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The Impact of the 13-Valent Pneumococcal Conjugate Vaccine on Pneumococcal Carriage in the Community Acquired Pneumonia Immunization Trial in Adults (CAPiTA) Study

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Background. The impact of pneumococcal conjugate vaccination on the prevalence of nasopharyngeal carriage with pneumococci and other bacteria in adults is unknown. The direct effects of the 13-valent pneumococcal conjugate vaccine (PCV13) in community dwelling older adults was investigated as part of the randomized controlled Community Acquired Pneumonia immunization Trial in Adults (CAPiTA).

Methods. We determined the carriage of *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Moraxella catarrhalis* before and 6, 12, and 24 months after vaccination using polymerase chain reaction (PCR)–based methods and conventional cultures of nasopharyngeal and oropharyngeal swab samples in 1006 PCV13 recipients and 1005 controls. Serotyping of the 13 vaccine-type (VT) pneumococci was performed by PCR targeting capsular synthesis genes and Quellung reaction of isolates.

Results. Before randomization and based on PCR, 339 of 1891 subjects had nasopharyngeal carriage with any pneumococci (17.9%), and 114 of 1891 (6.0%) carried VT pneumococci. At 6 months after vaccination, VT pneumococcal carriage was significantly lower in PCV13 recipients than in the placebo group (relative risk, 0.53; 95% confidence interval, .35–.80; $P = .04$). There was no difference between the groups at 12 and 24 months after vaccination. Carriage of non-VT pneumococci, *S. aureus*, *H. influenzae*, and *M. catarrhalis* did not change between groups.

Conclusions. In community-dwelling adults aged ≥65 years, a single dose of PCV13 seems to elicit a small and temporary reduction in VT carriage 6 months after vaccination. Neither replacement by non-VT serotypes nor impact on other nasopharyngeal bacteria was observed.

Keywords. nasopharyngeal carriage; PCV13; adult; RCT.

The incidence of invasive pneumococcal disease (IPD) and community-acquired pneumonia (CAP) peaks in early childhood and older adults [1]. In both age groups, *Streptococcus pneumoniae* is a significant cause of CAP, resulting in notable increases in morbidity and mortality rates [2, 3].

The human upper respiratory tract is the natural reservoir for *S. pneumoniae* and nasopharyngeal carriage of *S. pneumoniae* is seen as a prerequisite for disease. In young children, high pneumococcal nasopharyngeal colonization rates up to 60%–90% coincide with high pneumococcal infection rates [4]. In older community-dwelling adults, pneumococcal carriage rates, based on detection with conventional culture of nasopharyngeal and oropharyngeal swab samples, are usually <5%, despite high infection rates that increase with age [5–7]. The low pneumococcal carriage prevalence as determined by conventional culture in older adults is thought to be due to short carriage duration and low-density carriage. When using molecular techniques with higher sensitivity, *S. pneumoniae* colonization rates in older adults are up to 20% [8, 9], and in case of repeated carriage measurement, time prevalence over 6 months may go up to 48% [8].

The success of the 7-valent pneumococcal polysaccharide conjugate Prevenar-7 (7-valent pneumococcal conjugate vaccine [PCV7]; Wyeth) in young children is based on protection of the target group against vaccine-type (VT) disease and reduction of VT carriage [10]. Because young children with high pneumococcal carriage rates and high colonization density are the key transmitters of infection in the population, all other age groups also benefitted from herd protection after introduction

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of pneumococcal conjugate vaccine (PCV) in national immunization programs with ongoing reduction of VT pneumococcal disease [11–14]. However, over time, non-VT (NVT) serotypes filled in the biological gap in nasopharyngeal colonization created by PCV in children, and subsequent replacement disease continues to reduce the overall impact of PCV immunization. This is in particular true for older adults, who seem most susceptible to disease regardless of serotype [12, 13, 15]. Furthermore, PCV7 vaccination in children also resulted in carriage shifts of other nasopharyngeal bacteria, such as *Staphylococcus aureus* and *H. influenzae* in vaccinees and their unvaccinated parents [16–20]. The direct effects of PCV vaccination on nasopharyngeal pneumococcal carriage and other bacteria in older adults are unknown.

