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Changes in pathogens and pneumococcal serotypes causing community-acquired pneumonia in The Netherlands

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ABSTRACT

Background: In 2006 a 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in the immunisation programme for infants in The Netherlands and replaced by PCV10 in 2011. Limited data exist about the impact of PCV on the aetiology of CAP as a whole. The aim of the present study is to describe the overall changes in microbial aetiology, pneumococcal burden (including non-bacteraemic pneumococcal pneumonia) and its serotypes in adult community-acquired pneumonia (CAP) after the introduction of these PCVs.

Methods: Hospitalised adult CAP patients who participated in three consecutive trials were studied (2004–2006 (n = 201), 2007–2009 (n = 304) and 2012–2016 (n = 300) and considered as pre-PCV7, PCV7 and PCV10 period). Extensive conventional microbiological testing was applied for all patients. In addition, patients with a serotype-specific pneumococcal antibody response were diagnosed with pneumococcal CAP. Changes in proportions of causative pathogens and distributions of pneumococcal serotypes were calculated.

Results: The proportion of pneumococcal CAP decreased from 37% (n = 74/201) to 26% (n = 77/300) comparing the pre-PCV7 period with the PCV10 period ($p = 0.01$). For other pathogens, including *Legionella* spp., *Mycoplasma pneumoniae, S. aureus, H. influenzae*, and respiratory viruses, no sustained shifts were observed in their relative contribution to the aetiology of CAP. Within the pneumococcal CAP patients, we observed a decrease in PCV7 and an increase in non-PCV10 serotype disease. PCV10-extra type disease did not decrease significantly comparing the PCV10 period with the pre-PCV7 and PCV7 period, respectively. Notably, PCV7 type disease decreased both in bacteraemic and non-bacteraemic patients.

Conclusions: Our findings confirm that PCV introduction in infants impact the microbial aetiology of adult CAP and suggest herd effects in adults with CAP after introduction of PCVs in children.

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1. Introduction

Streptococcus pneumoniae is the most common causative agent of community-acquired pneumonia (CAP) in adults [1,2]. With conventional microbiological methods, *S. pneumoniae* is identified in 12–40% of adults hospitalised with CAP [3–5]. With extensive diagnostics, including the detection of serotype-specific pneumococcal antigens in urine or antibodies in blood, percentages of *S. pneumoniae* as causative agent in up to 54% have been estimated [6].

In June 2006, The Netherlands introduced a 7-valent conjugate vaccine (PCV7) in the national immunisation programme for infants. From May 2011 onwards, PCV7 was replaced by a 10-valent vaccine (PCV10). Vaccine coverage in children has been around 95% since the start of this campaign [7]. In contrast, in

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Dutch adults aged 65 and over the uptake of the 23-valent-pneumococcal-polysaccharide vaccine (PPV23) is less than 1% [8,9]. Since PCV7 and PCV10 introduction, the incidence of vaccine-type invasive pneumococcal disease (IPD) decreased both in infants and adults [10]. Besides these beneficial effects, also a serotype replacement by non-vaccine serotypes has been observed in adults and children [10].

In contrast to IPD, the impact of PCV programmes on the incidence and serotype distribution of non-invasive/non-bacteraemic pneumococcal pneumonia is less well established. This category of pneumococcal disease is the majority (up to 80%) of the pneumococcal disease incidence in adults [11,12] . Recently, Werkhoven et al. observed a reduction in PCV7-type non-bacteraemic pneumococcal pneumonia, parallel to the reduction in PCV7-type IPD [13] . In a UK surveillance study using a similar approach, also a decline in PCV13-type non-bacteraemic pneumococcal disease was observed after PCV13 introduction [14] . To our knowledge, no such data are available after the introduction of PCV10. Furthermore, limited data exist about the impact of PCV on the aetiology of CAP as a whole. Shifts in nasopharyngeal ecology, for example, may impact the risk of other pathogens to cause pneumonia [15] . To the best of our knowledge, there is only one study that reported an overall decrease in proportion of pneumococcal CAP in adults after introduction of childhood vaccination with PCV7 [16] . The latter study, however, identified patients based on ICD coded hospital discharge records, which have limited reliability regarding aetiology [17] . Studies using extensive microbiological diagnostics are needed to be able to assess this in more depth.

The aim of the present study was to describe the changes in overall microbial aetiology, pneumococcal burden and its serotypes in hospitalised adult CAP over the pre-PCV7, PCV7, and PCV10 periods in The Netherlands.

