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Follow-up of serotype distribution and antimicrobial susceptibility of *Streptococcus pneumoniae* in child carriage after a PCV13-to-PCV10 vaccine switch in Belgium

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ABSTRACT

Background: A three year pneumococcal carriage study was set up in Belgium when the vaccination programme switched from a 13-valent (PCV13) to a 10-valent (PCV10) vaccine. We compared the first follow-up period (October 2016 – June 2017, year 2, Y2) for nasopharyngeal carriage, serotype distribution and antimicrobial susceptibility of *S. pneumoniae* with the baseline (January-July 2016, year 1, Y1).
Materials/methods: A single nasopharyngeal swab was taken in children (6–30 months), either attending one of the 112 day-care centres (DCCs), or visiting one of the 21 physicians for an acute otitis media (AOM). *S. pneumoniae* were cultured, screened for antimicrobial susceptibility, and serotyped.
Results: In Y2, 1218 samples were collected. The majority of the Y2-children (>85%) was vaccinated appropriately for their age. Children in Y2 received either PCV13 only (DCC: 23.5%; AOM: 24.6%), PCV10 only (DCC: 29.8%; AOM: 37.7%), or a mix of both vaccines (DCC: 31.9%; AOM: 25.4%). Pneumococcal carriage rates were high (Y2, DCC: 68.2%; AOM: 64.8%). Among carriers, prevalence of PCV13 serotypes was low (Y2 vs Y1, DCC: 3.5% vs 5.4%; AOM: 7.6% vs 7.7%). Although prevalence of PCV13-non-PCV10 serotypes did not increase significantly compared to Y1 (Y2 vs Y1, DCC: 1.6% vs 0.9%; Y2 vs Y1, AOM: 5.1% vs 0.0%), the proportion of serotypes 3, 6A, 19A among PCV13 serotype carriers in DCC was significantly higher in Y2 (46.2% vs Y1: 16.0%, p-value = 0.034). Serotypes 23B and 15B were the predominant non-vaccine serotypes (Y2). Among detected strains, non-susceptibility to at least one of five antibiotics tested (penicillin, tetracycline, erythromycin, levofloxacin, cotrimoxazole) was comparable to Y1 (Y2 vs Y1, DCC: 41.3% vs 42.4%; AOM: 49.4% vs 48.1%).
Conclusion: After completion of the PCV13-to-PCV10 vaccine switch in Belgium, the proportion of PCV13-non-PCV10 serotypes (mainly 19A) significantly increased among PCV13 serotype carriers in DCC, stressing the need for strengthened surveillance as the PCV10-vaccinated population grows.

1. Introduction

The nasopharyngeal niche is colonized by a variety of potential pathogens, including *S. pneumoniae*. Usually, *S. pneumoniae* resides asymptomatically in the upper respiratory tract. However, this symptomless pneumococcal carriage may evolve to a respiratory infection or even an invasive disease. Besides elder adults, young children are a risk group for (invasive) pneumococcal disease (IPD) [1–5]. Each year, one million children under the age of five die from pneumococcal disease worldwide [6]. The introduction of pneumococcal conjugate vaccines (PCVs) has impacted upon pneumococcal disease and carriage [1,2,7–11].

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The Belgian infant pneumococcal vaccination programme reached high coverage in 2015–2016 (Wallonia: 96.9% [12], Flanders: 94.9% [13], Brussels: 90.1% [14]) and is characterised by a unique sequence of conjugated vaccine introductions over time: from a 7-valent vaccine in 2007 (PCV7, comprising serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) to a 13-valent PCV in 2011 (PCV13, comprising the PCV7 serotypes plus 1, 5, 7F, 3, 6A, 19A) and to a 10-valent vaccine in 2015–2016 (PCV10, comprising the PCV7 serotypes plus 1, 5, 7F). PCV10 was introduced at different time points in the three Belgian regions (Flanders: 1 July 2015, Wallonia: 1 May 2016; Brussels: Flemish or Walloon programme, depending on the consulted physician).

According to the Belgian IPD surveillance data of 2017, the proportion of IPD caused by PCV13 serotypes has decreased in children under two years of age; from 84% in 2006 to 22% in 2017 [15–17]. At the same time, the emergence of IPD caused by non-PCV13 serotypes was observed, especially in children under two years of age. The predominant serotypes in child IPD were 12F, 10A and 33F, followed by serogroups 24 and 15 [16,18].

To monitor the carriage of *S. pneumoniae* in children, a pneumococcal carriage study was started in 2016 (January-July 2016, year 1, Y1), targeting (1) children in day-care centres (DCCs) and (2) children presenting with acute otitis media (AOM; Fig. 1) [19]. We compared the first follow-up results (October 2016 – June 2017, year 2, Y2) of nasopharyngeal carriage, serotype distribution and antimicrobial susceptibility of *S. pneumoniae* with the baseline results (year 1). In addition, we compared the AOM-results with the DCC-results and we looked at the regional differences among the DCC-children.