The Community Acquired Pneumonia immunization Trial in Adults (CAPiTA), a randomized, double-blind clinical trial in 84 496 participants aged ≥65 years in the Netherlands, demonstrated a 45.6% efficacy of PCV13 against first episodes of VT CAP and 75.0% against first episodes of VT IPD per protocol [21]. In a substudy, we assessed the effects of PCV13 on nasopharyngeal carriage of *S. pneumoniae*, *S. aureus*, *H. influenzae*, and *Moraxella catarrhalis* in the first 24 months after vaccination in 2011 pneumococcal vaccine-naive older adults.

METHODS

The collection of carriage data was part of a substudy within the CAPiTA study. The study was a collaboration between University Medical Center Utrecht (UMCU) and the study sponsor, Wyeth, a Pfizer company. The study design and primary and secondary end points including safety data were previously published [21, 22].

The study was conducted according to a written protocol and in compliance with Good Clinical Practice guidelines, and was approved by the Central Committee on Research Involving Human Subjects and Ministry of Health, Welfare, and Sport in the Netherlands. The UMCU, Julius Clinical (an academic research organization affiliated with the UMCU), and Linnaeus Institute (an academic research organization of the Spaarne Hospital) conducted the substudy and gathered all data. The sponsor performed data management and polymerase chain reaction (PCR) and statistical analysis. Standard confidentiality agreements were in place between the sponsor, Spaarne Hospital, and UMCU, which allowed full access to all data and the right to publish.

Study Design and Population

The study was a parallel-group, randomized, placebo-controlled, double-blind trial [21]. Use of a placebo was appropriate because no pneumococcal vaccine is recommended in the Netherlands for routine use in older adults [23, 24]. Two years before the study (June 2006) PCV7 had been introduced for all newborns in the Netherlands (without a catch-up campaign) [25]. PCV7 was replaced by the 10-valent pneumococcal vaccine PHiD-CV10 (GlaxoSmithKline, Rixensart, Belgium) in March 2011 [26] and was in use during the last 2 months of our study. At the start of the study in 2008, no herd protection in older adults was yet observed in IPD [27], but a progressive decline in VT IPD by PCV7 serotypes was observed in the following years [13, 28].

A subset of 2011 community-dwelling older adults aged ≥65 years was enrolled at home visits in a single region in the Netherlands for the substudy between 15 September 2008 and 20 March 2009. The key eligibility criteria were no previous pneumococcal vaccination and immune competence. All subjects provided written informed consent. Full details of the eligibility criteria are provided in the supplementary appendix.

Subjects were vaccinated at home visit 1 (baseline) and followed up for 2 years with 3 home visits to collect nasopharyngeal samples for carriage data at baseline and 6, 12, and 24 months after vaccination. All visits were performed by trained study personnel. Comorbid conditions, such as asthma, diabetes mellitus with or without insulin use, heart disease, liver disease, lung disease, and splenectomy, were reported by the subjects at baseline.

Investigational Products

Subjects were randomly assigned in a 1:1 ratio to receive PCV13 or placebo by intramuscular injection in the right deltoid. PCV13 contains polysaccharides from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, as described elsewhere [29]. The placebo contained 5.0 mmol/L succinate buffer, 0.15 mol/L sodium chloride, 0.02% polysorbate 80, and 0.125 mg of aluminum as aluminum phosphate per 0.5-mL dose and was identical in appearance to PCV13.

Nasopharyngeal Sampling

The upper respiratory tract was sampled using flexible nylon flocked sterile E-swabs (Copan) with 2 nasopharyngeal and 2 separate oropharyngeal swab samples. Samples were collected by trained study personnel, immediately stored in Amies transport medium (Copan), and transferred within 8 hours at room temperature to the Regional Laboratory (Haarlem, the Netherlands). The first of each swab sample type was tested for the presence of *S. pneumoniae, H. influenzae, M. catarrhalis,* and *S. aureus* by means of conventional culture methods, as described elsewhere [30]. Identification was done by colony morphology and conventional methods of determination. Pneumococcal serotyping was performed using the capsular swelling method (Quellung reaction) with type-specific antisera (Statens Serum Institut).

