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In-depth analysis of pneumococcal serotypes in Belgian children (2015–2018): Diversity, invasive disease potential, and antimicrobial susceptibility in carriage and disease

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ABSTRACT

Background: Changes in serotype distribution have been described after the switch from the 13-valent pneumococcal conjugate vaccine (PCV13) to the 10-valent pneumococcal conjugate vaccine (PCV10) in Belgium.
Aim: To describe serotype's invasive disease potential and the detailed evolution of serotype distribution and antimicrobial susceptibility of pneumococcal isolates (carriage and IPD) in children up to 30 months of age over a period during and after the vaccine switch (2015–2018).
Methods: *S. pneumoniae* strains isolated from the nasopharynx of healthy children attending day-care centres (DCCs) and strains from normally sterile sites of children with IPD were serotyped (Quellung-reaction) and antimicrobial susceptibility testing was performed. Invasive disease potential was defined as the serotype-specific odds ratio (OR).
Results: The highly invasive (OR > 1) serotypes 12F, 1, 3, 24A/B/F, 33F, 19A, and 9N were not frequently carried (<7.5% of carriage strains). Different serotypes dominated in carriage (23B, 23A, 11A, 15B) versus IPD (12F, 19A, 10A, 33F). PCV13 vaccine serotypes increased in carriage (5.4% (25/463) in period 1 vs 10.3% (69/668) in period 3) and in IPD (7.3% (8/110 in period 1 vs 23.9% (34/142) in period 3) due to an increase ($p < 0.01$) in serotype 19A. The penicillin non-susceptibility of 19A was lower ($p = 0.02$) in carriage (6.8%) than in IPD (23.5%). Erythromycin and tetracycline non-susceptibility were more frequent ($p < 0.01$) in IPD (26.0%; 23.0%) compared to carriage strains (18.2%; 14.5%) and penicillin non-susceptibility increased over the three year study period (carriage: 13.4%, 19.8%, 18.5%, $p = 0.05$; IPD: 11.8%, 15.0%, 20.4%, $p = 0.02$).
Conclusion: Only some of the serotypes with high invasive disease potential (serotype 1, 3, 19A) in Belgium are included in PCV10 and/or PCV13. This reinforces the need for continuous monitoring, both in healthy children as in children with IPD, to better understand the dynamics of pneumococcal disease, to optimise the composition and implementation of PCVs.

1. Introduction

S. pneumoniae often resides as a commensal in the human upper respiratory tract [1]. Nevertheless, asymptomatic carriage may evolve to respiratory infections such as otitis media and pneumonia or invasive diseases such as bacteraemia and meningitis. These diseases are a serious health concern among children in which the highest carriage prevalence occurs [2]. In 2000, before pneumococcal conjugate vaccines (PCVs) were introduced, the global annual number of serious pneumococcal disease cases (pneumonia, meningitis, and bacteraemia) in children under five years of age was estimated to be 14.5 million [3]. In 2015, when PCVs had been

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implemented in 129 countries, this number had decreased to 9.2 million [4].

More than ninety-five pneumococcal serotypes exist and they vary in their capacity to colonise, invade, and activate the host immune system [5]. Furthermore, some serotypes are strongly associated with antimicrobial non-susceptibility [2]. Immunisation with PCVs provides direct protection against a number of clinically relevant serotypes [5]. In addition, indirect protection of unvaccinated individuals (herd effect) against pneumococcal disease is accomplished through reduced nasopharyngeal carriage and hence transmission of pneumococcal vaccine serotypes (VTs). However, several studies on carriage and invasive pneumococcal disease (IPD) reported on serotype replacement, i.e. VTs being largely replaced by non-VTs, which diminishes the magnitude of the vaccine effect [6–8].

Belgium initiated a free of charge universal paediatric immunisation PCV-programme according to a two plus one schedule (at 8 and 12 weeks, and 12 months of age) in 2007. The seven-valent vaccine (PCV7) was replaced by the thirteen valent (PCV13) in 2011, which was in turn replaced by the ten-valent vaccine (PCV10) in 2015–2016 [9]. The programme rapidly achieved high three dose coverage in children (2008–2009: >80%; 2015–2016: >94%) [10–13].

