High invasiveness of pneumococcal serotypes included in the new generation of conjugate vaccines

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Abstract

The implementation of the seven-valent pneumococcal conjugate vaccine, PCV7, has resulted in significant changes in the pneumococcal population being carried and causing disease. We aimed to determine the invasive disease potential of serotypes causing invasive paediatric disease in the era of conjugate vaccines in Catalonia, Spain, and their potential coverage by the 13-valent pneumococcal conjugate vaccine, PCV13. As a secondary objective, we evaluated whether implementation of PCV7 had resulted in significant changes in the invasive disease potential of the most frequent serotypes circulating in the area. Two pneumococcal collections obtained from children admitted to the University Hospital Sant Joan de Déu (Barcelona, Spain) between 2007 and 2011 were compared: a first set of 159 invasive disease isolates, and a second set of 209 nasopharyngeal isolates recovered from healthy children admitted for minor surgery. The most common invasive serotypes were 1 (24.5%, $n = 39$), 19A (21.2%, $n = 34$), 5 (8.8%, $n = 14$), 7F (8.8%, $n = 14$) and 3 (5%, $n = 8$). The most common serotypes in carriage were 19A (10%, $n = 21$), 6C (9%, $n = 19$), 23B (8.1%, $n = 17$), 6A (7.6%, $n = 16$) and 19F (6.2%, $n = 13$). A significantly higher propensity to cause invasive disease was observed for serotypes 1, 3, 5, 7F and 19A, all of which are included in PCV13. After false-discovery-rate correction, the results were robust for serotypes 1, 5, 7F and 19A. Non-PCV13 serotypes had a low invasive disease potential. Our data reinforce the need for continuous surveillance and should encourage efforts to introduce universal vaccination with PCV13 in children in our region.

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Introduction

Streptococcus pneumoniae is a major cause of invasive disease in children and a common cause of pneumonia, meningitis and sepsis [1]. Despite being such a virulent pathogen, the main ecological niche of the pneumococcus is the nasopharynx of healthy children, which is colonized asymptomatically [2]. Although almost all children carry pneumococci during early childhood, very few develop disease. The capsule of the pneumococcus has been considered its main virulence factor and at least 94 serotypes have been described [3]. Despite this diversity, a relatively small number of serotypes are associated with most invasive disease worldwide [4].

Recent epidemiological studies in different countries have compared nasopharyngeal carriage rates of serotypes with rates of invasive pneumococcal disease (IPD) to estimate the invasive disease potential of individual serotypes and clones. The results have shown that the potential to cause invasive disease differs by serotype (and even by genotype). For example, serotypes 6B, 19F and 23F have a low disease potential while serotypes 1, 7F and 14 have a high disease potential [5–11].

The seven-valent pneumococcal conjugate vaccine (PCV7), has proven to be remarkably effective in reducing overall carriage and disease caused by PCV7 vaccine serotypes, leading to its virtual extinction in some regions [12,13]. As a result, in countries where PCV7 has been extensively used, the population and distribution of serotypes causing invasive disease and detected in nasopharyngeal carriers has changed, potentially affecting the invasive disease potential of specific serotypes.

In Spain, PCV7 was licensed in 2001, PCV10 in April 2009 and PCV13 in January 2010; however, in our community, Catalonia, these vaccines are not subsidized by the Spanish Public Health System. It is estimated that <50% of children of our geographical area were vaccinated with PCV7 during the period 2007–2009 [14].

The main goal of this study was to determine the invasive disease potential of serotypes causing invasive paediatric disease in the era of conjugate vaccines in Catalonia, Spain, and evaluate its potential coverage by the most recent conjugate vaccine. As a secondary objective we evaluated whether implementation of PCV7 had resulted in significant changes in the invasive disease potential of the most frequent serotypes circulating in the area.

Materials and Methods

Study design and sample collection

Two sample collections were compared. The first included all invasive pneumococcal isolates (n = 159) obtained from children admitted to the University Hospital Sant Joan de Déu (Barcelona, Spain) between 2007 and 2011. The second collection (n = 209) included pneumococcal isolates obtained from the nasopharynx of healthy children who attended for minor surgical procedures in our hospital during the same time period. IPD was defined as the presence of clinical findings of infection together with the isolation by culture of *S. pneumoniae* in blood, cerebrospinal fluid or any other sterile fluid. In both collections, subjects were children up to 6 years old. The mean ages of patients with IPD and carriers was 27 months (SD 19.0) and 34 months (SD 17.7), respectively.

The University Hospital Sant Joan de Déu is a tertiary teaching children's hospital that annually captures ~ 19% of all paediatric admissions recorded in Catalonia. This region has a population of around 7 million people, of which 1.2 million are <18 years of age [15].