2. Materials & methods

The study design and baseline results (January-July 2016, year 1, Y1) were previously published in detail and are briefly summarized here for year 2 (October 2016 – June 2017, Y2) [19].

2.1. Study population, sampling and sample processing

Recruitment took place between January 2016 and June 2017. Healthy children between ≥6 months and ≤30 months were recruited from DCCs randomly selected over the three Belgian regions. Children of the same age group with AOM were recruited by trained general practitioners (GPs), paediatricians or paediatric outpatient services throughout Belgium.

After written informed consent of at least one parent, a trained nurse or a GP/paediatrician collected a parent questionnaire regarding demographic and clinical characteristics and vaccination status. A single nasopharyngeal swab was taken, transported in 1 ml STGG (Skim milk – Tryptone – Glucose – Glycerol) and cultured or stored at −80 °C within 24 h.

At the Belgian national Reference Centre for invasive *Streptococcus pneumoniae*, nasopharyngeal samples were cultured, *S. pneumoniae* strains were serotyped and antimicrobial susceptibility (penicillin, levofloxacin, tetracycline, erythromycin, cotrimoxazole) was determined as previously described [19]. Non-meningitis breakpoints (oral administration) were applied for the classification of penicillin non-susceptibility. A minimal inhibitory concentration (MIC) of ≥2 mg/l was interpreted as penicillin non-susceptible.

2.2. Statistical analysis

In year 2, a sample size of 900 children in DCCs was aimed for. In this way, we could detect 4% changes in the carriage prevalence of *S. pneumoniae* serotypes 19A or 6A with 80% power and assuming a baseline carriage prevalence below 2%.

To test the statistical significance (at a level of 5%) of differences between groups, the Chi-Square (Chi^2) or Fisher's Exact Test and the Mann-Whitney U Test (MWU) were used. A continuity correction was applied for 95% confidence intervals on proportions. Missing values were not replaced.

3. Results

3.1. Recruitment and characteristics of the study population

In total, 112 DCCs (34 in Wallonia, 60 in Flanders, 18 in Brussels; Table 1) and 21 GPs/paediatricians (8 in Wallonia, 10 in Flanders, 3 in Brussels) recruited study participants in year 2 of the study. They collected nasopharyngeal samples from a total of 1237 children. Eighteen children in DCCs and one child with AOM were excluded due to inappropriate age, use of oral antibiotics in the week prior to sampling, or because of an incomplete questionnaire. The remaining 1218 children (1096 healthy children from DCC and 122 children with AOM) were included in the *per protocol* analysis. Demographic and clinical characteristics of the study population are shown in the Table 1. In year 2, healthy children in DCCs differed in several demographic and clinical characteristics from children with AOM, including age, living with sibilings in the same household, breastfeeding, attending day-care, and number of previous AOM-episodes. In addition, regional differences for some variables were observed (Table 1).

3.2. Main findings

Carriage prevalence – In year 2, pneumococcal carriage prevalence determined by culture techniques in children attending DCCS

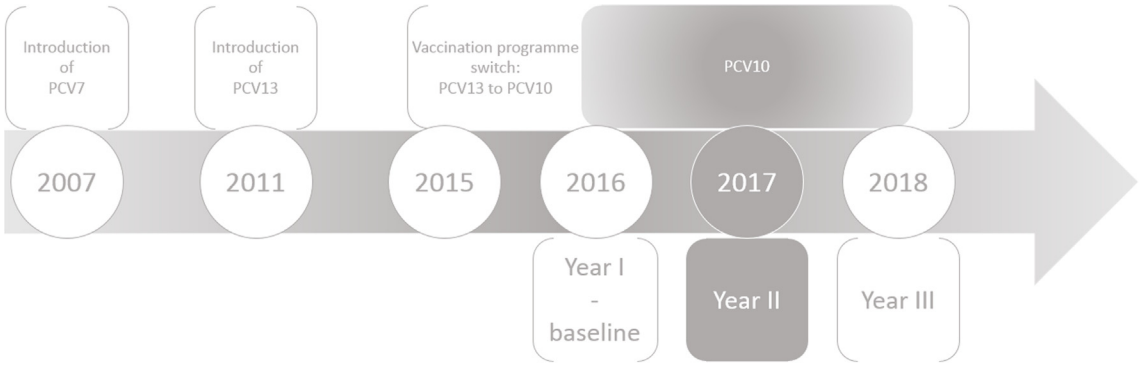

Fig. 1. Study flow: chronological representation of changes in the pneumococcal conjugate vaccine (PCV) programme in Belgium (upper part) and data collection periods (lower part).

Table 1
Demographic and clinical characteristics of included children per study group and per region, 2017 (Y2).