The second nasopharyngeal and oropharyngeal swab samples were stored in transport medium at −70°C until transferred to Pfizer's laboratories for further analyses. After thawing, DNA was extracted from 100 μL of each specimen using an ABI 6700 Nucleic Acid Workingstation (Applied Biosystems) and eluted in a final volume of 100 μL. *S. pneumoniae* was detected by means of quantitative PCR targeting conserved regions of the autolysin (*lytA*) gene [31]. Ten microliters of purified DNA was added to 10 μL of 2X Fast PCR mix (Applied Biosystems) containing

primers and probe, and DNA fragments were amplified using the 7900 Fast Real-Time PCR System (Applied Biosystems). Samples were considered positive for *S. pneumoniae* when the detected *lytA*-specific signal was <45 cycle threshold (C_T) [32]. Serotyping was done by single-plex PCR assays for detection of the 13 VT capsular synthesis genes. Three of the single-plex assays were only serogroup specific (serogroups 7, 9, and 18).

Statistical Analyses

The main carriage end point of the study was the prevalence of nasopharyngeal VT pneumococcal carriage based on molecular methods before and after vaccination (baseline, 6, 12, and 24 months after vaccination) within each vaccine group. As secondary carriage end points, the point prevalence of nasopharyngeal carriage of *S. pneumoniae, H. influenzae, M. catarrhalis,* and *S. aureus* was assessed for each visit (baseline, 6, 12, and 24 months after vaccination) within each vaccine group based on conventional culture. Subjects were considered a *S. pneumoniae* carrier if 1 of the 4 samples collected at a single time point was positive. If all 4 samples with a valid result were negative, a subject was considered a noncarrier. In case of more than 1 serotype, all serotypes were included in the analysis.

A post hoc analysis was conducted on the concordance between PCR analyses and conventional culture techniques. A relative risk with 2-sided 95% confidence intervals (CIs) was assessed, and differences were considered significant if the nominal 95% CI excluded 0 for an end point of a difference between the compared groups (or 1 for an end point of a ratio of the compared groups) without adjusting for multiplicity due to multiple end points, time points, and comparisons. Missing assay results were not replaced or imputed.

RESULTS

Of the 2011 subjects enrolled in the substudy, 1006 received PCV13 and 1005 received placebo. Of these subjects, 112 (5.6%) were excluded from analyses, evenly divided between the 2 groups (Supplementary Figure S1). The most common reason for exclusion was the absence of a valid postvaccination sample within the required time period (4.1% for PCV13 and 4.3% for placebo recipients). At each collection time point, ≥93.9% of samples were collected within the required time period. Missing values were well balanced between the 2 groups. Rates of discontinuation over the course of the study were 4.7% (n = 47) in the PCV13 group and 5.9% (n = 59) in the placebo group. Discontinuation was due mainly to death (n = 35; 1.7%).

The mean age at vaccination was 72.5 years; 11.6% of the subjects (n = 226) were ≥80 years of age (Table 1). Baseline characteristics and patient-reported comorbid conditions were well balanced between the 2 groups, with heart disease the most commonly reported (n = 428; 22.5%).

Table 1. Baseline Characteristics

Characteristic	Subjects, % (No.)[a]	
	PCV13 Group (n = 950)	Placebo Group (n = 949)
Age, y		
Mean (SD)	72.5 (5.5)	72.5 (5.6)
Median (range)	71.5 (65.1–92.3)	71.1 (65.0–94.0)
Male sex	52.8 (502)	56.3 (534)
White race	98.8 (939)	98.6 (936)
Current smoker	8.1 (77)	11.0 (104)
Patient-reported comorbid conditons[b]		
Any	42.6 (405)	39.3 (373)
Heart disease	23.1 (219)	22.2 (209)
Diabetes mellitus	11.6 (110)	11.6 (110)
Insulin use	2.0 (19)	2.3 (22)
No insulin use	9.6 (91)	9.3 (88)
Lung disease	10.4 (99)	10.1 (96)
Asthma	5.3 (50)	4.5 (43)
Liver disease	0.5 (5)	0.4 (4)
Splenectomy	0.1 (1)	0.1 (1)

Abbreviation: PCV13, 13-valent pneumococcal conjugate vaccine; SD, standard deviation.
[a]Data represent % (No.) of subjects unless otherwise specified. Baseline characteristics did not differ significantly between the 2 groups except for smoking status ($P = .04$ Fisher exact test).
[b]The conditions were not mutually exclusive (ie, some subjects had >1 condition).

