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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.

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Table 1
Baseline characteristics of all four studies.

	11 Months children				24 Months children			
	2005 Pre-PCV7	2009 Post-PCV7 3 yr	2010/2011 Post-PCV7 4.5 yr	2012/2013 Post-PCV10 1.5 yr	2005 Pre-PCV7	2009 Post-PCV7 3 yr	2010/2011 Post-PCV7 4.5 yr	2012/2013 Post-PCV7 6.5 yr
	No (%) $n=319$	No (%) $n=329$*	No (%) $n=330$	No (%) $n=330$	No (%) $n=321$	No (%) $n=330$	No (%) $n=330$	No (%) $n=330$
Male sex	156 (49)	181 (55)	173 (52)	176 (53)	155 (48)	**187 (57)[a]**	171 (52)	**145 (44)[e]**
Mean age in months (SD)	12.0 (0.3)	**10.9 (0.3)[a]**	**10.7 (0.4)[b,c]**	**10.8 (0.3)[d,e,f]**	24.2 (0.6)	**24.0 (0.3)[a]**	**23.8 (0.5)[b,c]**	**24.2 (0.4)[e,f]**
Presence of siblings < 5 yr§	126 (40)	**84 (26)[a]**	**145 (44)[c]**	**152 (46)[e]**	127 (40)	135 (41)	154 (47)	130 (39)
Day care attendance§	208 (65)	226 (69)	222 (67)	237 (72)	224 (70)	233 (71)	**259 (79)[b]**	253 (77)
Passive smoke exposure¶	21 (7)	**5 (2)[a]**	**9 (3)[b]**	8 (2)	26 (8)	16 (5)	**12 (4)[b]**	14 (4)
Symptoms of RTI and/or AOM#	95 (30)	95 (29)	112 (34)	93 (28)	82 (26)	69 (21)	**114 (35)[b,c]**	**89 (27)[f]**
Antimicrobial drug use**	20 (6)	24 (7)	**27 (8)[b]**	35 (11)	10 (3)	**23 (7)[a]**	**15 (5)[b]**	21 (6)
Period of sampling:								
October–March	149 (47)	**82 (25)[a]**	**274 (83)[b,c]**	330 (100)	156 (48)	86 (26)[a]	**299 (91)[b,c]**	330 (100)
April–September	170 (53)	247 (75)	56 (17)¥	–	166 (52)	244 (74)	31 (9)¥	–
	Parents of 24-month-old children (unvaccinated)							
	2005 Pre-PCV7	2009 Post-PCV7 3yr	2010/2011 Post-PCV7 4.5yr	2012/2013 Post-PCV7 6.5yr				
	n (%) $n=296$	n (%) $n=324$	n (%) $n=326$	n (%) $n=322$				
Male sex	51 (17)	53 (16)	58 (18)	61 (19)				
Mean age in years (SD)	34.7 (4.9)	35.1 (4.4)	35.3 (4.5)	35.8 (4.6)				
Active smoking	40 (14)	34 (11)	41 (13)	45 (14)				
Antimicrobial drug use**	9 (3)	20 (6)	16 (5)	15 (5)				

(a) p-values <0.05; 2009 vs. 2005
(b) p-values <0.05; 2010/2011 vs. 2005
(c) p-values <0.05; 2010/2011 vs. 2009
(d) p-values <0.05; 2012/2013 vs. 2005
(e) p-values <0.05; 2012/2013 vs.2009
(f) p-values <0.05; 2012/2013 vs.2010/2011

Abbreviations: PCV7; all serotypes included in 7-valent pneumococcal conjugate vaccine. PCV10; all serotypes included in 10-valent pneumococcal conjugate vaccine. SD; standard deviation; p-values were calculated with chi-square test or Fisher's exact test (2-sided) or independent-samples t-test where appropriate.

* Swabs were taken just before the booster vaccination at 11 months of age or within one week after the booster vaccination.
§ Defined as more than 4 h per week with at least 1 child from a different household.
¶ Defined as passive tobacco smoke exposure indoors at least 1 cigar or cigarette during 5 days/week.
# The presence of symptoms of a respiratory tract infection (RTI) and/or acute otitis media (AOM) as defined by evaluation of parents.
** Defined as use of oral or intravenous antibiotics within 1 month before sampling.
¥ Samples were collected between 15th and 30th of September.

