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Nasopharyngeal carriage of *Streptococcus pneumoniae* and other bacteria in the 7th year after implementation of the pneumococcal conjugate vaccine in the Netherlands

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ABSTRACT

After introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in the infant national immunization program (NIP) in the Netherlands in 2006, *Streptococcus pneumoniae* strains of the non-vaccine serotype 19A emerged and became the dominant serotype in carriage in children and their parents. Similar patterns were observed in other European countries and the United States. Increases in carriage rates of *Staphylococcus aureus* and non-typeable (NT) *Haemophilus influenzae* were also observed. After switching of PCV7 to 10-valent vaccine (PCV10) in 2011, a new carriage surveillance study was performed in the winter of 2012/2013. Nasopharyngeal carriage of *S. pneumoniae*, *H. influenzae*, *S. aureus*, and *Moraxella catarrhalis* was determined by conventional culture in 330 PCV10-vaccinated 11-month-old children, 330 PCV7-vaccinated 24-month-old children, and their parents. Carriage prevalence was compared with similar carriage studies conducted in 2005, 2009, and 2010/2011. Although serotype 19A remained the most frequently carried pneumococcal serotype in children, prevalence of 19A significantly declined in PCV7-vaccinated 24-month-old children (14% to 8%, $p = 0.01$), but less in PCV10-vaccinated 11-month-old children (12% to 9%, $p = 0.31$). Carriage of *H. influenzae* remained stable at an elevated level (65% in 11-month-olds and 69% in 24-month-olds), while the carriage of *S. aureus* returned to pre-PCV7 levels in 11-month-old children (14% in 2010/2011 to 7% in 2012/2013), but not in 24-month-olds (remained at 7%). Our results might indicate a new balance between replacing non-vaccine pneumococcal serotypes and other potential pathogenic bacteria in nasopharyngeal carriage. Carriage studies are valuable tools in assessing vaccine effects on pathogens circulating in the population, for evaluation of PCV impact, and in predicting changes in respiratory and invasive disease.

1. Introduction

Streptococcus pneumoniae (pneumococcus) is a leading cause of respiratory infections like pneumonia and acute otitis media, as well of invasive disease including septicaemia and meningitis. Pneumococcal disease is preceded by nasopharyngeal acquisition. Surveillances on asymptomatic colonization and carriage prevalence are therefore important tools in monitoring effects and predicting impact of vaccines targeting disease [1–4].

The upper respiratory tract in children is an important reservoir for common respiratory bacterial pathogens like *S. pneumoniae, Staphylococcus aureus, (non-typeable) Haemophilus influenzae*, and *Moraxella catarrhalis*, which usually behave like commensals but occasionally cause respiratory or invasive infectious disease [5]. In particular young children with high colonization prevalence and high carriage density are considered to be a major source for transmission and spread of respiratory pathogens in the community [6,7]. There are clear differences in carriage prevalence [8] and invasiveness [9,10] between the more than 90 different pneumococcal serotypes that are currently identified. Around 20 serotypes caused 70–80% of all invasive pneumococcal disease (IPD) in children before the introduction of pneumococcal conjugate vaccines(PCV)

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[11]. Reduction of pneumococcal vaccine serotype carriage through PCV vaccination of children has led to important herd protection against disease caused by vaccine type pneumococci in all age groups [12–15].

In the Netherlands, PCV7 was introduced in the national immunization program (NIP) in June 2006 for all newborns at the age of 2, 3, 4, and 11 months without a catch-up for older infants and toddlers. In 2011, the 10-valent pneumococcal conjugate vaccine (PCV10) replaced PCV7 for all children born after March 1st without catch-up. PCV10 includes serotypes 1, 5 and 7F next to the PCV7 vaccine serotypes; eight of the ten PCV10 serotypes are conjugated to Protein D, a conserved outer membrane protein of non-typeable *H. influenzae*, whereas the two remaining are conjugated to either tetanus toxoid or diphtheria toxoid as carrier protein.

In our previous carriage monitoring studies in infants, we found an initial decline of 20% in overall pneumococcal carriage prevalence due to a strong drop of vaccine serotype carriage [12]. Over time, the vacant nasopharyngeal niche was gradually filled in by non-vaccine serotypes in PCV7-vaccinated toddlers as shown in our carriage surveillance studies from 2009 [16] and 2010/2011 [17], and by others [18,19]. In particular, carriage of serotype 19A showed high carriage peaks in infants with carriage prevalence up to 14% in 2010/2011 and became the primary colonizing serotype in children in the Netherlands [16,17]. Dominance of serotype 19A in both carriage and disease after the implementation of PCV7 was also observed in other European countries and the USA [19,20,21,22,23,24]. Next to a shift in pneumococcal serotypes, increased carriage prevalence of *H. influenzae* and *S. aureus* were observed after PCV7 implementation [16,17]. This raises concerns about the potential impact of PCV7 on non-pneumococcal infections.