Overall Pneumococcal Carriage

During the 24-month follow-up, 41.6 % of subjects in the placebo group (354 of 850 subjects with a valid result at all time points; Supplementary Figure S2) had pneumococcal carriage at ≥1 of the 4 time points, as detected by means of PCR targeting *lytA* (39.7%; 337 of 850 subjects) and/or conventional culture (13.0%; 110 of 845). Across the 2-year study period, for subjects with both test results available, <2% of subjects with pneumococci identified by conventional culture (range, 0.7%–1.8%) did not have a positive PCR result targeting *lytA*.

Among the placebo group, 34 subjects (4%) had *S. pneumoniae* carriage detected at all 4 time points; another 56 subjects (6.6%) were *S. pneumoniae* carriers at 3 time points. Four subjects (0.5%) had the same VT serotype at all 4 time points. During 24-month follow-up, the PCR–based overall prevalence of *S. pneumoniae* carriage in the placebo group was highest in the youngest age group (65–69 years): 48.2% (95% CI, 43.1%–53.3%; 184 of 382 subjects); this prevalence was 35.6% (30.9%–40.6%; 139 of 390) for those aged 70–79 and 39.6% (30.2%–49.6%; 42 of 106) for those aged ≥80 years.

In the placebo group, based on PCR analysis, *S. pneumoniae* was more often observed in oropharyngeal than in nasopharyngeal samples at each time point (Table 2), with mean C_T values of 34.9–35.6 in oropharyngeal and 30.6–31.5 in nasopharyngeal samples (Supplementary Table S1). Based on culture, detection rates for *S. pneumoniae* were higher in nasopharyngeal than in oropharyngeal samples at each time point (Table 2). The concordance between culture and PCR at the different time points was higher in nasopharyngeal than in oropharyngeal samples at each time point (Table 2).

Table 2. Conventional Culture and PCR (*LytA*) Detection of *Streptococcus pneumoniae* in Nasopharyngeal and Oropharyngeal Samples From Community-Dwelling Older Adults in the Netherlands—Placebo Group[a]

	Conventional Culture			PCR (*LytA*)			Concordance[b]
		Subjects Carrying *Streptococcus pneumoniae*[d]			Subjects With Pathogen[d]		
Timing and Sample Type	N[c]	No.	% (95% CI)	Subjects Tested[c]	No.	% (95% CI)	% (95% CI)
Before vaccination	948	47	5.0 (3.7–6.5)	948	170	17.9 (15.5–20.5)	84.1 (81.6–86.3)
Nasopharyngeal	949	41	4.3 (3.1–5.8)	947	59	6.2 (4.8–8.0)	94.7 (93.1–96.1)
Oropharyngeal	948	10	1.1 (.5–1.9)	948	140	14.8 (12.6–17.2)	84.8 (82.4–87.0)
6 mo after vaccination	929	44	4.7 (3.5–6.3)	932	185	19.8 (17.3–22.6)	83.4 (80.9–85.8)
Nasopharyngeal	930	43	4.6 (3.4–6.2)	930	79	8.5 (6.8–10.5)	92.9 (91.1–94.5)
Oropharyngeal	931	3	0.3 (.1–.9)	931	146	15.7 (13.4–18.2)	84.2 (81.7–86.5)
12 mo after vaccination	905	45	5.0 (3.6–6.6)	909	176	19.4 (16.8–22.1)	84.0 (81.4–86.3)
Nasopharyngeal	905	39	4.3 (3.1–5.8)	906	57	6.3 (4.8–8.1)	95.9 (94.4–97.1)
Oropharyngeal	908	8	0.9 (.4–1.7)	908	164	18.1 (15.6–20.7)	82.2 (79.6–84.7)
24 mo after vaccination	876	31	3.5 (2.4–5.0)	880	165	18.8 (16.2–21.5)	84.0 (81.4–86.4)
Nasopharyngeal	877	24	2.7 (1.8–4.0)	875	41	4.7 (3.4–6.3)	96.0 (94.5–97.2)
Oropharyngeal	879	8	0.9 (.4–1.8)	878	149	17.0 (14.5–19.6)	82.7 (80.0–85.1)

Abbreviations: CI, confidence interval; PCR, polymerase chain reaction.

