Vaccine 37 (2019) 2704–2711

Contents lists available at ScienceDirect

Vaccine

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

Significant impact of pneumococcal conjugate vaccination on pediatric parapneumonic effusion: Italy 2006–2018

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ARTICLE INFO

Article history:
Received 23 July 2018
Received in revised form 1 April 2019
Accepted 4 April 2019
Available online 10 April 2019

Keywords:
Parapneumonic effusion
Streptococcus pneumoniae
Molecular surveillance
Realtime PCR
Vaccination impact
Child

ABSTRACT

Etiology and serotyping of parapneumonic effusion (PPE) and the impact of vaccination was evaluated over a 12-year period, before and after the PCV13 introduction (2011) for Italian children From 0 to 16 years of age.

Five hundred and two children were evaluated; 226 blood and 356 pleural fluid samples were obtained and tested using Realtime-PCR and culture. In the pre-PCV13 era *S. pneumoniae* was the most frequent pathogen identified (64/90; 71.1%) with a large predominance of serotypes 1 (42.4%), 3 (23.7%), 7F (5.1%) and 19A (11.9%).

The impact of vaccination, calculated on children 0–8 years of age, demonstrated a significant reduction of PPE: with an incidence rate of 2.82 (95%CL 2.32–3.41) in the pre-PCV13 era and an age-standardized rate (ASR) of 0.66 (95% CL 0.37–1.99) in the post-PCV13 era, $p < 0.0001$. No increase in non-PCV13 serotypes was recorded. *S. pneumoniae* remained the most frequent pathogen identified in the post-PCV13 era in unvaccinated children with an unchanged serotype distribution: respectively 26/66 (39.4%), 25/66 (37.9%), 5/66 (7.6%), and 4/66 (6.1%) for 1, 3, 7F and 19A. On the other hand 7F and 19A disappeared in vaccinated children and serotype 1 and 3 decreased by 91.8% and 31.5%, respectively. Realtime PCR was significantly more sensitive than culture both in pleural fluid (79.7% vs 12.5%) and in blood (17.8% vs 7.4%).

In conclusion, our findings indicate that routine immunization with PCV13 has significantly reduced the burden of childhood PPE in vaccinated children, without increasing PPE due to other bacteria and without serotype shift. Moreover, the impact of PCV13 may be underestimated due to the increase in pneumococcal surveillance in Italy. Data has also shown that Real-time PCR is an essential tool to better define the etiology of PPE and to monitor vaccination plans. Longer studies will be necessary to evaluate the role of herd protection in PPE prevention.

Abbreviations: PPE, parapneumonic effusion; PE, pleural empyema; CAP, community-acquired pneumonia; PCV7, 7-valent pneumococcal conjugate vaccine; IPD, invasive pneumococcal disease; PCV13, 13-valent pneumococcal conjugate vaccine; RT-PCR, realtime polymerase chain reaction.

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https://doi.org/10.1016/j.vaccine.2019.04.012
0264-410X/

1. Introduction

Parapneumonic effusion (PPE) and pleural empyema (PE) are severe complications of community-acquired pneumonia (CAP) [1]. Over the last twenty years, a marked increase in the worldwide incidence of complicated pneumonia both in children and adults has been described [2,3]. *Streptococcus pneumoniae* is the main causal agent of complicated pneumonia in childhood [4].

The introduction of 7-valent pneumococcal conjugate vaccine (PCV7) into pediatric immunization programs has led to a dramatic reduction in overall and PCV7-type incidence of pneumonia and invasive pneumococcal disease (IPD). This benefit has also been observed among unvaccinated age groups in countries where PCV-7 is routinely used, which suggests that PCV-7 provides herd protection [5,6].

Despite this apparent benefit, the empyema-associated hospitalization rates have increased over time [7], mainly due to non PCV-7 serotypes such as 1, 3, 7F, and 19A [4,8].

The introduction of 13-valent pneumococcal conjugate vaccine (PCV13) has further reduced the IPD incidence in childhood in many countries [9–12]. A decrease in empyema incidence [13] and hospitalization rates [14–16] of children after the introduction of PCV13 has recently been reported, but the role of pneumococcal serotypes in this evolution trend is still unclear. Moreover, data are still lacking on herd-protection or a shift to other etiologies in long-term studies in the PCV13 era.

The aims of our study were to describe the etiology of PPE and PE in Italian children over a period of ten-years, to evaluate the impact of pneumococcal conjugate vaccination using a population-based molecular surveillance, and to determine the sensitivity of RT-PCR compared to the culture method in the diagnosis of PPE.

2. Patients and methods

2.1. Study design

Our observational study evaluated retrospectively all children 0–16 years of age included into the national Molecular Surveillance Register and who had been admitted to Italian hospitals with the diagnosis of PPE complicating CAP from September 2006 to October 2018. The Molecular Surveillance Register was opened at the Immunology and Infectious Diseases Lab, Meyer Children's Hospital, Florence, Italy, (hereinafter "central Lab") in 2006 and has been expanded with dedicated funds from the Italian Center for Disease Control (CCM) [17]. All pediatric hospitals or pediatric units in Italy were invited to participate in the register. To include a patient in the register, at least one biological sample had to be tested at the central Lab by using Realtime-PCR. A culture-based test was not a criterion for inclusion but culture results were recorded when available. Available clinical and laboratory data were also recorded, using a standardized report form.

Children with severe concomitant diseases (i.e. cystic fibrosis, immunodeficiency, neurological impairment) or suspected nosocomially acquired infections (i.e. hospitalization and/or admission to emergency departments and/or access to ambulatory services over the previous 14 days) were excluded from the study. The study was approved by the Institutional Review Board. All data and samples included in this study were collected as part of the routine clinical activity and evaluated retrospectively and anonymously in the study. For this reason, a specific approval by the regional ethical committee was not required.

2.2. Case definition

Patients with clinical suspicion of pneumonia complicated by PPE/PE were evaluated. Pneumonia was suspected on the basis of clinical signs such as abnormal breath sounds and/or tachypnea; confirmation was obtained by chest X-ray and/or chest ultrasonogram and/or computed tomography. A pediatric pulmonologist with experience in radiology, in collaboration with radiologists, evaluated radiographs and assigned standardized and mutually exclusive diagnoses of focal, segmental, or lobar consolidation with or without pleural effusion, interstitial pneumonia, atelectasis, or necrotizing pneumonia, as previously described [8,18]. In particular, diagnosis of parapneumonic effusion (PPE) was assigned in the presence of loculated pleural fluid on a chest X-ray, a chest ultrasonogram, or a computed tomography [19]. Diagnosis of pleural empyema (PE) was assigned in the presence of pleural fluid parameters consistent with empyema [19]. Only patients with a confirmed radiological diagnosis of PPE/PE were included in the study. Clinical information was sent, together with biological samples, using a standardized report form for each patient. Updates on the clinical conditions of the patients and on culture results were obtained by phone interviews to the sending hospitals.