Epidemiological surveillance of pneumococcal carriage is an effective tool to monitor PCV-induced changes and to predict vaccine effects on pneumococcal disease [3,4]. As part of our on-going pneumococcal surveillance program, we evaluated the long-term impact of PCVs on both pneumococcal carriage and carriage of other respiratory bacterial pathogens. We performed the current study seven years after PCV7 introduction in the Dutch national immunization program and 1.5 years after PCV7 was replaced by PCV10.

2. Methods

2.1. Study population

Nasopharyngeal carriage of *S. pneumoniae, S. aureus, H. influenzae*, and *M. catarrhalis* was investigated from October 1st, 2012 through March 5th, 2013 in two age cohorts from the open population of children: (1) 330 11-month-old infants who had received PCV10 at 2, 3, and 4 months of age according to the Dutch national immunization program (NIP) (born after March 1st 2011), and before the booster vaccination at 11 months of age, (2) 330 24-month-old children who were immunized with PCV7 at 2, 3, 4, and 11 months of age (born before March 1st 2011). (3) Additionally, nasopharyngeal and oropharyngeal samples were collected from one of the parents of each 24-month-old child. All participants were non-febrile (i.e. <38.5 °C body temperature) at sampling. Exclusion criteria were known or suspected immunodeficiency, craniofacial or chromosomal abnormalities, coagulation disorders, or use of anticoagulant medication. Participants did not receive any financial compensation. An acknowledged national ethics committee in the Netherlands (METC Noord-Holland, committee on research involving human subjects) approved the study (NL40288.094.12). The study was conducted in accordance with the European Statements for Good Clinical Practice.

Current data was compared with historical data from PCV-unvaccinated children at 12 and 24 months of age and one parent of each 24-month-old child, derived from a previous longitudinal randomized controlled trial (RCT) in the Netherlands (NCT00189020) which was executed between 2005 and 2008 [12]. In addition, current data from 2012/2013 were compared with previous carriage data from similarly designed cross-sectional carriage surveillance studies executed in 2009 and 2010/11, i.e. 3 years [16] and 4.5 years after implementation of PCV7 in the NIP in 2006 [17], the latter immediately prior to introduction of PCV10 for children born after March 1, 2011. All studies were conducted in an open population living in the Western part of the Netherlands and performed by the same study team.

2.2. Nasopharyngeal swabs

Nasopharyngeal swabs were obtained transnasally by trained study personnel with a flexible, sterile swab according to World Health Organization standard procedures as previously described [25]. From parents, both transnasal and transoral nasopharyngeal samples were collected, as the pneumococcal yield is known to be higher in adults when taking both samples [26]. After sampling, swabs were immediately placed in liquid Amies transport medium and cultured within 12 h. All swabs in present and previous studies were processed by the same microbiological laboratory and according to the same study procedures, as described earlier [12,27]. Briefly, pneumococcal isolates were identified using conventional methods; one pneumococcal colony per plate was subcultured and serotyped by capsular swelling method (Quellung reaction). For *S. aureus, H. influenzae*, and *M. catarrhalis*, swabs were cultured according to standard diagnostic procedures.

2.3. Questionnaire

Research nurses completed a survey of each child and parent on possible predictors of nasopharyngeal bacterial carriage: age, sex, season of sampling, recent antibiotic use within one month prior to sampling, symptoms of a respiratory tract infection and/or acute otitis media during sampling, presence of siblings in the household, day care attendance of the participating child, passive smoke exposure indoors, and active smoking of the participating parent.

2.4. Statistical analyses

The sample size of the present surveillance study was similar to the previous cross-sectional studies [16,17], on the assumption that in all studies at least similar but presumably significantly larger differences would be observed in carriage of vaccine serotypes compared to the unvaccinated historical cohort from 2005.

Differences in baseline characteristics were statistically tested using 2-sided Chi-square or Fisher's exact test for dichotomous outcomes and Student's *t*-test for continuous outcomes. Differences in prevalence of pneumococcal serotypes and other respiratory bacteria were statistically tested using 2-sided Chi-square or Fisher's exact test, where appropriate. *p*-Values <0.05 were considered significant. A backward stepwise logistic regression (with backward variable selection based on likelihood ratio test) was used to determine adjusted estimates of the association between the bacterial carriage and pneumococcal vaccination, as given by adjusted odds ratios (aORs) and their corresponding 95% confidence intervals (CIs). Entered possible predictors included above-mentioned possible predictors of nasopharyngeal carriage. Due to the low amount of missing data (<0.1%) no imputation methods were used.