[a]This was a post hoc analysis.

[b]Proportion of samples with corresponding results in both tests, over the total number of samples tested.

[c]Subjects with a valid and determinant result at the given time point.

[d]Subjects carrying the specific pathogen at the given time point.

Postvaccination Pneumococcal Carriage

PCR (lytA)

Before vaccination, any pneumococcal carriage based on PCR targeting *lytA* was similar in both groups (17.9% for placebo and 17.8% for PCV13 group) and did not vary greatly over time (Table 3). Based on PCR, VT pneumococcal carriage in both placebo and PCV13 recipients was lower 24 months after vaccination than at baseline (Table 3). At 6 months after vaccination, VT pneumococcal carriage in PCV13 recipients was 1.8% lower than baseline and had declined compared with placebo recipients (relative risk, 0.53; 95% CI, .35–.80), who showed similar carriage prevalences at baseline and 6 months after vaccination (6.7%) (Table 3). This difference in VT carriage between PCV13 and placebo recipients was no longer observed at 12 and 24 months after vaccination.

Carriage prevalence of individual VT and NVT serotypes are listed in Supplementary Table S2. There was no clear change in carriage density between the 2 groups after vaccination (Supplementary Table S1).

Conventional Culture

Based on conventional culture, rates of any pneumococcal carriage and VT pneumococcal carriage in the placebo group before vaccination were 5.0% and 2.3% respectively (Table 4). In PCV13 recipients, these rates were already lower before vaccination than in the placebo group (Table 4). In both PCV13 recipients and the placebo group, VT pneumococcal carriage was lower 24 months after vaccination than at baseline. Carriage of NVT *S. pneumoniae* did not vary greatly over time in either group (Table 4). During the follow-up period, there was a decrease in the percentage of untypable pneumococci by Quellung reaction (decrease in placebo group, from 0.9% before vaccination to 0.2% 24 months after vaccination) (Table 4). Carriage prevalence rates for individual VT and NVT serotypes are listed in Supplementary Table S3.

Carriage of Other Bacteria

Before vaccination, overall carriage rates based on culture of *H. influenzae*, *M. catarrhalis*, and *S. aureus* in the placebo group were 10.2%, 7.2%, and 21.0%, respectively (Table 5). There was no detectable impact of PCV13 on the carriage of any of these bacteria during the follow-up period (Table 5).

DISCUSSION

In immunocompetent, community-dwelling adults aged ≥65 years, a single dose of PCV13 seems to elicit a limited and temporary reduction from 5.4% to 3.6% in VT carriage prevalence 6 months after vaccination, as determined with PCR. Differences in carriage prevalence of VT pneumococci did not remain significant 12 and 24 months after vaccination. No changes were observed in the prevalence of NVT pneumococci or other bacteria after vaccination with PCV13. These findings are relevant for interpreting the potential direct and indirect effects of PCV13 vaccination in older adults.

This is the first study evaluating pneumococcal carriage after PCV13 vaccination in a large group of community-dwelling

Table 3. Effect of PCV13 on PCR-Based Pneumococcal Nasopharyngeal Carriage Rates in Community-Dwelling Older Adults in the Netherlands