2. Methods

2.1. Study population and data collection

Samples and clinical data were used from adult patients with CAP who required hospitalisation and participated in one of three consecutive clinical trials conducted in The Netherlands. The first trial was a single centre study on polymorphisms in host immune response genes and included patients between October 2004 and August 2006 (n = 201, cohort (1) [18] . The other two trials, both multi-centre placebo-controlled trials investigating dexamethasone as adjunctive treatment in CAP, included patients between November 2007 and June 2009 (n = 304, cohort (2) [4], and between October 2012 and October 2016 (n = 300, cohort (3). The 300 patients from the third trial are the first 50% of patients recruited (ClinicalTrials.gov Identifier NCT01743755 with the aim to include 600 patients). We consider the inclusion periods mentioned above as pre-PCV7, a PCV7 (and pre-PCV10), and a PCV10 period, respectively. All three clinical trials were approved by the medical ethics committee of St. Antonius Hospital and all patients provided informed consent before participation.

2.2. Clinical characteristics

All three clinical trials applied similar inclusion and exclusion criteria providing a homogeneous overall study population. In short, the trials included patients aged ≥18 years hospitalised with CAP that was defined as presence of a new infiltrate on a chest radiograph and at least two of the following criteria (1) cough; (2) sputum production; (3) temperature of >38.0 °C or <35.0 °C; (4) auscultatory findings consistent with pneumonia; (5) elevated C-reactive protein concentration (>15 mg/dl); (6) leucocytosis (>10×10^9 cells per L), more than 10% of bands in leucocyte differentiation or leucopenia (<4×10^9 cells per L). Patients with congenital or acquired immunodeficiency, haematological malignant disease or immunosuppressive treatment in the last 6 weeks were excluded. In addition, patients that required immediate ICU admission were excluded from the trials with exception of the first trial (PCV7 period).

For all patients, the following characteristics were prospectively collected: age, gender, pneumonia severity index (PSI [19]) and two comorbidities not included in the PSI (chronic obstructive pulmonary disease and diabetes mellitus). Besides clinical data, serum samples were collected at day 1 (day of admission), day of discharge and at day 30 in all three trials. All samples were immediately stored at −80 °C.

2.3. Microbial aetiology

A standard microbiological work-up was applied for all patients. This included a set of conventional methods at the time of hospitalisation plus additional measurement of serotype-specific pneumococcal antibodies in serum in persons with an early and a late serum sample available (respectively drawn at day 1–3 and 7–100 after hospital admission).

2.3.1. Conventional methods

Blood cultures were obtained (drawn before the start of in-hospital antibiotic treatment) at time of admission. Sputum specimens (if applicable) were Gramme stained and cultured. In addition, TaqMan real-time PCRs (in-house assay) were performed on sputum to detect DNA of atypical pathogens (*Mycoplasma pneumoniae, Legionella pneumophila*, *Coxiella burnetii*, *Chlamydophila pneumoniae*, and *Chlamydophila psittaci*). Serological testing (in cohort 1 and 2) on day 1–3 and day 10–21, respectively, was used to detect antibodies to *M. pneumoniae*, *C. burnetii*, *Chlamydophila* spp. or respiratory viruses (adenovirus, influenza virus A and B, parainfluenza and respiratory syncytial virus). Pharyngeal samples at time of admission were taken for viral culture on influenza (cohort 1) or PCR for detection of (para)influenza, adenovirus, respiratory syncytial virus (cohort 2 and 3) and PCR for detection *Legionella pneumophila, Mycoplasma pneumoniae* and *Chlamydophila pneumoniae/psittaci* (for cohort 3). Urine antigen tests (UAT) were performed for the detection of *L. pneumophila* serogroup 1 and *S. pneumoniae* (BinaxNOW®).

2.3.2. Serotype-specific pneumococcal antibodies in serum

As an additional indication for the involvement of pneumococci, an early and a late serum sample were tested for development of serotype-specific pneumococcal antibodies as described previously [6]. Samples were diluted 100× in sample buffer composed of phosphate-buffered saline (PBS), pH 7.3, 5% antibody depleted human serum (ADHS) pneumococcal cell wall polysaccharide (CWPS), to inhibit nonspecific binding of anti-cell wall polysaccharides I and II. Diluted sera were incubated with a mixture of microsphere types, each coated with polysaccharides representing the serotype. After incubation, non-bound antibodies were washed away and incubated with phycoerythrin (PE)-conjugated goat anti-human IgG. Bead suspensions were analyzed on a Bio-Plex 200 (IS 2.3). The standard used was calibrated against the 89SF reference serum, were used to generate a standard curve for quantification of antibody concentrations. Three assay controls 007sp (NIBSC) in 3 dilutions were taken along in duplicate with the test samples in each assay as internal control. For the PCV10 period, a 25-plex immunoassay panel was used (including the 14 serotypes mentioned before plus 11 additional serotypes; 2, 5, 6A, 10A, 11A, 12A, 15B, 20, 22F, 33F and 45).

