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Nasopharyngeal *S. pneumoniae* carriage and density in Belgian infants after 9 years of pneumococcal conjugate vaccine programme

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ABSTRACT

Background: In Belgium, the infant pneumococcal conjugate vaccine (PCV) programme changed from PCV7 (2007–2011) to PCV13 (2011–2015) and to PCV10 (2015–2016). A 3-year nasopharyngeal carriage study was initiated during the programme switch in 2016. Main objective of the year 1 assessment was to obtain a baseline measurement of pneumococcal carriage prevalence, carriage density, serotype distribution and antibiotic resistance.

Materials/methods: Two infant populations aged 6–30 months and without use of antibiotics in the seven days prior to sampling were approached: (1) attending one of 85 randomly selected day-care centres (DCC); (2) presenting with AOM at study-trained general practitioners and paediatricians. Demographic and clinical characteristics were documented and a single nasopharyngeal swab was taken. *S. pneumoniae* were cultured, screened for antibiotic resistance and serotyped, and quantitative Taqman real-time PCR (qRT-PCR) targeting *LytA* was performed.

Results: Culture-based (DCC: 462/760; 60.8% – AOM: 27/39; 69.2%) and *LytA*-based (DCC: 603/753; 80.1% – AOM: 32/39; 82.1%) carriage prevalence was high. Average pneumococcal DNA load in *LytA*-positive day-care samples was 6.5×10^6 copies/µl (95%CI = 3.9–9.2 $\times 10^6$, median = 3.5×10^5); DNA load was positively associated with signs of common cold and negatively with previous antibiotic use. Culture-based frequency of 13 pneumococcal vaccine (PCV) serotypes was 5.4% in DCC and 7.7% in AOM, with 19F and 14 being most frequent, and frequencies below 0.5% for serotypes 3, 6A, 19A in both populations. Predominant non-PCV serotypes were 23B and 23A in day-care and 11A in infants with AOM. In day-care, resistance to penicillin was rare (<0.5%) and absent against levofloxacin; 32.7% and 16.9% isolates were cotrimoxazole- and erythromycin-resistant respectively.

Conclusion: Four years after PCV13 introduction in the vaccination programme, PCV13 serotype carriage was rare in infants throughout Belgium and penicillin resistance was rare. Continued surveillance in the context of a PCV programme switch is necessary.

Abbreviations: AOM, acute otitis media; CRO, clinical research organisation; Chi^2, Chi-Square test; DCC, day-care centre; GP, general practitioner; IPD, invasive pneumococcal disease; MWU, Mann-Whitney *U* test; NP, nasopharyngeal; PCV, pneumococcal conjugate vaccine; STGG, skim milk-tryptone-glucose-glycerol.

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1. Introduction

More than 90 *S. pneumoniae* serotypes exist and reside in the human upper respiratory tract [1]. Pneumococcal carriage is a highly dynamic process with subsequent episodes of acquisition, carriage and clearance [2]. Reported asymptomatic carriage is between 30% and 62% in infants under two years of age in Western countries [3]. Higher carriage rates (up to 93% in Gambian babies < 1 month) were reported in non-Western countries [4]. In circumstances such as host-pathogen imbalance, pneumococci may spread and reach respiratory organs or even the bloodstream to cause infections, including otitis media, pneumonia, sepsis and meningitis [3,5,6].

Infants under two years of age constitute the major reservoir and source of transmission [7]. Furthermore, they are at high risk of pneumococcal disease of which the most common is acute otitis media (AOM) [2]. Therefore, this age group is an interesting population to be studied for nasopharyngeal (NP) carriage.

Pneumococcal disease risk increases if infants reside in day-care centres (DCC), a setting associated with enhanced pneumococcal carriage and antimicrobial resistance [8,9]. Also, the crowded DCC environment facilitates transmission from one infant to another.

With an overall incidence of 299/1000 person-years in infants up to two years of age as reported from five European countries [10], AOM is the major reason for paediatric consultation and antibiotic prescription [11,12]. Worldwide, *S. pneumoniae* causes 28–55% of AOM episodes, making it the dominant bacterial pathogen implicated in the development of AOM [13–16]. The extensive prescription and use of antibiotics promotes antimicrobial resistance, compromising the ability to effectively treat pneumococcal infection and emphasising the importance of preventing pneumococcal disease via vaccines [17].