	PCV13 Group			Placebo Group			
		Subjects With Pathogen[c]			Subjects Carrying *Streptococcus pneumoniae*[c]		
Serotype and Timing	N[b]	No.	% (95% CI)	Subjects Tested[b]	No.	% (95% CI)	RR (95% CI)[a]
Any pneumococcal serotype							
Before vaccination	950	169	17.8 (15.4–20.4)	948	170	17.9 (15.5–20.5)	0.99 (.82–1.20)
6 mo after vaccination	932	143	15.3 (13.1–17.8)	932	185	19.8 (17.3–22.6)	0.77 (.63–.94)
12 mo after vaccination	909	165	18.2 (15.7–20.8)	909	176	19.4 (16.8–22.1)	0.94 (.77–1.14)
24 mo after vaccination	888	146	16.4 (14.1–19.0)	880	165	18.8 (16.2–21.5)	0.88 (.72–1.07)
Any VT pneumococcal serotype[d]							
Before vaccination	949	51	5.4 (3.9–6.8)	942	63	6.7 (5.1–8.3)	0.80 (.56–1.15)
6 mo after vaccination	928	33	3.6 (2.4–4.7)	928	62	6.7 (5.1–8.3)	0.53 (.35–.80)
12 mo after vaccination	901	46	5.1 (3.7–6.5)	895	51	5.7 (4.2–7.2)	0.90 (.61–1.32)
24 mo after vaccination	875	33	3.8 (2.5–5.0)	871	40	4.6 (3.2–6.0)	0.82 (.52–1.29)

Abbreviations: CI, confidence interval; PCR, polymerase chain reaction; PCV13, 13-valent pneumococcal conjugate vaccine; RR, relative risk; VT, vaccine-type.

[a]RR is the proportion of subjects from the vaccine group who carried the pathogen(s) relative to the proportion from the placebo group who carried the same pathogen(s) at the given time point.

[b]Subjects with a valid and determinant result at the given time point.

[c]Subjects carrying the specific pathogen at the given time point.

[d]Three of the single-plex assays were only serogroup specific (serogroups 7, 9, and 18).

Table 4. Effect of PCV13 on Pneumococcal Nasopharyngeal Carriage Rates Based on Conventional Culture in Community-Dwelling Older Adults in the Netherlands

	PCV13 Group			Placebo Group			
		Subjects Carrying *Streptococcus pneumoniae*[c]			Subjects With Pathogen[c]		
	N[b]	No.	% (95% CI)	Subjects Tested[b]	No.	% (95% CI)	RR (95% CI)[a]
Any pneumococcal serotype							
Before vaccination	950	36	3.8 (2.6–5.0)	948	47	5.0 (3.6–6.3)	0.76 (.50–1.17)
6 mo after vaccination	929	30	3.2 (2.1–4.4)	929	44	4.7 (3.4–6.1)	0.68 (.43–1.07)
12 mo after vaccination	906	27	3.0 (1.9–4.1)	905	45	5.0 (3.6–6.4)	0.60 (.37–.95)
24 mo after vaccination	881	16	1.8 (.9–2.7)	876	31	3.5 (2.3–4.8)	0.51 (.27–.92)
Any VT pneumococcal serotype[d]							
Before vaccination	950	15	1.6 (.8–2.4)	948	22	2.3 (1.4–3.3)	0.68 (.34–1.31)
6 mo after vaccination	929	7	0.8 (.2–1.3)	929	14	1.5 (.7–2.3)	0.50 (.17–1.21)
12 mo after vaccination	906	8	0.9 (.3–1.5)	905	18	2.0 (1.1–2.9)	0.44 (.16–1.00)
24 mo after vaccination	881	4	0.5 (.0–.9)	876	6	0.7 (.1–1.2)	0.66 (.14–2.59)
Any NVT pneumococcal serotype[d]							
Before vaccination	950	21	2.2 (1.3–3.1)	948	26	2.7 (1.7–3.8)	0.81 (.45–1.42)
6 mo after vaccination	929	23	2.5 (1.5–3.5)	929	30	3.2 (2.1–4.4)	0.77 (.44–1.32)
12 mo after vaccination	906	20	2.2 (1.3–3.2)	905	27	3.0 (1.9–4.1)	0.74 (.40–1.33)
24 mo after vaccination	881	12	1.4 (.6–2.1)	876	25	2.9 (1.8–4.0)	**0.48 (.22–.93)**
Any untypable pneumococcal serotype[d,e]							
Before vaccination	950	6	0.6 (.1–1.1)	948	9	0.9 (.3–1.6)	0.67 (.22–1.86)
6 mo after vaccination	929	9	1.0 (.3–1.6)	929	6	0.6 (.1–1.2)	1.50 (.54–4.48)
12 mo after vaccination	906	1	0.1 (–.1 to .3)	905	3	0.3 (.0–.7)	0.33 (.01–3.30)
24 mo after vaccination	881	1	0.1 (–.1 to .3)	876	2	0.2 (–.1 to .5)	0.50 (.02–5.56)