Table 1
Patient characteristics from the three cohorts.

	pre-PCV7 2004–2006 (n = 201)	PCV7 2007–2009 (n = 304)	PCV10 2012–2016 (n = 300)
Male sex	124 (62)	171 (56)	178 (59)
Age (years)	64 (17)	63.7 (18)	64.4 (16)
Comorbidities			
Chronic renal failure	10 (5)	30 (10)	41 (14)
Diabetes mellitus	35 (17)	43 (14)	75 (25)
Liver disease	0 (0)	2 (1)	3 (1)
Neoplastic disease	26 (13)	19 (6)	12 (4)
Chronic heart failure	19 (10)	48 (16)	26 (9)
COPD	64 (32)	34 (11)	60 (20)
PSI class			
Classes 1–3	117 (58)	161 (53)	177 (59)
Classes 4–5	84 (42)	143 (47)	123 (41)
Days ill before admission	5.2 (4.9)	5.7 (5.3)	5.4 (5.4)
Pretreated with antibiotics at home	48 (24)	82 (27)	83 (28)

Data are presented as number (%) or mean (SD). Abbreviations: PSI, pneumonia severity index; COPD, chronic obstructive pulmonary disease.

A positive immune response was defined as at least a 2-fold increase in serotype-specific antibodies between the early and late serum sample (with an end concentration >0.35 μg/ml). The fold increase in antibody concentration against a given single pneumococcal serotype had to be at least two times greater than the fold increase against any other serotype (with exception of a ≥2-fold increase for serotypes within the same serogroup (e.g. 19A/19F), for which the serotype with the highest fold increase was regarded as infecting serotype). Only patients in whom no causative pathogen was detected using conventional methods but did have a positive serotype-specific antibody response, were diagnosed as pneumococcal CAP so mixed infections were not taken into account.

2.3.3. Final microbial aetiology

The information from the conventional work-up plus the serotype-specific antibody measurement (only taking into account the 14 serotypes common to the two multiplex immunoassay panels applied) was used to categorise the patients as (1) pneumococcal CAP, (2) CAP due to an atypical pathogen, (3) CAP due to another identified pathogen or (4) CAP with no causative agent detected by methods used herein. In case multiple pathogens were detected by conventional methods the main causative agents was determined by a consensus panel, consisting of two medical microbiologists who reviewed all microbiological results. Subsequently, for pneumococcal CAP patients the infecting serotype was determined based on Quellung for isolates cultured from blood and, in a subselection of patients, from sputum (using specific antisera from Statens Serum Institute SSI, Denmark) and based on the serotype-specific antibody measurement in case blood culture was negative (based on the 14-plex assay common for all patients). Within patients with pneumococcal CAP and an infecting serotype identified, we discriminated PCV7 (4, 6B, 9V, 14, 18C, 19F, 23F), PCV10-extra (present in PCV10 but not PCV7; 1, 5, 7F), non-PCV10 serotype disease (all serotypes not present in PCV10) and non-PCV7 (all serotypes not present in PCV7).

For pneumococcal CAP patients with known serotype, pneumococcal CAP was divided further in bacteraemic (*S. pneumoniae* cultured from blood) and non-bacteraemic pneumococcal pneumonia (defined as a positive serotype-specific antibody response against a single serotype and/ or *S. pneumoniae* cultured from sputum in the absence of a positive blood culture).

2.4. Statistical analyses

Proportions of the causative pathogens in CAP were calculated for comparisons of pre-PCV7 (October 2004 to August 2006, cohort 1), PCV7 (and pre-PCV10, November 2007 to June 2009, cohort 2), and PCV10 (October 2012 to October 2016, cohort 3) periods.

