Vaccine 31 (2013) 3187–3190

Contents lists available at SciVerse ScienceDirect

Vaccine

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

Distribution of carried pneumococcal clones in UK children following the introduction of the 7-valent pneumococcal conjugate vaccine: A 3-year cross-sectional population based analysis

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ARTICLE INFO

Article history:
Received 9 January 2013
Received in revised form 3 April 2013
Accepted 26 April 2013
Available online 13 May 2013

Keywords:
Pneumococci
Pediatric
Carriage, PCV-7
Sequence types
Serotype

ABSTRACT

The success of *Streptococcus pneumoniae* (pneumococcus) in both colonisation and disease is associated with the increased prevalence of genetic clones expressing virulence factors that assist host invasion. We studied the distribution of pneumococcal clones in paediatric carriage as part of an ongoing longitudinal study of pneumococcal carriage in children less than 5 years of age. Across three years, 87 different sequence types (STs) were found amongst 310 pneumococci. A decline in PCV-7 related STs was observed during the study period. STs 62, 199, 433 and 1692 increased after the implementation of PCV-7 and were related to increases in serotypes 11A, 19A, 22F, and to serotype 6C, respectively. Overall, a strong correlation was observed between ST and serotype. Thirteen STs contained multiple serotypes and 74 STs were associated with only one serotype. On-going molecular epidemiological surveillance of pneumococcal carriage is warranted during the implementation of pneumococcal conjugate vaccines.

1. Introduction

Streptococcus pneumoniae (the pneumococcus) is a common resident in the human nasopharynx. In rare occasions, underlying factors such as young age, certain immune deficiencies and environmental factors may lead to pneumococcal infection [1]. Children younger than 5 years and the elderly carry the highest burden for pneumococcal colonisation and progression to disease. The ninety-three pneumococcal serotypes identified to date are spread across 46 serogroups and consist of more than 6000 genetic clones. However, only 10 serogroups have been associated with pneumococcal disease. A seven-valent pneumococcal conjugate vaccine (PCV-7) was introduced in the UK in 2006, which targeted serotypes responsible for ≥75% of invasive pneumococcal disease in children ≤5 years. The vaccine was implemented in the children vaccination programme as a 2 + 1 dose schedule at two, four and 13 months with a catchup protocol for children ≥2 years. One year after implementation, the vaccine reduced the incidence of vaccine type IPD in children ≤5 years by 65.7%, and by ∼90% in epidemiologic year 2009–2010. However, the decline of vaccine type IPD was mirrored by the increase of non-vaccine type IPD and in 2010 PCV-7 was replaced by PCV-13 that provided additional predicted IPD coverage of >60% primarily due to serotypes 7F, 19A and 22F [2]. Pneumococcal conjugate vaccination has been related to increases in the circulation of certain genetic clones that are associated with antibiotic resistance [3,4] and enhanced virulence [5–7]. The clonal pneumococcal composition in the era of pneumococcal vaccination is now routinely analysed.

The pneumococcal population biology is complex. Healthy individuals provide the optimum sample for studying pneumococcal clonality and the circulating genetic pool [8]. Pneumococci that are commonly carried in the nasopharynx would more often have the temporal opportunity for a random horizontal gene transfer. It is then expected, and indeed observed, that pneumococcal strains that are often carried will be more genetically diverse, and more invasive isolates will be more conserved in their clonality [9].

In the UK, only six studies so far have examined the pneumococcal clonal composition. Four studies from Scotland examined the genetic distribution of pneumococci primarily isolated from invasive disease before the implementation of PCV-7 [10–13]. The UK's Health Protection Agency has also studied the pneumococcal clonal distribution during PCV-7 introduction [14]. The UK studies of pneumococcal genetic diversity demonstrated that the pneumococcal clonal composition is stable and genetic clones associated

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http://dx.doi.org/10.1016/j.vaccine.2013.04.075

Table 1
Percentage change in the distribution of major vaccine (white) and non-vaccine (grey) – related clones. In the present study, ST176 and ST138 were strongly associated with serotype 6B. ST162 was related to serotypes 19F and 9V, and ST36 to serotype 23F. Of the non-vaccine types, ST62 represented serotype 11A solely; ST1692 – serotype 6C only in years 2 and 3, and serotype 6A and 6C in year 1. ST199 expressed in the most diverse cps background represented by serotypes 14, 15A, 15B, 15C and 19A.

	No isolates			% change			*p*-value
ST	Y	Y+1	Y+2	Y vs Y+1	Y+1 vs Y+2	Y vs Y+2	overall
176	10	6	3	−40.0	−49.0	−69.4	0.08
162	9	1	1	−88.9	2	−88.7	0.02
138	6	4	1	−33.3	−74.5	−83.0	0.12
36	4	7	2	75.0	−70.9	−49.0	0.68
199	7	6	13	−14.3	120.9	89.4	0.16
1692	1	9	8	800.0	−9.4	715.7	0.02
62	2	6	7	200.0	18.9	256.9	0.09

with PCV-7 serotypes were the most common [10–12]. Temporal fluctuations in the clonal diversity and a strong association between ST and serotype was observed [13].