Abbreviations: CI, confidence interval; NVT, non–vaccine-type; PCV13, 13-valent pneumococcal conjugate vaccine; RR, relative risk; VT, vaccine-type.

[a]RR is the proportion of subjects from the vaccine group who carried the pathogen(s) relative to the proportion from the placebo group who carried the same pathogen(s) at the given time point.

[b]Subjects with a valid and determinant result at the given time point.

[c]Subjects carrying the specific pathogen at the given time point.

[d]These categories were not mutually exclusive.

[e]Untypable *Streptococcus pneumoniae* serotype by Quellung reaction.

Table 5. Effect of PCV13 on Nasopharyngeal Carriage Rates of Other Bacteria Based on Conventional Culture in Community-Dwelling Older Adults in the Netherlands

	PCV13 Group			Placebo Group			
		Subjects Carrying Pathogen			Subjects With Positive Sample		
	N[b]	No.	% (95% CI)	Subjects Tested[b]	No.	% (95% CI)	RR (95% CI)[a]
Haemophilus influenzae							
Before vaccination	950	67	7.1 (5.4–8.7)	948	97	10.2 (8.3–12.2)	0.69 (.51–.93)
6 mo after vaccination	929	62	6.7 (5.1–8.3)	929	65	7.0 (5.4–8.6)	0.95 (.68–1.34)
12 mo after vaccination	906	66	7.3 (5.6–9.0)	904	92	10.2 (8.3–12.1)	0.72 (.53–.97)
24 mo after vaccination	881	81	9.2 (7.3–11.1)	877	92	10.5 (8.5–12.5)	0.88 (.66–1.17)
Moraxella catarrhalis							
Before vaccination	950	76	8.0 (6.3–9.7)	948	68	7.2 (5.5–8.8)	1.12 (.81–1.53)
6 mo after vaccination	929	56	6.0 (4.5–7.6)	929	36	3.9 (2.7–5.3)	1.56 (1.04–2.36)
12 mo after vaccination	906	63	7.0 (5.3–8.6)	904	63	7.0 (5.3–8.6)	1.00 (.71–1.40)
24 mo after vaccination	882	64	7.3 (5.5–9.0)	876	56	6.4 (4.8–8.0)	1.14 (.80–1.61)
Staphylococcus aureus							
Before vaccination	950	199	20.9 (18.4–23.5)	949	199	21.0 (18.4–23.6)	1.00 (.84–1.19)
6 mo after vaccination	929	201	21.6 (19.0–24.3)	930	215	23.1 (20.4–25.8)	0.94 (.79–1.11)
12 mo after vaccination	906	210	23.2 (20.4–25.9)	905	213	23.5 (20.8–26.3)	0.98 (.83–1.16)
24 mo after vaccination	882	195	22.1 (19.4–24.8)	879	215	24.5 (21.6–27.3)	0.90 (.76–1.07)

Abbreviations: CI, confidence interval; PCV13, 13-valent pneumococcal conjugate vaccine; RR, relative risk.

[a]RR is the proportion of subjects from the vaccine group who carried the pathogen(s) relative to the proportion from the placebo group who carried the same pathogen(s) at the given time point.

[b]Subjects with a valid and determinant result at the given time point.

adults aged ≥65 years over a long time period of 24 months, using both molecular and conventional culture methods to detect pneumococcal presence in nasopharyngeal and oropharyngeal samples. Our results show a high prevalence of any pneumococcal carriage of up to 41.7% over 2 years in older adults based on PCR targeting *lytA* without previous culture enrichment, with conventional cultures contributing about 2% on top of the lytA results. This 24-month pneumococcal carriage prevalence is in the same range as reported in a similar study in community-dwelling older adults with molecular pneumococcal carriage detection after culture enrichment [8], which is currently considered the most sensitive method for pneumococcal detection [33].