2.3. Laboratory methods

Laboratory confirmation was obtained by RT-PCR and/or culture methods as previously described [8].

For culture purposes, standardized procedures were used for collection and shipment of biological samples to local laboratories.

Samples for molecular tests were sent by participant centers to the central Lab using an overnight freepost carrier and tests were performed within 2 h of delivery. For routine diagnosis, a panel of primers and probes for the following 14 pathogens was used: *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Escherichia coli, Klebsiella pneumoniae, Streptococcus agalactiae, Listeria monocytogenes, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Salmonella spp, Mycoplasma pneumoniae, Fusobacterium spp,* (the most frequent in invasive bacterial diseases in Italy) and *Adenovirus*. Etiological diagnosis was made if RT-PCR and/or culture was positive in blood or pleural fluid as previously described [8]. When RT-PCR was negative for all primers/probes included in the panel, amplification and sequencing of the 16S rRNA bacterial gene were performed. All samples positive for *S. pneumoniae* were serotyped by RT-PCR using 33 primer couples and probes. Part of the primer-probe sets are published [8,19], the others are available upon request. As previously published [18], Realtime PCR reliability in pneumococcal serotyping has been demonstrated by testing serotyped pneumococcal isolates from ATCC as controls. Pneumococcal serotypes were classified as PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F), PCV13 serotypes (PCV7 plus 1, 3, 5, 6A, 7F, 19A), and non-PCV13 serotypes (not included in the PCV13). If no increase in fluorescent signal was observed after 40 cycles for any of the serotype-specific primer/probe sets, in spite of a positive result with both RT-PCR (*lytA gene*) and end-point PCR (*cpsA gene*), the sample was reported as non-typeable.

2.4. Vaccine uptake

Mean vaccination coverage for PCV13 in Italian target populations in the post-PCV13 era was obtained from the Italian Ministry of Health (http://www.salute.gov.it/portale/documentazione/p6_2_8_3_1.jsp?lingua=italiano&id=20; accessed April 1, 2019) and is shown in the supplementary file, Table 1. No catch-up campaign for children >1 years of age was planned after the introduction of PCV13.

2.5. Evaluation of PPE/PE incidence and impact of vaccination

Since participation in the Molecular Surveillance Register and in the present study was dependent on voluntary instead of mandatory surveillance, the incidence of PPE cases calculated using the whole Italian pediatric population as denominator does not reflect the true incidence of disease in Italy but allows the impact of the vaccination to be evaluated. The overall incidence and incidence for each serotype in the pre- and post-PCV13 eras were calculated as a crude incidence rate in the pre-PCV13 era (reference population) and as an age standardized incidence rate (ASR) in the post-PCV13 era (study population).

Since PCV13 was included in the vaccination schedule in the last quarter of 2010, only children 0–8 years of age were evaluated in the pre- vs post-PCV13 era comparison. Children 0–8 years of age who were born before 2011 and had pneumococcal PPE either before or after 2011 were considered as reference population and were compared with children of the same age range born after 2011 (study population). Details are given in the supplementary file, Tables 2 and 3

The number of samples received for molecular surveillance from all Italian hospital for patients <16 years of age was evaluated in order to ascertain potential bias associated with improvement of surveillance that might interfere with the evaluation of the impact of PCV13.

2.6. Statistical analysis

Data were processed with the SPSS release 21 statistical package and the freely available "epitools" R package (https://www.r-project.org/). Results were expressed as mean and standard deviations or as a median; $p < 0.05$ was considered to be statistically significant and 95% confidence intervals were shown when appropriate. Incidences were calculated as crude age-specific incidences and age-standardized incidence rates (ASR). ASR was calculated through direct age standardization considering the pre-PCV13 population as the reference population and the post-PCV13 population as the study population. Confidence intervals were calculated using the exact methods. Differences between rates were calculated using the Rothman methods. Standardized incidence ratio (SIR) was calculated as the ratio between the sum of observed cases of the post-PCV13 era and the sum of expected cases of the post-PCV 13 era (see supplementary file for details). The Pearson chi-square test or Fisher's exact test were used to assess group differences in categorical variables. For comparison of two different etiologic tests (with comparison of proportions) a two-sample test of proportions was used to determine significance and confidence intervals.

Cohen's kappa coefficient and the McNemar test were used to measure agreement between tests.

For continuous variables, Student's *t* test was used.

3. Results

Data from 502 children with PPE were available (286 males, 57.0%; mean age, 5.61 ± 3.67 years; median age, 4.57 years, range IQ, 3.05–7.44 years). Patients were recruited from regions uniformly distributed over the country and covering highly populated areas in Italy, so that they represent 91.9% of the national population <16 years of age (http://demo.istat.it). The number of samples received for molecular surveillance from all Italian hospitals for patients <16 years of age increased over the years (post-PCV13 era vs pre-PCv134 era: +102%). Because of this bias, the impact of PCV13 on PPE/PE might be underestimated.

3.1. Vaccine uptake

PCV13 was included in a national immunization plan with a 3-dose-schedule (3-5-12 months) in replacement of PCV7 in the last quarter of 2010. Mean annual vaccination coverage for PCV13 in Italian target populations in the post-PCV13 era has always been over 80% (http://www.salute.gov.it/portale/documentazione/p6_2_8_3_1.jsp?lingua=italiano&id=20; accessed April 1, 2019; see supplementary file, Table 1 for detailed data). No catch-up campaign for children >1 years of age has been carried out since the introduction of PCV13.

3.2. Etiological diagnosis

Overall, etiological diagnosis was achieved in 214/502 (42.6%) patients. Pleural fluid and blood samples were obtained from 226 and 356 children, respectively. Pleural fluid was significantly more informative than blood in revealing etiology: respectively 179/226 (79.2%) for pleural fluid and 67/356 (18.8%) for blood (difference between proportions 0.60; $p < 10^{-5}$; 95% CI 0.54–0.67). *S. pneumoniae* was the most frequent pathogen identified (156/214; 72.8%), followed by *Streptococcus pyogenes* (22/214; 10.2%) and *Staphylococcus aureus* (8/214; 3.7%). The distribution of all pathogens identified is shown in Fig. 1.