Likewise, distributions of pneumococcal serotypes (according to the PCV7, PCV10-extra, non-PCV10 and non-PCV7 group) were compared for the pre-PCV7, PCV7, and PCV10 period within all CAP patients, within all pneumococcal CAP patients and within all pneumococcal CAP patients with serotype known. Last, proportions of serotypes within bacteraemic and non-bacteraemic pneumococcal pneumonia were compared.

Differences in proportions were tested with $\chi 2$ or Fisher exact test, where appropriate. Relative risks (RR) and 95% confidence intervals (CI) were calculated using 2×2 tables (z-distribution) [20] . Means were compared using Student's t-test. A p-value of <0.05 was considered to represent a statistically significant differ-

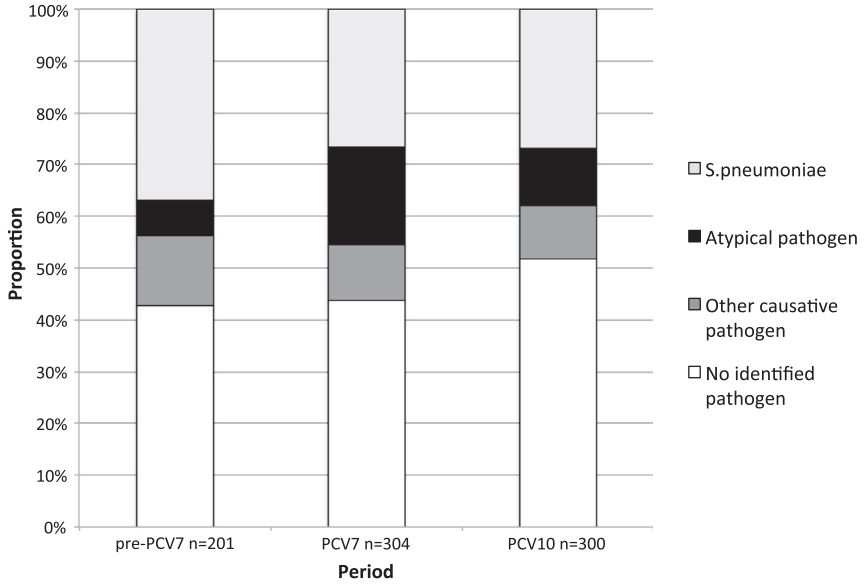

Fig. 1. Proportion of causative pathogens in CAP patients in the pre-PCV7, PCV7 (and pre-PCV10) and PCV10 period.

ence. Microsoft Excel and SPSS software (version 22.0) were used for statistical analyses. The data of the 11 additional serotypes from the 25-plex panel were used as a sensitivity analysis for identification of pneumococci in the PCV10 period (because the 14-plex panel does not cover all non-vaccine serotypes which potentially increased due to replacement disease).

3. Results

3.1. Patient characteristics

In total, 805 patients were included in the analyses (201 pre-PCV7, 304 in the PCV7 and 300 in the PCV10 period). Table 1 shows the patient characteristics of the three cohorts. Mean age of the patients varied between 63 and 64 years, and the proportion of patients categorised as PSI class 4–5 ranged from 41 to 47%.

3.2. Shifts in pathogens causing CAP

Comparing the PCV7 with pre-PCV7 period, the proportion of patients with pneumococcal pneumonia decreased from 37 to 27% ($p = 0.01$) (Fig. 1, Table 2). This coincided with a significant increase in proportion of patients with CAP due to atypical pathogens from 7 to 19%, primarily Q-fever and an increase in patients diagnosed with *Chlamydophila* spp.

In the PCV10 period, the proportion of atypical pathogens decreased back to 11% (similar to pre-PCV7). This, however, did not result in an increase in proportion of pneumococcal pneumonia, which remained significantly lower (26%) compared to pre-PCV7 ($p = 0.01$). The proportion of patients with no identified pathogen increased from 44 to 53% comparing the PCV7 and PCV10 periods. For other pathogens, including *Legionella* spp., *Mycoplasma pneumoniae, S. aureus*, *H. influenzae*, influenza and other respiratory viruses causing CAP, no shifts were observed for their relative contribution over time (Supplementary Table 1). The data from the 11 additional serotypes tested in the 25-plex assay, resulted in 5 additional cases of pneumococcal pneumonia in the PCV10 period. Including these cases in the analysis did not impact the findings (an overall proportion of patients with pneumococcal pneumonia in the PCV10 period (27%) remained significantly lower compared with the pre-PCV7 period ($p = 0.03$)).