In Belgium, a PCV7 programme with free of charge vaccine was started in 2007 (2+1 schedule). It quickly reached high 3-dose coverage in infants: from 89.1% in 2008 to 96.5% in 2012 in Flanders (Northern part of Belgium) and from 80.7% in 2009 to 89.2% in 2012 in Wallonia (Southern part of Belgium) [18–21]. Four years later, PCV13 was introduced and recently replaced by PCV10, with regional difference in timing: (1) Flemish infants were PCV13 vaccinated up to the end of June 2015 and thereafter received PCV10 for their further schedule; (2) Walloon infants were PCV13 vaccinated until the end of April 2016 and thereafter received PCV10; (3) In Brussels-Capital-Region, the individual vaccine offer depended on the particular general practitioner (GP) or paediatrician consulted (Flemish or Walloon programme). This change in the Belgian universal vaccination programme was an outcome of the regular tender processes for all vaccines (after expiry of a previous contract), during which the Belgian regions decide autonomously, based on different vaccine characteristics, including price [22].

The introduction of PCV10, lacking PCV13 serotypes 3, 6A, 19A, in European countries such as Austria and Finland resulted in a decreased incidence of invasive pneumococcal disease (IPD), but with a high proportion (70–86% in children) of PCV13-preventable cases among remaining invasive IPD, dominated by serotypes 19A and 3 [23–26]. Although case-control studies in several countries have shown early effectiveness of PCV10 against 19A-IPD, continued surveillance data suggest waning of this cross-protection [27]. In Belgium (up to 2016), remaining IPD in infants after PCV13 introduction was dominated by non-PCV13 serotypes [28].

Pneumococcal conjugate vaccines (PCV) do not only generate direct protection, but also reduce carriage of pneumococcal serotypes present in the vaccine. Such indirect effects of PCV vaccination in infants have been suggested to protect older age groups too and several IPD-surveillance data from other countries are supportive, but the effect size varies and is being downgraded through replacement by non-vaccine serotypes causing IPD [29–31]. Carriage data are essential to understand pneumococcal biology and transmission and to anticipate changes in IPD. The unique Belgian situation of a PCV programme that altered from PCV7 to PCV13 and then to PCV10 and reached high coverage in infants, created the opportunity for an observational follow-up study on NP carriage of *S. pneumoniae* in infants. The baseline results of this three-year carriage study which started in 2016 are presented here. Specific objectives were: to obtain a baseline measurement of pneumococcal carriage prevalence, carriage density, serotype distribution and antibiotic resistance; to compare pneumococcal carriage between regions and between infants with AOM and healthy infants in day-care; and to identify other predictors of carriage and density.

2. Materials and methods

The current cross-sectional study investigates carriage in two infant populations with high reported carriage of *S. pneumoniae*: those attending DCC and those with AOM. The protocol of the AOM part was based on that of a similar 13-year survey by Cohen et al. [32]. Since pneumococcal carriage and AOM incidence are both lower in summer, recruitment is restricted to non-summer seasons (October to June) from 2016 to 2018 at least. The first sampling period was between January and June 2016, i.e. during and shortly after the switch in vaccination programme (Fig. 1). Protocol details are added in the Supplement and summarised here.

2.1. Study population

After ethics approval of the study, recruitment started in: (1) healthy infants residing in one of 85 DCC randomly selected over the three Belgian regions and (2) infants with AOM visiting one of 55 trained GP's or paediatric outpatient services (private or hospital) throughout Belgium.

Age limits for inclusion were $\geq$6 months (to limit inclusion of unvaccinated infants) and $\leq$30 months and for the baseline assessment in the first recruitment season, region-specific age criteria were added to include only infants who were offered PCV13 for both primary vaccine doses in the first year of life.

AOM was defined by the acute onset (i.e. within the preceding seven days) of symptoms and specified otoscopic criteria.

Exclusion criteria were: (1) second inclusion in the same winter season, (2) use of oral antibiotics in the past seven days, (3) presence of a chronic and severe pathology.

2.2. Nasopharyngeal samples and questionnaire

After parents gave written informed consent, a trained nurse or a GP/paediatrician collected a single NP swab and a parent questionnaire about demographic and clinical characteristics and pneumococcal vaccination status. Signs of common cold were defined as coughing and/or running nose.

