Invasive disease potential of pneumococci before and after the 13-valent pneumococcal conjugate vaccine implementation in children

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ABSTRACT

Background: Changes in serotype distribution have been induced after pneumococcal conjugate vaccines (PCV) implementation, and non-vaccine serotypes are now circulating. Among these latter serotypes, we aimed to distinguish those with high invasive disease potential before (2008–2009) and after PCV13 implementation (2012–2013).

Methods: Invasive pneumococcal disease (IPD) serotypes isolated from children 6 to 24 months were compared with nasopharyngeal-colonizing serotypes in healthy children. To assess the invasive potential of a given serotype, odds ratios (ORs) were calculated. For each serotype, OR > 1 indicated increased probability of association with IPD and OR < 1 decreased probability.

Results: In 2008/2009 and 2012/2013, 355 pneumococci were isolated from 1212 healthy children and from 569 IPD, including 166 meningitis, 114 pneumonia, and 289 other IPDs. In period 1, serotypes 7F, 3, 1, 24F, and 19A showed highly significant invasive disease potential whereas in period 2, only serotype 24F was associated with a significant high OR (6.6 [95% CI 2.6; 16.2]). Of note, for serotype 12F, OR could not be calculated because of no carrier recorded, however, if there had been a single 12F carrier, the OR would be among the highest, in period 2, 15.7 [95% CI 3.4; 73.0]). Only two serotypes appeared negatively associated with IPD, 11A and 23B in the period 2 as compared with nine in period 1. In the second period, pneumococcal penicillin non-susceptible isolates were mostly represented by serotypes 19A, 15A, 19F, 35B and 24F both in carriers and IPD. Only one strain was resistant to penicillin with MIC = 4 μg/ml (serotype 19A) during the first period.

Conclusion: In children <2 years old, compared to the previous period, the number of serotypes having a high disease potential decreased after PCV13 implementation, only two non-vaccine serotypes, 24F and 12F, had high invasive disease potential.

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1. Introduction

Nasopharyngeal (NP) flora is the ecological niche of Streptococcus pneumoniae (Sp) and the reservoir of strains implicated in invasive pneumococcal disease (IPD) and non-invasive infection [1]. Each pneumococcal infection is preceded by the acquisition of Sp strains in NP [2,3]. More than 94 serogroups have been recognized and could be implicated in disease and carriage. However, before pneumococcal conjugate vaccines implementation (PCVs), only five to 11 were responsible for 90% of childhood IPD cases in developed countries and serotypes reported from carriage studies were distributed differently from those involved in invasive pneumococcal disease [4,5].
The concept of invasive disease potential in pneumococci infection was developed from the early 2000s with the implementation of the seven-valent pneumococcal conjugate vaccine (PCV7) [6]. A few studies have compared, for a given serotype, the case carrier ratio to determine the invasiveness of specific serotypes [6–8]. In these studies, conducted in different countries, vaccine serotypes 4, 14, and 18C (included in PCV7 and PCV13) and 1, 5, 7F, and 19A (included in PCV13) had significant odds ratios (ORs) or invasive indices for IPD, which suggested their invasive disease potential. After the implementation of PCVs, the incidence of IPD markedly decreased in all countries where these vaccines were introduced [9–12]. The reduction in IPD incidence is related to the decrease in vaccine-type (VT) IPD (from 79% to 100%), but the global efficacy against pneumococcal infections was partially reduced in many countries by the increase in non-VT (NVT) IPD [10,13,14].

Most VT PCVs have disappeared from NP flora, and in this ecological niche, the almost complete replacement by NVTs is accompanied by minimal changes in overall pneumococcal carriage [15–17]. One of the main hypotheses to explain the discrepancy between carriage and disease is the lesser disease potential of most of the NVTs carried after PCV implementation. However, little is known about the invasive disease potential of NVTs, so we need to elucidate which serotypes are prone to give more frequently invasive diseases [10,18,19].

In France, since 2008, PCV7 coverage (immunization schedule 2 + 1) for children <2 years old has been high (≥86%) [20]. In June 2010, the French authorities recommended routine vaccination with PCV13 for infants at 2, 4 and 12 months old to replace PCV7, without catch-up for older children except those at high risk of IPD [21]. The PCV vaccination coverage in France for children <2 years old after replacement of PCV7 with PCV13 was >92% [20]. In parallel, taking advantage of our ongoing surveys concerning both IPD and pneumococcal carriage before and after PCV13 implementation, we aimed to estimate the invasive disease potential of serotypes, with a special attention to emerging serotypes, in the PCV13 era.

2. Patients and methods

The serotype specific relationship between NP carriage and IPD was investigated by comparing the serotype specific prevalence in carriage and in invasive infections in two periods, before and after PCV13 implementation.