3.3. Shifts in distribution of pneumococcal serotypes

Shifts in pneumococcal serotype distribution are shown in Table 3. The serotype distribution of pneumococcal CAP changed after PCV7 implementation. Overall, within all CAP patients, the proportion of PCV7-serotypes decreased from 12 to 4% (RR 0.34 95% CI: 0.18–0.66) comparing the pre-PCV7 to the PCV7 period. For non-PCV10 and non-PCV7 serotype disease, the overall proportions remained similar (from 7 to 9% and 12 to 13%, respectively).

Within all pneumococcal CAP patients with serotype known (pre-PCV7 n = 50, PCV7 n = 51 and PCV10 n = 44), after PCV7 introduction PCV7-serotypes decreased from 50 to 25% (RR 0.51 95% CI: 0.3–0.88) and-non-PCV10-type serotypes increased from 28 to 51% (RR 1.82 95% CI: 1.08–3.06) whereas PCV10-extra type disease remained stable (from 22 to 24% RR 1.07 95% CI: 0.52–2.2). These relative changes were quite similar for both bacteraemic and non-bacteraemic patients (Fig. 2).

In the PCV10 period, the overall decrease in PCV7-type disease within all CAP patients continued with an additional absolute reduction of 3% in proportion (from 4 to 1%; $p < 0.01$), comparing the PCV7 and PCV10 periods. Within pneumococcal CAP patients with known serotype, the relative decrease in the proportion of PCV7 type disease was from 25 to 5% (RR 0.18 95% CI: 0.04–0.75) whereas non-PCV10 type disease increased from 51 to 77% (RR 1.52 95% CI: 1.11–2.07). PCV10-extra type disease did not decrease significantly (RR 0.77 95% CI: 0.35–1.72). No decrease in potentially cross-reactive PCV10-related serotype 19A was observed (from 1 to 3 to 6 cases in the pre-PCV7, PCV7 and PCV10 period, respectively). The most prevalent serotypes in the PCV10 period in descending order were 3, 8, 19A, 7F, 9N and 12F.

4. Discussion

In this study in over 800 hospitalised adults with CAP from 2004 to 2016, we observed a significant reduction in proportion CAP due to *S. pneumoniae* following introduction of PCV in children in The Netherlands. Furthermore, within the pneumococcal pneumonia patients, we observed a continuing decrease in the proportion of cases due to PCV7 serotypes and an increase in non-PCV10 serotypes. These findings confirm that PCV introduction in infants impact the microbial aetiology of adult CAP.

Our study compared pre- and post-vaccine periods for shifts in overall microbial aetiology of adults hospitalised with CAP including pneumococcal serotype-specific antibody detection in serum. In the PCV7 and PCV10 periods, the proportion of patients with pneumococcal CAP clearly decreased coinciding with an increase in proportion of CAP patients without a causative pathogen identified in the PCV10 period. No increase in proportion of CAP due to *H. influenzae* or *S. aureus* was observed. The latter suggests that the potential increases in nasopharyngeal colonisation in adults, as has been observed in parents of vaccinated children [15,21], does not result in more disease caused by these pathogens in adults. The higher proportion of patients with Q-fever observed in the PCV7 period is likely to be linked to an epidemic in the Netherlands, which ended in 2010 [21]. The transient increase in number of *Chlamydophila* pneumonia cases is possibly related to a to a clus-

Table 2
Proportions of causative pathogens in CAP patients pre-PCV7, PCV7 (and pre-PCV10) and PCV10 period.

	Pre-PCV7 2004–2006 (n = 201) No. (%)	PCV7 2007–2009 (n = 304) No. (%)	PCV10 2012–2016 (n = 300) No.(%)	PCV7 vs pre-PCV7 RR 95% CI[a]	*p*-value	PCV10 vs PCV7 RR 95% CI[b]	*p*-value	PCV10 vs pre-PCV7 RR 95% CI[c]	*p*-value
S. pneumoniae	74 (37)	81 (27)	77 (26)	0.72 (0.56–0.94)	**0.02**	0.96 (0.74–1.26)	**0.79**	0.70 (0.54–0.91)	**0.01**
Atypical pathogens	14 (7)	57 (19)	34 (11)	2.69 (1.54–4.70)	**<0.01**	0.60 (0.41–0.9)	**0.01**	1.63 (0.90–2.95)	**0.10**
Other causative pathogen	27 (13)	33 (11)	31 (10)	0.81 (0.50–1.30)	**0.38**	0.95 (0.60–1.51)	**0.84**	0.77 (0.47–1.25)	**0.28**
No identified pathogen	86 (43)	133 (44)	158 (53)	1.02 (0.83–1.25)	**0.83**	1.20 (1.02–1.42)	**0.03**	1.23 (1.02–1.49)	**0.03**

Abbreviations: PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; No.: number of cases; RR: Relative risk; 95% CI: 95% Confidence interval. We assessed the proportion of causative pathogens in CAP patients comparing the post-PCV to pre-PCV periods. Differences in proportions were tested with $\chi 2$ test and relative risks and 95% confidence intervals were calculated.