2.3. Cultures – Serotyping – Antibiotic susceptibility

At the Reference Centre for Pneumococci at the University Hospitals in Leuven, NP samples were cultured, *S. pneumoniae* strains were serotyped and antibiotic susceptibility to penicillin, tetracycline, erythromycin, levofloxacin and cotrimoxazole was determined following CLSI 2016 guidelines [33].

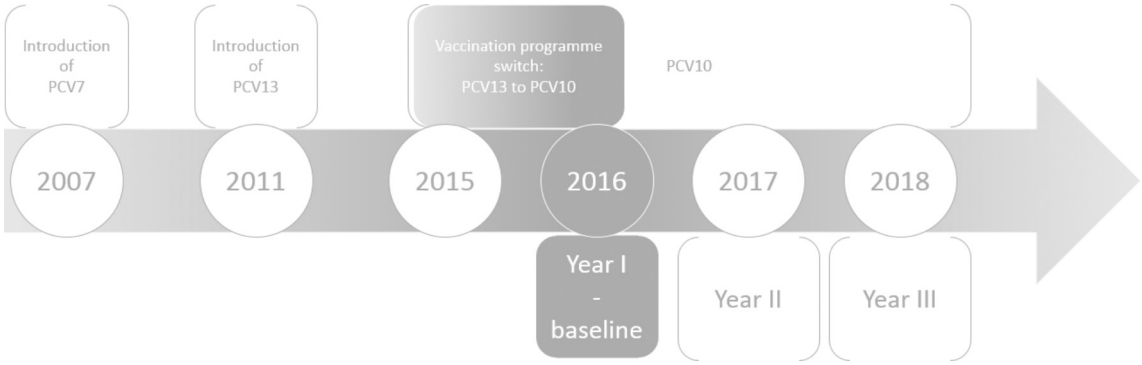

Fig. 1. Study flow: chronological representation of changes in pneumococcal conjugate vaccine (PCV) programme in Belgium (upper part) and planned data collection periods (lower part).

2.4. PCR – Density assessment

Concentrations of pneumococcal DNA were determined using quantitative Taqman real-time PCR (qRT-PCR) targeting *LytA* [34]. Bacterial densities were determined based on a standard curve that was set up using 10-fold serially diluted *LytA* PCR product of *S. pneumoniae* strain ATCC 49619 [35]. Samples and standard curves were run in triplicate.

2.5. Statistical analysis

The current baseline assessment presents the Walloon, Brussels and Flemish cohorts. The results of Brussels infants are shown in order to present complete results, but they were not separately taken into account for comparative analyses.

The Chi-Square test (Chi^2) and the Mann-Whitney *U* test (MWU) were used to test significance at a level of 5%. 95% confidence intervals on proportions were calculated with continuity correction. Missing values were not replaced.

3. Results

3.1. Recruitment and infant characteristics

A total of 856 NP samples were collected of which 760 *per protocol* DCC samples and 39 *per protocol* AOM samples were included in the statistical analyses. The 57 remaining samples were excluded due to inappropriate age or antibiotic treatment in the seven days prior to sampling. Because of the low number of infants with AOM, less detailed results are given for AOM than for DCC infants (Table 1).

3.2. Quality check of sampling procedure

3.2.1. Sampling depth

The majority of the samples (73.0% for DCC, 38/39 for AOM) was taken at a depth of at least half the distance between the nostril and the ear lobe and only 3.0% of the DCC samples and one AOM sample were taken at a depth of less than 1 cm from the nostril. In DCC infants, carriage prevalence and carriage density were not statistically different between samples taken at more or less than half the distance between nostril and ear lobe, excluding <1 cm.

3.2.2. Freeze-thaw cycle prior to culture

AOM samples were immediately cultured upon arrival, whereas the majority of DCC samples (85.3%, n = 112) was frozen at −80 °C prior to culture and within 12 h after sampling. Detection rate of *S. pneumoniae* was similar in frozen (60.3%) and in non-frozen DCC samples (63.3%) (Chi^2, P = .541). Pneumococcal loads were also similar among frozen (7.6×10^6 copies/μl, 95%CI = $4.2–10.9 \times 10^6$, median = 0.5×10^6) and non-frozen (1.0×10^6 copies/μl 95%CI = $0.6–1.4 \times 10^6$, median = 0.4×10^6) samples.

