Vaccine 37 (2019) 2200–2207

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Impact of 13-valent pneumococcal conjugate vaccination on invasive pneumococcal disease in children under 15 years old in Madrid, Spain, 2007 to 2016: The HERACLES clinical surveillance study

Juan J. Picazo [a,*], Jesús Ruiz-Contreras [b,c], Juan Casado-Flores [d,e], Sagrario Negreira [b,c], Fernando Baquero-Artigao [e,f], Teresa Hernández-Sampelayo [c,g,h], Enrique Otheo [i,j], María del Amo [k], Cristina Méndez [k], On behalf of Heracles Study Group [1]

[a] Medicine Department, School of Medicine, Universidad Complutense, Madrid, Spain
[b] PediatricDepartment, Hospital Universitario 12 de Octubre, Madrid, Spain
[c] Pediatric Department, School of Medicine, Universidad Complutense, Madrid, Spain
[d] Pediatric ICU, Hospital Universitario Infantil Niño Jesús, Madrid, Spain
[e] Pediatric Department, School of Medicine, Universidad Autónoma, Madrid, Spain
[f] Pediatric Department, Hospital Universitario La Paz, Madrid, Spain
[g] Pediatric Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain
[h] CIBER of Respiratory Diseases, CIBERES, Madrid, Spain
[i] Pediatric Department, Hospital Universitario Ramón y Cajal, Madrid, Spain
[j] Faculty of Medicine, Universidad de Alcalá, Madrid, Spain
[k] MedicalDepartment, Pfizer SLU, Madrid, Spain

ARTICLE INFO

Article history:
Received 11 October 2018
Received in revised form 7 March 2019
Accepted 11 March 2019
Available online 19 March 2019

Keywords:
Streptococcus pneumoniae
Invasive pneumococcal disease (IPD)
PCV13
Regional Immunization Programme (RIP)
Serotype 19A
Antibiotic resistance

ABSTRACT

Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide. Using the data from the HERACLES clinical surveillance study (2007–2016), we describe the population impact of the 13-valent pneumococcal conjugate vaccine (PVC13) on invasive pneumococcal disease (IPD) in children <15 years of age in the Community of Madrid, Spain. After six years of the inclusion of PCV13 in the vaccination calendar (2010–2016), and despite changes in the Regional Immunization Programme that limited its availability, the net benefit incidence rate (IR) of IPD fell by 70.1% (IRR 0.3 [95% CI: 0.22–0.4]; $p \leq 0.001$), mainly due to a significant reduction (91%) in the PCV13 serotypes (IRR 0.09 [95% CI: 0.05–0.16], $p \leq 0.001$). Furthermore, no significant changes were detected in the IR of IPD caused by non-PCV13 serotypes. The IRs of the aggressive, resistant and most prevalent serotype in the analysed population, the 19A serotype, dramatically decreased from the beginning to the end of the study (98%) [IRR 0.03 (95% CI: 0.00–0.19), $p \leq 0.001$], to its almost total disappearance. Remarkably, this reduction led to a pronounced decline in the percentage of cefotaxime-resistant isolates and the incidence of meningitis cases. Assessment of the clinical impact revealed a reduction in the number of all clinical presentations of IPD, confirming the effectiveness of the PCV13. Finally, PCV13 detected by PCR is predicted to have a stronger impact than the one based on culture methods, which can overlook more than 20% of cases of IPD, mainly pleural empyemas.

1. Introduction

Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide. The World Health Organization (WHO) estimated that 476 000 out of a total of 8.8 million deaths among children younger than five years of age in 2008 were caused by pneumococcal diseases [1,2]. Furthermore, acute respiratory infections constitute an important cause of mortality in children, responsible for more than 1500 million deaths globally [3]. The diagnostic approach in pneumococcal disease is difficult. Currently, the isolation of *S. pneumoniae* from usually sterile body fluids remains the reference standard for diagnosis, but the reliability of fluid cultures is controversial, since some factors, such as the

* Corresponding author at: Medicine Department, School of Medicine, Universidad Complutense, Facultad Medicina. Pabellón I, 1ª planta. Ciudad Universitaria, 28040 Madrid, Spain.
E-mail address: jpicazo@picazo.net (J.J. Picazo).

[1] Membership of the Heracles Study Group is provided in the Acknowledgements section.

https://doi.org/10.1016/j.vaccine.2019.03.015
0264-410X/

administration of antibiotics prior to the assay, clearly reduce the frequency of culture of pneumococci [4].

Following the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in 2000–2001, the incidence of invasive pneumococcal disease (IPD) due to PCV7-serotypes (4, 6B, 9 V, 14, 18C, 19F and 23F) decreased dramatically among children, but also among adults due to herd protection [5]. This change was accompanied by a general decline in antibiotic resistance [6], although in the late PCV7 period, new emerging serotypes were detected [7–9]. Among these, serotype 19A — one of the most prevalent serotypes prior to the introduction of PCV7 in both invasive isolates [10,11] and colonising strains [12] — increased significantly in Spain [10] and other countries [13] (the use of antimicrobials and resistance to oral penicillin remained unchanged over the course of the present study). The reason for this could be its ability to fill the ecologic niche left by the reduction in the number of PCV7 types after vaccine introduction [14].

In 2010, the 13–valent pneumococcal conjugate vaccine (PCV13) was licensed for children aged 6 weeks to 71 months for the prevention of IPD caused by the 13 pneumococcal serotypes included in the vaccine (PCV7 serotypes + serotypes 1, 3, 5, 6A, 7F and 19A) [15]. In the Community of Madrid, Spain, PCV7 was implemented in October 2006. In June 2010, and in compliance with the fully-government funded Regional Immunization Programme (RIP), it was replaced by the PCV13 (2 + 1 schedule) but was later excluded from the RIP in 2012 because of changes in vaccination policies and economical constraints, for children born after May 2012 (except boosters for primary vaccinations). PCV13 was eventually reintroduced in March 2015 (2 + 1 schedule) for infants born after January 2015 (including catch-up doses for children following vaccination programmes that had already started). Therefore, between May 2012 and January 2015, the vaccine was only available for private purchase.

The HERACLES study aimed to estimate the effect of PCV13 on IPD incidence in children ≤15 years in the Community of Madrid, Spain. Results corresponding to the 2007–2015 period were recently published, demonstrating the efficacy of a fully government-funded programme for universal vaccination against pneumococci, as well as its impact on vaccine uptake levels and consequently on IPD [16].

Here, we provide a final analysis of this nine-year surveillance study. Thus, we evaluated the impact of PCV13 on the incidence of IPD in children <15 years of age since its introduction in the RIP of the Community of Madrid until the end of the HERACLES study (2016), as well as the specific impact on serotype 19A and on antibiotic resistances. The effect of IPD diagnostic methods was also evaluated.