		Children attending DCC								Children with AOM		Statistical significance (P-value Chi²)
		Wallonia	Missing	Flanders	Missing	Brussels	Missing	Belgium	Missing	Belgium	Missing	Belgium (DCC vs AOM)
Number of actively participating centres		34		60		18		112		21		
Number of *per protocol* inclusions[1]		315		605		176		1096		122		
Sex (% male)		49.2		52.7		51.7		51.6		54.2	4	0.579
Age in months (mean)		18.3		18.5		17.7		18.3		14.8	4	<0.001[2]
Age group	6–12 months (%)	26.0		23.6		26.1		24.7		48.3	4	<0.001
	13–24 months (%)	51.1		49.4		53.4		50.5		39.0		
	25–30 months (%)	22.9		26.9		20.5		24.7		12.7		
Preterm delivery (%)		8.3	1	8.0	3	6.3	1	7.8	5	11.2	6	0.200
Breastfeeding >6 months (%)		34.4	1	27.4	2	50.3	1	33.1	4	20.7	6	0.007
Siblings at home (%)[3]	1	37.3	1	47.8	3	38.3	1	43.3	5	35.7	7	<0.001
	≥2	22.0		14.6		24.6		18.3		38.3		
At least two days in day-care (%)		89.9	9	90.3	6	89.1	2	90.0	17	71.2	4	<0.001
At least one parent smokes (%)		20.1	2	19.4	2	26.6	3	20.8	7	22.4	6	0.676
Number of AOM episodes (%)[3,4]	1	10.4	6	8.8	11	5.9	7	8.8	24	53.0	7	<0.001[5]
	≥2	22.7		15.8		10.7		17.0		47.0		
AB in previous 3 months (%)[3]	1	28.8	30	19.1	35	16.9	16	21.5	81	18.3	7	0.203
	≥2	15.8		7.4		4.4		9.3		5.2		
Age-appropriate vaccinated (%)[6]		84.8		86.4		82.4		85.3		87.9	6	0.493

Missing values were due to incomplete questionnaires.
DCC = day-care centre, AOM = acute otitis media, AB = antibiotics, PCV = pneumococcal conjugate vaccine.
[1] Denominator of percentages is number of *per protocol* inclusions.
[2] Mann-Whitney U Test.
[3] Category 0 not shown, but included in statistical analysis.
[4] For the AOM-population, the current AOM-episode (at the time of sampling) is included.
[5] Fisher's Exact Test.
[6] Age appropriate vaccinated = complete pneumococcal vaccination schedule of 2 or 3 doses if ≤12 months of age or booster received (not earlier than 351 days of age) if >12 months of age, vaccination status was based on vaccination documentation or parental reporting, taking into account the date of vaccine switch (1 July 2015 in Flanders, 1 May 2016 in Wallonia).

(68.2%, 95%CI = 65.4%–71.0%) was similar to children with AOM (64.8%, 95%CI = 55.5%–73.0%; Table 2). Overall, carriage prevalence in healthy children was significantly higher in year 2 (71.7%) than in year 1 (62.3%; p-value = 0.003). This finding was independent of sampling time (calendar month), sampling nurse and size of DCC. In addition, pneumococcal carriage prevalence in year 2 was higher in Flemish DCC-children (71.7%) compared to DCC-children from other regions (Wallonia: 61.6%, Brussels: 68.2%; p-value = 0.007; Appendix: Table 1). In the Flemish AOM-children, carriage prevalence was similar for Y2 and Y1 (Y2 vs Y1 66.2% vs 62.5%; p-value = 0.834).

Table 3 summarizes the vaccination status of children in DCCs. More than 89% of the children had received two or more PCV-doses (DCC: 91.0%; AOM: 89.8%) and the majority of the children were vaccinated appropriately for their age (DCC: 85.3%; AOM: 87.9%), i.e. participating infants aged 6–11 months had received at least 2 doses and toddlers had received at least two priming doses and a booster dose. A large proportion of children vaccinated appropriately for their age had received mixed PCV13-PCV10 schedules (31.9%). The highest frequency of vaccine serotype carriage was found in children who had received a mixed schedule (4.3%), whereas the lowest frequency was found in children who received PCV13 only (1.3%; p-value = 0.174). Among children older than twelve months in year 2, 45.9% had received PCV10 for their booster dose and 17.9% had received PCV10 for the complete 2 + 1 schedule.

In a univariate analysis, symptoms of common cold and living with siblings were risk factors for pneumococcal carriage in children attending DCCs, while AOM-history and antibiotic treatment in the three months prior to sampling were protective factors (Appendix: Table 2). In children with AOM, parental smoking was negatively associated with pneumococcal carriage.

Serotype distribution – Among *S. pneumoniae* carriers in year 2, 37 different serotypes were identified in DCC-children (42 in Y1; Fig. 2), and 26 in AOM-children (versus 19 in Y1). The prevalence of PCV13 serotypes in year 2 tended to be lower in the DCC-children (3.5%) than in the AOM-children (7.6%; p-value = 0.071; Table 2 and Appendix: Table 1). A similar non-significant trend was observed in year 1 (DCC: 5.4%; AOM: 7.7%). In year 2, four different PCV13 serotypes were identified in children in DCCs (19F, 19A, 3 and 14) and three in children with AOM (3, 19A, 19F), whereas in Y1, vaccine serotypes 23F, 1 and 6A were also identified in DCC-children. Among the 630 DCC-children who were appropriately vaccinated for their age and carrying *S. pneumoniae* in year 2 (Table 2), twenty-one carried PCV13 serotypes (10x 19A, 9x 19F, 1x14, 1x 3). Of these children, two had received PCV13 only (Table 3). The data of year 1 showed 22 PCV13 serotype carriers among 394 vaccinated carriers. The proportion of PCV13 serotype carriers among DCC-children with an age-appropriate PCV13-vaccination was lower in Y2 (n = 2, 0.8%; Table 3) than in Y1 (n = 19, 3.4%). In Y2, eleven 19A carriers were identified among children in DCC: one was unvaccinated, two received a complete schedule, but the administered vaccine type was unknown, eight were age-appropriately vaccinated, either with PCV10 only (n = 4) or with PCV13 for their primary doses and PCV10 for their booster dose (n = 4).