3.3. Main findings

3.3.1. Carriage prevalence

Culture-based carriage prevalence was similar in DCC (60.8%) and in AOM (69.2%). For infants in day-care, *LytA*-based carriage prevalence was 80.1% and similar in Wallonia (78.3%) and in Flanders (83.2%). *LytA*-based carriage prevalence in infants with AOM was 82.1%. Two characteristics were negatively associated with carriage in DCC infants: parental smoking with culture-positivity of the sample and antibiotic use in the three months prior to sampling with *LytA*-positivity of the sample (Table 2). Antibiotic use was not associated with culture-negativity of *LytA*-positive samples (Chi^2, P > .1).

3.3.2. Carriage density

Average pneumococcal DNA load in *LytA*-positive samples of infants in day-care was 6.5×10^6 copies/μl (95%CI = $3.9–9.2 \times 10^6$, median = 3.5×10^5), similar among culture-positive (6.6×10^6 copies/μl) and culture-negative (6.3×10^6 copies/μl) samples and seven times higher in DCC infants than in AOM infants (0.9×10^6 copies/μl, 95%CI = $0.31–1.5 \times 10^6$, median = 0.2×10^6) (MWU, P = .042).

In infants in day-care, significantly higher pneumococcal loads were found when signs of common cold (27.8%) were present at sampling (Table 3). In contrast, antibiotic use in the previous three months was associated with lower pneumococcal loads. PCV13 serotype carriage was not related to carriage density in DCC infants.

3.3.3. Serotype distribution

Among culture-positive samples, frequency of PCV13 serotypes was low. Serotype 19F was most frequent (half of the cases in DCC and in AOM), followed by serotype 14 (16.0% of DCC cases, half of AOM cases, only identified in Wallonia) and in DCC by 23F and 19A. PCV13 serotype carriage was similar in Wallonia and in Flanders for each setting. Among the unvaccinated DCC infants (4.4%, n = 33), one 19F was detected, resulting in a PCV13 serotype frequency (4.5%) similar to the vaccinated population. For non-PCV13 serotypes, 23B and 23A were predominant in DCC and 11A and 23B in AOM. Regarding the PCV13 non-PCV10 serotypes (3, 6A, 19A), extremely low frequencies were detected (0.9%; 95%CI = 0.3–2.4% in DCC) (Table 4).

Table 1
Basic characteristics of participating infants per setting and per region, 2016.

		DCC				AOM			
		Wallonia	Flanders	Brussels	Belgium	Wallonia	Flanders	Brussels	Belgium
No. of actively participating centres		24	48	13	85	6	3	3	12
No. of *per protocol* inclusions		353	329	78	760	27	8	4	39
Sex (% male)		55.2	46.4	50.7	50.9	44.4	75.0	50.0	51.3
Age in months (mean)		18.2	23.2	23.9	21.0	14.3	20.5	23.0	16.5
Age group	6–12 months (%)	22.7	0.0	0.0	10.5	40.7	0.0	0.0	28.2
	12–24 months (%)	50.1	54.2	46.7	51.6	44.4	87.5	50.0	53.8
	24–31 months (%)	27.2	45.8	53.3	37.9	14.8	12.5	50.0	17.9
Premature (%)		6.6	7.5	17.6	8.0	0.0	0.0	0.0	0.0
Breast-feeding (%)		32.8	25.7	40.0	30.4	11.1	37.5	25.0	17.9
At least one parent smokes (%)		27.8	17.0	21.3	22.4	37.0	62.5	0.0	38.5
Siblings at home (%)	1	46.2	42.3	45.2	44.4	40.7	25.0	25.0	35.9
	≥2	20.6	16.1	19.2	18.5	51.9	62.5	75.0	56.4
No. of AOM episodes (%)	1	10.4	6.8	10.0	8.8	25.9	37.5	75.0	33.3
	≥2	21.1	28.0	10.0	23.0	29.6	37.5	25.0	30.8
AB received in previous 3 months (%)	1	27.7	26.0	15.1	25.6	33.3	12.5	0.0	25.6
	≥2	13.5	7.3	2.7	9.6	3.7	0.0	0.0	2.6
PCV13 doses received (%)[a]	1	1.4	1.5	1.3	1.5	0.0	0.0	0.0	0.0
	≥2	98.0	89.7	96.0	94.2	96.2	87.5	100.0	94.7
Age-appropriate vaccinated (3 PCV-doses at age 14 months, %)		94.6	95.3	91.8	94.6	100.0	100.0	100.0	100.0

DCC = day-care centre, AOM = acute otitis media, No. = number, AB = antibiotics, PCV = pneumococcal conjugate vaccine.
[a] Vaccination status is based on vaccination documentation or parental reporting.