2. Materials and methods

2.1. Study design

The HERACLES study was a prospective, active study consisting of a yearly clinical surveillance of all paediatric laboratory-confirmed IPD requiring hospitalization of children <15 years of age (912 cases identified over the 9 years in a total paediatric population of 9 016 186). The study involved all hospitals with paediatric departments located in the Community of Madrid, Spain; it started on 1 May 2007 (6 months after the introduction of the 7-valent PCV7) and concluded on 30 April 2016 (9 years). A network of all paediatricians, paediatric infectious disease specialists and clinical microbiologists from all public and private hospitals in Madrid (27 hospitals) was created to carry out the active surveillance. According to quality standards for data collection, a supervisor oversaw study performance and controlled protocol compliance, as stated in the monitoring plan. This plan included a comparison of the data in the case report form (CRF) with the original data sources. Any discrepancy was discussed with the hospital staff and resolved accordingly. In compliance with Spanish legislation, the Research Ethics Committee of Hospital Universitario Clínico San Carlos (Madrid) approved the present study, and the approval was communicated to the research ethics committees of all participating centres, offering them the opportunity for further review/clarifications. Written informed consent was obtained from parents/guardians before inclusion.

2.2. Data collection

Basic demographic data (age, sex, and vaccination status) and clinical presentation (meningitis, pleural empyema, bacteraemic pneumonia, primary bacteraemia and others) were recorded. IPD was defined as the presence of *S. pneumoniae* in normally sterile fluids, demonstrated by culture and/or polymerase chain reaction (PCR). Samples were sent to the clinical microbiology laboratory of each hospital for microbiological culture and/or PCR detection. A total of 912 pneumococcal isolates were sent to a single reference laboratory (Hospital Universitario Clínico San Carlos) for serotyping by Quellung reaction. When pleural or cerebrospinal fluids from children with meningitis or pleural empyema did not yield positive cultures, samples of these fluids were also sent to the reference laboratory for confirmation of the serotype using a real-time PCR assay (Light Cycler® SYBR green format analysis; Serotypes identified by PCR assay included: serotype 1, 3, 4, 5, 6A/B/C, 7F, 14, 19A and 19F).

The PCV13 group encompasses the serotypes included in the PCV13 vaccine [15]. All other serotypes were considered non-PCV13 serotypes.

Susceptibilities to penicillin, cefotaxime, erythromycin, clindamycin and tetracycline were determined by microdilution according to Clinical and Laboratory Standards Institute (CLSI) recommendations [17]. Current CLSI breakpoints were considered for susceptibility interpretation [18].

2.3. Data sources and measurable outcomes

Annual population data on children <15 years old and estimated person-years data for different age groups were obtained from the Spanish *Instituto Nacional de Estadística* (Spanish Statistical Office) [19] (Age groups: <5 years and ≥5 years). Incidence rates (IRs), defined as the number of cases per 100 000 inhabitants of the corresponding group, were calculated for total IPD (children ≤15 years of age), total IPD stratified by age groups, IPD caused by PCV13 serotypes, IPD caused by serotypes not included in PCV13, IPD caused by the additional serotypes included in the vaccine (not included in PCV7) and IPD for every additional serotype in PCV13 (six in total) for all study periods (a period was considered from the beginning of May to the end of April of the following year. The period from May 2007 to April 2010 is referred to as the PCV7 period; the period from May 2010 to April 2016 is referred to as the PCV13 period). The impact population was defined as the total number of children registered in the Community of Madrid in each age group for each period.

Disposition data for vaccine doses were obtained from IMS (Intercontinental Marketing Services Iberica S.A., Madrid, Spain). Yearly estimated vaccine uptake (assuming a 2 + 1 dose schedule) was calculated by dividing total doses by the number of children < 2 years of age and expressed in units/1000 inhabitants <2 years old/year.

Incidence rate ratios (IRRs), with their respective 95% confidence intervals (CI), were calculated as ratios between IR in 2015–16/IR in 2007–10 (PCV7 period). Note that, since IRRs are

ratios obtained by dividing rates and, in some cases calculated rates at the end of the study were equal to 0 (0 cases reported), the statistical software automatically adjusted those values to $0.5 \approx 1$ case to avoid obtaining any IRR = 0. All calculations were performed with the EPIDAT version 3.1.

3. Results

3.1. Incidence of invasive pneumococcal disease

Data from the PCV7 period (2007/2010) were used as a baseline to estimate the real impact of the implementation of PCV13. Fig. 1 shows the evolution of the PCV13 impact on IPD. Thus, after inclusion of the vaccine in June 2010, the IR of IPD (all serotypes considered)decreased from 16.96 to 5.35 cases per 100 000 children < 15 years in May 2013. It is important to highlight that, between 2012 and 2015, a reduction in vaccine uptake (at least 95% in 2011/2012 down to 73% in 2014/2015) occurred, due to the exclusion of PCV13 from the RIP (2011–2014 mixed funding period; 2014–2015 private funding period). When the study concluded in 2016, a final IR of 5.1 was achieved, constituting a decrease of 70.1% compared to the PCV7 period (IRR 2015/2016 *vs*. 2007/2010: 0.3 [95% CI: 0.22–0.4], $p \leq 0.001$). A similar trend was observed when analysing the IR of the serotypes included in the PCV13, with an overall significant decrease of 91% from the PCV7 period to the end of the study (IRR 2015/2016 *vs*. 2007/2010: 0.09 [95% CI: 0.05–0.16], $p \leq 0.001$). Notably, no significant changes in the evolution of the non-PCV13 serotypes were reported (IRR: 1.08[95% CI: 0.76–1.57], $p = 0.72$).

3.2. Serotype distribution

IPD case distribution by serotype, age group and epidemiological period is shown in Table 1. In accordance with the overall decrease reported in the IPD of PCV13 serotypes in the whole analysed population, significant reductions of the six additional serotypes included in PCV13 (not included in PCV7) were reported in both children < 5 years (IRR 2015/2016 *vs*. 2007/2010: 0.11[95% CI: 0.06–0.21], $p \leq 0.001$) and ≥ 5 years (IRR 2015/2016 *vs*. 2007/2010: 0.06 [95% CI: 0.01–0.22], $p \leq 0.001$) at the end of the study. In the last year (2015/2016), only 9 PCV13 cases (8 serotype 3 and one serotype 19A) and one PCV7 case (serotype 23F) were reported in children <5 years, while no serotype 3 or 19A cases were reported in children ≥5 years.

The evolution of the most relevant additional PCV13 serotypes are shown in Fig. 2.