In the pre-PCV13 era (2006–2010), 239 cases of PPE were found. Etiology was identified in 90/239 (37.6%) patients, and *S. pneumoniae* was found in 64/90 (71.1%).

In the post-PCV13 era (2011–2018), 263 cases of PPE were found. Etiology was identified in 124 (47.1%). As in the pre-PCV13 era, *S. pneumoniae* was the most frequent pathogen (92/124; 74.2%). However, the large majority 75/92 (81.5%) of affected children had not been vaccinated with PCV13: 58/92 (63.0%) because they were born before 2011, 17/92 (18.4%) due to their young age (3.2%) or their parents' decision (15.2%); on the other hand 17/92 had received PCV13 (18.4%).

3.3. Streptococcus pneumoniae serotyping

RT-PCR analysis allowed serotyping in 140/151 (92.7%) patients; in 5 patients the sample amount was not enough to perform serotyping. Serotype distribution in the pre-PCV13 era (n = 59) and in the post-PCV13 era (n = 81) is shown in Fig. 2. In the pre-PCV13 era, PCV13 serotypes accounted for 98.3% (n = 57/59). Serotypes 1, 3, 7F and 19A were the most frequent (n = 49; 83.0%).

In the post-PCV13 era, only serotypes 1 (2/15, 13.3%) and 3 (13/15 86.6%) were found present in children who experienced pneumococcal PPE in spite of vaccination (n = 15/81) (Fig. 2). On the other hand, the serotype distribution in children who had not received the PCV13 vaccination, due to being born before PCV13 was available (n = 66/81), was as follows: PCV13 serotypes still accounted for 92.4% (n = 61/66) with one case of serotype 14 and serotypes 1, 3, 7F and 19A causing all other (60/61, 90.9%) cases.

The proportion of non-PCV13 serotypes did not significantly increase over the years: 2/59 (3.4%) cases in the pre-PCV13 period (one each serotype 8 and 20), and 5/81 (6.2%) in the post-PCV13 period (two serotypes 12 and one each serotype 24, 32, and 35F) were found ($p = 0.70$).

3.4. The impact of vaccination (pre- vs post-PCV13 era)

The incidence of pneumococcal PPE/PE cases in children 0–8 years of age born in the pre- or post-PCV13 era is shown in Fig. 3. Data demonstrates a significant impact of vaccination with a decrease in pneumococcal PPE in the post-PCV13 era. The Standardized Incidence Ratio (SIR) decreased (data and calculations

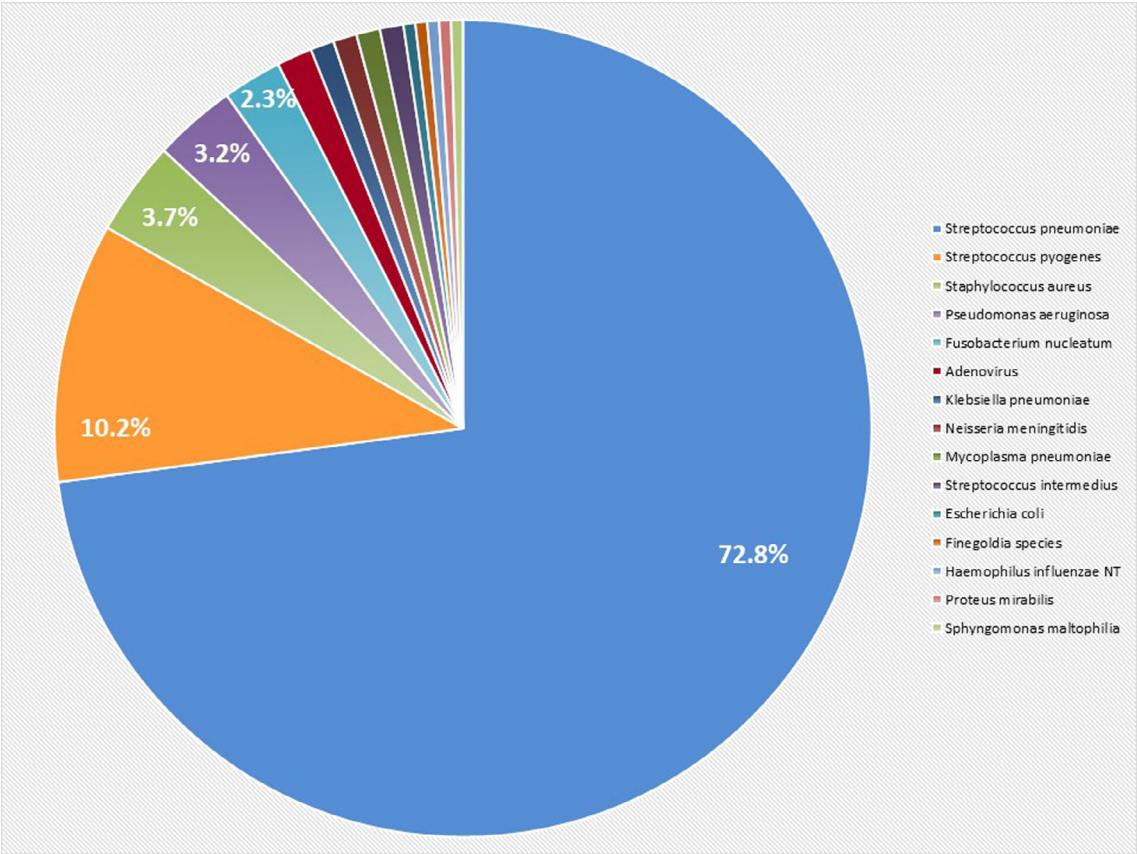


Fig. 1. Etiology of pleural empyema in 214 children <16 years of age.

are shown in Table 2 of the supplementary file). Comparing the two groups, the crude incidence rate was 2.82 per 1,000,000 person-years (95% CL 2.32–3.41) for children born before 2011 while the ASR was 0.66 per 1,000,000 person-years (95% CL 0.37–1.99) for children born after 2011 and vaccinated with PCV13; $p < 0.0001$ (Fig. 3).