Recent studies investigating paediatric IPD epidemiology and pneumococcal nasopharyngeal carriage in Belgium, reported an increase in PCV13 non-PCV10 serotypes shortly after the PCV13 to PCV10 vaccine switch [14–18].

The current study directly compares the evolution over time of pneumococcal serotype distribution and antimicrobial non-susceptibility of pneumococci in healthy children and children with IPD, up to 30 months of age, between July 2015 and June 2018, and focuses on serotype's invasive disease potential.

2. Methods

2.1. Study design

2.1.1. Carriage monitoring

The design of the carriage study was previously described in detail [15–17] and is summarised here. Healthy children between six and thirty months of age were recruited in day-care centres (DCCs) randomly selected in the three Belgian regions (Wallonia, Flanders, Brussels). Nasopharyngeal sampling was performed from March up to and including the first week of July in period 1 (2016) and from November up to the end of March in period 2 (2016–2017) and 3 (2017–2018). Trained nurses collected demographic and clinical characteristics and the vaccination status of the participating child. A single nasopharyngeal swab was taken with a flocked nylon fiber swab, transported in 1 ml STGG (Skim milk-Tryptone-Glucose-Glycerol), and cultured or stored at −80 °C within 24 h. At the National Reference Centre (NRC) for pneumococci, nasopharyngeal samples were plated both directly and following BHI-enrichment on blood agar plates for detection of *S. pneumoniae*. If children carried different serotypes, all serotypes were taken into account.

2.1.2. IPD surveillance

All *S. pneumoniae* isolates sent to the NRC from July 2015 to June 2018 and collected from a normally sterile site (e.g. blood culture, cerebrospinal fluid, pleural fluid or synovial fluid) in children up to 30 months old were included in the study. Data collection was part of the passive surveillance network. From 2015 to 2018, the estimated representativeness of the surveillance for all Belgian IPD cases ranged between 89 and 92% [18]. In total, 110 labs participated in this period, which are located over the whole country. Results are analysed per epidemiological year (from July to June).

2.2. Pneumococcal serotyping and antimicrobial susceptibility testing

For all strains, *S. pneumoniae* identification was confirmed and serotyping performed by phase-contrast microscopy using the Quellung-reaction with serotype/serogroup-specific sera obtained from SSI Diagnostica (Hillerød, Denmark). Antimicrobial susceptibility testing was carried out by disk diffusion according to CLSI (2015–2017) [19] and EUCAST (2017–2018) [20] guidelines for oxacillin (penicillin), erythromycin, levofloxacin, and tetracycline. In case of positive oxacillin screen, minimal inhibitory concentration (MIC) was determined by Etest (BioMérieux, France) for penicillin. Penicillin MIC > 0.064 mg/L was interpreted as non-susceptible.

2.2.1. Statistics

Chi-Square (Chi2) or Fisher's Exact Test (FET) were used in IBM SPSS Statistics 25 to compare variables between different groups, Spearman's Correlation Test was used to test the strength and direction of associations between variables; p-values less than 0.05 were considered to be statistically significant. To investigate the diversity of serotypes identified over the three year study period, the Simpson index of diversity and its 95% confidence interval (95%CI) was calculated via an online tool available at http://www.comparingpartitions.info and using the formula:

$$Simpson\ index\ of\ diversity = 1 - \frac{1}{N(N-1)}\sum_{i=1}^{S} n_i(n_i - 1)$$ [36];

with N = total number of isolates, S = total number of different serotypes, n_i = number of isolates with serotype i. This index can be interpreted as the probability that two random pneumococcal isolates are different serotypes. Indices close to 1 indicating high diversity. Invasive disease potential was defined as the serotype-specific odds ratio (OR):

$$OR = \frac{Number\ of\ IPD\ isolates\ of\ serotype\ x * Number\ of\ carriage\ isolates\ that\ are\ not\ serotype\ x}{Number\ of\ carriage\ isolates\ of\ serotype\ x * Number\ of\ IPD\ isolates\ that\ are\ not\ serotype\ x}$$