As can be observed, in the PCV7 period, IRs corresponding to all analysed PCV13 serotypes increased, especially 19A serotype (previously reported by Picazo et al. [16]). When PCV13 was introduced in the vaccination calendar in 2010, a significant reduction in the IRs of IPD caused by serotypes 1, 19A and 7F (2015/2016 *vs*. 2007/2010; IRR ST1: 0.04 [95% CI: 0.01–0.17], $p \leq 0.001$; ST19A: 0.03 [95% CI: 0.00–0.19], $p \leq 0.001$; IRR ST7F: 0.00 [95% CI: 0.00–0.53], $p \leq 0.05$) was determined, while no significant changes were detected in the incidence of serotype 3 (IRR: 0.95[95% CI: 0.43–2.12], $p = 0.93$) (Table 1 and Supplementary Table S1). It is worth highlighting that the incidence of serotype 19A, an especially antibiotic-resistant serotype and the most prevalent one in the analysed population [14], decreased by 97% between May 2007-April 2010 (just before the introduction of PCV13) and May 2015-April 2016, from 3.77 cases per 100 000 children to 0.1 cases (no cases detected in 2013/2014 and one single case detected in both 2014/2015 and 2015/2016, coinciding with a decline in vaccine uptake). Other PCV13 serotypes also displayed similar trends (Serotype 5 [ST5], serotype 6 [ST6A] and PCV7 serotypes), although their effect on IPD was generally low over the course of the study (no serotype 5 or 6A cases reported between 2011 and 2016) (Supplementary Table S1).

3.3. Antibiotic resistance

Antimicrobial susceptibility was tested for the 912 *S. pneumoniae* isolates detected over the 9-year study. Results stratified by

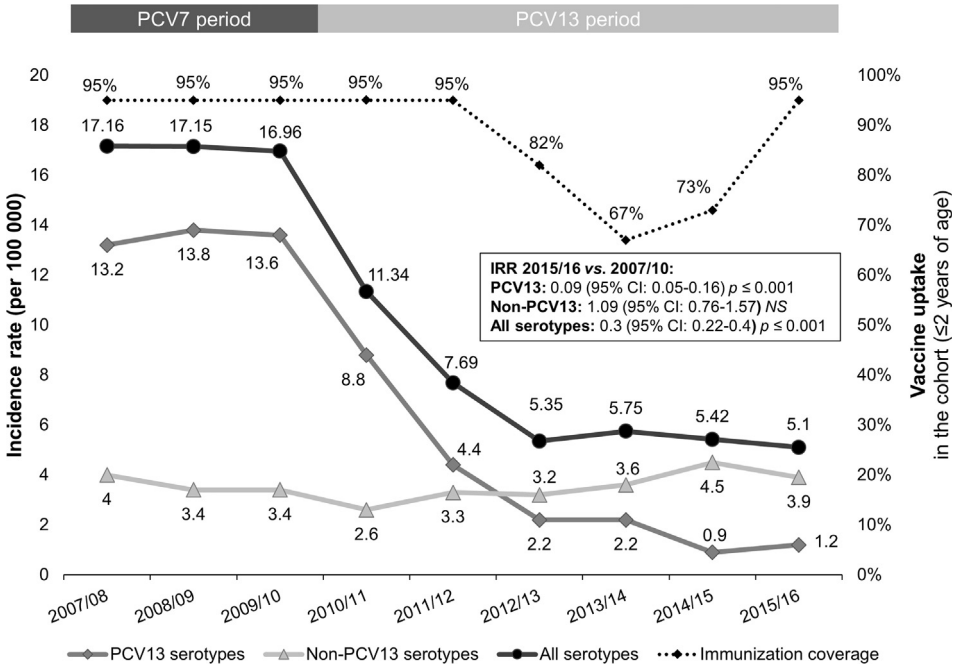


Fig. 1. PCV13 impact on the incidence rates (IRs) of invasive pneumococcal disease (IPD) in children <15 years of age. Incidence rates (IR) for every period analysed and for all groups are shown next to the specific dot representing each time point. Incidence rate ratios (IRRs) 2015/2016 *versus* 2007/2010 (95% confidence interval, CI) for PCV13 serotypes, non-PCV13 serotypes and all serotypes are shown. Percentages of vaccine uptake are shown as dotted lines.

Table 1
Number of cases, incidence of invasive pneumococcal disease and incidence rate ratios (2015/2016 *vs.* 2007/2010) by age and serotype group.

	2007–2010 IR(*n*)			2015–2016 IR (*n*)			IRR (95% CI) 2015/2016 *vs.* 2007/2010		
	Children <5 years	Children ≥ 5 years	Total < 15 years	Children < 5 years	Children ≥ 5 years	Total < 15 years	Children < 5 years	Children ≥ 5 years	Total < 15 years
Total IPD cases	35.08 (3 8 5)	6.25 (1 1 4)	17.09 (4 9 9)	12.49 (42)	1.46 (10)	5.09 (52)	0.36 (0.26–0.49) ***	0.23 (0.12–0.44) ***	0.30 (0.22–0.40) ***
PCV7 serotypes	1.00 (11)	0.55 (10)	0.72 (21)	0.30 (1)	0.00 (0)	0.10 (1)	0.3(0.01–2.04)	0.00 (0.00–1.87)	0.14 (0.02–1.01) *
PCV13 serotypes	27.06 (2 9 7)	5.32 (97)	13.49 (3 9 4)	2.97 (10)	0.29 (2)	1.17 (12)	0.11 (0.06–0.21) ***	0.06 (0.01–0.22) ***	0.09 (0.05–0.16) ***
Non-PCV13 serotypes	8.02 (88)	0.93 (17)	3.60 (1 0 5)	9.52 (32)	1.17 (8)	3.91 (40)	1.19 (0.79–1.78)	1.25 (0.54–2.90)	1.09 (0.76–1.57)
Serotype 1	7.84 (86)	2.96 (54)	4.79 (1 4 0)	0.00 (0)	0.29 (2)	0.20 (2)	0.00 (0.00–0.27) ***	0.1 (0.02–0.40) ***	0.04 (0.01–0.17) ***
Serotype 3	2.00 (22)	0.11 (2)	0.82 (24)	2.38 (8)	0.00 (0)	0.78 (8)	1.19 (0.53–2.67)	0.00 (0.00–25.52)	0.95 (0.43–2.12)
Serotype 5	3.64 (40)	0.82 (15)	1.88 (55)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0.00–0.59) **	0.00 (0.00–1.34)	0.00 (0.00–0.38) ***
Serotype 6A[#]	0.36 (4)	0.05 (1)	0.17 (5)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0.00–8.25)	0.00 (0.00–208.533)	0.00 (0.00–5.11)
Serotype 7F	3.10 (34)	0.27 (5)	1.34 (39)	0.00 (0)	0.00 (0)	0.00 (0)	0.0 (0.00–0.70) **	0.00 (0.00–4.75)	0.00 (0.00–0.53) **
Serotype 19A	9.11 (1 0 0)	0.55 (10)	3.77 (1 1 0)	0.30 (1)	0.00 (0)	0.10 (1)	0.03 (0.00–0.23) ***	0.00 (0.00–1.87)	0.03 (0.00–0.19) ***
Impact population	1,097,603	1,822,743	2,920,346	336,203	686,125	1,022,328			