[a] RR comparing proportion of the PCV7 (and pre-PCV10) to the pre-PCV7 period.

[b] RR comparing proportion of the PCV10 to the PCV7 period.

[c] RR comparing proportion of the PCV10 to the pre-PCV7 period.

ter of *C. psittaci* patients who visited a bird fair (November 2007) [22] . The frequencies of pathogens like *Legionella* spp., *Mycoplasma pneumoniae, S. aureus* and respiratory viruses causing CAP did not change over time. Since serological tests to detect atypical pathogens and respiratory viruses (and viral culturing) were replaced by PCRs, which generally have a higher sensitivity [23,24], our comparisons are not likely to be biased by decreased detection chances over time. Likewise, the detection probability for *S. pneumonia* did not decrease over time whereas a constant rate of (conventional) diagnostics able to detect *S. pneumoniae was* applied in the three periods (Supplementary Table 2). Furthermore, our sensitivity analyses resulted in only 5 additional pneumococcal pneumonia cases detected through the additional 11 serotypes that were absent in the 14-plex assay applied to the samples of the first two trials.

The most likely explanation for the significant reduction in proportion pneumococcal CAP are herd protection effects of PCVs resulting in a decline in vaccine-serotypes and replacement by non-vaccine-serotypes with an overall lower (invasive) disease potential. This has been observed in many previous studies [10,25–28] . Regarding the serotypes, the present study showed a decline in the relative contribution of vaccine serotypes after PCV7 and after PCV10 introduction. PCV7 type disease decreased both in bacteraemic patients and non-bacteraemic patients. For PCV10-extra serotype disease no decline was observed in non-bacteraemic pneumococcal CAP patients. This finding aligns with Werkhoven et al. who also observed a reduction in PCV7-type non-bacteraemic pneumonia in the Netherlands, but no impact on PCV10 serotypes [13] . The latter study had an observation period of only until ≈2.5 years after PCV10 introduction, which is too

Table 3
Serotype distribution in overall pneumococcal CAP (with serotype available) and bacteraemic vs non-bacteraemic pneumococcal CAP.