The incidence rates of the most frequent serotypes in the pre- or post-PCV13 era are shown in Fig. 4. In vaccinated children in the post-PCv13 era, serotypes 19A and 7F completely disappeared, serotype 1 decreased by 91.8% and serotype 3 decreased by 31.5% (statistical analysis is shown in Fig. 4).

3.5. Sensitivity of RT-PCR vs. Culture

RT-PCR analysis and culture on pleural fluid samples were positive respectively in 177/222 (79.7%) and 8/64 (12.5%) children. Overall, in pleural fluid RT-PCR analysis was 6.4 times more sensitive than culture in achieving etiological diagnosis (difference between proportions 0.67; $p < 10^{-5}$; 95% CI 0.58–0.77). RT-PCR analysis and culture on blood samples were positive in 64/359 (17.8%) and 15/204 (7.4%) of children, respectively. Overall, in blood samples RT-PCR analysis was 2.4 times more sensitive than culture in achieving etiological diagnosis (difference between proportions 0.11; $p < 0.00058$; 95% CI 0.05–0.16). The results did not change in the subgroup of samples in which culture and RT-PCR analyses, either on pleural fluid (n = 63) or in blood (n = 173) samples, were performed on the same sample (respectively for pleural fluid OR 42.4, 95%CI 13.63–139.89; Cohen's K coefficient 0.008, McNemar $p < 10^{-5}$; for blood OR 4.03, 95% 1.95–8.50; Cohen's k coefficient 0.162; McNemar $p < 10^{-5}$).

4. Discussion

To the best of our knowledge, the present study is one of the largest studies on children with PPE in Europe. Our study confirms that, as in the USA and Australia [3,4], *S. pneumoniae* is the most frequent pathogen involved in PPE (73%). A variety of other organisms was detected by RT-PCR. After *S. pneumoniae*, *S. pyogenes* was the next most common pathogen and that is consistent with the results of a recent study in Canadian children [20].

By using the year 2011 as a watershed between the pre- and post-PCV13 eras, the proportion of cases due to *S. pneumoniae* in PPE was similar when the two periods were compared (71.1% vs 73.9%). However, over 85% of pneumococcal PPE diagnosed after 2011 occurred in children who had not received the PCV13 vaccination, mainly because they were too old when PCV13 was included in newborn vaccinations in Italy and no catch-up program for older children had been planned in Italy, even though demonstrated as highly effective in other countries.

Our study shows a significant impact of the PCV13 vaccination on PPE complicating CAP: the age standardized incidence rate was significantly lower in children born in the post-PCV13 era and vaccinated with PCV13 when compared to children born in the pre-PCV13 era. That impact is probably even greater, since samples received and diagnosed by the central Lab increased over time during the study period and that bias might have obviously caused an underestimation of the impact of PCV13.

Our data demonstrated a significant impact of the PCV13 vaccination on the incidence of most frequent serotypes: in the post-PCV13 era serotypes 7F and 19A completely disappeared, serotype 1 decreased by 92%, and serotype 3 by 32%. The lower impact of the

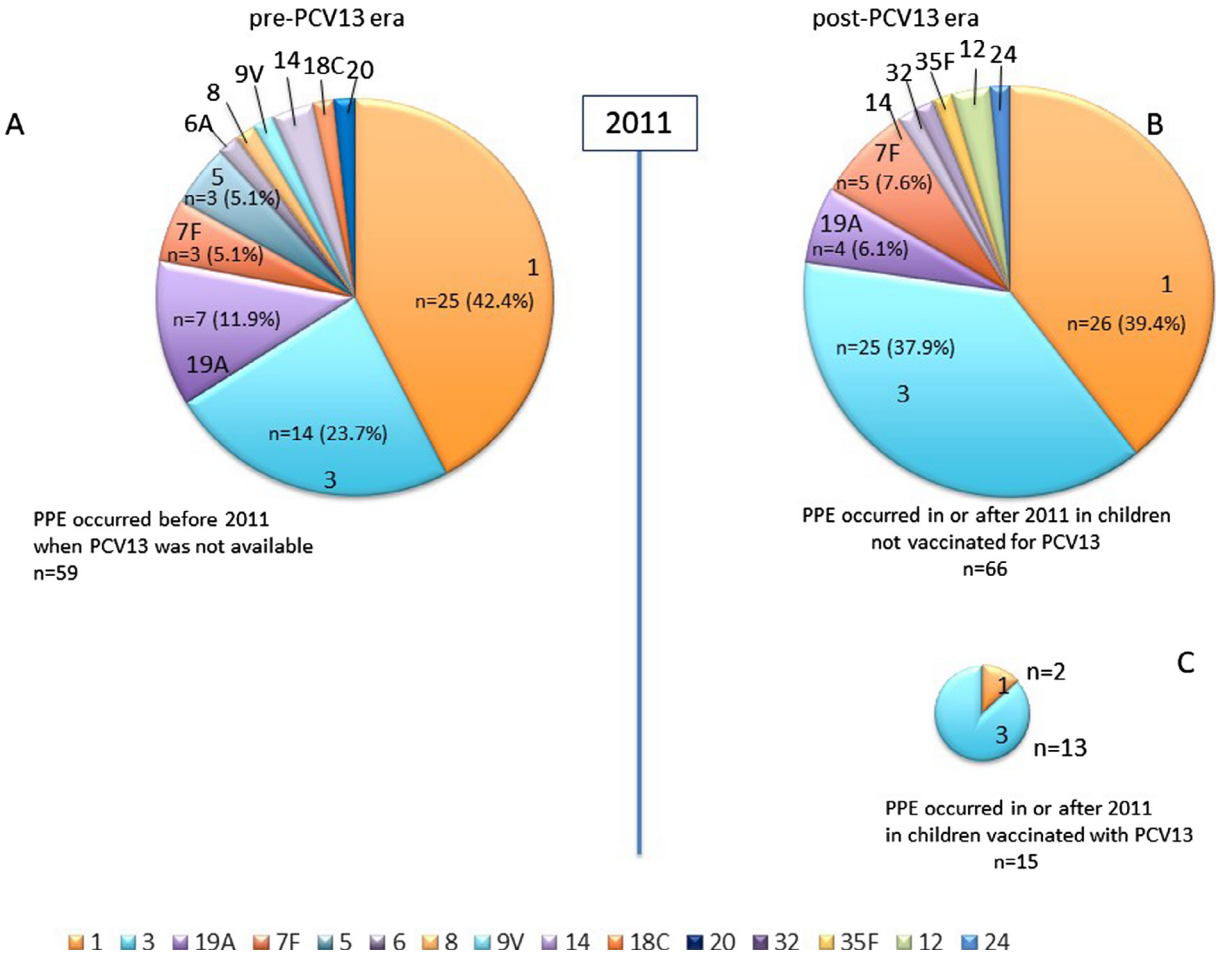
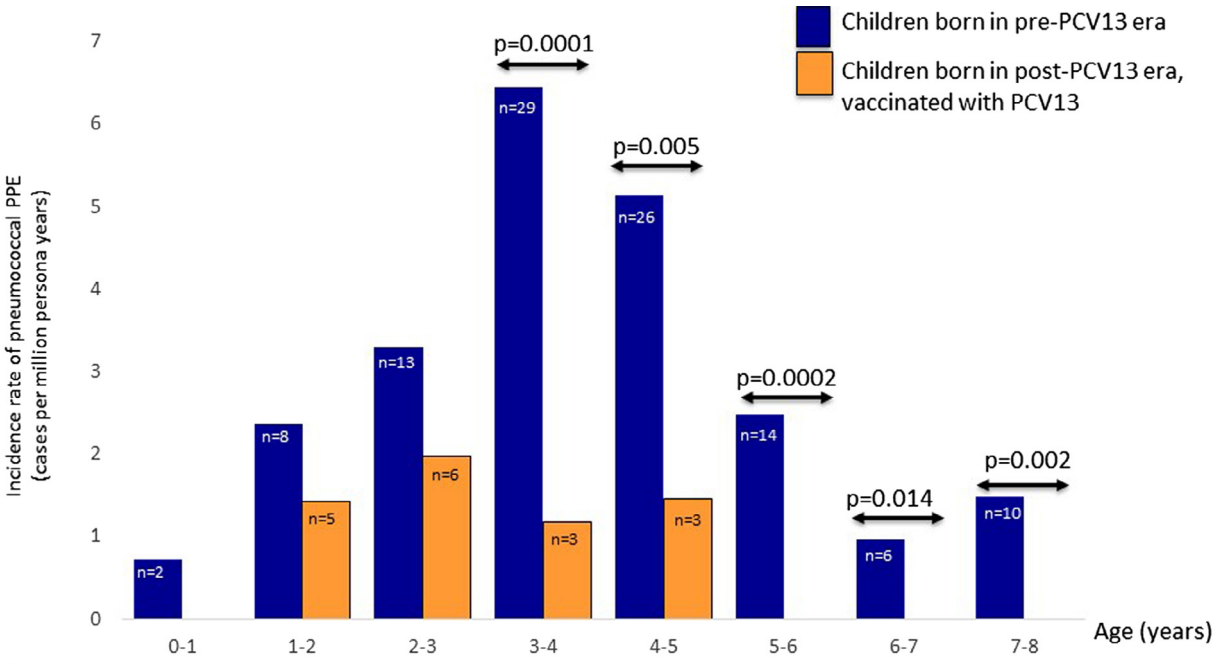