The combined proportions of PCV13-non-PCV10 serotypes (3 + 6A + 19A) were 1.6% among the DCC-carriers and 5.1% in the AOM-carriers, which was not significantly different from the Y1-proportion (Y1; DCC: 0.9%, p-value = 0.314; AOM: 0.0%, p-value = 0.570; Table 2 and Appendix: Table 1). However, the proportion of these serotypes among vaccine serotype carriers in DCCs was significantly higher in Y2 than in Y1 (Y2: 46.2%; Y1: 16.0%; p-value = 0.034).

Table 2
Serotype distribution of carried *S. pneumoniae* in Belgium per study group, 2016 (Y1) and 2017 (Y2).

		Children attending DCC[1]		Children with AOM[1]	
		Y1 n = 760	Y2 n = 1096	Y1 n = 39	Y2 n = 122
Sp carriage (%)		462 (60.8)	748 (68.2)	27 (69.2)	79 (64.8)
Number of different serotypes identified		42	37	19	26
PCV7 VT prevalence (%)	19F	13 (2.8)	13 (1.7)	1 (3.8)	2 (2.5)
	14	4 (0.9)	1 (0.1)	1 (3.8)	0 (0.0)
	23F	3 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
PCV10-non-PCV7 VT prevalence (%)	1	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
PCV13-non-PCV10 VT prevalence (%)	3	1 (0.2)	1 (0.1)	0 (0.0)	2 (2.5)
	6A	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
	19A	2 (0.4)	11 (1.5)	0 (0.0)	2 (2.5)
PCV13 VT prevalence (%)	All VT	25 (5.4)	26 (3.5)	2 (7.7)	6 (7.6)
non-PCV13 VT prevalence (%)[2]	23B	64 (13.8)	133 (17.7)	3 (11.5)	13 (16.5)
	15B	33 (7.1)	62 (8.3)	2 (7.4)	8 (10.1)
	10A	27 (5.8)	59 (7.9)	1 (3.7)	4 (5.1)
	23A	49 (10.6)	57 (7.6)	1 (3.8)	3 (3.8)
	21	24 (5.2)	54 (7.2)	0 (0.0)	4 (5.1)
	11A	40 (8.6)	51 (6.8)	4 (15.4)	5 (6.3)

DCC = day-care centre, AOM = acute otitis media, PCV = pneumococcal conjugate vaccine, Sp = *S. pneumoniae*, PCV7 VT = vaccine serotypes included in PCV7 (4, 6B, 9V, 14, 18C, 19F, 23F), PCV13 VT = vaccine serotypes included in PCV13 (4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, 7F, 3, 6A, 19A), PCV10-non-PCV7 VT = serotypes included in PCV10 but not in PCV7 (1, 5, 7F), PCV13 non-PCV10 VT = serotypes included in PCV13 but not in PCV10 (3, 6A, 19A), non-PCV13 VT prevalence = vaccine serotypes not included in PCV13.
[1] None of the differences in serotype frequency over time or between study groups (except for serotype 3 in Y2, AOM vs DCC) were significant at a level of 0.05 by Chi^2 or Fisher's Exact Test.
[2] Only serotypes identified at a frequency ≥6% in children in day-care are shown separately.

Table 3
S. pneumoniae carriage per vaccination status in children attending day-care, Belgium, 2017 (Y2).

Vaccination status[1]		Total number of children n (%)	Sp-carriers n (% of total children)	PCV13 serotype carriers n (% of Sp-carriers)
Primary[2]	Booster[3]			
PCV10	PCV10	327 (29.8)	235 (71.9)	8 (3.4)
PCV13	PCV13	258 (23.5)	159 (61.6)	2 (1.3)
PCV13	PCV10	234 (21.4)	162 (69.2)	7 (4.3)
PCV10/PCV13	PCV10/PCV13	116 (10.6)	74 (63.8)	4 (5.4)
Incomplete[4]		161 (14.7)	118 (73.3)	5 (4.2)

Sp = *Streptococcus pneumoniae,* VT = vaccine serotype included in PCV13, PCV13 = 13-valent pneumococcal conjugate vaccine, PCV10 = 10-valent pneumococcal conjugate vaccine, PCV13-non-PCV10-carriers = carriers of vaccine serotypes included in PCV13, but not in PCV10, i.e. carriers of serotypes 3, 6A or 19A.
[a] Vaccination status was based on vaccination documentation or parental reporting, taking into account the date of vaccine switch (1 July 2015 in Flanders, 1 May 2016 in Wallonia), the national pneumococcal vaccination schedule is 2 primary doses + 1 booster or 3 primary doses + 1 booster if preterm delivery.
[2] Priming series may have contained two or three doses.
[3] Booster was defined as dose administered at the age of ≥351 days, children too young to having received their booster were grouped based on their primary doses.
[4] >12 months of age and no booster[2] or ≤12 months of age and <2 doses/parents do not know/unvaccinated (0 doses).