3. Results

In total, 330 children aged 11 months, 330 children aged 24 months, and 322 parents were sampled. Baseline characteristics of children and parents of the current study (2012/2013) and the previous studies are shown in Table 1. The most noticeable difference among these four time-points is the season of sampling. The current study and the follow-up study in 2010/2011 were both performed during the autumn/winter whereas samples in the studies of 2005 and 2009 were also collected during the spring and summer months. We observed no significant effect of season on pneumococcal carriage prevalence by conventional culture, except for carriage of *M. catarrhalis*, which seemed to be higher during the summer months (data not shown). We corrected for this in the multivariate analyses.

In 2012/2013, pneumococcal carriage prevalence in children was lower than the pre-vaccination level of 2005 (respectively 59% vs. 67% in 11-months-olds ($p=0.03$) and 56% vs. 66% in 24-months-olds ($p=0.01$)). In parents, the overall pneumococcal carriage prevalence was lower as compared to all previous years (Fig. 1A and Table 2).

Both in children and parents, we observed a nearly complete disappearance of PCV7-serotypes (carriage < 2%) as compared to 2005. The carriage of the additional PCV10 serotypes (serotypes 1, 5 and 7F) was below 2% in children in all four consecutive studies (Fig. 1A and B, and Supplemental Table 1).

Non-PCV7 serotype carriage prevalence had almost doubled over time after introduction of PCV7; from 29% in 2005 to 57% in 2012/2013 in 11-month-old children and from 30% to 55% in 24-month-old children. In parents, all except one (serotype 6B) of 31 cultured pneumococci were non-PCV7 serotypes in 2012/2013 (Fig. 1A and B and Supplemental Table 1).

Carriage of serotype 19A increased over the years after PCV7 introduction. In 2012/2013, 19A remained the predominant serotype in carriage in children, although the prevalence of 19A had significantly declined since 2010/2011 in the 24-month-olds (14% to 8%, $p=0.01$), whereas serotype 19A carriage prevalence had remained stable at around 10% in PCV10-vaccinated 11-month-old children (Fig. 1B and Supplemental Table 1). In infants, 19A was followed by the serotypes 6C, 23B, 23A, and 11A. In parents, serotype 10A was the most predominant serotype, followed by 23B, 19A, and 3 (Fig. 1B and Supplemental Table 1). After implementation of PCV7, carriage of 6A declined rapidly and remained low (around 1%) in 2012/2013 in children and parents. Carriage of serotype 3, also covered by the 13-valent vaccine but not in PCV10, remained low in all study groups. Serotype 6C had increased after PCV implementation in both PCV10-vaccinated 11-month-olds (from 2% in 2005 to 6% in 2012/2013) and PCV7-vaccinated 24-month-olds (from 2% in 2005 to 7% in 2012/2013).

For *S. aureus*, after a stepwise progressive rise in 2009 and 2010/2011 to up to 14% in 11-month-old children, the prevalence had returned to pre-PCV7 levels of around 7% in 2012/2013. In parents, *S. aureus* carriage remained at a higher level of 32% as compared to 20% in 2005. In 24-month-old children, no changes in *S. aureus* carriage were observed between 2005 and 2012/2013 (Table 2 and Fig. 2A).

Carriage prevalence of *H. influenzae* had increased in the years after PCV7 implementation in all age groups and remained at a