Microbiological identification, antimicrobial susceptibility and serotyping

Pneumococcal strains were identified by standard microbiological methods that included the optochin sensitivity test and an antigenic test targeting the capsular polysaccharide (Slidex pneumo-kit; BioMérieux, Marcy-l'Etolie, France). The MICs to penicillin, cefotaxime, erythromycin, tetracycline, chloramphenicol and levofloxacin were determined by Etest (BioMérieux). Antibiotic susceptibilities were defined according to the breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [16]. Multidrug resistance was defined as non-susceptibility to three or more antimicrobial agents.

Pneumococcal isolates were serotyped by the Quellung reaction at the National Pneumococcal Reference Centre of Majadahonda (Madrid, Spain). Since 2010, a fragment analysis technique based on fluorescent PCR and subsequent fragment analysis by capillary electrophoresis that allows the detection of 40 serotypes/serogroups (1, 2, 3, 4, 5, 6A/6B, 6C, 7C/(7B/40), 7F/7A, 8, 9N/9L, 9V/9A, 10A, 10F/(10C/33C), 11A/11D, 12F/(12A/44/46), 13, 14, 15A/15F, 15B/15C, 16F, 17F, 18/(18A/18B/18C/18F), 19A, 19F, 20, 21, 22F/22A, 23A, 23B, 23F, 24/(24A/24B/24F), 31, 33F/(33A/37), 34, 35A/(35C/42), 35B, 35F/47F, 38/25F, 39) has been implemented in our laboratory [17] and the Quellung reaction has been used as a complementary assay when the serotype cannot be determined by the PCR-based method.

Statistical analysis

Simpson's Index of Diversity was used to measure the collections' diversity through the website www.comparingpartitions.info [18].

The invasive disease potential of serotypes was estimated using ORs with 95% CI, as described by Brueggeman *et al.* [5]. The equation used for calculations of ORs was OR = $(ad)/(bc)$, where a was the number of invasive X serotypes, b was the number of carriage X serotypes, c was the number of invasive non-X serotypes, and d was the number of carriage non-X serotypes. An OR of 1 indicated that the serotype was equally likely to be recovered from invasive disease and carriage, whereas an OR >1 indicated an increased probability to cause invasive disease. OR significance was tested with the two-tailed Fisher exact test, using a cut-off p value of ≤0.05 (two-tailed) for all statistical analyses. The resulting p values were corrected for multiple testing by controlling the false discovery rate (FDR) to ≤0.05 through the Benjamini and Hochberg method as previously described [9,19].

Results

Serotype distribution in disease and colonization

Among the 159 pneumococcal strains causing IPD in young children, collected in our laboratory between 2007 and 2011, 26 serotypes were identified. The most common were

serotypes 1 (24.5%, *n* = 39), 19A (21.2%, *n* = 34), 5 (8.8%, *n* = 14), 7F (8.8%, *n* = 14) and 3 (5%, *n* = 8). All of these serotypes are included in the most recently approved pneumococcal conjugate vaccine, PCV13. Overall, 13.8% (*n* = 22) of the isolates were covered by PCV7, 55.9% (*n* = 89) were covered by PCV10, and 84.9% (*n* = 135) were covered by PCV13 (see Supplementary material, Table S1).

Among the 209 strains from carriage collected between 2007 and 2011, 37 serotypes were identified. The most common were 19A (10%, *n* = 21), 6C (9%, *n* = 19), 23B (8.1%, *n* = 17), 6A (7.6%, *n* = 16) and 19F (6.2%, *n* = 13). When looking for the most common serotypes in invasive disease, serotypes 1 and 3 (apart from 19A described above), were also found among carriage strains (*n* = 2); serotypes 5 and 7F were not detected. One strain was non-typeable. Overall, 14.3% (*n* = 30) of the strains expressed serotypes included in PCV7, 15.3% (*n* = 32) expressed serotypes included in PCV10, and 33.9% (*n* = 71) expressed serotypes included in PCV13.

Comparison of the serotype diversity between the two collections using the Simpson's Index of Diversity, showed that the invasive disease collection was significantly less diverse than the carriage collection: 0.874 (95% CI 0.844–0.904) versus 0.953 (95% CI 0.945–0.962).

Antimicrobial susceptibility in disease and colonization

The percentage of penicillin non-susceptible strains according to EUCAST penicillin breakpoints (≥0.12 mg/L) was similar in both collections: 30.2% (*n* = 48) in the invasive collection and 28.2% (*n* = 59) in the carriers collection (p 0.7).