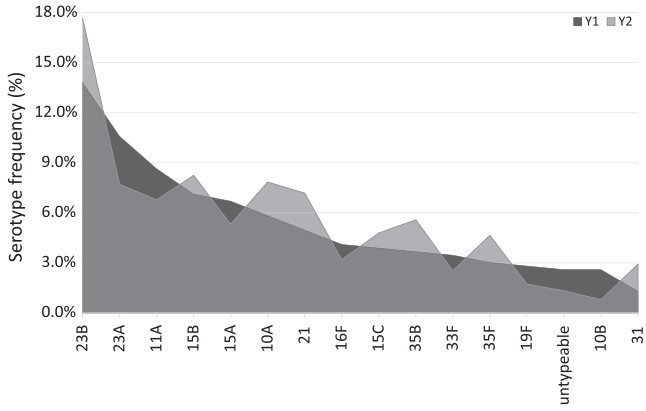

Fig. 2. Serotype frequency of carried *S. pneumoniae* in infants attending day-care, 2016 (Y1) and 2017 (Y2). Dark grey area – Y1, light grey area – Y2; Only serotypes with a frequency ≥2% are shown; The frequency of serotype 10B significantly decreased (p-value = 0.012).

Serotypes 23B, 15B and 10A were the predominant non-PCV13 serotypes in children in DCCs, accounting for 33.8% of all serotypes identified (Table 2 and Appendix: Table 1). In the AOM-population, serotypes 23B (16.5%), 15B (10.1%), 11A (6.3%) and 15C (6.3%) were the predominant non-PCV13 serotypes, similar to Y1, and accounted for 39.2% of all serotypes identified in Y2. Other non-vaccine serotypes were summarized in Table 2 and Appendix: Table 1.

Antimicrobial susceptibility – In Y2, non-susceptibility of *S. pneumoniae* strains to at least one of the five antibiotics (penicillin, levofloxacin, tetracycline, erythromycin, cotrimoxazole) was similar for children in DCCs and for children with AOM (DCC: 41.3%; AOM: 49.4%; p-value = 0.168; Table 4 and Appendix: Table 3). Non-susceptibility to cotrimoxazole was detected most frequently (DCC: 34.3%; AOM: 40.5%; p-value = 0.272). Within the cotrimoxazole non-susceptible DCC-strains, serotypes 23B (37.9%), 11A (16.0%) and 15B (12.1%) dominated. Within the cotrimoxazole non-susceptible AOM-strains, serotype 23B was predominant (37.5%). No strains were found non-susceptible to penicillin or levofloxacin. Multiple non-susceptibility (≥2 antibiotics) was also similar between the DCC-population and the AOM-population (11.3% and 6.3% respectively; p-value = 0.186). The results of Y1 were within the same range (DCC: 12.1%; AOM: 7.4%).

Table 4
Antibiotic non-susceptibility[1] of *S. pneumoniae* per setting and per region, 2017 (Y2).

		Children attending DCC n = 749[2]	Children with AOM n = 79	Statistical significance (P-value for DCC vs AOM)[*]
Non-susceptibility for ≥1 antibiotic tested[1]		307 (41.3)	39 (49.4)	0.168
Non-susceptibility for ≥2 antibiotics tested[1]		84 (11.3)	5 (6.3)	0.175
Penicillin non-susceptibility		0 (0.0)	0 (0.0)	–
Levofloxacin non-susceptibility		0 (0.0)	0 (0.0)	–
Tetracycline non-susceptibility		79 (10.6)	4 (5.1)	0.120
Dominating serotypes within tetracycline non-susceptible strains	15A	16 (20.3)	0 (0.0)	1.000[4]
	35B	11 (13.9)	2 (50.0)	0.053
	15B[3]	10 (12.7)	0 (0.0)	1.000[4]
Erythromycin non-susceptibility		115 (15.5)	10 (12.7)	0.510
Dominating serotypes within erythromycin non-susceptible strains	15A	17 (14.8)	0 (0.0)	0.355[4]
	15B	15 (13.0)	0 (0.0)	0.607
	33F	12 (10.4)	2 (20.0)	0.311[4]
Cotrimoxazole non-susceptibility		256 (34.3)	32 (40.5)	0.272
Dominating serotypes within cotrimoxazole non-susceptible strains	23B	97 (37.9)	12 (37.5)	0.966
	11A	41 (16.0)	4 (12.5)	0.606
	15B	31 (12.1)	3 (9.4)	0.651

DCC = day-care centre, AOM = acute otitis media.
[*] Chi^2 Test or [4] Fisher's Exact Test.
[1] Five antibiotics were tested: penicillin, levofloxacin, tetracycline, cotrimoxazole and erythromycin, non-meningitis breakpoints (oral administration) were applied for penicillin, a MIC of ≥2 mg/l was interpreted as non-susceptible.
[2] Missing values were due to unavailable antibiotic non-susceptibility profile.
[3] 15C was equally frequent in DCC.
[4] Fisher's Exact Test.