[22–24]; serotypes with an OR > 1 were considered to have an increased probability to cause invasive disease. For carriage, in children attending DCCs, the following serotype clustering factor (SCF) was introduced: $SCF = \frac{Number\ of\ pneumococcal\ strains\ with\ serotype\ x}{Number\ of\ DCCs\ where\ serotype\ x\ was\ identified}$ in order to assess the distribution of serotypes over the different DCCs, and their tendency to cluster in particular DCCs. This factor was calculated per serotype, over the three year study period. SCF equals 1 for serotypes that were not found more than once per day-care centre in a season, or not more than once per season, and increases if a serotype is found in several children in the same day-care centre.

3. Results

3.1. Study population

Over the three successive periods, 1883 carriage isolates out of a total of 2817 samples from children attending DCCs and 365 isolates from children with IPD were included in the analyses. The gender and age distribution are shown in Table 1 (elaborate descriptives of the carriage study population were previously published [17], more details on both study populations can be found in Supplementary Table 1, 2). Regarding children attending DCCs, age distribution changed over the study period due to the inclusion of (on average) older children in Flanders in period 1 [15–17].

Table 1
Characteristics of children providing *S. pneumoniae* positive carriage samples and IPD samples by period, from 2016 till 2018. (IPD: invasive pneumococcal disease).

		Carriage monitoring [1]						IPD surveillance					
		Period 1: spring 2016 (n = 463)		Period 2: winter 2016–2017 (n = 752)		Period 3: winter 2017–2018 (n = 668)		Period 1: July 2015-June 2016 (n = 110)		Period 2: July 2016-June 2017 (n = 113)		Period 3: July 2017-June 2018 (n = 142)	
		n	%	n	%	n	%	n	%	n	%	n	%
Number of centres [2]		85	–	112	–	102	–	99	–	94	–	94	–
Gender	male	236	51.0	373	49.6	310	46.4	59	53.6	61	54	82	58
Age (months)	<6	0	0	0	0	0	0	22	20.0	24	21.2	27	19.0
	6–12	56	12.1	183	24.3	151	22.6	45	40.9	39	34.5	58	40.8
	13–24	267	57.7	387	51.5	375	56.1	34	30.9	42	37.2	43	30.3
	25–30	140	30.2	182	24.2	142	21.3	9	8.2	8	7.1	14	9.9

[1] Only children carrying pneumococci were taken into account, [2] Carriage monitoring: randomly selected per Belgian region (population-proportionate for Wallonia, Flanders, Brussels), IPD surveillance: participating labs.

3.2. Serotype distribution, overall study period

Over the entire study period, 1883 carried strains representing 49 different serotypes and 365 IPD strains representing 46 different serotypes were detected. In both carriage and IPD a high proportion of serotypes not included in PCV13 (non-PCV13 serotypes) was found, respectively 93.6% (1763/1883) and 83.0% (303/365). 10.0% (188/1883) of carriage strains and 27.1% (99/365) of IPD strains were PCV15 serotypes (vaccine in development [25]; PCV13 serotypes plus serotype 22F and 33F). 36.0% (678/1883) of carriage strains and 59.7% (218/365) of IPD strains were PCV20 serotypes (vaccine in development [5]; PCV13 serotypes plus serotype 8, 10A, 11A, 12F, 15B, 22F, 33F).