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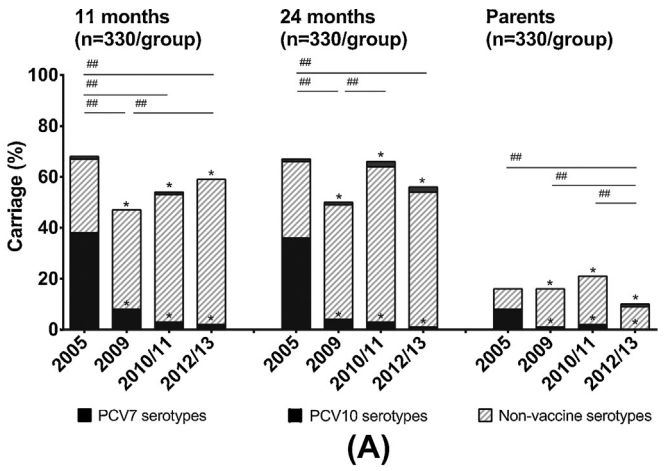
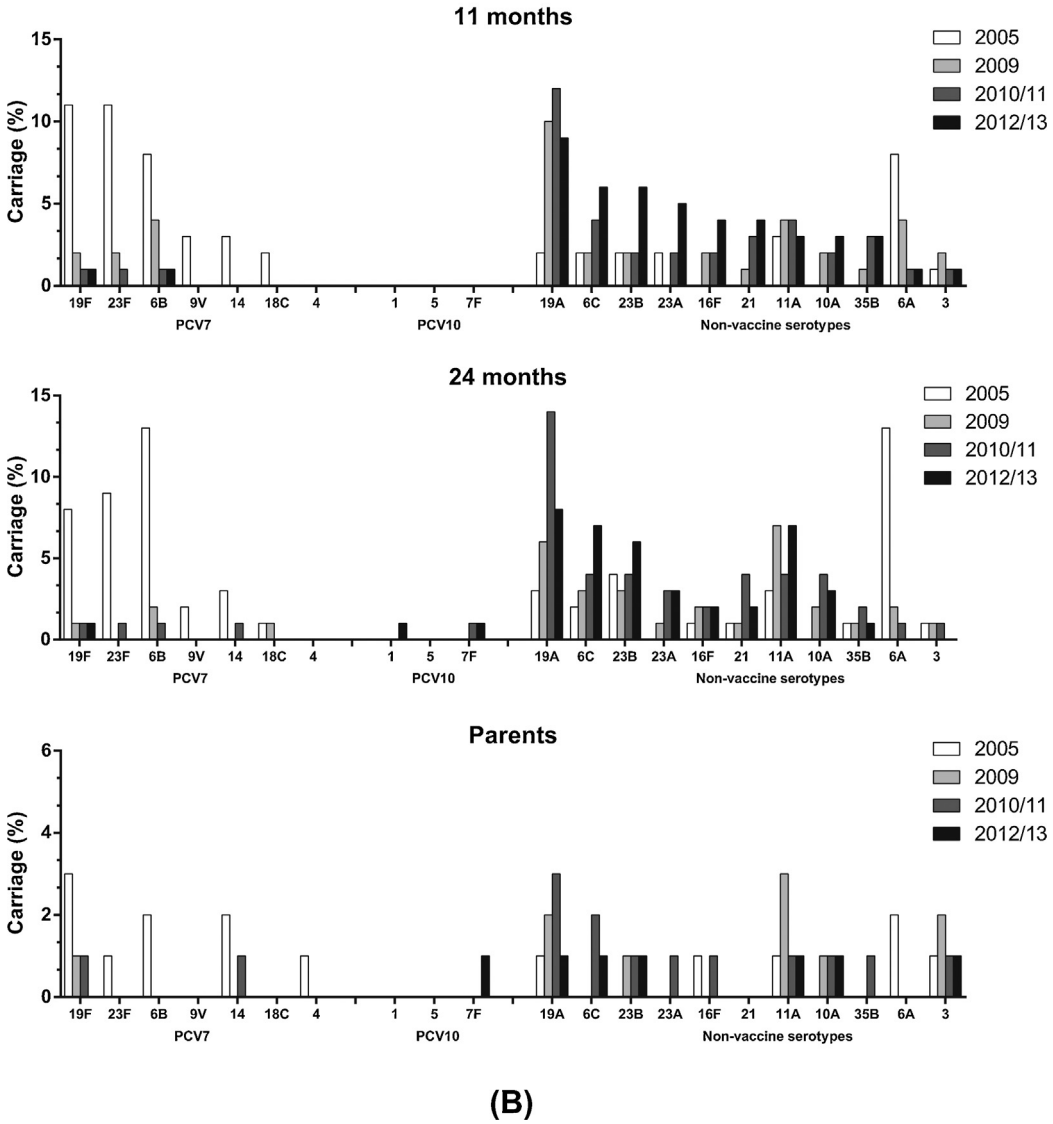


(A)



(B)