	Pre-PCV7 2004–2006 No. (%)	PCV7 2007–2009 No. (%)	PCV10 2012–2016 No. (%)	PCV7 vs pre-PCV7 RR 95% CI[a]	PCV10 vs PCV7 RR 95% CI[b]	PCV10 vs pre-PCV7 RR 95% CI[c]
Within all CAP patients	201 (100)	304 (100)	300 (100)			
PCV7	25 (12)	13 (4)	2 (1)	0.34 (0.18–0.66)	0.16 (0.04–0.68)	0.05 (0.01–0.22)
PCV10 extra	11 (5)	12 (4)	8 (3)	0.72 (0.32–1.6)	0.68 (0.28–1.63)	0.49 (0.2–1.19)
Non-PCV10	14 (7)	26 (9)	34 (11)	1.23 (0.66–2.29)	1.33 (0.82–2.15)	1.63 (0.9–2.95)
Non-PCV7	25 (12)	38 (13)	42 (14)	1.01 (0.63–1.61)	1.12 (0.74–1.69)	1.13 (0.71–1.79)
Within all pneumococcal CAP patients	74 (100)	81 (100)	77 (100)			
PCV7	25 (34)	13 (16)	2 (3)	0.48 (0.26–0.86)	0.16 (0.04–0.69)	0.08 (0.02–0.31)
PCV10 extra	11 (15)	12 (15)	8 (10)	1 (0.47–2.12)	0.7 (0.3–1.62)	0.7 (0.3–1.64)
Non-PCV10	14 (19)	26 (32)	34 (44)	1.7 (0.96–2.99)	1.38 (0.92–2.06)	2.33 (1.37–3.98)
Non-PCV7	25 (34)	38 (47)	42 (55)	1.39 (0.94–2.06)	1.16 (0.85–1.58)	1.61 (1.11–2.36)
Within all pneumococcal CAP patients with serotype known	50 (100)	51 (100)	44 (100)			
PCV7	25 (50)	13 (25)	2 (5)	0.51 (0.3–0.88)	0.18 (0.04–0.75)	0.09 (0.02–0.36)
PCV10 extra	11 (22)	12 (24)	8 (18)	1.07 (0.52–2.2)	0.77 (0.35–1.72)	0.83 (0.37–1.87)
Non-PCV10	14 (28)	26 (51)	34 (77)	1.82 (1.08–3.06)	1.52 (1.11–2.07)	2.76 (1.72–4.43)
Non-PCV7	25 (50)	38 (75)	42 (95)	1.49 (1.08–2.05)	1.28 (1.08–1.52)	1.91 (1.44–2.54)
Within bacteraemic *S. pneumoniae* with serotype known	17 (100)	24 (100)	19 (100)			
PCV7	11 (65)	7 (29)	0 (0)	0.45 (0.22–0.92)	NA	NA
PCV10 extra	5 (29)	8 (33)	4 (21)	1.13 (0.45–2.87)	0.63 (0.22–1.78)	0.72 (0.23–2.24)
Non-PCV10	1 (6)	9 (38)	15 (79)	6.38 (0.89–45.73)	2.11 (1.19–3.71)	13.42 (1.98–91.14)
Non-PCV7	6 (35)	17 (71)	19 (100)	2.01 (1–4.01)	1.41 (1.09–1.82)	2.83 (1.49–5.39)
Within non-bacteraemic *S. pneumoniae* with serotype known	33 (100)	27 (100)	25 (100)			
PCV7	14 (42)	6 (22)	2 (8)	0.52 (0.23–1.18)	0.36 (0.08–1.62)	0.19 (0.05–0.76)
PCV10 extra	6 (18)	4 (15)	4 (16)	0.81 (0.26–2.6)	1.08 (0.3–3.86)	0.88 (0.28–2.79)
Non-PCV10	13 (39)	17 (63)	19 (76)	1.6 (0.96–2.67)	1.21 (0.84–1.74)	1.93 (1.2–3.11)
Non-PCV7	19 (58)	21 (78)	23 (92)	1.35 (0.95–1.93)	1.18 (0.94–1.49)	1.6 (1.17–2.19)

Abbreviations: PCV7, 7-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; No.: number of cases; RR: Relative risk; 95% CI: 95% Confidence interval. We assessed the proportion of pneumococcal serotypes (according to the PCV7, PCV10-extra, non-PCV10 and non-PCV7 group) in (pneumococcal) CAP patients comparing the post-PCV to pre-PCV periods. Differences in proportions were tested with χ^2 test and relative risks and 95% confidence intervals were calculated.

[a] RR comparing proportion of the PCV7 (and pre-PCV10) to the pre-PCV7 period.

[b] RR comparing proportion of the PCV10 to the PCV7 period.

[c] RR comparing proportion of the PCV10 to the pre-PCV7 period.