Over the three year study period, the most frequently detected pneumococcal serotypes in carriage were 23B (15.6%, 293/1883), 23A (8.3%, 157/1883), and 11A (7.8%, 146/1883) whereas in IPD serotype 12F (15.9%, 58/365), 19A (9.3%, 34/365), and 10A (8.5%, 31/365) were most frequent. Serotypes 6A, 7C, 11F, 12A, 15F, 16C, 18C, 35A, 38F, 42 were only detected in carriage and serotypes 5, 8, 9, 11, 15, 16, 19B, 19C, 27, 35 exclusively in IPD. None of the IPD strains were serologically non-typeable, versus 2.0% (37/1883) of carriage strains.

In carriage, 30 serotypes had an SCF > 1, of which the ten with the highest SCF (>1.5; range: 1.6–2.0) were 15A, 35B, 14, 31, 10A, 23A, 12F, 19A, 21, and 23B. Of these serotypes, 35B, 15A, 10A, 21, 23A, and 23B were frequently carried serotypes (range: 5.0–15.6% of carriage isolates). According to Spearman's Correlation Test the SCF was associated with serotype carriage proportion (correlation coefficient = 0.81; $p < 0.01$).

3.3. Invasive disease potential

In total 9 of the 36 serotypes detected both in carriage and IPD (12F, 1, 3, 24B, 24F, 24A, 33F, 19A, 9 N) had an OR significantly higher than 1 (Fig. 1). The highest invasive disease potential was found for serotype 12F (OR = 23.5, 95%CI = 13.2–42.0), one of the

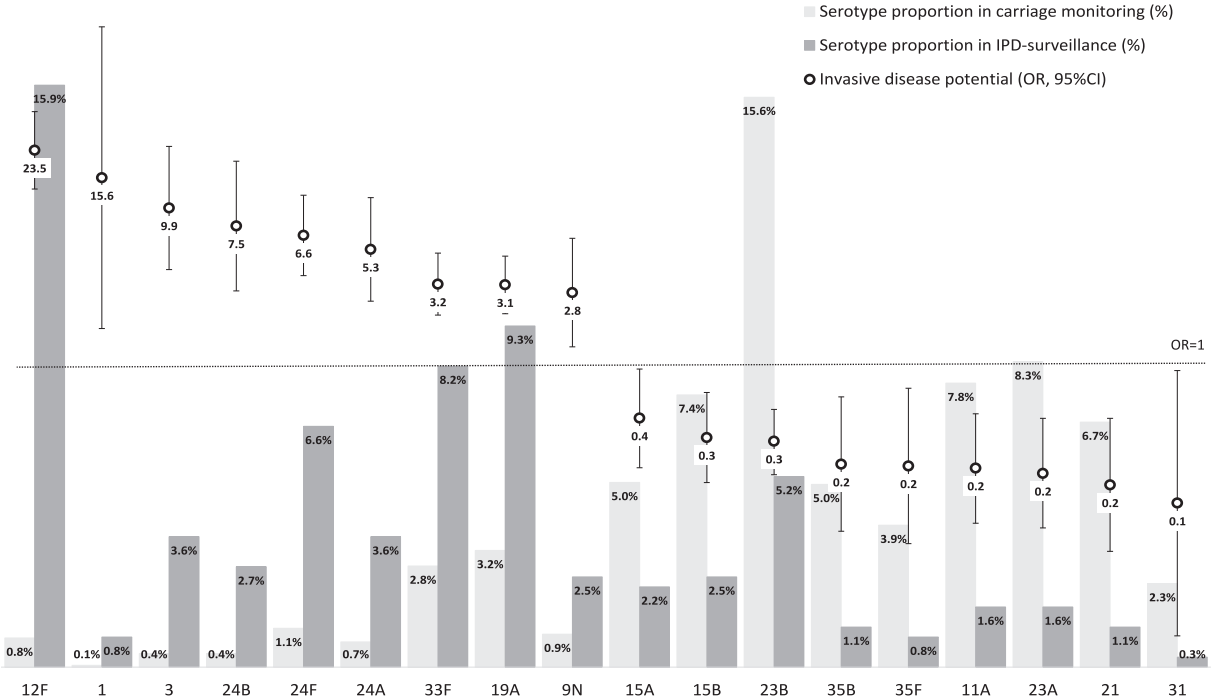

Fig. 1. Serotype proportion among pneumococcal isolates and invasive disease potential (OR) of serotypes identified in carriage monitoring and in IPD surveillance, over the three year study period from 2015 till 2018; OR displayed on logarithmic scale, error bars indicate 95% confidence intervals; only serotypes with OR significantly different from 1 are shown, ORs of the other serotypes can be found in Supplementary Table 1 (OR and 95%CI of serotype 10A: 1.5 (1.0–2.2)).

