sampelayo T, Bueno M, Calvo C, Ríos E, Méndez C, HERACLES Study Group. 2011. Laboratory-based, 2-year surveillance of pediatric parapneumonic pneumococcal empyema following heptavalent pneumococcal conjugate vaccine universal vaccination in Madrid. *Pediatr. Infect. Dis. J.* 30:471–474.
- Picazo J, Ruiz-Contreras J, Casado-Flores J, Giangaspro E, García-de-Miguel MJ, Hernández-Sampelayo T, Otheo E, Méndez C. 2013. Impact of introduction of conjugate vaccines in the vaccination schedule on the incidence of pediatric invasive pneumococcal disease requiring hospitalization in Madrid 2007 to 2011. *Pediatr. Infect. Dis. J.* 32:656–6561.
- Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, Reingold A, Thomas A, Schaffner W, Craig AS, Smith PJ, Beall BW, Whitney CG, Moore MR, Active Bacterial Core Surveillance/Emerging Infections Program Network. 2010. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J. Infect. Dis.* 201:32–41.
- Fitzwater SP, Chandran A, Santosham M, Johnson HL. 2012. The worldwide impact of the seven-valent pneumococcal conjugate vaccine. *Pediatr. Infect. Dis. J.* 31:501–508.
- Choi YH, Jit M, Flasche S, Gay N, Miller E. 2012. Mathematical modelling long-term effects of replacing Prevnar7 with Prevnar13 on invasive pneumococcal diseases in England and Wales. *PLoS One* 7:e39927. doi:10.1371/journal.pone.0039927.
- Isaacman DJ, McIntosh ED, Reinert RR. 2010. Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. *Int. J. Infect. Dis.* 14:e197–e209. doi:10.1016/j.ijid.2009.05.010.
- Gladstone RA, Jefferies JM, Faust SN, Clarke SC. 2012. Pneumococcal 13-valent conjugate vaccine for the prevention of invasive pneumococcal disease in children and adults. *Expert Rev. Vaccines* 11:889–902.
- Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB. 2001. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. *J. Clin. Microbiol.* 39:1553–1558.
- Carvalho MdaGS, Tondella ML, McCaustland K, Weidlich L, McGee L, Mayer LW, Steigerwalt A, Whaley M, Facklam RR, Fields B, Carlone G, Ades EW, Dagan R, Sampson JS. 2007. Evaluation and improvement of real-time PCR assays targeting *lytA*, *ply*, and *psaA* genes for detection of pneumococcal DNA. *J. Clin. Microbiol.* 45:2460–2466.
- Sanz JC, Culebras E, Ríos E, Rodríguez-Avial I, Wilhelmi I, Ramos B, Ordoñas M, Picazo JJ. 2010. Direct serogrouping of *Streptococcus pneumoniae* strains in clinical samples by use of a latex agglutination test. *J. Clin. Microbiol.* 48:593–595.
- Enright MC, Spratt BG. 1998. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. *Microbiology* 144:3049–3060.
- Clinical and Laboratory Standards Institute. 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically—seventh edition. Approved standard M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2009. Performance standards for antimicrobial susceptibility testing; 19th informational supple-

- ment. CLSI M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
16. Ministerio de Sanidad y Consumo. 2010. Clasificación internacional de enfermedades: CIE 9 MC (7ª ED). Ministerio de Sanidad y Consumo, Madrid, Spain.
17. Roberts J, Chandra M, Pebody R, Stuart J. 2007. Variation in incidence of pneumococcal and meningococcal disease across Europe. *Euro Surveill.* 12:pii=3310. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3310>.
18. Hanquet G, Perrocheau A, Kissling E, Bruhl DL, Tarragó D, Stuart J, Stefanoff P, Heuberger S, Kriz P, Vergison A, de Greeff SC, Amato-Gauci A, Celentano LP, ECDC Country Experts for Pneumococcal Disease. 2010. Surveillance of invasive pneumococcal disease in 30 EU countries: towards a European system? *Vaccine* 28:3920–3928.
19. Aristegui J, Bernaola E, Pocheville I, García C, Arranz L, Durán G, Pérez L, Bastida M, Canduela C, Herranz Aguirre M, Garrote E, Fletcher MA, Pérez C. 2007. Reduction in pediatric invasive pneumococcal disease in the Basque Country and Navarre, Spain, after introduction of the heptavalent pneumococcal conjugate vaccine. *Eur. J. Clin. Microbiol. Infect. Dis.* 26:303–310.
20. Calbo E, Díaz A, Cañadell E, Fábrega J, Uriz S, Xercavins M, Morera MA, Cuchi E, Rodríguez-Carballeira M, Garau J, Spanish Pneumococcal Infection Study Network. 2006. Invasive pneumococcal disease among children in a health district of Barcelona: early impact of pneumococcal conjugate vaccine. *Clin. Microbiol. Infect.* 12:867–872. doi:10.1111/j.1469-0691.2006.1502_1.x.
21. de Sevilla MF, García-García JJ, Esteva C, Moraga F, Hernández S, Selva L, Coll F, Ciruela P, Planes AM, Codina G, Salleras L, Jordan I, Domínguez A, Muñoz-Almagro C. 2012. Clinical presentation of invasive pneumococcal disease in Spain in the era of heptavalent conjugate vaccine. *Pediatr. Infect. Dis. J.* 31:124–128.
22. Gutiérrez Rodríguez MA, Ordobás Gavín M, Ramírez Fernández R, García Comas L, García Fernández C, Rodero Garduño I. 2008. Incidence of pneumococcal disease in the autonomous region of Madrid (1998–2006). *Med. Clin. (Barc.)* 130:51–53. (In Spanish.)
23. Ardanuy C, Rolo D, Fenoll A, Tarragó D, Calatayud L, Liñares J. 2009. Emergence of a multidrug-resistant clone (ST320) among invasive serotype 19A pneumococci in Spain. *J. Antimicrob. Chemother.* 64:507–510.
24. Muñoz-Almagro C, Esteva C, de Sevilla MF, Selva L, Gene A, Pallares R. 2009. Emergence of invasive pneumococcal disease caused by multidrug-resistant serotype 19A among children in Barcelona. *J. Infect.* 59:75–82.
25. Rodríguez MA, González AV, Gavín MA, Martínez FM, Marín NG, Blázquez BR, Moreno JC. 2011. Invasive pneumococcal disease: association between serotype, clinical presentation and lethality. *Vaccine* 29:5740–5746.
26. Selva L, Ciruela P, Esteva C, de Sevilla MF, Codina G, Hernandez S, Moraga F, García-García JJ, Planes A, Coll F, Jordan I, Cardenosa N, Batalla J, Salleras L, Domínguez A, Muñoz-Almagro C. 2012. Serotype 3 is a common serotype causing invasive pneumococcal disease in children less than 5 years old, as identified by real-time PCR. *Eur. J. Clin. Microbiol. Infect. Dis.* 31:1487–1495.
27. Fenoll A, Granizo JJ, Aguilar L, Giménez MJ, Aragoneses-Fenoll L, Hanquet G, Casal J, Tarragó D. 2009. Temporal trends of invasive *Streptococcus pneumoniae* serotypes and antimicrobial resistance patterns in Spain from 1979 to 2007. *J. Clin. Microbiol.* 47:1012–1020.
28. Díez-Domingo J, Ridao-López M, Gutiérrez-Gimeno MV, Puig-Barberá J, Lluch-Rodrigo JA, Pastor-Villalba E. 2011. Pharmacoeconomic assessment of implementing a universal PCV-13 vaccination programme in the Valencian public health system (Spain). *Vaccine* 29:9640–9648.