Similar to those previous findings, *S. pneumoniae* was more often detected with PCR targeting *lytA* than with conventional culture, especially in oropharyngeal samples. The concordance between culture and PCR was ≥80% at each time point. The discordance between PCR and culture most likely results from the fact that separate swab samples were obtained for culture and PCR, the low pneumococcal carriage density found in older adults [9], and the small volume of transport medium used for PCR. Based on previous reports on the high specificity of this method [8, 32, 34, 35], we believe that our results accurately reflect the presence of live pneumococcal bacteria in the specimens tested with positive pneumococcal culture isolates obtained after repeated cultures of *lytA*-positive samples [34]. Furthermore, Krone et al [8] recently showed that detection of *S. pneumoniae* could be further increased by adding molecular testing of saliva.

Based on PCR results, there seem to be different dynamics in the decline of VT carriage after vaccination. The decreasing prevalence rates of VT carriage over time observed in both placebo and PCV13 recipients coincide with a gradual decline in PCV7 serotype IPD in older adults, probably caused by the implementation of PCV7 vaccination for newborns in the Netherlands in 2006 [13, 28]. In our present study, we also observed a significantly lower VT carriage 6 months after PCV13 vaccination, whereas carriage remained similar in the placebo group, although the difference in decline in VT serotypes between the 2 groups was no longer seen 12 and 24 months after vaccination. The clinical relevance of this temporary decline in VT carriage after PCV13 in older adults is unknown, because clinical protection against VT pneumonia and IPD did not seem to decline within the first years after PCV13 vaccination [21].

During the follow-up period, the small decline in VT carriage based on PCR was seen in combination with a stable overall pneumococcal carriage in both study groups (Table 3). In contrast to findings in children, in whom the decline in VT *S. pneumoniae* comes with a concomitant increased carriage of NVT *S. pneumoniae* [18], this was not observed in older adults. The exact mechanism of NVT replacement in children is unknown but seems mainly due to unmasking of NVT after PCV [36]. Simultaneous carriage of multiple serotypes in children makes outgrowth of cocolonizing NVT pneumococci after PCV a

likely prime mechanism of NVT replacement in this age group [37], although capsular switches and secular changes might also play a role [38]. Furthermore, the carriage shifts of other nasopharyngeal bacteria entering the niche created by PCV in children [16–19], such as *S. aureus* and *H. influenzae*, is not seen in vaccinated older adults. This is probably owing to the lower-density pneumococcal colonization in older adults [9]. Because the upper respiratory tract is the likely source of pathogens causing lower respiratory infections such as CAP, the absence of shifts in both NVT and other pathogens can be seen as reassuring.

Caution is needed when interpreting the results of the current study. First, 3 single-plex PCR assays were only serogroup specific (groups 7, 9, and 18), which could have led to an overestimation of VT carriage detected with PCR. Because this was the case for both PCV13 and placebo groups, this had limited effect on our results. Second, during the study period the proportion of untypable pneumococci by Quellung reaction decreased (Table 4). We cannot explain this phenomenon, because the study personnel did not change during the study period. Finally, because PCV7 was introduced for all newborns 2 years before the start of our study, the decline in VT serotypes can partly be attributed to herd effects, with decline in PCV7 serotypes also seen in the placebo group. The difference in impact on the remaining PCV13 serotypes between PCV13 and placebo groups is assumed to be directly due to PCV13 vaccination.

In conclusion, in immunocompetent, community-dwelling adults aged ≥65 years, a single dose of PCV13 may elicit a small and temporary reduction in VT nasopharyngeal pneumococcal carriage, based on PCR findings 6 months after vaccination. There were no clear changes in the prevalence of NVT pneumococci or other bacteria after PCV13 vaccination.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

Author contributions. All authors made substantial contributions to the conception/design of the study or to data collection/analysis, participated in data interpretation, participated in writing this report, jointly decided to publish, and assume accountability.

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