dominating serotypes in IPD. For serogroup 24, the invasive disease potential was also high (range OR 5.3–7.5 for 24A, 24B, and 24F). The four serotypes most frequently identified in carriage (11A, 15B, 23A, 23B) had an OR < 1, indicating low invasive disease potential. Among PCV13 vaccine serotypes, a high invasive disease potential was found for serotypes 1 (OR = 15.6, 95%CI = 1.6–150.4), 3 (OR = 9.9, 95%CI = 3.9–25.0), and 19A (OR = 3.1, 95%CI = 2.0–4.8), whereas the ORs of the remaining PCV13 vaccine serotypes, were not significantly different from 1 (Table 2) or could not be calculated.

3.4. Serotype diversity and trends in time

The evolutions in serotype distribution from period 1 to period 3 are summarized in Table 3. Simpson index of diversity showed higher levels of serotype diversity in each of the three periods for carriage strains than for IPD strains. No significant differences in serotype diversity were observed between carriage and IPD when comparing each of the three periods, except for period 1 in which the diversity of serotypes identified in IPD was lower than in carriage (see 95% CI in Table 3).

At serotype level, serotype 6C was rare, but its carriage proportion significantly increased over the study period ($p < 0.01$); from 0.9% (4/463) to 1.7% (13/752) and to 5.8% (39/668) and a similar but non-significant trend was seen among IPD (from 0.9%; 1/110 in period 1 to 2.1%; 3/142 in period 3). Additionally, a decrease in the proportion of serotype 15A was seen both in carriage and in IPD, which was only significant in carriage ($p = 0.04$); from 6.7% (31/463) to 5.5% (41/752) to 3.4% (23/668) in carriage and from 4.5% (5/110) to 0.9% (1/113) to 1.4% (2/142) in IPD.

However, the overall proportion of serotypes not included in PCV13 (Table 3) decreased significantly ($p < 0.01$) in both carriage (from 94.6% (438/463) to 89.7% (599/688)) and IPD (from 92.7% (102/110) to 76.1% (108/142)). The proportion of PCV10 vaccine serotypes, was already low at start and further decreased in carriage, from 4.5% (21/463) to 2.5% (17/668) ($p < 0.01$), whereas the proportion of PCV13-non-PCV10 vaccine types (3, 6A, 19A) significantly increased ($p < 0.01$) both in carriage and IPD due to a significant increase ($p < 0.01$) in serotype 19A over the study period; from 0.4% (2/463) to 1.5% (11/752) to 7.0% (47/668) in carriage and from 2.7% (3/110) to 5.3% (6/113) to 17.6% (25/142) in IPD. Apart from 6C, 15A and 19A, no other serotype proportions significantly changed over the study period.

3.5. Antimicrobial non-susceptibility

Non-susceptibility of pneumococcal strains against levofloxacin was inexistent in both carriage and in IPD (Table 4). Over the three

Table 2
Invasive disease potential (OR) of serotypes identified in carriage monitoring and in IPD surveillance, over the three year study period from 2015 till 2018.