Fig. 1. *Carriage of S. pneumoniae in children and parents depicted over time.* The first bar in each age group depicts the carriage prevalence before the implementation of PCV (2005). The following three bars depict the carriage prevalence as observed during the follow-up studies; respectively 3 years after PCV implementation (2009), 4.5 years after PCV implementation (2010/2011) and in the 7th year after PCV implementation (2012/2013).
(A) Overall pneumococcal carriage, ## adjusted Odds Ratio does not include 1, compared with linked bar on overall pneumococcal carriage. * Adjusted Odds Ratio does not include 1, compared to pre-PCV7 data on carriage of PCV7 serotypes and carriage of non-PCV7 serotypes (panel A). (B) Carriage of pneumococcal serotypes. Abbreviations: PCV7; all serotypes included in 7-valent pneumococcal conjugate vaccine. PCV10; all serotypes included in 10-valent pneumococcal conjugate vaccine.

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Table 2
Multivariate analysis of nasopharyngeal carriage of *S. pneumoniae, S. aureus, H. influenzae* and *M. catarrhalis* before, 3, 4.5 and 6.5 years after PCV implementation among children and their parents.

	2005 Pre-PCV7	2009 Post-PCV7 3 yr	2010/2011 Post-PCV7 4.5 yr	2012/2013 Post-PCV10 1.5 year	2009 vs 2005	2010/2011 vs 2005	2012/2013 vs 2005	2010/2011 vs 2009	2012/2013 vs 2009	2012/2013 vs 2010/2011
	No (%)	No (%)	No (%)	No (%)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
11 months	*n* = 319	*n* = 329	*n* = 330	*n* = 330						
S. pneumoniae	214 (67)	154 (47)	173 (52)	193 (59)	***0.43 (0.30–0.60)***	***0.48 (0.34–0.67)***	***0.63 (0.45–0.89)***	1.02 (0.68–1.53)	***1.49 (1.07–2.09)***	1.34 (0.96–1.88)
PCV7-serotypes	122 (38)	25 (8)	10 (3)	7 (2)	***0.14 (0.09–0.23)***	***0.04 (0.02–0.08)***	***0.03 (0.01–0.06)***	***0.38 (0.18–0.80)***	***0.26 (0.11–0.62)***	0.83 (0.28–2.38)
Non-PCV7-serotypes	92 (29)	129 (39)	163 (50)	186 (57)	***1.75 (1.24–2.46)***	***2.59 (1.85–3.62)***	***3.58 (2.54–5.05)***	1.39 (1.00–1.93)	***1.92 (1.37–2.69)***	1.40 (1.00–1.96)
PCV10-serotypes	124 (39)	26 (8)	12 (4)	7 (2)	***0.14 (0.09–0.23)***	***0.05 (0.03–0.09)***	***0.03 (0.01–0.06)***	***0.44 (0.55–0.89)***	***0.26 (0.11–0.60)***	0.58 (0.23–1.50)
S. aureus	16 (5)	31 (9)	46 (14)	23 (7)	1.79 (0.95-3.38)	***3.27 (1.78–5.98)***	1.54 (0.79–3.00)	***1.88 (1.14–3.13)***	0.88 (0.49–1.57)	***0.47 (0.28–0.80)***
H. influenzae	146 (46)	212 (64)	213 (65)	209 (63)	***2.69 (1.90–3.80)***	***2.22 (1.58–3.13)***	***1.96 (1.40–2.75)***	0.83 (0.59–1.17)	0.73 (0.52–1.03)	0.89 (0.64–1.25)
M. catarrhalis	218 (68)	210 (64)	251 (76)	263 (80)	0.81 (0.57–1.15)	1.45 (1.00–2.10)	1.33 (0.87–2.04)	***1.82 (1.27–2.61)***	***2.09 (1.43–3.04)***	0.80 (0.53–1.19)