Fig. 2. Serotype distribution in 140 pediatric (0–16 years of age) pneumococcal PPE occurring before 2011 (A), occurring after 2011 in children not vaccinated or because they were born before PCV13 was available (2011), or due to their young age or their parents' decision(B), and occurring in children born after 2011 and vaccinated with PCV13(C). The diameter of circles is proportional to the number of cases. Number of cases is not indicated where a single case for serogroup was found.

serotype 3 vaccination on complicated pneumonia is not surprising: a similar study on pneumococcal pneumonia in the USA demonstrated a decrease of 38% [16] and recent studies on other IPD showed that PCV13 effectiveness against serotype 3 is lower than other serotypes, such as 7F or 19A [9,10,21]. In addition, prevention of pneumonia and its complications requires higher levels of anti-capsular polysaccharide antibody concentration, which may not be reached for some serotypes with the standard vaccination protocol [21]. On the other hand, the impact of PCV13 on other invasive pneumococcal infections due to serotype 3 such as sepsis or meningitis seems to be significantly higher (personal data, submitted).

Data from previous studies on pediatric IPD showed a significant overall reduction in invasive pneumococcal disease, while data on serotype replacement (increased incidence of the non-PCV13 serotypes) are conflicting. Evidence of increasing invasive pneumococcal disease due to non-PCV13 serotypes has been found in the UK and in Israel [10,22] while serotype replacement has not been reported in the USA [23], where incidence of IPD due to non-vaccine serotypes in young children has remained unchanged since the introduction of PCV13. Data from the present study show that serotype replacement was not observed in pediatric PPE in Italy. One possible explanation is that, due to the known geographical variations in serotype replacement [23], non-PCV13 serotypes are not increasing in Italy. However, data obtained in the present study in a specific disease (PPE/PE) cannot be generalized to all pediatric IPD. Actually, other possible explanations for the lack of serotype replacement in PPE/PE should also be considered and, particularly, the fact that pneumococcal PPE are mainly caused by a limited number of serotypes [24] -among them 1, 3 and 19A- which have a strong tropism for lung and pleural tissue as demonstrated by previous reports [2–4,8,24]. Emerging serotypes recently found in other IPD might have a lesser tropism for lung and pleural tissue and therefore their impact on PPE incidence could be limited. However, the number of non-vaccine serotypes are still too low to see a significant difference and close monitoring of non PCV13 cases is mandatory.

Data on PCV7 showed clear evidence of herd protection, with a significant decrease in PCV7 serotypes in all age groups [6]. Differently from what has been shown with PCV7 [5,6], herd protection for the six additional serotypes included in PCV13 (1, 3, 5, 6A, 7F, 19A) is debatable [25–27]. Our data show that, in non-vaccinated children, the distribution of pneumococcal serotypes did not change after the introduction of PCV13 and, at a first superficial glance, that might suggest a lack of herd protection in non-vaccinated groups. Actually, in non-vaccinated children, serotypes 1, 3, 7F, and 19A remained the most represented in the post-PCV13 era (83% before 2011, 90.9% after 2011). On the contrary, in the limited number of children that experienced PE/PPE in spite of the PCV13 vaccination, only serotype 1 (two cases) and 3 (thirteen cases) were detected, showing a completely different distribution of pneumococcal serotypes. However, it must be remarked that even though the PCV13 coverage was high (over 85%) in Italian newborns (target population) in the post-PCV13 era (http://