Serotype		Carriage monitoring (N = 1883)		IPD surveillance (N = 365)		OR	95% CI	
		n	%	n	%		Lower bound	Upper bound
12F	PCV20 VT	15	0.8	58	15.9	**23.5**	**13.2**	**42.0**
1	PCV10 VT	1	0.1	3	0.8	**15.6**	**1.6**	**150.4**
3	PCV13 VT	7	0.4	13	3.6	**9.9**	**3.9**	**25.0**
24B	NVT	7	0.4	10	2.7	**7.5**	**2.9**	**20.0**
24F	NVT	20	1.1	24	6.6	**6.6**	**3.6**	**12.0**
24A	NVT	13	0.7	13	3.6	**5.3**	**2.4**	**11.6**
12A	NVT	1	0.1	1	0.3	5.2	0.3	82.9
7B	NVT	1	0.1	1	0.3	5.2	0.3	82.9
12B	NVT	3	0.2	2	0.5	3.5	0.6	20.7
23F	PCV7 VT	3	0.2	2	0.5	3.5	0.6	20.7
33F	PCV15 VT	52	2.8	30	8.2	**3.2**	**2.0**	**5.0**
19A	PCV13 VT	60	3.2	34	9.3	**3.1**	**2.0**	**4.8**
14	PCV7 VT	5	0.3	3	0.8	3.1	0.7	13.1
9N	NVT	17	0.9	9	2.5	**2.8**	**1.2**	**6.3**
29	NVT	4	0.2	2	0.5	2.6	0.5	14.2
9L	NVT	2	0.1	1	0.3	2.6	0.2	28.6
22F	PCV15 VT	16	0.8	7	1.9	2.3	0.9	5.6
38	NVT	23	1.2	9	2.5	2.0	0.9	4.5
33A	NVT	6	0.3	2	0.5	1.7	0.4	8.6
10A	PCV20 VT	111	5.9	31	8.5	1.5	1.0	2.2
17F	NVT	11	0.6	2	0.5	0.9	0.2	4.3
19F	PCV7 VT	42	2.2	6	1.6	0.7	0.3	1.7
15C	PCV20 VT	78	4.1	10	2.7	0.7	0.3	1.3
34	NVT	8	0.4	1	0.3	0.6	0.1	5.2
16F	NVT	64	3.4	6	1.6	0.5	0.2	1.1
6C	NVT	56	3.0	5	1.4	0.5	0.2	1.1
15A	NVT	95	5.0	8	2.2	**0.4**	**0.2**	**0.9**
10B	NVT	30	1.6	2	0.5	0.3	0.1	1.4
15B	PCV20 VT	140	7.4	9	2.5	**0.3**	**0.2**	**0.6**
23B	NVT	293	15.6	19	5.2	**0.3**	**0.2**	**0.5**
35B	NVT	94	5.0	4	1.1	**0.2**	**0.1**	**0.6**
35F	NVT	73	3.9	3	0.8	**0.2**	**0.1**	**0.7**
11A	PCV20 VT	146	7.8	6	1.6	**0.2**	**0.1**	**0.5**
23A	NVT	157	8.3	6	1.6	**0.2**	**0.1**	**0.4**
21	NVT	126	6.7	4	1.1	**0.2**	**0.1**	**0.4**
31	NVT	43	2.3	1	0.3	**0.1**	**0.0**	**0.9**

No OR determined for the following serotypes (number), present in carriage monitoring only: non-typeable (37), 35A (4), 7C (3), 10F (3), 11F (3), 15F (2), 28 (1), 42 (1), 6A (1), 16C (1), 18C (1), 21E (1), 35C (1), 38F (1), and in IPD surveillance only: 27 (3), 5 (1), 8 (5), 9 (1), 11 (1), 15 (1), 16 (1), 24 (2), 35 (1), 19B (1), 19C (1); 95% CI = 95% confidence interval, NVT = non-PCV20 serotype, PCV10 VT = PCV10-non-PCV7 vaccine serotype, PCV13 VT = PCV13-non-PCV10 vaccine serotype, PCV15 VT = PCV15-non-PCV13 vaccine serotype, PCV20 VT = PCV20-non-PCV15 vaccine serotype, OR = odds ratio, in **bold** if significant, an OR > 1 indicates increased invasive disease potential, and an OR < 1 indicates decreased invasive disease potential

Table 3
Proportion of PCV vaccine serotypes, non-vaccine serotypes and dominating serotypes (accounting for > 50% of identified serotypes) as well as the Simpson index of diversity for identified serotypes, among children attending day-care centres (carriage monitoring) or with invasive pneumococcal disease (IPD surveillance), per period, from 2015 till 2018.