IPD, invasive pneumococcal disease; **PCV7**(4, 6B, 9 V, 14, 18C, 19F and 23F), 7-valent pneumococcal conjugate vaccine; **PCV13** (PCV7 serotypes + serotypes 1, 3, 5, 6A, 7F and 19A), 13-valent pneumococcal conjugate vaccine; **IR**, incidence rate; **IRR**, incidence rate ratio; **CI**, confidence interval.
[#] serotype 6A was determined by culture methods. PCR assays were unable to differentiate between serotypes 6A/B/C.
* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$.

meningitis and non-meningitis cases (Fig. 3) revealed an important general decrease in the percentage of both penicillin- and cefotaxime-resistant strains, with total disappearance of the cefotaxime-resistant isolates from 2012 onwards, except for a minimal number of non-meningitis cases recorded in 2014/2015. Disappearance of the non-meningeal penicillin-resistant isolates followed a similar trend (Fig. 3). Interestingly, in the PCV7 period, 53% of penicillin-resistant meningeal isolates belonged to the 19A serotype, while in the PCV13 period, only 13% of resistant strains belonged to this serotype. By the end of the study, all cefotaxime-resistant isolates causing meningitis, as well as penicillin-resistant isolates responsible for respiratory infections (non-meningitis cases), had disappeared.

Regarding the other antibiotic-resistant isolates analysed, an erythromycin resistance of 31% was observed in the last year of the PCV7 period (2009/2010). This was mainly associated with the 19A serotype (72%). At the end of the study (2015/2016), this number was reduced to 24% (although not a statistically significant reduction), and non-PCV serotypes accounted for 89% of the isolates. Similar results were found for clindamycin and tetracycline resistance (Supplementary Figure S1).

3.4. Clinical impact

Fig. 4 and Table 2 show the evolution of the IRs of the main IPD clinical presentations in children < 15 years. An overall significant reduction of all clinical manifestations was observed: bacteraemic pneumonia (IRR 2015/2016 *vs.* 2007/2010: 0.27 [95% CI: 0.16–0.46], $p \leq 0.001$) pleural empyema (IRR 2015/2016 *vs.* 2007/2010: 0.29 [95% CI: 0.18–0.48], $p \leq 0.001$), meningitis (IRR 2015/2016 *vs.* 2007/2010: 0.41 [95% CI: 0.2–0.82], $p \leq 0.05$) and primary bacteraemia (IRR 2015/2016 *vs.* 2007/2010: 0.48 [95% CI: 0.24–0.93], $p \leq 0.05$). Specifically, the percentage of cases of meningitis due to PCV13-only serotypes was higher in the PCV7 period (52.3%) than in the PCV13 period (16.0%), and the IR decreased from 1.16 cases per 100 000 children at the beginning of the study to 0.2 cases at the end, with no detected cases between 2011 and 2015 and two cases reported in the last period of the study (2015/2016) (Table 2). Furthermore, serotype 19A was the most common for meningitis, accounting for 18.5% of all isolates before the inclusion of PCV13, while a heterogeneous distribution was found for the remaining serotypes. Other clinical manifestations, although minimal, also disappeared completely (IRR 2015/2016 *vs.* 2007/2010: 0.06 [95% CI: 0.01–0.43], $p \leq 0.001$). Specifically, of the 68 reported cases caused by serotype 3 throughout the study period, 73.5% ($n = 50$) were pleural empyema, 10.3% ($n = 7$) bacteraemic pneumonia, 7.4% ($n = 5$) primary bacteraemia, 1.5% ($n = 1$) meningitis and 7.4% ($n = 5$) other clinical presentations.

3.5. Invasive pneumococcal disease diagnosis

Between May 2007 and April 2016, a total of 912 cases of IPD in children <15 years were reported in the Community of Madrid, of which pleural empyema was the most frequent clinical presentation ($n = 311$, 34.1%), followed by bacteraemic pneumonia ($n = 250$, 27.4%), primary bacteraemia ($n = 131$, 14.4%), meningitis ($n = 113$, 12.4%) and others ($n = 107$, 11.7%). Importantly, 210 out of the 912 cases identified were culture-negative but positive in the PCR assay, with pleural empyema being the most frequently underdiagnosed IPD (Supplementary Table S2). In fact, 194 cases with this clinical presentation (62.4% of the total) were culture-negative and were only diagnosed by real-time PCR. PCR assay was negative in one single case only.

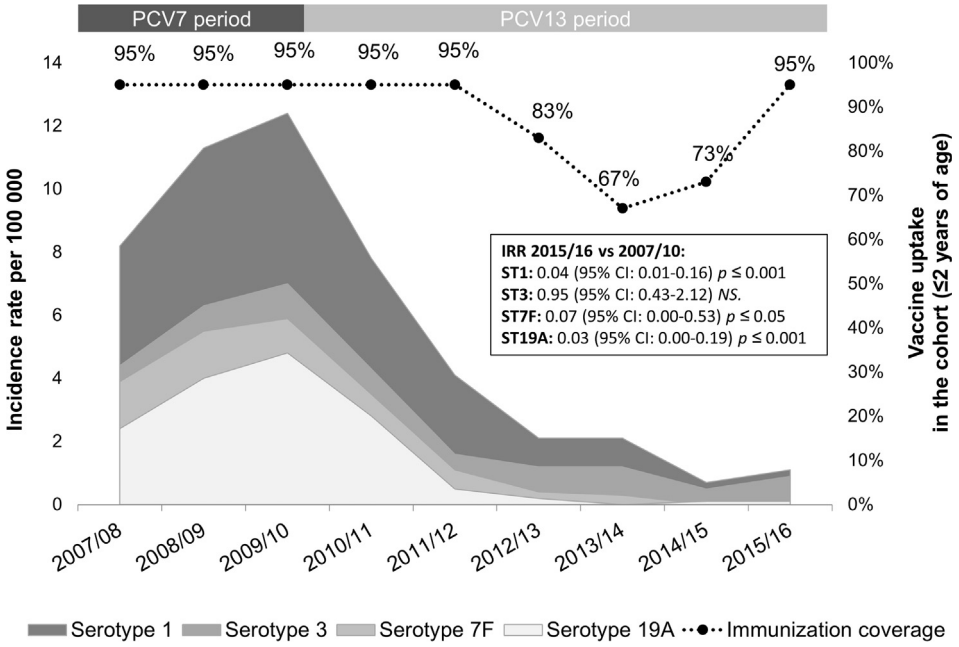


Fig. 2. Evolution of the main PCV13 serotypes in children <15 years of age. Cumulative incidences of serotypes 1, 3, 7F and 19A are presented. Incidence rate ratios (IRRs) 2015/2016 *versus* 2007/2010 (95% confidence interval, CI) for each serotype are shown. Percentages of vaccine uptake are shown as dotted lines.