Table 2
Basic characteristics of infants in day-care by pneumococcal carrier status per method, Belgium, 2016.

	Culture-based carriers (%)	Denominators[a]	P-value Chi^2	*LytA*-based carriers (%)	Denominators[a]	P-value Chi^2
Sex (male; female)	60.7; 60.9	387; 373	.970	80.9; 79.2	383; 370	.548
Premature (no; yes)	60.6; 63.3	686; 60	.682	80.1; 81.4	680; 59	.823
Breast-feeding (no; yes)	59.4; 63.5	527; 230	.290	79.9; 80.3	522; 228	.905
Siblings at home (no; yes)	56.8; 63.3	271; 460	.085	78.4; 81.1	269; 455	.386
At least one parent smokes (no; yes)	63.1; 52.9	588; 170	.017	80.6; 78.6	583; 168	.558
History of AOM (no; yes)	62.9; 56.2	483; 258	.074	81.5; 77.2	480; 254	.167
AB treatment in last 3 months (no; yes)	62.9; 57.8	453; 249	.186	84.6; 72.5	448; 247	<.001
Age-appropriate vaccinated (no; yes)[b]	56.4; 60.7	39; 685	.591	76.3; 80.0	38; 680	.582
Sampling depth (<1/2; ≥1/2)[c]	59.9; 61.2	182; 554	.755	78.3; 80.3	180; 549	.563
Age group 6–12 months	58.8	80	.118	73.8	80	.234
12–24 months	64.3	392		81.9	392	
24–31 months	56.6	288		79.4	281	

[a] Missing values were due to incomplete questionnaires and/or *LytA*-result not available (n = 7).
[b] 3 PCV-doses at age 14 months.
[c] <1/2 = sample was taken at a depth of >1 cm from the nostril, but less than half the distance nostril-ear lobe, >1/2 = sample was taken at a depth of at least half the distance nostril-ear lobe; AOM = acute otitis media, AB = antibiotics.

Table 3
Density assessment via *LytA*-based qRT-PCR in *S. pneumoniae* carrying infants in day-care, 2016.

		Average pneumococcal loads (10^6 copies/μl)	95%CI (10^6 copies/μl)	P-value MWU-test[a]
Common cold	Yes	9.2	2.0–16	<.001
	No	4.4	2.6–6.2	
PCV13 VT carriers[b]	Yes	4.6	1.4–7.8	.560
	No	5.8	3.3–8.3	
AB treatment in last 3 months	Yes	3.9	1.7–6.1	<.001
	No	6.7	3.3–10	

95%CI = 95% confidence interval, PCV13 VT = vaccine serotypes included in PCV13, AB = antibiotics.
[a] MWU-test = Mann-Whitney *U* Test.
[b] Assessed in culture-positive samples only.

3.3.4. Antimicrobial susceptibility

Among the carried *S. pneumoniae,* culture-based resistance against any of the five tested antibiotics was 42.4% in DCC and 48.1% in AOM infants. Antibiotic resistance against cotrimoxazole was most frequent (32.7% in DCC; 40.7% in AOM), followed by tetracycline-resistance (16.9% in DCC; 3.7% in AOM). Sub-analysis in DCC infants showed 12.1% multidrug-resistance (resistance ag ainst ≥ 2 tested classes of antibiotics) and similar occurrence of resistance in the two regions. In contrast, proportions of erythromycin-resistant pneumococci were twice as high in the

Table 4
Culture-based carriage prevalence of *S. pneumoniae* per setting and per region, 2016.