	2005 Pre-PCV7	2009 Post-PCV7 3 yr	2010/2011 Post-PCV7 4.5 yr	2012/2013 Post-PCV7 6.5 year	2009 vs 2005	2010/2011 vs 2005	2012/2013 vs 2005	2010/2011 vs 2009	2012/2013 vs 2009	2012/2013 vs 2010/2011
	No (%)	No (%)	No (%)	No (%)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
24 months	*n* = 321	*n* = 330	*n* = 330	*n* = 330						
S. pneumoniae	211 (66)	162 (49)	211 (64)	185 (56)	***0.50 (0.36–0.70)***	0.81 (0.58–1.13)	***0.62(0.44–0.86)***	***1.61 (1.16–2.23)***	1.25 (0.91–1.72)	0.79 (0.57–1.10)
PCV7-serotypes	114 (36)	14 (4)	11 (3)	2 (1)	***0.08 (0.05–0.15)***	***0.06 (0.03–0.12)***	***0.01 (0.00–0.04)***	0.76 (0.34–1.70)	***0.14 (0.03–0.61)***	***0.18 (0.04–0.80)***
Non-PCV7-serotypes	97 (30)	148 (45)	200 (61)	183 (55)	***1.99 (1.43–2.77)***	***3.35 (2.40–4.67)***	***3.14 (2.25–4.38)***	***1.67 (1.21–2.30)***	***1.46 (1.06–2.01)***	0.89 (0.64–1.24)
PCV10-serotypes	118 (37)	15 (5)	16 (5)	7 (2)	***0.08 (0.05–0.15)***	***0.09 (0.05–0.15)***	***0.04 (0.02–0.08)***	1.00 (0.49–2.07)	0.45 (0.18–1.11)	0.43 (0.17–1.05)
S. aureus	18 (6)	25 (8)	24 (7)	22 (7)	1.39 (0.74–2.63)	1.61 (0.84–3.07)	1.29 (0.67–2.48)	1.09 (0.60–1.97)	0.94 (0.51–1.72)	0.81 (0.44–1.49)
H. influenzae	168 (52)	240 (73)	252 (76)	227 (69)	***2.70 (1.91–3.82)***	***2.68 (1.88–3.82)***	***2.00 (1.42–2.82)***	0.99 (0.68–1.44)	0.76 (0.53–1.09)	0.77 (0.53–1.11)
M. catarrhalis	189 (59)	199 (60)	264 (80)	251 (76)	1.37 (0.97–1.94)	***1.75 (1.18–2.61)***	1.29 (0.86–1.98)	1.37 (0.85–2.21)	0.96 (0.58–1.61)	1.01 (0.66–1.55)
	2005	2009	2010/2011	2012/2013	2009 vs 2005	2010/2011 vs 2005	2012/2013 vs 2005	2010/2011 vs 2009	2012/2013 vs 2009	2012/2013 vs 2010/2011
	No (%)	No (%)	No (%)	No (%)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	aOR (95% CI)	aOR (95% CI)
Parents	*n* = 296	*n* = 324	*n* = 326	*n* = 322						
S. pneumoniae	50 (17)	51 (16)	68 (21)	31 (10)	0.91 (0.59–1.40)	1.27 (0.84–1.91)	***0.52 (0.32–0.84)***	1.35 (0.90–2.02)	***0.57 (0.35–0.92)***	***0.42 (0.27–0.66)***
PCV7-serotypes	25 (8)	2 (1)	7 (2)	1 (0)	***0.07 (0.02–0.29)***	***0.24 (0.10–0.55)***	***0.03 (0.01–0.25)***	4.09 (0.83–20.1)	0.50 (0.04–5.54)	0.22 (0.03–2.00)
Non-PCV7-serotypes	25 (8)	49 (15)	61 (19)	30 (9)	***1.93 (1.16–3.23)***	***2.43 (1.48–4.00)***	0.95 (0.53–1.68)	1.25 (0.82–1.89)	***0.48 (0.29–0.80)***	***0.38 (0.23–0.91)***
S. aureus	60 (20)	104 (32)	111 (34)	104 (32)	***1.85 (1.36–2.83)***	***1.96 (1.36–2.83)***	***1.86 (1.29–2.69)***	1.07 (0.77–1.49)	0.79 (0.49–1.28)	0.82 (0.59–1.15)
H. influenzae	67 (23)	96 (30)	130 (40)	111 (35)	***1.49 (1.03–2.14)***	***2.26 (1.58–3.33)***	***1.85 (1.29–2.64)***	***1.52 (1.10–2.11)***	1.22 (0.88–1.71)	0.79 (0.58–1.09)
M. catarrhalis	28 (10)	43 (13)	71 (22)	61 (19)	1.44 (0.87–2.39)	***2.54 (1.59–4.08)***	***2.22 (1.38–3.58)***	***1.77 (1.17–2.69)***	1.29 (0.71–2.33)	0.80 (0.54–1.17)