2.1. Population

2.1.1. Carriage study

Between January 2008 and December 2013, 90 pediatricians throughout France took part in this prospective study. Each year, 300 healthy children of both sexes, aged 6 to 24 months, consulting for well-baby visits (generally for routine immunization) and without fever or respiratory symptoms were enrolled. We excluded children receiving antibiotic treatment within 7 days before enrolment, with severe underlying disease. In addition, we excluded children already enrolled during the last 12 months.

After written informed consent was obtained from parents, we queried parents or guardians regarding the child’s medical record to document the status of PCV vaccination. The study was approved by the Saint Germain en Laye Hospital Ethics Committee. NP specimens were obtained by using cotton-tipped wire swabs. The swabs were inserted into the nares, gently rubbed on the NP wall, removed, and immediately placed in transport medium (Copan Venturi Transys®, Brescia, Italy). The samples were transferred within 48 h to central laboratories (French National Reference Centre for Pneumococci [NRCP] and Robert Debre Hospital).

2.1.2. IPD study

Pneumococcal invasive cases were defined by the isolation of pneumococcus from a normally sterile site (cerebrospinal fluid or blood or pleural fluid). IPD surveillance relies on data generated by the NRCP. Invasive strains were collected as part of the French national survey program of pneumococcal infections, via the "Observatoires Régionaux du Pneumocoque", a network of 400 laboratories located in the 22 regions of France and covering at least 70% of the admissions in medical wards [22]. Data collected included age, sex, site of isolation, and the disease.

Analysis was performed on data for patients 6 to 24 months old that was extracted from this database during the carriage study (January 2008 to December 2013).

For both studies, S. pneumoniae was identified by standard methods and serotyped at the NRCP by latex agglutination testing with antiserum provided by Statens Serum Institut (Copenhagen, Denmark) [23]. Serotypes 15B and 15C were considered the single serotype 15B/C because the capsule is quickly interchangeable [24]. Susceptibility of S. pneumoniae isolates to penicillin G was determined by minimal inhibitory concentration (MIC) by the agar-dilution method. Isolates were classified as penicillin-susceptible (PSP; MIC ≤ 0.06 μg/ml), penicillin–non-susceptible (PNP; MIC ≥ 0.12 μg/ml), or penicillin-resistant (PRP; MIC > 2 μg/ml) according to the European Committee on antimicrobial susceptibility testing. (http://www.eucast.org/clinical_breakpoints/)

2.2. Statistical analysis

Two periods were defined according to PCV13 implementation in France: the first, before the PCV13 era (January 2008 to December 2009) and the second, the PCV13 era (January 2012 to December 2013). Data for years 2010 and 2011, corresponding to PCV13 implementation in France, were excluded from the analysis. Four groups were defined: healthy carriers and those with meningitis, pneumonia or other IPDs. The first analysis compared healthy carriers to the whole IPD group, including those with meningitis, pneumonia and other IPDs. The second one applied to healthy carriers versus meningitis versus pneumonia versus other IPDs.

Quantitative data were analyzed by one-way ANOVA and categorical data by chi-square test or Fisher’s exact test. Statistical significance was set at two-sided p < 0.05. Stata SE 12.1 (Statacorp, College Station, TX) was used for analysis.

To estimate the invasive disease potential of serotypes, we compared the serotype-specific rates of isolation with carriage and invasive diseases. To assess the invasive disease potential of a given serotype, odds ratios (ORs) were calculated by reference to the other serotypes. For each serotype, OR > 1 indicated an increased probability to cause IPD and OR < 1 decreased probability.

3. Results

3.1. Epidemiology

During the two periods studied (2008/2009 and 2012/2013), among 1212 healthy children, 355 pneumococci strains were isolated and 569 IPD were recorded: 166 meningitis, 114 pneumonia, and 289 other IPDs. For this pooled dataset (n = 924), mean ± SD age was 13.4 ± 5.3 months (median 12.3). Table 1 shows the distribution of cases by type of IPD, period and age.

For healthy controls, among the 32 serotypes found in period 1, the most common were 19A (14.5%), 15B/C (11.9%), 15A (6.7%), and 6C (6.7%). In period 2, among the 30 serotypes found, the main ones were 15B/C (13.6%), 23B (9.3%), 15A (8.6%), 11A (8.0%), and 10A (6.8%) (Fig. 1). For IPD, among the 40 serotypes found in period...
1, three, 19A, 7F, and 1 were implicated in 54.4% of cases; among the 31 serotypes isolated in period 2, the three leading serotypes (24F, 19A, and 12F) accounted for 37.6% of cases (p < 0.001 between the two periods). Of note, serotypes 1 and 7F in period 1 and serotype 12F in period 2 were quasi-exclusively found among IPD isolates and not in carriers (Fig. 1). In the first period, PNSP isolates were mostly represented by serotypes 19A, 15A, 15B/C, NT, 19F and 35B in carriers and 19A, 24F, 19F, 15A and 14 in IPD cases. Only one strain was resistant to penicillin with MIC = 4 μg/mL (serotype 19A) during the first period. In the second period, PNSP isolates were mostly represented by serotypes 19A, 15A, 19F, 35B and 24F both in carriers and IPD.