4. Discussion

We present the first follow-up of the Belgian nasopharyngeal carriage study which was started in 2016. Carriage prevalence, serotype distribution and antimicrobial susceptibility of *S. pneumoniae* were investigated after the infant pneumococcal vaccination programme changed from PCV13 to PCV10. Two child populations (healthy children in day-care centres (DCCs) and children with acute otitis media (AOM)) were surveyed in parallel.

After the implementation of the PCV-programme in 2007 (PCV7), a high child PCV-coverage was achieved in Belgium. As a result, the carriage prevalence of PCV13 serotypes was low in both studied populations (Y2 vs Y1, DCC: 3.5% vs 5.4%; AOM: 7.6% vs 7.7%). The PCV13 serotypes most often identified in Y2 (19A and 19F in both populations) were the same as in Y1. The proportion of PCV13-non-PCV10 serotypes among vaccine serotype carriers was higher in Y2 than in Y1 (for both child populations). This finding was mainly due to the higher prevalence of serotype 19A and it was significant in children in DCCs. Nevertheless, this finding should not be over-interpreted. The number of vaccine serotype carriers (n = 26) was low in DCC in Y2 and further follow-up is needed to see if the increase in PCV13-non-PCV10 serotype carriage will persist after a longer period of PCV10-programme implementation. Reports from other countries that have replaced PCV13 by PCV10 are limited [20]. Some PCV13-administering countries, such as Italy (2011) and Norway (2013) reported vaccine serotypes 3, 14 and 19A/F as predominant in children in DCCs [21,22]. PCV10-administering countries such as Brazil (2010–2013) and Bulgaria (2011–2016) reported 6A/C and 19A as predominant vaccine serotypes in children in the post-PCV10 era [23,24]. Surveillance data from The Netherlands and Finland (also PCV10-administering countries) mentioned serotypes 19F, 3 and 19A as most frequent in vaccine serotype IPD [25,26].

The Belgian IPD surveillance data of 2017 indicated a 10-fold increase in the number of IPD-cases caused by serotype 19A in children under two years of age (from 2/121 to 21/154) [27]. A trend that was not yet seen in the first part of 2017, which was the sampling period of the year 2 DCC and AOM samples. Similar findings were reported in IPD-surveillance data of Swedish counties using PCV10 [28]. Contrasting data showing a reduction of 19A-caused IPD, were reported in Canada where PCV10 replaced PCV13 after 15 months [29]. It should be kept in mind that natural variation occurs and could cause a temporary 19A-increase.

Overall, the same (predominant) serotypes were carried in year 1 and in year 2. Although no season-dependence of serotype distribution has been reported, the trends observed in the DCC-population from year 1 to year 2 are to be cautiously interpreted until data of year 3 samples, which will be collected in a similar calendar period as year 2, will be available. Carriage prevalence, serotype distribution and antimicrobial susceptibility were similar in both studied populations, but the relatively low sample size of the AOM-population might have limited the detection of differences.

The associations that we have found between child characteristics in the DCC-population and culture-based pneumococcal carriage were similar to other studies [30–32] and to our year 1 report (symptoms of common cold and parental smoking) [19].

Similar to year 1, non-susceptibility of *S. pneumoniae* to erythromycin and penicillin was low in year 2 (erythromycin, DCC: 16.9%, AOM: 11.1%; penicillin, DCC: 0.2%, AOM: 0.0%). Cotrimoxazole non-susceptibility was the most frequent in our population (DCC: 34.3%; AOM: 40.5%), and its frequency was within the range identified in other PCV-using countries (8.9%–39.8%) [22,23,33,34]. Within the cotrimoxazole non-susceptible strains, serotype 23B (DCC: 37.9%; AOM: 37.5%) was most often identified in both studied populations, similar to Y1. This was in contrast with the Belgian IPD-surveillance data of 2017, which reported serotype 12F as the most frequent cotrimoxazole-resistant strain. A difference that might be explained by the higher relative invasive disease potential of serotype 12F compared to serotype 23B [35].