Age	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	Overall (ASR)
Incidence 95%CL	0.72 0.09-2.59	2.36 1.02-4.66	3.30 1.76-5.64	6.45 4.32-9.27	5.14 3.36-7.53	2.48 1.36-4.17	0.97 0.36-2.11	1.48 0.71-2.72	2.82 2.32-3.41
Incidence 95%CL	0 0.00-0.94 p=0.1573	1.42 0.46-3.32 p=0.3709	1.97 0.72-4.28 p=0.2734	1.17 0.24-3.41 p=0.0001	1.45 0.30-4.24 p=0.005	0 0.00-2.38 p=0.0002	0 0.00-3.64 p=0.014	0 0.00-7.91 p=0.002	ASR=0.66 0.37-1.99 P<0.0001

Fig. 3. Impact of PCV13 vaccination on pneumococcal PPE in children 0–8 years of age, according to age. Incidence is calculated as cases for million person years. Crude incidence rate and 95% CL of pneumococcal PPE are shown in blue for children born before PCV13 was available; age standardized incidence rate (ASR) and 95% CL are shown in orange for children born after PCV13 era and vaccinated with PCV13. Number of cases an p values are shown on bars. *p is calculated for overall incidence. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

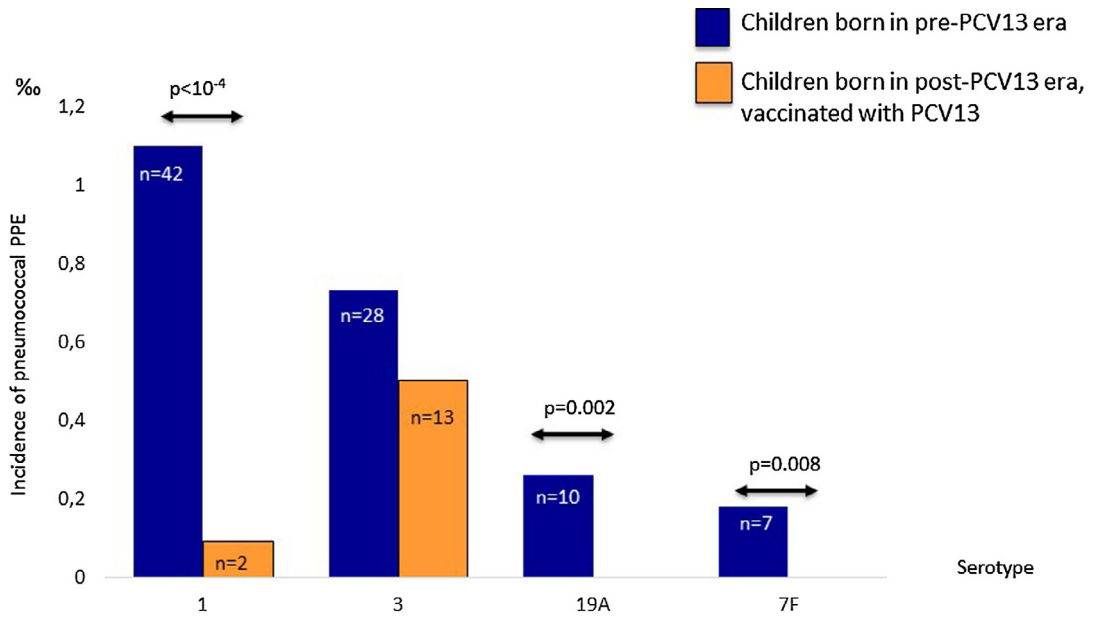
www.epicentro.iss.it/temi/vaccinazioni/dati_Ita), immunization was only offered to newborns and no catch up program was carried out. Therefore, the proportion of vaccinated children in the 0–16 years of age group was very limited (about 5–6% in the first year after implementation of PCV13) and increased by the same amount every year. For this reason, an impact of the vaccinated population on the protection of older non-vaccinated children could not be expected in the first years after PCV13 was introduced and should be evaluated in a longer follow-up. Therefore, the evaluation of herd protection was not included in the aims of the present study and conclusions on that point cannot be drawn.

Pleural fluid analysis was significantly more sensitive than blood in revealing etiology; this was not unexpected, since pneumonia is uncommonly associated with persistent bacteremia [8]. RT-PCR appeared significantly more sensitive than culture in achieving etiologic diagnosis, both in blood (17.8% vs 7.4%) and especially in pleural fluid samples (79.7% vs 12.5%), thus confirming the results of our and other authors' previous reports [8,12,28–31]. Early antibiotic therapy may have, in part, affected the results [32], but other interfering factors associated with sample collection, shipment, and storage may also have contributed to the low sensitivity of blood culture. Being DNA stable at room temperature for a long time, RT-PCR suffers the effect of those factors to a much lesser extent.

Our study has some limitations. First of all, since participation to molecular surveillance is voluntary and not based on a mandatory surveillance, cases of PPE in hospitals not included in the present study might have occurred; therefore, the data does not reflect the real incidence of PPE in Italy and the data cannot be compared with incidences in other countries. However, the evaluation of real PPE incidence was not the goal of the present study, which aimed instead to evaluate the impact of the PCV13 vaccination on pediatric PPE. In this regard, the present study -including 502 cases of PPE and, among them, 214 with etiological diagnosis and serotyping- was, to the best of our knowledge, the largest collection of pediatric PPE cases in Europe and allowed the change in etiology and the impact of the PCV13 vaccination over a decade to be evaluated.

Secondly, the number of samples received by the central Laboratory increased by over 100% over time during the study period. This increase, due to the increase in attention paid by clinicians in Italy to the need for surveillance, confirms the data collected by the National Institute of Health (http://old.iss.it/mabi/index.php?lang=1&id=5&tipo=16, accessed April 1, 2019) as well as the difficulties associated with regionalism in Healthcare. As a consequence of surveillance improvement, diagnoses of monitored infectious diseases (such as pneumococcal and meningococcal infection) increased over time and that bias may reflect negatively on the impact of PCV13 and its effectiveness can result underestimated.