Multidrug resistance (MDR), defined as non-susceptibility to three or more antibiotics, was detected in 22.6% (*n* = 36) of the invasive isolates and 17.7% (*n* = 37) of the carriage isolates (p 0.2). Among the MDR invasive strains (*n* = 36), 21 expressed serotype 19A (58.3%) and four expressed serotype 19F (11.1%). Serotypes 6B, 15A, 16F and 24F were detected in two strains each and serotypes 1, 23B and 24B in one strain each. Among the MDR carrier strains (*n* = 37), 11 expressed serotype 19A (29.7%), six expressed serotype 23A, five serotype 19F, four serotype 23F and three serotype 15A. Serotypes 6A and 6B were detected in two strains each, and serotypes 6C, 16F, 24F and a non-typeable isolate were detected in one strain each. Overall, among the MDR invasive strains 80.6% expressed serotypes included in PCV13. The corresponding percentage among carrier strains was significantly lower (54%, p 0.003).

Association with antimicrobial susceptibility profile and invasiveness

The putative association between antimicrobial resistance and invasiveness was explored (Table 1). An apparent but non-significant association was found between penicillin non-susceptibility and/or MDR strains and invasiveness.

Association of serotypes with invasiveness

There were significant differences in the serotype-specific ORs of the pneumococci characterized in this study (Table 2).

TABLE 1. Invasive disease potential according to antimicrobial susceptibility

Antimicrobial susceptibility	No. of invasive isolates (total: 159)	No. of carriage isolates (total: 209)	OR	95% CI	p value
Penicillin non-susceptible strains	48	59	1.1	0.7–1.7	0.7
Multidrug-resistant strains[a]	36	37	1.3	0.8–2.3	0.2

[a]Defined as non-susceptibility to three or more antimicrobial agents tested (penicillin ≥0.12 mg/L, cefotaxime >0.5 mg/L, erythromycin >0.25 mg/L, tetracycline ≥2 mg/L and cloramphenicol ≥8 mg/L).

TABLE 2. Invasive disease potential of serotypes

Serotype[a]	No. of isolates		OR[b]	95% CI[b]	p value[c]
	Invasive	Carriage			
PCV7	22	30	0.9	0.5–1.7	0.89
6B	2	4	0.6	0.1–3.7	0.71
14	6	4	2.0	0.5–8.2	0.47
19F	7	13	0.7	0.2–1.8	0.59
23F	4	7	0.7	0.2–2.6	0.69
Other PCV7[d]	3	2	–	–	–
PCV10	89	32	**7.0**	**4.3–11.5**	**<0.01***
1	39	2	**33.4**	**9.3–208.8**	**<0.01***
5	14	0	∞	**5.9–∞**	**<0.01***
7F	14	0	∞	**5.9–∞**	**<0.01***
PCV13	135	71	**10.8**	**6.5–18.5**	**<0.01***
3	8	2	**5.5**	**1.2–38.1**	0.10
6A	4	16	**0.3**	**0.1–0.9**	0.12
19A	34	21	**2.4**	**1.4–4.4**	**0.02***
Non PCV13	24	138	**0.1**	**0.1–0.2**	**<0.01***
6C	1	19	**0.1**	**0.003–0.4**	**<0.01***
10A	2	10	0.3	0.04–1.1	0.14
11A	0	9	**0**	**0.0–0.5**	**0.03***
15A	2	6	0.4	0.1–2.1	0.45
15B	1	9	**0.1**	**0.006–0.9**	0.11
15C	2	4	0.7	0.1–3.7	0.71
16F	2	5	0.5	0.1–2.7	0.58
17F	0	5	0	0.1–1.1	0.15
21	0	5	0	0.1–1.1	0.15
22F	3	8	0.5	0.1–1.8	0.53
23A	0	9	0	**0.0–0.5**	**0.03***
23B	5	17	0.4	0.1–1.0	0.14
34	0	6	0	**0.0–0.8**	0.11
35B	0	5	0	0.0–1.1	0.15
Other[d]	6	21	–	–	–
Total	159	209			

[a]PCV7: Serotypes included in 7-valent conjugate vaccine (4, 6B, 9V, 14, 18C, 19F and 23F); PCV10: serotypes included in 10-valent conjugate vaccine (PCV7 plus serotypes 1, 5, 7F); PCV13: serotypes included in 13-valent conjugate vaccine (PCV10 plus serotypes 3, 6A, 19A); non-PCV13 serotypes: other serotypes not included in any conjugate vaccine.
[b]OR and 95% CI are shown for serotypes that had at least five isolates. An OR >1 indicates increased invasive disease potential, and an OR <1 indicates decreased invasive disease potential. ORs and 95% CIs in bold type are statistically significant.
[c]p values that were significant after false detection rate correction are marked by asterisks.
[d]Other: serotypes with <5 isolates were (with the corresponding number of isolates in disease and carriage, respectively): 4 (1/0), 8 (1/0), 9N (0/1), 9V (0/1), 11D (0/1), 12F (0/1), 13 (0/1), 18C (2/1), 24B (1/0), 24F (2/1), 28 (1/0), 29 (0/3), 31 (0/4), 33F (0/2), 35A (0/1), 35F (0/2), 37 (0/1), 38 (1/1), 47 (0/1), and NT (0/1).