The predominant non-vaccine serotypes in our study (23B, 15B, 10A, 11A and 15C) did cause IPD in children in Belgium in 2016 [16]. Serotype 12F, which was the most prevalent one in IPD reported in 2016 and 2017 (10.1% of child IPD), was only carried at very low frequency in both populations in year 2 (DCC: 1.5%; AOM: 3.8%). As reported in other studies, 12F has a high invasive disease potential and is thus more prevalent in IPD than in carriage [35,36]. The second, third and fourth most prevalent non-vaccine

serotypes in child IPD in Belgium in 2017 were 10A, 33F and 16F. These serotypes circulated in both populations at levels between 2.5% and 7.8%. The serotypes 3, 24A, 24B and 24F, which each caused between 4.3% and 5.1% of child IPD, were carried at levels below 0.6% and below 2.5% in DCC and AOM respectively. In the AOM-population, the IPD-serotypes 24A and 24F were not detected. Since non-vaccine serotypes represent the majority of the strains detected in IPD, it is of importance to recognise any significant increase in the carriage frequency of these serotypes, especially the ones that are often seen in IPD, such as serotype 10A.

The presented data have several limitations. First, the period of sampling differed between year 1 (DCC: March 2016 – July 2016; AOM: January 2016 – June 2016) and year 2 (DCC: November 2016 – April 2017; AOM: October 2016 – May 2017) due to a delayed start in year 1. The covered follow-up period is thus less than one year, and changes in vaccine serotype carriage frequency related to the vaccine programme change might need more time to become detectable. Second, the timing of the vaccine switch was different between the Belgian regions, but the sample size does not allow sensitive comparison between the regions. Existing differences in serotype distribution could thus be missed. Nevertheless, we detected a higher culture-based carriage prevalence in Flemish DCC-children in year 2 compared to year 1 which was not related to differences in sampling time (calendar month), sampling nurse or size of DCC. Further follow-up will show if this difference persists [19]. Finally, recruitment targets were not reached for children with AOM, but the serotype distribution in this group was similar as in DCC-children in spite of detectable differences in characteristics.

5. Conclusion and perspectives

In the first year after completion of the PCV13-to-PCV10 vaccine switch in Belgium, culture-based pneumococcal carriage of PCV13 serotypes remained rare. For non-vaccine serotypes, pneumococcal carriage remained high in healthy children in day-care centres and in children with acute otitis media. The proportion of PCV13-non-PCV10 serotypes (mainly 19A) increased significantly among vaccine serotype carriers in day-care centres. Serotypes 19F and 19A were the predominant PCV13 vaccine serotypes in a population vaccinated with PCV13, PCV10, or a mix of both, which stresses the need for strengthened surveillance as the PCV10-vaccinated population grows.

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Conflict of interest

None.

Disclosure

All authors have approved the final article.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2018.12.068.

References

[1] Faden H et al. Relationship between nasopharyngeal colonization and the development of otitis media in children. Tonawanda/Williamsville Pediatrics. J Infect Dis 1997;175(6):1440–5.
[2] Gray BM, Converse 3rd GM, Dillon Jr HC. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. J Infect Dis 1980;142(6):923–33.
[3] Millar MR et al. Outbreak of infection with penicillin-resistant *Streptococcus pneumoniae* in a hospital for the elderly. J Hosp Infect 1994;27(2):99–104.
[4] Principi N et al. Risk factors for carriage of respiratory pathogens in the nasopharynx of healthy children. Ascanius Project Collaborative Group. Pediatr Infect Dis J 1999;18(6):517–23.
[5] Reichler MR et al. Spread of multidrug-resistant *Streptococcus pneumoniae* among hospitalized children in Slovakia. J Infect Dis 1996;173(2):374–9.
[6] O'Brien KL et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet 2009;374 (9693):893–902.
[7] Simell B et al. The fundamental link between pneumococcal carriage and disease. Expert Rev Vaccines 2012;11(7):841–55.
[8] Cohen R. The need for prudent use of antibiotics and routine use of vaccines. Clin Microbiol Infect 2009;15(Suppl 3):21–3.
[9] Loughlin AM et al. Direct and indirect effects of PCV13 on nasopharyngeal carriage of PCV13 unique pneumococcal serotypes in Massachusetts' children. Pediatr Infect Dis J 2014;33(5):504–10.
[10] Moore MR et al. Effectiveness of 13-valent pneumococcal conjugate vaccine for prevention of invasive pneumococcal disease in children in the USA: a matched case-control study. Lancet Respir Med 2016;4(5):399–406.
[11] van Hoek AJ et al. Pneumococcal carriage in children and adults two years after introduction of the thirteen valent pneumococcal conjugate vaccine in England. Vaccine 2014;32(34):4349–55.
[12] Robert E, Swennen B. Enquête de couverture vaccinale des enfants de 18 à 24 mois en Fédération Wallonie-Bruxelles (Bruxelles exceptée). Bruxelles: ULB; 2015.
[13] Vandermeulen C, Hoppenbrouwers K, Roelants M, Theeten H, Braeckman T, Maertens K, et al. Studie van de vaccinatiegraad in Vlaanderen, 2016. Vlaamse Overheid, Vlaams Agentschap Zorg en Gezondheid; 2017.
[14] Mendes da Costa E et al. Infectieziekten bij kinderen, die voorkomen kunnen worden door vaccinatie, jaarrapport 2015. Brussel: Wetenschappelijk Instituut Volksgezondheid; 2016.
[15] Verhaegen J et al. Pneumococcal serotype evolution/replacement 4 years after the introduction of the 13 valent conjugate vaccine into the infant vaccination program in Belgium. 10th International symposium on pneumococci and pneumococcal disease (ISPPD), Glasgow, UK, 2016.
[16] Verhaegen J. Surveillance van de pneumokokkeninfecties in België. Verslag voor 2016. UZ Leuven: Nationaal Referentiecentum voor invasieve pneumokokkeninfecties; 2017.
[17] Verhaegen J. Surveillance van de pneumokokkeninfecties in België. Verslag voor 2017. UZ Leuven: Nationaal Referentiecentum voor invasieve pneumokokkeninfecties; 2018.
[18] Sabbe M, Hue D, Quoillin S. Surveillance van infectieziekten bij kinderen in België, PediSurv – Jaarrapport 2010. Brussel: Wetenschappelijk Instituut voor Volksgezondheid; 2011.
[19] Wouters I et al. Nasopharyngeal *S. pneumoniae* carriage and density in Belgian infants after 9 years of pneumococcal conjugate vaccine programme. Vaccine 2018;36(1):15–22.
[20] Diawara I et al. Invasive pneumococcal disease among children younger than 5 years of age before and after introduction of pneumococcal conjugate vaccine in Casablanca, Morocco. Int J Infect Dis 2015;40:95–101.
[21] Steens A et al. Decreased carriage and genetic shifts in the *Streptococcus pneumoniae* population after changing the seven-valent to the thirteen-valent pneumococcal vaccine in Norway. Pediatr Infect Dis J 2015;34(8):875–83.
[22] Zuccotti G et al. Serotype distribution and antimicrobial susceptibilities of nasopharyngeal isolates of *Streptococcus pneumoniae* from healthy children in the 13-valent pneumococcal conjugate vaccine era. Vaccine 2014;32 (5):527–34.
[23] Setchanova L et al. Serotype changes and antimicrobial nonsusceptibility rates of invasive and non-invasive *Streptococcus pneumoniae* isolates after