Abbreviations: PCV7; all serotypes included in 7-valent pneumococcal conjugate vaccine. Non-PCV7; all other serotypes not included in 7-valent pneumococcal conjugate vaccine. PCV10; all serotypes included in 10-valent pneumococcal conjugate vaccine. aOR; adjusted odds ratio. CI; confidence interval. In children; aOR-values were adjusted by multivariate analysis for sex; presence of siblings <5 yr in the household; day care attendance; antibiotic use; passive smoke exposure; season; and the presence of URTI and/or AOM using binary logistic regression with backward LR. In parents; aOR-values were adjusted by multivariate analysis for sex; smoking; antibiotics use; and season using binary logistic regression with backward LR. Bold indicates the significant aORs.

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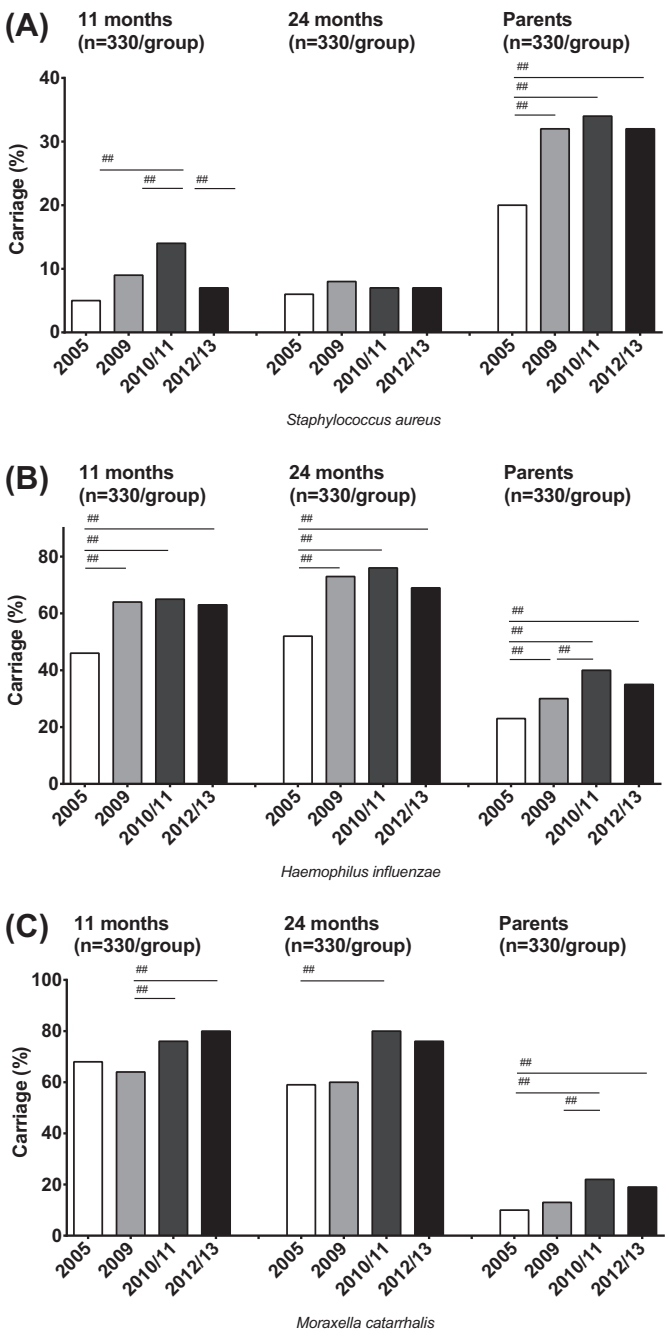

Fig. 2. Carriage of *S. aureus*, *H. influenzae* and *M. catarrhalis* in children and parents depicted over time. (A) Carriage of *S. aureus*. (B) Carriage of *H. influenzae*. (C) Carriage of *M. catarrhalis*, ## adjusted Odds Ratio does not include 1 compared with linked bar on carriage of *S. aureus* (panel B), *H. influenzae* (panel C) and *M. catarrhalis* (panel D).

higher level of 63–69% in children and 35% in parents. Carriage of *M. catarrhalis* remained stable over time after PCV introduction (Table 2 and, respectively Fig. 2B and C).