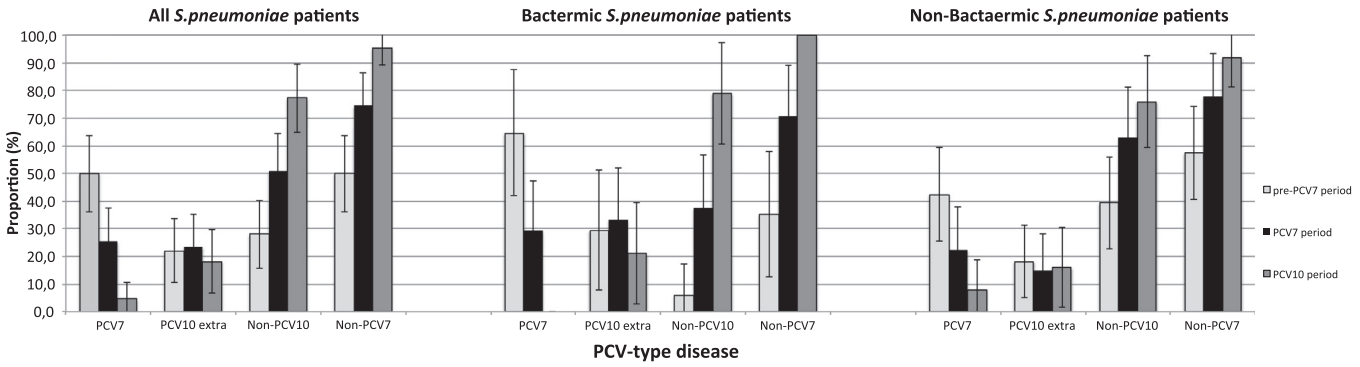

Fig. 2. Serotype distribution in pneumococcal pneumonia patients with serotype known. Proportions in the pre-PCV7, PCV7 (and pre-PCV10) and PCV10 period. Bars are divided in serotypes included in the 7-valent pneumococcal conjugate vaccine (PCV7); serotypes 1, 5, 7F (PCV10 extra); and all serotypes not included in previous groups (non-PCV10 and non-PCV7 and, respectively) and error bars represent the 95% confidence intervals.

short to detect possible herd effects (and take around 2 years to establish for IPD without catch-up campaigns [29,30]). Our study period, with sampling up to 5 years after introduction of PCV10, should have been long enough to observe potential herd effects on non-bacteraemic pneumococcal pneumonia.

A strength of this study is that we used data from three prospective studies in adults requiring hospitalisation for CAP that was diagnosed by clear and consistent criteria. In all patients, the standard microbial diagnostic workup included an extensive effort to detect the causative pathogen including a reliable serotype-specific immunoassay to diagnose pneumococcal CAP (in only 1 out of 27 cases with serotype identified by both Quelling and serology, there was a discordant result i.e. Quellung of blood isolate; 12F, with a positive (12-fold) immune response against serotype 8). MIA identified 13, 16 and 22 additional pneumococcal CAP cases in the pre-PCV7, PCV7 and PCV10 period, respectively, as compared with the conventional methods (blood cultures, sputum culture, urinary antigen testing). The sensitivity of MIA to detect pneumococcal pneumonia (with conventional methods as gold standard) was 42% (95% CI: 0.34–0.50). Furthermore, our study period extended to over 10 years after PCV7 introduction and over 5 years after PCV10 introduction.

Our study is limited by its observational design. Non-vaccine related factors might have contributed to the shifts in aetiology of CAP, as was illustrated by the Q-fever epidemic. Also the study excluded patients with congenital or acquired immunodeficiency, haematological malignant disease or recent immunosuppressive treatment, which are important risk groups for CAP [31] . The number of patients with a history of chronic obstructive pulmonary disease (COPD) differed significantly between the three periods ($p < 0.001$ with a chi-square test). To explore COPD as potential confounding factor, we have therefore conducted a sensitivity analysis by excluding all COPD patients. This analysis, showed very similar findings compared to the original analysis and excludes COPD as important confounding factor (*S. pneumoniae* decreased from 34% to 26% in the sensitivity analysis versus from 37% to 27% in the original analysis). In addition, patients that required immediate ICU admission were excluded from the trials with exception of the first trial (PCV7 period). However, this only concerned 5 patients of whom one had pneumococcal pneumonia so this does not impact our findings. Consequently, these two restrictions might impact the generalisability of our findings to all patients with CAP. Nevertheless, the comparison between periods remains valid since these restrictions were applicable to all three cohorts. In addition, there were no substantial changes over time in (1) resistance patterns for common causative agents of CAP (including *S. pneumoniae* penicillin resistance, which remained <1%) and (2) the empirical treatment of CAP so these factors are not likely to impact our results. Lastly, the study time frame of the post-PCV periods coincided with the CAPiTA trial, in which 42.240 Dutch elderly persons received PCV13 [32] . The influence, however, is expected to be negligible, because less than 2% of the Dutch population ≥65 years old participated in that trial. The same applies to PPV23 vaccination of Dutch elderly, in which the uptake is less than 1% [8,9].

5. Conclusions

The proportion of *S. pneumoniae* as causative agent in hospitalised adults with CAP (both bacteraemic and non-bacteraemic) decreased following the introduction of the PCV programme in children in The Netherlands. In addition, there has been a shift to non-vaccine serotypes. These findings suggest herd effects in adults with CAP after introduction of PCVs in the national immunisation programme in children. Ongoing monitoring of CAP aetiology, including *S. pneumoniae* serotype distribution, is needed to evaluate the long-term effects of pneumococcal conjugate vaccination programmes.

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Conflict of interest statement

G.H.J.W. has received a lecturing fee from Pfizer.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.06.049.

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