TABLE 3. Invasive disease potential of non-PCV13 serotypes according to age

Non-PCV13 serotypes	No. of invasive isolates (total: 24)	No. of carriage isolates (total: 138)	OR[a]	95% CI[a]	p value[b]
Age group					
<2 years old	16	38	**0.18**	**0.08–0.4**	<0.0001*
≥2 years old	8	100	**0.05**	**0.02–0.1**	<0.0001*

[a]Data shown in bold are OR estimates whose confidence interval does not include 1.
[b]p values that were significant are marked by asterisks.

Serotypes 1, 3, 5, 7F and 19A, all included in the most recent vaccine (PCV13), were associated with high invasiveness with an OR significantly higher than 1. These results were robust after FDR correction for serotypes 1, 5, 7F and 19A. In contrast, non-PCV13 serotypes were associated with low invasive disease potential (overall, OR 0.1, 95% CI 0.1–0.2) (Table 2). This was also noted for the recently described serotype 6C (OR 0.1, 95% CI 0.003–0.4, p 0.001). Significantly low invasiveness of non-PCV13 serotypes was observed both in children younger than 2 years old and in the older group (Table 3).

Discussion

Since the implementation of PCV7, the rank order of the most frequent serotypes causing IPD and being carried has changed significantly in Catalonia, Spain [15,20,21]. We aimed to evaluate whether this has resulted in changes in the invasive disease potential of the most frequent serotypes circulating in the area.

The results of the present study showed that serotypes 1, 5, 7F and 19A, which became more prevalent in invasive disease in Spain and Portugal during the last decade [22–24], have a high invasive disease potential. Previous studies, conducted before the introduction of conjugate vaccines, had also identified these serotypes as highly invasive [5,6,10,25]. In addition, the observation that, among serotype 19A, MDR strains have expanded, is worrying [26]. These four serotypes are now included in the most recent conjugate vaccine, PCV13.

Of interest, the results obtained for serotype 3 suggested a high invasive disease potential (OR 5.5, 95% CI 1.2–38.1, p 0.02 before FDR correction). However, this result was not significant after FDR correction (p 0.10, Table 2), a statistical test that has seldom been used in most published studies of this nature and that aims to correct for the risk of detecting significant associations due to chance alone when multiple comparisons are performed [9,19]. The invasive disease potential of this serotype remains controversial. Brueggemann *et al.*, [6] in an extended meta-analysis that included data of >4000 isolates from carriers and >700 isolates of patients with IPD recruited in seven datasets, indicated that serotype 3 had an OR significantly <1 and, consequently, a low propensity to cause invasive disease. Sá-Leão *et al.* [9] in a study conducted during 2001–2003 in Portugal, Yildirim *et al.* [27] in a study conducted during 2003–2009 in Massachusetts and Rivera-Olivero *et al.* [28] in a study conducted during 2006–2008 in Caracas associated serotype 3 with a high invasive disease potential. Of note, serotype 3 has become the third most frequent paediatric serotype in children with IPD in Barcelona [29]. The reasons for these contrasting findings may be multifactorial. For example, a difference in the lineages associated with this serotype in each study may be distinct. In fact, recent studies have shown that apart from the serotype, the genotype may also affect the disease potential of a lineage [9,10,30]. In addition, it is conceivable that removal of serotypes included in PCV7 from the pneumococcal population may have resulted in a competitive release of some non-PCV7 serotypes. This could in turn lead, ultimately, to changes in the invasive disease potential of selected non-PCV7 serotypes [31]. Clearly, further studies will be needed to fully address these questions.

In our study, a low attack rate was observed in all non-PCV13 serotypes, the recently described serotype 6C among them. In recent years, serotype 6C has expanded, becoming one of the most prevalent serotypes in carriage among healthy children [32,33]. Nevertheless, this serotype was a rare cause of IPD in our study. Given that serotype 6C was not analysed in the pre-vaccine era, our data regarding the low attack rate of this specific serotype are novel and of interest.

In conclusion, we have found that all serotypes with high attack rate are included in PCV13 and that the remaining serotypes circulating in our regions have a low invasive disease potential. Our data reinforce the need for continuous surveillance and should encourage efforts to introduce universal vaccination with PCV13 in children.

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Transparency Declaration

The authors declare no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Proportion of the different invasive serotypes

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