4. Discussion

After introduction of PCV7 in the NIP in 2006, carriage of PCV7 serotypes had rapidly declined toward a very low level, both in vaccinated children and their parents. Simultaneously, an almost complete replacement with non-vaccine pneumococcal strains occurred over the years, with strains of serotype 19A becoming the predominant pneumococci in carriage and progressively increasing prevalence until 2010/2011. In 2012/2013, this serotype 19A expansion was no longer observed; 19A carriage had declined significantly in the 24-month-old children who had received four PCV7 vaccinations, but not in 11-months-olds vaccinated with three doses of PCV10. IPD cases with serotype 19A in children under five years of age also declined since 2011 in the Netherlands [28]. Several studies have reported on potential cross-protection against serotype 19A by PCV10 in IPD [29,30]. Our data does not support PCV10-induced cross-protection in carriage since the decline in 19A prevalence was more outspoken in the PCV7-vaccinated 24-month-old children than in the PCV10-vaccinated 11-month-old children who showed similar 19A carriage levels as compared to 2009. This is in line with a randomized controlled trial that compared 19A carriage in PCV10 versus PCV7-vaccinated children [31] and with data from a post-PCV10 surveillance study on carriage by Hammitt et al. which both found no effect of PCV10 on 19A carriage [32]. A possible explanation for the stabilization or even decline in 19A carriage may be a new balance in competition between the replacing non-PCV7 serotypes several years following implementation of PCV7 with no single serotype clearly dominating in carriage, as reported by Hanage and colleagues six to eight years after PCV7 introduction in Massachusetts [21]. Since the extra PCV10 serotypes 1, 5, and 7F were rare (≤2%) in our studies, we speculate that introduction of PCV10 may have had limited effect on further serotype replacement in carriage between 2010/2011 and 2012/2013. However, follow-up studies need to confirm whether our data reflect a temporal serotype fluctuation, transitory state, or indeed a newly established balance between serotypes.

Following implementation of PCV7, the overall IPD cases caused by serotypes 1, 5, and 7F had slightly increased as observed in the Netherlands and Germany [19,28]. Three years after the switch from PCV7 to PCV10, which covers the serotypes 1, 5, and 7F, the number of IPD cases caused by these serotypes significantly decreased by 42–47% in (unvaccinated) adults aged 18–64 years old, which likely represents herd effects of infant immunization. A similar trend was observed in PCV10-vaccinated infants under the age of two, though not significant possibly due to the low number of cases [28]. Whilst serotype 6A declined over time following PCV7 implementation, carriage of serotype 6C gradually increased from 2% to 6–7% in infants, thereby becoming the second most dominant serotype in carriage in 2012/2013. Also an increased involvement of serotype 6C in AOM was seen in the years after PCV7 implementation [33]. Although 6C is not included in the PCV13 vaccine, 6A in PCV13 possibly elicits cross-protection against serotype 6C by induction of functional opsonophagocytic responses against 6C [34].

After PCV7 introduction in the NIP, we also observed an increased carriage of other potential pathogenic bacteria, especially *S. aureus* [17,27], which next to non-vaccine pneumococcal serotypes, may have contributed to the rise in some respiratory diseases like empyema [35]. Previously, we reported on increased *S. aureus* prevalence in healthy children after PCV7 vaccinations in a randomized controlled setting [36] and increased involvement of *S. aureus* after pneumococcal conjugate vaccination in acute otitis media (AOM) in children with a history of recurrent otitis who participated in a randomized controlled trial [37]. Our current data show a return in carriage prevalence of *S. aureus* toward (almost) pre-PCV levels in 11-month-old children. Since interactions between *S. aureus* and pneumococcal serotypes have been described in literature [5,38,39], this decline may also be in line with a new balance in the pneumococcal serotypes and other colonizers of the nasopharyngeal tract and warrants further investigation.