We are also aware of the fact that we included, in our molecular surveillance, only 14 pathogens, so that other etiologies (bacterial or viral) could have been missed. However, with the set of primer/probes included in the study, it was possible to find the causative



	Serotype 1	Serotype 3	Serotype 19 A	Serotype 7F
Incidence 95%CL	1.10 0.79-1.48	0.73 0.49-1.06	0.26 0.13-0.48	0.18 0.07-0.38
ASR 95%CL	0.09 0.03-0.23 p<0.0001	0.50 0.32-0.78 p=0.1903	0 0.00-0.10 p=0.002	0 0.00-0.10 p=0.008

Fig. 4. Impact of PCV13 vaccination on the frequency of the most frequent serotypes in children 0–8 years of age in Italy. Incidence is calculated as cases for million person years. Crude incidence rate and 95% CL of pneumococcal PPE are shown in blue for children born before PCV13 was available; Age standardized incidence rate (ASR) and 95% CL are shown in orange for children born after PCV13 era and vaccinated with PCV13. Number of cases and p values are shown on bars. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pathogen in about 80% of pleural fluids. Moreover, the use of 16S gene sequencing, even if less sensitive than Realtime PCR because based on an end-point PCR technology [19], could have found other bacterial etiologies. Further studies will be necessary to evaluate the role of viral infections or co-infections on PPE/PE epidemiology in Italy.

In conclusion, our findings indicate that routine immunization with PCV13 has significantly reduced the burden of childhood PPE in vaccinated children, without increasing PPE due to other bacteria and without a serotype shift. The reduction may be significantly underestimated because of the improvement in the surveillance all over the country. Data has also shown that Real-time PCR in pleural fluids is an essential tool to better define the etiology of PPE and to monitor vaccination plans, while longer studies will be necessary to evaluate the importance of herd protection.

Funding

This work was supported partly by the Italian Center for Disease Control and Prevention [CCM-4393 117-2006] and partly by the University of Florence.

Conflict of interests

The authors have no conflict of interest.

Disclosure

All the authors participated in study design, analysis and interpretation of results, CA has prepared the manuscript, DS, ELo, FC, FMdB, LL, SR, GI, IC, RC and MR contributed to the acquisition of data, MM, FN, EdV, MC, AC have processed all samples in the lab, both for diagnosis and serotyping, ELu revised statistical analysis; FMdB and MR have critically revised the manuscript. All the authors have given final approval to the version to be submitted.

Acknowledgements

We acknowledge the significant contributions of the Italian Group for the Study of Invasive Pneumococcal Disease and all the group investigators.

Italian Group for the Study of Invasive Pneumococcal Disease:

Agostiniani R., Pistoia; Amarri S., Reggio Emilia; Bellettato M., Vicenza; Benetti G., Cecina ; Bergamaschi R., Bologna; Bernardini R., Empoli; Biban P., Verona; Boner A., Verona; Bossi G., Pavia; Bottone U., Massa; Brusa S., Imola; Cardinale F., Bari; Civitelli F., Montepulciano; Correra A., Napoli; de Luca F., Messina; Di Silvio R., Borgo San Lorenzo; Domenici R., Lucca; Facchin S., Latisana; Falorni S., Grosseto; Federici S., Rimini; Gadducci F., Livorno; Gagliardi L., Viareggio; Girasole A. Rho; Guala A., Verbania; Guastaferro N., Ascoli Piceno; Isola P., Livorno; Iughetti L., Modena; Landini MP., Bologna; Marchetti F., Ravenna; Martini M, Arezzo; Marzini S.,

Feltre; Memmini G., Carrara; Mesirca P., Montebelluna; Migliozzi L., Senigallia; Minasi D., Polistena; Mirri GP., Saronno; Montini G., Milano; Nunziata F., Avellino; Palatron S., Camposampiero; Passalacqua G., Terni; Perferi G., Firenze; Peroni D., Pisa; Petricci P., Livorno; Pezzati M., Firenze; Poggi G.M., Firenze; Poli S., Esine; Rapisardi G., Firenze; Rocca M., Cuneo; Sartor A. Udine; Tarantino D., Pontedera; Toffolo A., Oderzo; Tripodi S., Roma; Vasarri P., Prato; Vascotto M., Siena; Vispi L., Poggibonsi; Vocale C., Bologna.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.04.012.

References

[1] de Benedictis FM, Azzari C, Bernardi F. Pleural empyema, necrotising pneumonia and lung abscess. In: Eber E, Midulla F, editors. ERS Handbook of Paediatric Respiratory Medicine. ERS. p. 258–65. https://doi.org/10.1183/9781849840392.029612.

[2] Byington CL, Spencer LY, Johnson TA, et al. An epidemiological investigation of a sustained high rate of pediatric parapneumonic empyema: risk factors and microbiological associations. Clin Infect Dis 2002;34(4):434–40. https://doi.org/10.1086/338460.

[3] Grijalva CG, Nuorti JP, Zhu Y, Griffin MR. Increasing incidence of empyema complicating childhood community-acquired pneumonia in the United States. Clin Infect Dis 2010;50(6):805–13. https://doi.org/10.1086/650573.

[4] Strachan RE, Cornelius A, Gilbert GL, et al. Bacterial causes of empyema in children, Australia, 2007–2009. Emerg Infect Dis 2011;17(10):1839–45. https://doi.org/10.3201/eid1710.101825.

[5] Fitzwater SP, Chandran A, Santosham M, Johnson HL. The worldwide impact of the seven-valent pneumococcal conjugate vaccine. Pediatr Infect Dis J 2012;31(5):501–8. https://doi.org/10.1097/INF.0b013e31824de9f6.

[6] Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. Lancet Infect Dis 2011;11(10):760–8. https://doi.org/10.1016/S1473-3099(11)70090-1.

[7] Li ST, Tancredi DJ. Empyema hospitalizations increased in US children despite pneumococcal conjugate vaccine. Pediatrics 2010;125(1):26–33. https://doi.org/10.1542/peds.2009-0184.

[8] Resti M, Moriondo M, Cortimiglia M, et al. Community-acquired bacteremic pneumococcal pneumonia in children: diagnosis and serotyping by real-time polymerase chain reaction using blood samples. Clin Infect Dis 2010;51(9):1042–9. https://doi.org/10.1086/656579.

[9] Moore MR, Link-Gelles R, Schaffner W, et al. Effectiveness of 13-valent pneumococcal conjugate vaccine for prevention of invasive pneumococcal disease in children in the USA: a matched case-control study. Lancet Respir Med 2016;4(5):399–406. https://doi.org/10.1016/S2213-2600(16)00052-7.

[10] Waight PA, Andrews NJ, Ladhani SN, Sheppard CL, Slack MP, Miller E. Effect of the 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease in England and Wales 4 years after its introduction: an observational cohort study. Lancet Infect Dis 2015;15(5):535–43. https://doi.org/10.1016/S1473-3099(15)70044-7.