		DCC				AOM			
		Wallonia n = 353	Flanders n = 332	Brussels n = 75	Belgium n = 760	Wallonia n = 27	Flanders n = 8	Brussels n = 4	Belgium n = 39
PCV vaccinated population (≥2 vaccinations)[a]		346 (98.0)	295 (89.7)	72 (96.0)	713 (94.2)	26 (96.2)	7 (87.5)	4 (100.0)	37 (94.7)
Sp carriage (%)		205 (58.1)	207 (62.3)	50 (66.7)	462 (60.8)	19 (70.4)	5 (62.5)	3 (75.0)	27 (69.2)
PCV13 VT prevalence (%)	All (incl. 3, 6A, 19A)	11 (5.4)	11 (5.3)	3 (6.0)	25 (5.4)	1 (5.3)	1 (20.0)	0 (0.0)	2 (7.7)
	19F	4 (2.0)	6 (2.9)	3 (6.0)	13 (2.8)	0 (0.0)	1 (20.0)	0 (0.0)	1 (3.8)
	14	4 (2.0)	0 (0.0)	0 (0.0)	4 (0.9)	1 (5.3)	0 (0.0)	0 (0.0)	1 (3.8)
	23F	2 (1.0)	1 (0.5)	0 (0.0)	3 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Non-PCV13 VT prevalence (%)	23B	37 (18.0)	24 (11.6)	3 (6.0)	64 (13.9)	2 (10.5)	1 (20.0)	0 (0.0)	3 (11.5)
	23A	24 (11.7)	25 (12.1)	0 (0.0)	49 (10.6)	1 (5.3)	0 (0.0)	0 (0.0)	1 (3.8)
	11A	15 (7.3)	18 (8.7)	7 (14.0)	40 (8.7)	4 (21.1)	0 (0.0)	0 (0.0)	4 (15.4)
PCV13 non-PCV10 VT prevalence (%)	3	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	6A	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	19A	1 (0.5)	1 (0.5)	0 (0.0)	2 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

DCC = day-care centre, AOM = acute otitis media, PCV = pneumococcal conjugate vaccine, Sp = *S. pneumoniae*, (non-) PCV13 VT = vaccine serotypes (not) included in PCV13, PCV13 non-PCV10; VT = serotypes included in PCV13 but not in PCV10.
[a] Vaccination status is based on vaccination documentation or parental reporting.

Walloon DCC infants (23.9%) compared to those in Flanders (11.6%) (Chi^2, P = .001). Most prevalent erythromycin-resistant pneumococcal serotypes in DCC infants were 15A (18.4%) and 33F (16.3%) in Wallonia and 35B (20.8%) in Flanders (Fig. 2). For tetracycline, occurrence of resistant pneumococci was similar in both regions. Resistance to penicillin or levofloxacin was quasi inexistent in DCC and in AOM.

4. Discussion

We report the overall pneumococcal carriage, the serotypes involved and the antimicrobial susceptibility of the strains isolated in infants in day-care and in infants with AOM in Belgium.

To our knowledge, this is the first study combining carriage prevalence and carriage density results in two infant populations, in a country where PCV13 is being replaced by PCV10 in the infant vaccination programme.

NP carriage of *S. pneumoniae* in infants has been widely studied after PCV7 implementation [36–39], however, only few studies report on pneumococcal carriage after PCV13 was licensed: available reports are about diseased infants in France and in Spain [29,40], households in the UK [30] and healthy infants in Italy and in Norway [31,41]. None of these countries had a similar sequence of vaccines as Belgium.

Culture-based carriage rates reported in infants in day-care vary widely, from 8.3% in infants too young to have been vaccinated [42] to 89.5% in PCV7 vaccinated infants between 6 and 34 months of age [43]. In PCV vaccinated infants with AOM between 6 and 24 months of age, carriage rates between 11.8% [44] and 71.2% [32] were reported. A Belgian study performed before PCV7 introduction, demonstrated a 21% carriage in DCC infants between 3 and 36 months [45]. Culture-based carriage in the current study (60.8% in DCC infants and 69.2% in AOM infants) is at the higher-end of the mentioned ranges. Even though there are methodological differences, this finding suggests that carriage did not decrease after PCV implementation. Studies comparing carriage pre- and post-PCV indeed demonstrated no impact on overall pneumococcal carriage [31,46]. This was also supported by the 80% *LytA*-based carriage prevalence.