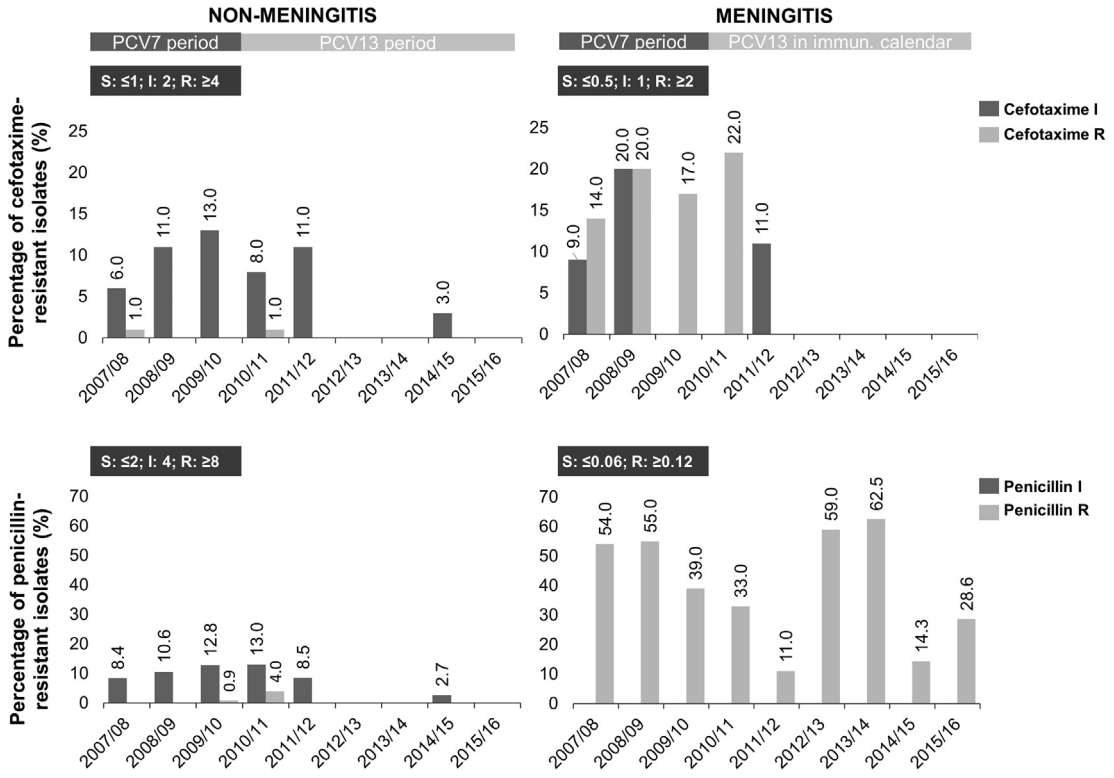
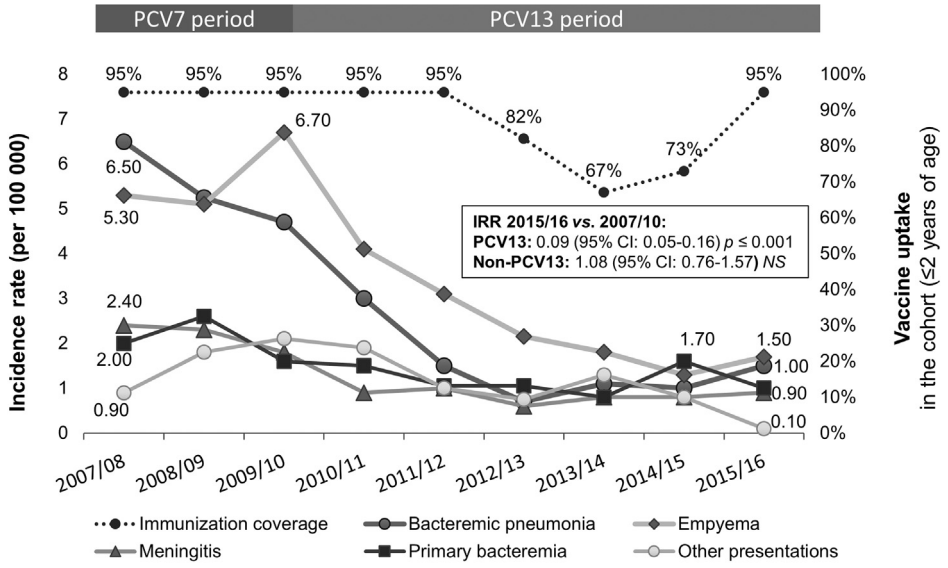


Fig. 3. Antibiotic susceptibility to penicillin and cefotaxime of *Streptococcus pneumoniae* isolates stratified by meningitis and non-meningitis cases. Clinical Laboratory Standards Institute (CLSI) breakpoints are shown in grey-coloured boxes. S, Susceptible; I, Intermediate; R, Resistant.

4. Discussion

The results of the HERACLES clinical surveillance study conducted over a 9-year period in the Community of Madrid showed a general benefit of PCV13 over PCV7, with interesting findings concerning certain serotypes (such as the 19A serotype) and their impact on antibiotic resistance and clinical manifestations. Furthermore, they also demonstrated the underdiagnosis phenomenon that underlies culture assays. In this regard, and consistent with previous findings [20], our data showed that real-time PCR is a more sensitive method for diagnosing IPD, identifying almost all cases of pleural empyema, the most common



Main decline of serotypes by clinical presentation		Meningitis	ST19A
Bacteraemic pneumonia	ST1, ST5	Primary bacteraemia	ST5, ST7F
Empyema	ST1, ST5, ST7F, ST19A	Other presentations	ST19A

Fig. 4. Clinical Impact of PCV13 on invasive pneumococcal disease (IPD) in children <15 years of age (all serotypes). Incidence rates (IR) for some periods and for all clinical manifestations are shown next to the specific dot representing each time point. Incidence rate ratios (IRRs) 2015/2016 *versus* 2007/2010 (95% confidence interval, CI) of the different clinical manifestations are shown. Percentages of vaccine coverage are shown as dotted lines.

underdiagnosed clinical manifestation. Consequently, surveillance studies in which IPD diagnosis is exclusively based on bacterial culture results are likely to underestimate the actual incidence and subsequent impact of PCV13.