implementation of 10-valent pneumococcal nontypeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) in Bulgaria. Braz J Infect Dis 2017;21(4):433–40.
[24] Brandileone MC et al. Distribution of invasive *Streptococcus pneumoniae* serotypes before and 5years after the introduction of 10-valent pneumococcal conjugate vaccine in Brazil. Vaccine 2018;36(19):2559–66.
[25] Alberts N. The National Immunisation Programme in the Netherlands – Surveillance and developments in 2015-2016. In: Reports R, editor. The National Immunisation Programme in the Netherlands – surveillance and developments. National Institute for Public Health and the Environment – Ministry of Health, Welfare and Sport; 2016.
[26] Jaakola S et al. Infectious diseases in Finland 2016, in infectious diseases in Finland. National Institute for Health and Welfare; 2017.
[27] Desmet S et al. Switch in a childhood pneumococcal vaccination programme from PCV13 to PCV10: a defendable approach? Lancet Infect Dis 2018;18(8):830–1.
[28] Naucler P et al. Comparison of the impact of pneumococcal conjugate vaccine 10 or pneumococcal conjugate vaccine 13 on invasive pneumococcal disease in equivalent populations. Clin Infect Dis 2017;65(11):1780–9.
[29] Deceuninck G et al. Effectiveness of three pneumococcal conjugate vaccines to prevent invasive pneumococcal disease in Quebec, Canada. Vaccine 2015;33(23):2684–9.
[30] Chavanet P et al. Nasopharyngeal carriage, antibiotic susceptibility and serotyping of *Streptococcus pneumoniae* and *Haemophilus influenzae* in children attending day care centers. Med Mal Infect 2011;41(6):307–17.
[31] Franco CM et al. Survey of nonsusceptible nasopharyngeal *Streptococcus pneumoniae* isolates in children attending day-care centers in Brazil. Pediatr Infect Dis J 2010;29(1):77–9.
[32] Vestrheim DF et al. Phenotypic and genotypic characterization of *Streptococcus pneumoniae* strains colonizing children attending day-care centers in Norway. J Clin Microbiol 2008;46(8):2508–18.
[33] Neves FPG et al. Pneumococcal carriage among children after four years of routine 10-valent pneumococcal conjugate vaccine use in Brazil: the emergence of multidrug resistant serotype 6C. Vaccine 2017;35(21):2794–800.
[34] Zhou JY et al. Prevalence of nasopharyngeal pneumococcal colonization in children and antimicrobial susceptibility profiles of carriage isolates. Int J Infect Dis 2015;39:50–2.
[35] Balsells E et al. The relative invasive disease potential of *Streptococcus pneumoniae* among children after PCV introduction: a systematic review and meta-analysis. J Infect 2018;77(5):368–78.
[36] Lindstrand A et al. Unaltered pneumococcal carriage prevalence due to expansion of non-vaccine types of low invasive potential 8 years after vaccine introduction in Stockholm, Sweden. Vaccine 2016;34(38):4565–71.