It is striking that, despite the high sample number and the high detected carriage rates, no associations could be demonstrated between carriage and demographic/clinical characteristics, except

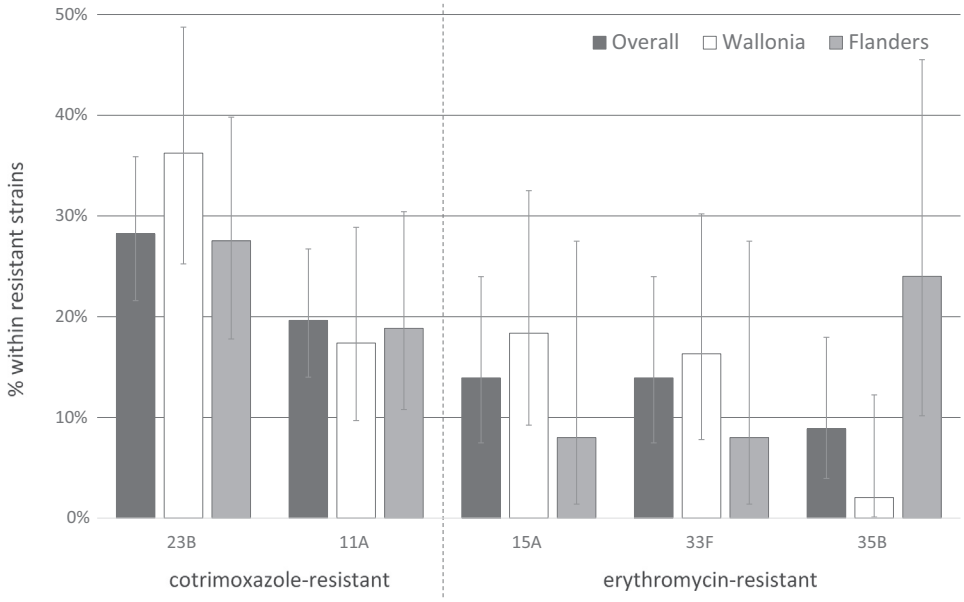

Fig. 2. Most prevalent cotrimoxazole-resistant (left) and erythromycin-resistant (right) pneumococcal serotypes per region in Belgium, 2016 (error bars depict 95%CI).

for antibiotic use in the past three months (*LytA*-based carriage) and parental smoking (culture-based carriage). This is in contrast with other carriage studies, reporting siblings [47], and/or recent antibiotic treatment [47,48], as significant carriage predictors among culture-based studies. However, these studies did not exclude infants who received antibiotics in the seven days prior to sampling. The unexpected negative association with parental smoking was only found in the culture-based analysis, reducing its acceptability.

The low frequency of PCV13 vaccine serotypes (5.4% in DCC, 7.7% in AOM), is probably the result of the high PCV coverage in Belgium (in 2015: 96.9% in Wallonia, in 2016: 94.9% in Flanders) and the PCV13 programme having been in place for four years [21,49]. The PCV13 serotypes most frequently identified were 19F (half of the cases) and 14. In DCC, serotypes 23F and 19A were also identified. These findings corroborate reports from other PCV13 using countries [31,41].

Among non-PCV13 serotypes, 23B (14.0% in DCC) and 11A (15.4% in AOM) were most prevalent, as reported in other European studies [29,31,41].

However, since pre-PCV carriage data from Belgium are limited to two studies with a different design (6–8 years before PCV13-implementation in 1–30 day-care centres) [45,50], no conclusions can be drawn on the impact of the previous years of PCV-use (up to 2016) on pneumococcal carriage in Belgian infants.

Notwithstanding differences in the timing of the Walloon and Flemish PCV immunisation programme switch, similar carriage prevalence (general and PCV13 serotypes) was found in both regions. Region-specific age criteria were applied in this first collection season in order to obtain a homogenous baseline measurement. However, if the different timing of the vaccine switch induced temporal differences in serotype distribution between the regions this will become clear in the next sampling periods. Also, a possible increase in the carriage of serotypes 3, 6A and 19A might become apparent when the cohort of PCV10 vaccinated infants grows over time.

The low frequency of antibiotic resistance among the carried strains in this study is probably another positive consequence of the low vaccine type carriage rate, since vaccine types that were formerly common were often antibiotic-resistant. Indeed, for each assessed antibiotic, resistance was at the lower edge of the range reported in other carriage studies in unvaccinated infants in day-care, which found resistance to penicillin, erythromycin and tetracycline up to 71.4%, 90.5% and 48.5% respectively [45,51]. In addition, the regional difference in erythromycin resistance reflects the regional difference in carriage of serotype 14, which is frequently macrolide resistant.