Table 2 presents the distribution of main serotypes by type of IPD and period (Supplemental Table 2 presents overall serotypes). Compared to period 1, during period 2, the number of pneumococcal isolates was reduced for overall IPD (53.4%), meningitis (37.2%), pneumonia (65.9%), and other IPDs (56.2%). PCV7 serotypes almost disappeared between the 2 periods, regardless of pathologic, except for serotype 19F, accounting for 3.9% of IPD isolates. The frequency of the six additional PCV13 serotypes significantly decreased between the 2 periods for overall IPD, meningitis, pneumonia, and other IPDs, from 60.8% to 12.2%, 48.2% to 11.0%, 67.0% to 17.2%, and 64.7% to 11.4%, respectively (p < 0.001 for each comparison). In overall IPD, the frequency of serotype 19A decreased between the 2 periods from 33% to 8.8% and ranked second in period 2 after the serotype 24F which accounted for 19.9% of cases.

3.2. Invasive disease potential

Serotype-specific ORs were compared in each period (Fig. 2). Of the 40 serotypes found in IPD in period 1, five showed highly significant invasive disease potential: 7F, 3, 1, 24F, and 19A. In period 2, only serotype 24F was associated with a significant high OR [6.6 (95% CI 2.6; 16.2)]. Of note, for ST 12F, considered a highly invasive serotype [8,25], ORs could not be calculated because of no carrier recorded in both periods. However, if there had been a single 12F carrier during each study period, 12F ORs would be among the highest, although significant only in the second period (OR period 1, 4.6 [95% CI 0.7; 30.3], and period 2, 15.7 [95% CI 3.4; 73.0]). Among the serotypes isolated in the period 1, nine were negatively associated with IPD, 15B/C, 15A, 35F, 21, 23A, 23B, 35B, 6A and 6C. In period 2, two serotypes appeared negatively associated with IPD, 11A and 23B.

4. Discussion

The design of this study allowed us to analyze a large national collection of isolates from invasive disease and carriage in children 6 to 24 months old to compare the invasive disease potential of predominant serotypes before and after PCV13 implementation. To estimate the serotype-specific invasive disease potential we used invasive ORs rather than invasive capacity or attack rate because invasive ORs have been used most frequently in the literature [7,8,26–28].

Before PCV7 implementation, serotypes 1, 4, 5, 14, 18C, and 12F were the most invasive serotypes [6,8]. After the widespread implementation of PCV7, serotypes 3, 7F, 18C, 19A, 22F and 33F were highly invasive [29]. To our knowledge, our large study is the first to analyze serotype pneumococci invasiveness before and after the PCV13 era. The number of serotypes with high invasive disease potential decreased from five (7F, 3, 1, 24F, and 19A) to one (24F) between the PCV7 and PCV13 periods. Of note, the serotypes 7F, 3, and 1, with very high invasiveness in the PCV7 period, disappeared in 2012–2013. Serotype 24F had the highest invasive disease potential [OR 6.5 (95% CI 2.6; 16.2)] during the PCV13 period. In light of our results, only this serotype maintained the same invasiveness before and after PCV13 implementation. For serotype 12F, the second predominant serotype in IPD in the PCV13 era, although no carrier was recorded, a strong invasive disease potential was expected. This expectation was confirmed because by estimating a single carrier, the OR was the highest [OR 15.7 (95% CI 3.4; 73.0)]. Of note, such a high OR was found only for serotype 7F [OR 15.1 (95% CI 11.6; 49.6)] during the PCV7 period. This result was expected because both 12F and 7F were reported to be hyperinvasive as compared with other IPD strains and found more often in meningitis [8,11,25,30–32].

These results were observed in the context of a decrease in IPD in several countries [9–11,18,19,33]. A marked decrease in IPD cases after PCV13 implementation in children <2 years old was also reported by different surveillance networks in France. Indeed, IPD and meningitis incidence significantly decreased by 30% and 20%, respectively, between 2008/2009 and 2012 (24.6 to 17.2/100,000 and 5.6 to 4.5/100,000) [10]. For children <2 years old, even if PCV13 coverage was >92%, PCV13 serotypes were not yet totally eliminated from IPD because they still accounted for 12.2% of cases in period 2. This finding is not surprising because few cases probably occurred in non-vaccinated children. Moreover the immunization schedule (2 + 1) used in France as compared with the US one (3 + 1) could have contributed to this absence of total disappearance. The last explanation could be the effectiveness of the vaccine measured only after 3 years of implementation.