Vaccination with PCV7 resulted in increased levels of *H. influenzae* in previous randomized controlled trials after PCV7

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vaccinations [40,41] and follow-up surveillance studies [16,17,42]. This coincided with increased involvement of (mainly non-typeable) *H. influenzae* in otitis media in a RCT on PCV7 and AOM [43] and after the widespread implementation of PCV7 [44,45]. Here we show that carriage of *H. influenzae* remained on a high level albeit stable on the long term compared with pre-PCV7 vaccination data. This was also observed in PCV10 vaccinated children even though in PCV10 eight of the ten serotypes are conjugated to Protein D, a conserved outer membrane protein of non-typeable *H. influenzae*. No effect on *H. influenzae* carriage, acquisition, and/or density was previously observed in randomized controlled trials comparing PCV10 to PCV7 [46] or meningococcal vaccine [47]. In contrast, a study performed in Kenya found a reduction in carriage of *H. influenzae* in the total population after introduction of PCV10 [32]. The authors, however, question the role of PCV10 as a causative agents since there was an overall reduction in *H. influenzae* carriage and no association between vaccination status and carriage of *H. influenzae* [32]. No consistent changes in carriage of *M. catarrhalis* overtime were found [45], also not on microbiota level both in PCV7 [41] and PCV10-vaccinated children [48].

Strengths of our study are the consistency of data collection in repeated large, identically designed, well-defined studies conducted overtime by the same study group in an open population of children and their parents. Moreover, vaccination coverage among Dutch infants of targeted age-groups is high (>95%) [49].

When interpreting our data, some potential limitations should be considered. Due to the design of the study, causality between implementation of PCV7/PCV10 and changes in carriage of well-known nasopharyngeal colonizers cannot clearly be established. The lower overall pneumococcal carriage in 2012/2013 in parents, but not in children, compared to previous years is remarkable and coincided with an increase in number of IPD cases caused by non-PCV10 serotypes in unvaccinated Dutch adults, most significantly in the elderly, in the same period [28]. However, children with high density colonization rates rather than parents with relatively low carriage prevalence are the key transmitters of non-vaccine pneumococci causing IPD in other age groups. The contribution of carriage monitoring by culture in healthy, younger adults appears therefore to be limited. Since there were no changes in the study protocols, research team, and laboratory handling, the overall lower carriage in parents compared to previous years may be explained by other potential confounders, including viral presence or annual variation. Another limitation of our study could be the use of conventional culture techniques allowing us to investigate only the dominating serotypes carried [50]. We may have missed possible additional (secondary) serotypes that might have been detected using more sensitive diagnostic methods [51,52]. Future surveillance studies on pneumococcal and serotype carriage using both conventional culture and molecular methods [51,53,54] and investigating not only children but also the elderly are warranted for a better overview of circulating serotypes in the most vulnerable groups at risk for IPD.

In conclusion, 7 years following implementation of PCV7, 19A remained the predominant pneumococcal serotype in carriage but the previous expansion was stopped and 19A carriage even had declined in PCV7-vaccinated children. Carriage of *H. influenzae* remained at a higher level, while the *S. aureus* carriage returned to pre-PCV7 levels in 11-month-old children. This might indicate a return of a balance between pneumococci and other potential pathogenic bacteria in nasopharyngeal carriage after significant shifts following PCV implementation in the Netherlands. Future carriage and disease surveillance studies are warranted to monitor the long-term impact, also of the broader coverage pneumococcal conjugate vaccines.

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Author contributions

Conceived and designed the experiments: MAH, AJWM, NYR, EAMS. Performed the study: AATMB, MAH, JPB. Analyzed the data: AATMB, EAMS. Contributed reagents/materials/analysis tools: KT, DB, JPB. Drafted of the manuscript: AATMB, MAH, AJWM, NYR, JPB, DB, KT, EAMS.

Conflict of interest statement

EAMS declares to have received unrestricted research support from Pfizer, grant support for vaccine studies from Pfizer and GSK and fees paid to the institution for advisory boards or participation in independent data monitoring committees for Pfizer and GSK. None of the fees or grants listed here was received for the research described in this paper. All other authors report no potential conflicts.

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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.2015.11.060.

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