Our results demonstrate that PCV13 inclusion in the vaccination calendar reduced the IR of IPD by 70.1%, despite a reduction in vaccine uptake from 2012 and 2015 due to a switch from public to private funding. Importantly, although this led to stagnation in the decline of IRs in that period, as we recently published in a preliminary analysis of the HERACLES study performed before its completion [16], PCV13 impact was sustained, with remarkable positive effects on IPD over the analysed period. The relevance of these results is also supported by the fact that the population of the region of Madrid was estimated at 6 507 184 inhabitants in 2017 [19], and consequently above the population of several European countries such as Denmark, Norway, Ireland and Finland [21]. This clearly reinforces the impact of our study.

When IRs were estimated by serotype group, a general decline in PCV13 serotypes was found in all age groups in every period analysed. Interestingly, in all children <15 years, we specifically observed a 91% decrease in PCV13 serotypes, demonstrating that, indeed, PCV13 serotype reduction accounted for the entire 70.1% reduction in IPD observed from 2010 to 2016. This also confirmed the net positive effect of the introduction of this vaccine in the RIP.

Rapid replacement by non-vaccine serotypes may mitigate the impact of pneumococcal conjugate vaccines on nasopharyngeal bacterial ecology (fundamental for pathogen spread) [22]. In this study, no substantial changes in the evolution of non-PCV13 serotypes were observed, which additionally demonstrates the effectiveness of PCV13. Specifically, evolution of non-PCV13 serotypes reached equilibrium, probably due to catch-up doses, which accelerate the build-up of herd protection and hence PCV impact [1]. Our data confirm previous observations reporting that overall reduction of IPD prevented by PCVs is only partly offset by serotype replacement [23,24].

An analysis of the evolution of the main serotypes covered by PCV13 over the time analysed reflected a significant decrease in the IRs of IPDs caused by serotypes 1, 19A and 7F. Other serotypes included in PCV13 showed similar behaviour. These results explain the overall 91% decrease in total PCV13 serotypes previously discussed. However, non-vaccine-related secular trends [10] could likewise have affected the decrease in some serotypes, such as has also been suggested by other authors for serotypes 1 and 5, both among children [23] and adults [25]. In particular, serotype 19A experienced a remarkable reduction, with its almost total disappearance by the end of the HERACLES study (one case in 2015/2016; IR = 0.1 cases per 100 000 children <15 years). Serotype 19A was not only the most prevalent serotype during majority of the study period [14], but it also caused nearly 20% cases of meningitis, being the most frequent multidrug resistance-associated serotype that also showed high-level resistance to cefotaxime in meningitis. Our data clearly demonstrate that PCV13 introduction in the vaccination calendar led to an almost complete elimination of this serotype, with the subsequent impact on the disease. In line with these results, an important reduction in the percentage of penicillin- and cefotaxime-resistant strains was reported, along with the total disappearance of cefotaxime resistance from 2012 onwards within the meningitis group. These results are especially relevant considering that serotype 19A is the most prevalent in meningitis cases [26] and that, as will be discussed later, the incidence of this clinical manifestation decreased accordingly. In the same way, the disappearance of cefotaxime-resistant strains suggests the elimination of combined treatments with major associated side effects, such as treatment with vancomycin, where nephrotoxicity is frequently reported in treated patients [27].

The impact on clinical manifestations of IPD (caused by both PCV13 and non-PCV13 serotypes) was evaluated as an additional and important indicative measure of the effectiveness of PCV13. Our data allow us to establish a clear connection between serotype trends over the 9-year study and the evolution of the IRs of the different clinical manifestations. Thus, the IR of bacteraemic pneumonia decreased mainly due to the reduction in serotypes 1 and 5. Pleural empyema reduction between 2009/2010 and 2015/2016

Table 2
Clinical presentation per age, number of cases, incidence rate (IR) and incidence rate ratio (IRR) [95% confidence interval, CI] of invasive pneumococcal disease (IPD) in children < 15 years stratified by PVC13 serotypes, non-PVC13 serotypes and total cases (PVC13 + non-PVC13 serotypes).

		2007–2010 IR (*n*)			2015–2016 IR (*n*)			IRR (95% CI) 2015/2016 *vs.* 2007/2010		
		Children < 5 years	Children ≥ 5 years	Total < 15 years	Children < 5 years	Children ≥ 5 years	Total < 15 years	Children < 5 years	Children ≥ 5 years	Total < 15 years
Bacteraemic pneumonia	**Total IPD**	10.3 (1 1 3)	2.63 (48)	5.51 (1 6 1)	3.87 (13)	0.29 (2)	1.47 (15)	0.38 (0.21–0.67) ***	0.11 (0.03–0.46) ***	0.27 (0.16–0.46) ***
	PCV13 serot.	8.47 (93)	2.52 (46)	4.76 (1 3 9)	0.00 (0)	0.15 (1)	0.10 (1)	0.04 (0.01–0.25) ***	0.06 (0.01–0.42) ***	0.02 (0.00–0.15) ***
	Non-PCV13 serot.	1.82 (20)	0.11 (2)	0.75 (22)	3.87 (13)	0.15 (1)	1.37 (14)	2.12 (1.06–4.27) *	1.33 (0.02–25.52)	1.82 (0.93–3.55)
Pleural empyema	**Total IPD**	11.30 (1 2 4)	2.36 (43)	5.72 (1 6 7)	2.97 (10)	1.02 (7)	1.66 (17)	0.26 (0.14–0.5) ***	0.43 (0.19–0.96) *	0.29 (0.18–0.48) ***
	PCV13 serot.	9.20 (1 0 1)	2.08 (38)	4.76 (1 3 9)	2.08 (7)	0.15 (1)	0.78 (8)	0.23 (0.11–0.49) ***	0.07 (0.01–0.51) ***	0.16 (0.08–0.34) ***
	Non-PCV13 serot.	2.10 (23)	0.27 (5)	0.96 (28)	0.89 (3)	0.87 (6)	0.88 (9)	(0.43–0.13–1.42)	3.19 (0.81–13.20)	0.92 (0.43–1.95)
Meningitis	**Total IPD**	4.65 (51)	0.66 (12)	2.16 (63)	2.68 (9)	0.00 (0)	0.88 (9)	0.58 (0.28–1.17) *	0.22 (0.01–1.5)	0.41 (0.2–0.82) **
	PCV13 serot.	2.64 (29)	0.27 (5)	1.16 (34)	0.59 (2)	0.00 (0)	0.20 (2)	0.23 (0.05–0.94) *	0.53 (0.01–4.75)	0.17 (0.04–0.7) **
	Non-PCV13 serot.	2.00 (22)	0.38 (7)	0.99 (29)	2.08 (7)	0.00 (0)	0.68 (7)	1.04 (0.44–2.43)	0.38 (0.01–2.95)	0.69 (0.3–1.57)
Primary bacteraemia	**Total IPD**	5.01 (55)	0.27 (5)	2.05 (60)	2.68 (9)	0.15 (1)	0.98 (10)	0.53 (0.26–1.08)	0.53 (0.01–4.75)	0.48 (0.24–0.93) *
	PCV13 serot.	3.92 (43)	0.16 (3)	1.58 (46)	0.3 (1)	0.00 (0)	0.1 (1)	0.08 (0.01–0.55) **	0.89 (0.02–11.03)	0.06 (0.01–0.45) ***
	Non-PCV13 serot.	1.09 (12)	0.11 (2)	0.48 (14)	2.38 (8)	0.15 (1)	0.88 (9)	2.18 (0.77–5.79)	1.33 (0.02–25.52)	1.84 (0.70–4.56)
Other presentations	**Total IPD**	3.83 (42)	0.33 (6)	1.64 (48)	0.3 (1)	0.00 (0)	0.10 (1)	0.08 (0.01–0.56) **	0.44 (0.01–3.65)	0.06 (0.01–0.43) ***
	PCV13 serot.	2.82 (31)	0.27 (5)	1.23 (36)	0.00 (0)	0.00 (0)	0.00 (0)	0.11 (0.01–0.77) *	0.53 (0.01–4.75)	0.08 (0.01–0.58) **
	Non-PCV13 serot.	1.00 (11)	0.05 (1)	0.41 (12)	0.3 (1)	0.00 (0)	0.10 (1)	0.3 (0.01–2.04)	2.66 (0.03–208.53)	0.24 (0.01–1.61)