In the PCV13 period, a relatively small number of pneumococcal serotypes were responsible for most cases of IPD in children 6 to 24 months old. Indeed, six serotypes, 24F, 19A, 12F, 15B/C, 10A, and 15A, represented 60.7% of IPD cases. PNSP isolates were mostly represented by the serotypes 24F, 19A, and 15A. Serotype 24F ranked first, both for meningitis and pneumonia, and serotypes 12F and 19A were the second predominant serotype in meningitis and pneumonia, respectively. Among the other most prevalent non-PCV13 serotypes (15B/C, 10A, and 15A, sorted by frequency) serotype 10A was invasive (OR > 1), whereas serotypes 15B/C and 15A appeared negatively associated with IPD (OR < 1), but not significantly. Browall et al. reported a lower invasive disease potential for NVT before PCV13 implementation, as did van Hoek et al. for the PCV13 era [34,35]. Interestingly, in our study, serotypes 11A and 23B showed low invasive disease potential [7,25,27,34,36].

For most of the other NVT, the risk of IPD cannot be assessed in our study because of the low number of IPD cases (22F and 33F). By contrast, serotypes 22F and 33F increased in frequency and
Fig. 1. *Streptococcus pneumoniae* serotype distribution by resistance profile in healthy carriers and in invasive diseases before (2008/2009) and after (2012/2013) 13 valent pneumococcal conjugate vaccine implementation. * Only one strain with penicillin MIC = 4 μg/ml (serotype 19A).
were among the most frequent serotypes in IPD in North America, England and Wales [37–39]. We could not assess the risk of IPD due to the similar frequency of serotypes 15B/C and 15A both in carriage and IPD. However, in children <2 years old, who have immature immunity, these carriage serotypes are known to be associated with IPD [23]. Because we enrolled only children <2 years, frequently pneumococcal carriers, our results cannot be completely extrapolated to older children and this is the first limitation of our study.

Some other limitations should be taken into account when interpreting our results. The estimated serotype invasiveness could be biased by variation in duration of carriage [3,25]. Sleeman et al. reported an inverse relationship between the attack rate of a capsular serotype and duration of carriage. Serotypes with a short...
duration of carriage were significantly more likely to be those with high attack rates [25]. This finding was well illustrated by serotype 12F (with probably the strongest invasive disease potential) reported to have one of the shortest carriage duration [25].

The third limitation is related to our distribution of IPD: we may have overestimated meningitis cases, and pneumonia cases may have been underrepresented. In period 2, meningitis represented 35.5% of cases and pneumonia 16% of cases. This IPD distribution is not the one usually observed in France because the French Institute for Public Health Surveillance reported that meningitis accounted for 26% of IPD cases in children <2 years old in 2012 [10]. Such discrepancies were reported, in the Kaplan et al. study, where meningitis and pneumonia accounted for 18.5% and 25% of cases, whereas in the Ben-Shimol et al. study, these rates were
9.4% and 35.5%, respectively [19,40]. Such differences in diagnosis distribution could be explained by the method of collecting data, use of electronic data capture, mandatory declaration of cases, and organization of public health programs for pneumococcal surveillance. Likewise, these differences could be due to blood culture practices for febrile children or indications for lumbar puncture [12,41]. Another explanation could be the difficulty in matching bacteremia with the source of infection, particularly for pneumonia in children. However, from our results, a more thorough meningitis case-reporting cannot be ruled out. However, if these difficulties in data collection represent a limitation of our study, we emphasize that our methodology did not change in the two periods.

The last limitation is the absence of analysis by multilocus sequence typing (MLST). Indeed, the invasive disease potential of pneumococci seems to be determined by capsular serotype rather than genotype, as determined by MLST [26]. However, the diversity in genotype contributes to the heterogeneity of invasive disease potential in a given serotype [7,36].

5. Conclusion

The number of serotypes with high invasive disease potential decreased after PCV13 implementation, only two non-vaccine serotypes, 24F and 12F, had high invasive disease potential in children <2 years old. By contrast, serotypes 11A and 23B appeared negatively associated with IPD. This study is valuable to improve our knowledge of the emerging serotypes, especially their invasive disease potential. Furthermore, such studies seem to be pertinent for accurately choosing the serotypes to add to the formulation of future vaccines. This study points out some changes occurring only three years after PCV13 implementation and suggests that a new equilibrium in the pneumococcal population is not yet reached. However, this reflects the epidemiology of pneumococci in France, and we are aware that post-PCV13 emerging serotypes could be different in other countries. Therefore, ongoing surveys performed concomitantly for carriage and IPD are needed to further characterize the little-known NVT, which are still poorly represented.

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The first author and the corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Conflict of interest statement

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Pr Cohen Robert reports personal fees from Pfizer, GSK, Sanofi and Novartis outside the submitted work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.10.015.

References