[11] Simonsen L, Taylor RJ, Schuck-Paim C, Lustig R, Haber M, Klugman KP. Effect of 13-valent pneumococcal conjugate vaccine on admissions to hospital 2 years after its introduction in the USA: a time series analysis. Lancet Respir Med 2014;2:387–94. https://doi.org/10.1016/S1473-3099(15)70044-7.

[12] Amodio E, Costantino C, Giuffrè M, Piccione M, Tramuto F, Vitale F. Invasive pneumococcal diseases in children aged 1–59 months in Sicily, Italy: importance of active family paediatrician surveillance and vaccination coverage. EuroMediterr Biomed J 2014;9(3):19–23. https://doi.org/10.3269/1970-5492.2014.9.3.

[13] Nath S, Thomas M, Spencer D, Turner S. Has the incidence of empyema in Scottish children continued to increase beyond 2005? Arch Dis Child 2015;100(3):255–8. https://doi.org/10.1136/archdischild-2014-306525.

[14] Saxena S, Atchison C, Cecil E, Sharland M, Koshy E, Bottle A. Additive impact of pneumococcal conjugate vaccines on pneumonia and empyema hospital admissions in England. J Infect 2015;71:428–36. https://doi.org/10.1016/j.jinf.2015.06.011.

[15] Wiese AD, Griffin MR, Zhu Y, Mitchel Jr EF, Grijalva CG. Changes in empyema among U.S. children in the pneumococcal conjugate vaccine era. Vaccine 2016;34:6243–9. https://doi.org/10.1016/j.vaccine.2016.10.062.

[16] Olarte L, Barson WJ, Barson RM, et al. Pneumococcal pneumonia requiring hospitalization in US children in the 13-valent pneumococcal conjugate vaccine era. Clin Infect Dis 2017;64(12):1699–704. https://doi.org/10.1093/cid/cix115.

[17] Azzari C, Nieddu F, Moriondo M, et al. Underestimation of invasive meningococcal disease in Italy. Emerg Infect Dis 2016;22(3):469–75. https://doi.org/10.3201/eid2203.150928.

[18] Balfour-Lynn IM, Abrahamson E, Cohen G, et al. Paediatric pleural diseases subcommittee of the BTS standards of care committee. BTS guidelines for the management of pleural infection in children. Thorax 2005;60. https://doi.org/10.1136/thx.2004.030676. Suppl 1:i1-21.

[19] Azzari C, Moriondo M, Indolfi G, et al. Realtime PCR is more sensitive than multiplex PCR for diagnosis and serotyping in children with culture negative pneumococcal invasive disease. PLoS ONE 2010;5(2):e9282. https://doi.org/10.1371/journal.pone.0009282.

[20] Pernica JM, Moldovan I, Chan F, Slinger R. Real-time polymerase chain reaction for microbiological diagnosis of parapneumonic effusions in Canadian children. Can J Infect Dis Med Microbiol 2014;25(3):151–4.

[21] Andrews NJ, Waight PA, Burbidge P, et al. Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: a postlicensure indirect cohort study. Lancet Infect Dis 2014;14(9):839–46. https://doi.org/10.1016/S1473-3099(14)70822-9.

[22] Ben-Shimol S, Givon-Lavi N, Grisaru-Soen G, Megged O, Greenberg D, Dagan R. Comparative incidence dynamics and serotypes of meningitis, bacteremic pneumonia and other-IPD in young children in the PCV era: insights from Israeli surveillance studies. Vaccine 2018;36(36):5477–84. https://doi.org/10.1016/j.vaccine.2017.05.059.

[23] Lewnard JA, Hanage WP. Making sense of differences in pneumococcal serotype replacement. Lancet Infect Dis 2019. https://doi.org/10.1016/S1473-3099(18)30660-1.

[24] Shigayeva A, Rudnick W, Green K, et al. Association of serotype with respiratory presentations of pneumococcal infection, Ontario, Canada, 2003-2011. Vaccine 2016;34(6):846–53. https://doi.org/10.1016/j.vaccine.2015.11.021.

[25] Nieddu F, Moriondo M, De Vitis E, et al. PCV13 serotype decrease in Italian adolescents and adults in the post-PCV13 era: Herd protection from children or secular trend? Vaccine 2017;13:1544–50. https://doi.org/10.1016/j.vaccine.2017.01.064.

[26] Corcoran M, Vickers I, Mereckiene J, et al. The epidemiology of invasive pneumococcal disease in older adults in the post-PCV era. Has there been a herd effect? Epidemiol Infect 2017;145(11):2390–9. https://doi.org/10.1017/S0950268817001194.

[27] Regev-Yochay G, Katzir M, Strahilevitz J, et al. The herd effects of infant PCV7/PCV13 sequential implementation on adult invasive pneumococcal disease, six years post implementation; a nationwide study in Israel. Vaccine 2017;35(18):2449–56. https://doi.org/10.1016/j.vaccine.2017.03.03.

[28] Azzari C, Cortimiglia M, Nieddu F, et al. Pneumococcal serotype distribution in adults with invasive disease and in carrier children in Italy. Should we expect herd protection of adults through infants' vaccination? Hum Vaccin Immunother 2016;12:344–50. https://doi.org/10.1080/21645515.2015.1102811.

[29] Pasinato A, Indolfi G, Marchisio P, et al. Pneumococcal serotype distribution in 1315 nasopharyngeal swabs from a highly vaccinated cohort of Italian children as detected by RT-PCR. Vaccine 2014;32(12):1375–81. https://doi.org/10.1016/j.vaccine.2014.01.023.

[30] Marimon JM, Morales M, Cilla G, Vicente D, Perez-Trallero E. Detection of bacteria and viruses in the pleural effusion of children and adults with community-acquired pneumonia. Future Microbiol 2015;10(6):909–15. https://doi.org/10.2217/fmb.14.143.

[31] Krenke K, Sadowy E, Podsiadły E, Hryniewicz W, Demkow U, Kulus M. Etiology of parapneumonic effusion and pleural empyema in children. The role of conventional and microbiological tests. Respir Med 2016;116:28–33. https://doi.org/10.1016/j.rmed.2016.05.009.

[32] Resti M, Micheli A, Moriondo M, et al. Comparison of the effect of antibiotic treatment on the possibility of diagnosing invasive pneumococcal disease by culture or molecular methods: a prospective, observational study of children and adolescents with proven pneumococcal infection. Clin Ther 2009;31(6):1266–73. https://doi.org/10.1016/j.clinthera.2009.06.010.