We have previously characterised the genetic composition of serotype 6C isolates from paediatric carriage in the UK and observed that PCV-7 implementation coincided with a significant increase of serotype 6C strains due to expansion of ST1692 [15] and further described the serotype prevalence of carried pneumococci [16].

Here we report the clonal distribution of pneumococci isolated during a nasopharyngeal carriage study in young children. We hypothesised that increasing PCV-7 coverage and vaccine uptake will result in a shift in the clonal distribution of carried pneumococci with the favourable expansion of non-vaccine serotype clones, and capsular polysaccharide (cps) replacement resulting in the expression of cps in alternative genetic background. Since the clonal structure of pneumococci has been related to their virulence, knowledge of the circulating clones is warranted.

2. Materials and methods

2.1. Study design and population

This study is part of a longitudinal cross-sectional analysis of pneumococcal nasopharyngeal carriage in children four years of age under. Pneumococci were isolated as previously described [16]. The main outcome measure in the initial carriage study was the prevalence of pneumococcal carriage. Sample size and power calculations were based on the lowest expected carriage rate of 10%. To detect an estimated 50% relative reduction in pneumococcal carriage after the introduction of PCV-7 with 80% power at a 5% significance level, we used a sample size of 100 pneumococci per study year. Fisher's exact test was used to determine significance ($p \leq 0.05$) between proportions, expressed as a difference of outcomes in different study years.

2.2. Genomic DNA extraction

Single α-hemolytic, optochin sensitive colonies were collected from an overnight Columbia blood agar plate culture. Genomic DNA (gDNA) was extracted from bacterial lysates using the QIAGEN QIAamp DNA Mini Kit (Crawley, UK) following the gDNA extraction protocol for tissue culture.

2.3. Multi locus sequence typing

Genetic characterisation of the pneumococcal population was performed using multi locus sequence typing (MLST). MLST was performed by Qiagen Genomic Services (Hilden, Germany). Relationships between sequence types (ST) as determined by MLST were characterised using the eBurst program (available at http://eburst.mlst.net/). Clonal complexes were defined on the basis of six out of seven identical alleles.

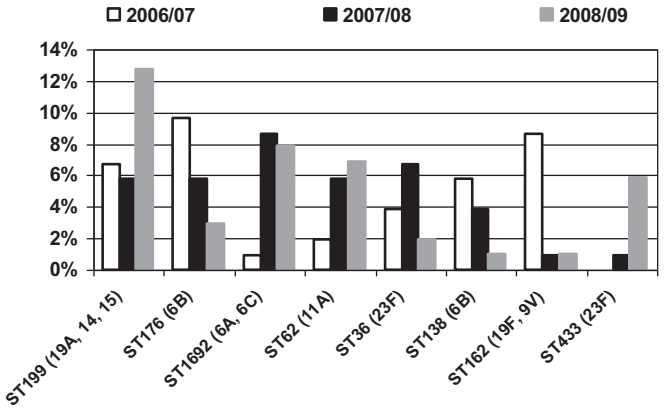

Fig. 1. Distribution of pneumococcal clones in paediatric carriage following PCV-7 introduction. The bars represent ST frequency (%) by study year. The serotypes expressing the relevant clones are in brackets.

3. Results

A total of 310 pneumococci were isolated during this three year study. MLST revealed that the isolates were distributed across 87 different STs. 47 STs were present in 2006/2007 (year 1), 45 in 2007/2008 (year 2) and 48 in 2008/2009 (year 3). Eight STs were newly identified, of which 75% ($n = 6/8$) were isolated in the second year of the study. 16 STs were exclusively related to year 1, 19 to year 2, 17 to year 3 and 30 were shared across the three years. During PCV-7 implementation (2006/07), the most common STs were ST176 and ST162, represented by serotypes 6B, 19F and 9V. In year 2, those were ST65 and ST1692, and in year 3 ST199 associated with serotypes 6A (ST65), 6C (ST1692) and 19A, 14 and serogroup 15 (ST199).

3.1. Distribution of PCV-7 related clones

A marked decrease in the number of PCV-7 related STs was observed over the three years (Table 1) paralleled by the decline of vaccine serotypes (Fig. 1). A strong concordance between ST and serotype was observed within the vaccine related strains.

In 2007/08 and 2008/09, a marked decline of ST176 and a significant decrease of ST162 occurred, which was associated with a decline in serotypes 6B and 19F, respectively (Fig. 1). The clonal complex with a founder ST176 (CC176), which was identified in the first two years, was not observed in year 3. CC176 was exclusively related to serotype 6B. A clonal complex with a founder ST42 (CC42) was identified in 2007/08 and persisted in 2008/09. In 2007/08, ST42, the founder of the complex, expressed serotype 6B capsular polysaccharide and the complex was shared by 5 different serotypes across 3 serogroups. In 2008/09, CC42 was only shared by 3 different serotypes within serogroup 23 in year 3. CC42 contained the majority of serotype 23A and 23B isolates in year 3, and