Few studies [52,53] have measured the density of pneumococcal carriage in infants, which is suggested to be a predictor of transmission probability and possibly even disease. The wide variety in absolute density we found (4.2×10–4.2×10^8 copies/μL in DCC, 3.0×10^2–7.4×10^6 copies/μL in AOM), is higher than the range mentioned in previous studies in a similar age group [53,54]. The higher density in infants in day-care compared to infants with AOM (of whom 12 of 32 carriers were not in day-care), would suggest that residing in day-care predicts higher density. This hypothesis will be tested when more data are available. In day-care infants, common cold was related to higher pneumococcal loads, as reported earlier [55]. This suggests interplay between viral infections and pneumococcal colonisation. We also found lower pneumococcal loads in infants treated with antibiotics more than seven days prior to sampling, but overall similar loads for infants carrying resistant and infants carrying susceptible strains, consistent with the results of Hanke et al. [56]. The latter finding suggests that antibiotic resistant strains circulating in healthy infants are as viable and reproductive as non-resistant strains. Surprisingly, age was not related to carriage density, but the narrow age range and the age distribution of the cohort studied might have limited the detection of an age effect.

The high culture-based detection rate of *S. pneumoniae* indicates that the established sampling protocol enables reasonably sensitive monitoring of *S. pneumoniae* colonisation in infants over time. Nevertheless, PCR screening of culture-negative samples was implemented to further increase sensitivity. Planned analyses include multiplex PCR and micro-array to detect multiple serotype carriage.

Another important strength of this study is the inclusion of both DCC and AOM infants in one study which allows comparison of a healthy and an ill population. This is informative, since differences in carriage rate, serotype distribution and antimicrobial susceptibility may occur. To improve the understanding of the relationship between carriage and disease, serotype distribution in national IPD surveillance data in infants under two years of age was also looked at and compared in an exploratory analysis: serotype distribution differed between IPD and carriers in DCC, because of differences in invasiveness. However, the frequency of vaccine serotypes was low in IPD and also in carriers in DCC. In 2016, serotypes most often identified in IPD were 12F, 10A and 33F, with a combined frequency of 29.5% (95%CI = 21.3–40.6%), but their combined frequency among carriers in DCC was clearly lower (9.7%, 95%CI = 7.3–12.9%). Furthermore, the combined frequency of the serotypes 23B, 23A and 11A, which were most often identified among carriers in DCC, was 33.1% (95%CI = 28.9–37.7%), which was higher compared to the combined frequency of these serotypes in IPD (6.5% 95%CI = 2.7–14.1%). The combined frequency of PCV13 vaccine serotypes was 12.9% (95%CI = 7.1–21.8%) in IPD and 5.4% (95 %CI = 3.6–8.0%) in carriers in DCC.

Regarding DCC, the randomised and population proportionate recruitment strategy ensures representativeness for the Belgian population. The number of AOM infants recruited in the first season was low, but increasing recruitment in consecutive seasons will allow more sensitive comparisons between both infant populations.

Recruiting in similar seasons enhances comparability of yearly sample collections, however, seasonality patterns cannot be derived from our data.

We also included a sampling protocol quality check and found no indication that carriage or density were influenced by sampling depth or an extra freeze-thaw cycle prior to culture. This suggests that a representative NP sample can be taken at a depth between 1 cm from the nostril and half the distance between nostril and ear lobe. Also, an extra freeze-thaw cycle prior to culture may create opportunities regarding the transport of NP samples. Nevertheless, it might be that the sample size was too small to notice any differences.

5. Conclusion and perspectives

At baseline in 2016, culture-based carriage of PCV13 vaccine serotypes was rare in infants in day-care and in infants with AOM throughout Belgium and it was similar in the three Belgian regions, as was the case for *LytA*-based pneumococcal carriage in DCC infants, despite the use of different vaccines in 2016. Carriage density (*LytA*-based) was related to antibiotic use and to clinical signs of common cold.

The continued surveillance as currently planned will demonstrate whether this situation will be maintained under the recent PCV programme change.

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Conflicts of interest

None.

Disclosure

All authors have approved the final article.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2017.11.052.

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