IR, incidence rate; **IRR**, incidence rate ratio; **PCV13**, 13-valent conjugate pneumococcal vaccine; **Serot.**, serotype; **CI**, confidence interval.
* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$.

correlated with the decrease in serotypes 1, 5, 7F and 19A, the most common ones detected in this clinical manifestation. Notably, in the PCV7 period (from 2007 to 2010), the incidence of pleural empyema increased, which can be attributed to the increment in serotypes 1 and 19A in the same years. A specific reduction in serotypes 5 and 7F was also the cause of decreased primary bacteraemia. In terms of meningitis, we observed almost total disappearance of this clinical manifestation caused by the abovementioned disappearance of 19A serotype cases, and the aforementioned almost total disappearance of cefotaxime-resistant isolates.

One study limitation is the estimation of vaccine uptake, assuming a "2 + 1" dose schedule instead of the recommended "3 + 1", which may falsely increase vaccination coverage. However, given the fact that adherence was probably suboptimal between 2012 and 2015 (privately funded period), a phenomenon already reported by other authors in Spain and other European countries [28], we believe that this calculation is the most appropriate in our study. Another limitation is related to the fact that, as demonstrated, many culture-negative cases considered as clinically suspicious were positive when tested by real-time PCR. Although additional testing was performed, it is likely that the disease burden was underestimated (especially regarding pleural empyema cases). Finally, only isolates belonging to serotypes 1, 3, 4, 5, 6, 7F, 14, 19A, and 19F could be serotyped by the real-time PCR assay used for serotyping of pneumococci identified by PCR; therefore, we cannot dismiss the possibility that the IPDs diagnosed by PCR were caused by pneumococci belonging to some PCV13 serotypes such as 9 V, 18C, and 23F.

In conclusion, the HERACLES study provides sufficient evidence to support, on the one hand, the additional benefits of PCV13 over PCV7 on the general incidence of IPD and, on the other hand, the effectiveness of the vaccine since its inclusion in the RIP of the Community of Madrid in 2010. Importantly, positive outcomes were achieved despite changes in vaccine funding policies, including the almost total elimination of the aggressive and resistant serotype 19A and the subsequent disappearance of 19A-related meningitis cases. Finally, PCV13 impact on IPD did not result in replacement by other serotypes not covered by the vaccine.

Conflict of interests

Study Sponsored by Pfizer

Acknowledgements

Medical writing support was provided by Almudena Fuster-Matanzo, PhD from Medical Statistics Consulting S.L. (Valencia, Spain).

Members of the Heracles Study Group are:

J. J.Picazo, J.T. Ramos, C. Betriu, E. Culebras, F. González, I. Rodriguez-Avial (H. Universitario Clínico San Carlos, Madrid, Spain), J. Ruiz-Contreras, S. Negreira, M.A. Orellana (H. Universitario 12 de Octubre, Madrid, Spain), A. Delgado-Iribarren, M. Bueno (H. Universitario Fundación Alcorcón, Madrid, Spain), A. Alhambra, M.T. García (H. Universitario HM Sanchinarro, Madrid, Spain), M.J. González, J. Cobos (H. San Rafael, Madrid, Spain), F.

Baquero, A. García (H. Universitario La Paz, Madrid, Spain), J. Casado, B. Hernández (H. Universitario Infantil Niño Jesús, Madrid, Spain), M. Zafra, J. Jaqueti (H. Universitario de Fuenlabrada, Madrid, Spain), C. Calvo, I. Wilhelmi (H. Universitario Severo Ochoa, Madrid, Spain), C. Serrano, E. García-Peñuela (H. Universitario Sanitas la Zarzuela, Madrid, Spain), E. Cercenado, T. Hernández-Sampelayo (H. General Universitario Gregorio Marañón, Madrid, Spain), E. Otheo, P. Ruiz (H. Universitario Ramón y Cajal, Madrid, Spain), N. Domínguez, I. Gadea (Fundación Jiménez Díaz, Madrid,

Spain), I. Romero (H. Universitario HM Torrelodones, Madrid, Spain), J.L. Gómez-Garcés, M.A. Roa (H. Universitario de Móstoles, Madrid, Spain), M. Ruíz, J. Cacho (H. Universitario de Getafe, Madrid, Spain), M.L. García-Picazo, S. Gallego (H. de El Escorial, Madrid, Spain), M.J. Cilleruelo, M.I. Sánchez (H. Universitario Puerta de Hierro, Madrid, Spain), M. Penin, P. Gómez (H. Universitario Príncipe de Asturias, Madrid, Spain), S. Salso, M. Corcino (H. Universitario HM Montepríncipe, Madrid, Spain), A. Rodríguez (H. Universitario del Sureste, Madrid, Spain), J. Clemente (H. del Henares, Madrid, Spain), J.L. Ruibal (H. Universitario Infanta Cristina, Madrid, Spain), B. Agúndez (H. Universitario Infanta Leonor, Madrid, Spain), A. Cañete (H. Universitario Infanta Sofía, Madrid, Spain), C. García-Vao (H. Universitario del Tajo, Madrid, Spain), A. Sánchez (BR Salud, Madrid, Spain), J.C. Sanz (LRSP, Madrid, Spain), C. Méndez, M. del Amo, C. Balseiro, and M.L. Samaniego (Pfizer S.L.U., Madrid, Spain).

Funding

Study Sponsored by Pfizer, Spain.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.03.015.

References

[1] (WHO) WHO. Pneumococcal vaccines WHO position paper–2012. Wkly Epidemiol Rec 2012;87:129–44.
[2] O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. Lancet 2009;374:893–902.
[3] Klugman KP, Black S, Dagan R, Malley R, Whitney CG. Vaccines. In: Stanley A. Plotkin WAOaPAO, W.B. Saunders, editor. Vaccines. Sixth edition ed. London2013. p. 504-41.
[4] Moine P, Vercken J-B, Chevret S, Chastang C, Gajdos P. Severe community-acquired pneumonia. CHEST 1994;105:1487–95.
[5] Whitney CG, Farley Mm Fau - Hadler J, Hadler J Fau - Harrison LH, Harrison Lh Fau - Bennett NM, Bennett Nm Fau - Lynfield R, Lynfield R Fau - Reingold A, et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. 2003.
[6] Kyaw MH, Lynfield R Fau - Schaffner W, Schaffner W Fau - Craig AS, Craig As Fau - Hadler J, Hadler J Fau - Reingold A, Reingold A Fau - Thomas AR, et al. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant Streptococcus pneumoniae. 2006.
[7] Beall BW, Gertz Re Fau - Hulkower RL, Hulkower Rl Fau - Whitney CG, Whitney Cg Fau - Moore MR, Moore Mr Fau - Brueggemann AB, Brueggemann AB. Shifting genetic structure of invasive serotype 19A pneumococci in the United States. 2011
[8] Hicks LA, Harrison Lh Fau - Flannery B, Flannery B Fau - Hadler JL, Hadler Jl Fau - Schaffner W, Schaffner W Fau - Craig AS, Craig As Fau - Jackson D, et al. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998-2004. 2007.
[9] Pai R, Moore Mr Fau - Pilishvili T, Pilishvili T Fau - Gertz RE, Gertz Re Fau - Whitney CG, Whitney Cg Fau - Beall B, Beall B. Postvaccine genetic structure of Streptococcus pneumoniae serotype 19A from children in the United States. 2005.
[10] Fenoll A, Granizo JJ, Aguilar L, Gimenez MJ, Aragoneses-Fenoll L, Hanquet G, et al. Temporal trends of invasive Streptococcus pneumoniae serotypes and antimicrobial resistance patterns in Spain from 1979 to 2007. J Clin Microbiol 2009;47:1012–20.
[11] Robinson KA, Baughman W, Rothrock G, Barrett NL, Pass M, Lexau C, et al. Epidemiology of invasive Streptococcus pneumoniae infections in the United States, 1995–1998: opportunities for prevention in the conjugate vaccine era. JAMA 2001;285:1729–35.
[12] Moore MR, Gertz Jr RE, Woodbury RL, Barkocy-Gallagher GA, Schaffner W, Lexau C, et al. Population snapshot of emergent Streptococcus pneumoniae serotype 19A in the United States, 2005. J Infect Dis 2008;197:1016–27.
[13] Reinert R, Jacobs MR, Kaplan SL. Pneumococcal disease caused by serotype 19A: review of the literature and implications for future vaccine development. Vaccine 2010;28:4249–59.
[14] Picazo J, Ruiz-Contreras J, Hernandez B, Sanz F, Gutierrez A, Cercenado E, et al. Clonal and clinical profile of Streptococcus pneumoniae serotype 19A causing pediatric invasive infections: a 2-year (2007–2009) laboratory-based surveillance in Madrid. Vaccine 2011;29:1770–6.
[15] (CDC) CfDCaP. Licensure of 13-valent pneumococcal conjugate vaccine for adults aged 50 years and older. MMWR Morb Mortal Wkly Rep 2012;61:394–5.
[16] Picazo J, Ruiz-Contreras J, Casado-Flores J, Negreira S, Baquero F, Hernandez-Sampelayo T, et al. Effect of the different 13-valent pneumococcal conjugate vaccination uptakes on the invasive pneumococcal disease in children: Analysis of a hospital-based and population-based surveillance study in Madrid, Spain, 2007–2015. PLoS One 2017;12:e0172222.
[17] (CLSI) CaLSI. Methods for dilution antimicrobial susceptibility tests for bacteria thar grow aerobically. Clinical and Laboratory Standards. 7th ed, approved standard M7-A& ed. Wayne, PA: Clinical and Laboratory Standards Institute (CLSI); 2006.
[18] (CLSI) CaLI. Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard M100-S24. Clinical Laboratory Standards. Wayne, PA: Clinical Laboratory Institute (CLSI); 2014
[19] (INE) INdE.
[20] Michelow IC, Lozano J, Olsen K, Goto C, Rollins NK, Ghaffar F, et al. Diagnosis of Streptococcus pneumoniae lower respiratory infection in hospitalized children by culture, polymerase chain reaction, serological testing, and urinary antigen detection. Clin Infect Dis 2002;34:E1–E11.
[21] Worldometers. European countries by population (2018). Worldometers; 2018.
[22] Kwambana-Adams B, Hanson B, Worwui A, Agbla S, Foster-Nyarko E, Ceesay F, et al. Rapid replacement by non-vaccine pneumococcal serotypes may mitigate the impact of the pneumococcal conjugate vaccine on nasopharyngeal bacterial ecology. Sci Rep 2017;7:8127.
[23] Aguiar SI, Brito MJ, Horacio AN, Lopes JP, Ramirez M, Melo-Cristino J. 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. Euro Surveill 2014;19:20750.
[24] Link-Gelles R, Taylor T, Moore MR. Forecasting invasive pneumococcal disease trends after the introduction of 13-valent pneumococcal conjugate vaccine in the United States, 2010–2020. Vaccine 2013;31:2572–7.
[25] Horacio AN, Diamantino-Miranda J, Aguiar SI, Ramirez M, Melo-Cristino J. The majority of adult pneumococcal invasive infections in Portugal are still potentially vaccine preventable in spite of significant declines of serotypes 1 and 5. PLoS One 2013;8:e73704.
[26] Picazo J, Ruiz-Contreras J, Casado-Flores J, Giangaspro E, Del Castillo F, Hernandez-Sampelayo T, et al. 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 2011;18:89–94.
[27] Bamgbola O. Review of vancomycin-induced renal toxicity: an update. Ther Adv Endocrinol Metab 2016;7:136–47.
[28] Gervaix A, Ansaldi F, Brito-Avo A, Azzari C, Knuf M, Martinon-Torres F, et al. Pneumococcal vaccination in Europe: schedule adherence. Clin Ther 2014;36 (802–12):e1.