	Carriage monitoring			IPD surveillance		
	Serotype	n	%	Serotype	n	%
Period 1: 2015–2016 [1]	all serotypes	463	100.0	all serotypes	110	100.0
	PCV10 vaccine serotypes	21	4.5	PCV10 vaccine serotypes	3	2.7
	PCV13 vaccine serotypes	25	5.4	PCV13 vaccine serotypes	8	7.3
	PCV13-non-PCV10 serotypes	4	0.9	PCV13-non-PCV10 serotypes	5	4.5
	Non-PCV13 serotypes	438	94.6	Non-PCV13 serotypes	102	92.7
	Dominating serotypes			Dominating serotypes		
	23B	64	13.8	12F	20	18.2
	23A	49	10.6	10A	13	11.8
	11A	40	8.6	33F	12	10.9
	15B	33	7.1	15B	6	5.5
	15A	31	6.7	24F	5	4.5
	10A	27	5.8	15A	5	4.5
	Simpson's diversity index (95% CI)	0.92 (0.89–0.96)		Simpson's diversity index (95% CI)	0.78 (0.70–0.86)	
Period 2: 2016–2017	all serotypes	752	100.0	all serotypes	113	100.0
	PCV10 vaccine serotypes	14	1.9	PCV10 vaccine serotypes	9	8.0
	PCV13 vaccine serotypes	26	3.5	PCV13 vaccine serotypes	20	17.7
	PCV13-non-PCV10 serotypes	12	1.6	PCV13-non-PCV10 serotypes	11	9.7
	Non-PCV13 serotypes	726	96.5	Non-PCV13 serotypes	93	82.3
	Dominating serotypes			Dominating serotypes		
	23B	133	17.7	12F	17	15.0
	15B	62	8.3	10A	11	9.7
	10A	59	7.9	33F	10	8.8
	23A	58	7.7	23B	6	5.3
	21	54	7.2	19A	6	5.3
	11A	51	6.8	24B	6	5.3
	Simpson's diversity index (95% CI)	0.91 (0.85–0.96)		Simpson's diversity index (95% CI)	0.80 (0.72–0.87)	
Period 3: 2017–2018	all serotypes	668	100.0	all serotypes	142	100.0
	PCV10 vaccine serotypes	17	2.5	PCV10 vaccine serotypes	3	2.1
	PCV13 vaccine serotypes	69	10.3	PCV13 vaccine serotypes	34	23.9
	PCV13-non-PCV10 serotypes	52	7.8	PCV13-non-PCV10 serotypes	31	21.8
	Non-PCV13 serotypes	599	89.7	Non-PCV13 serotypes	108	76.1
	Dominating serotypes			Dominating serotypes		
	23B	96	14.4	19A	25	17.6
	11A	55	8.2	12F	21	14.8
	23A	50	7.5	24F	15	10.6
	21	49	7.3	23B	9	6.3
	19A	47	7.0	33F	8	5.6
	15B	45	6.7	10A	7	4.9
	Simpson's diversity index (95% CI)	0.90 (0.84–0.96)		Simpson's diversity index (95% CI)	0.81 (0.74–0.88)	

PCV10 vaccine serotypes include serotypes: 4, 6B, 9V, 14, 18C, 19F, 23G, 1, 5, 7F, PCV13 vaccine serotypes include PCV10 vaccine serotypes plus: 3, 6A, 19A, 95% CI = 95% confidence interval.
[1] Period 1 represents spring 2016 in carriage and 1 July 2015 – 1 June 2016 in IPD.

Table 4
Antimicrobial non-susceptibility of pneumococcal strains in children attending day-care (carriage monitoring) or with invasive pneumococcal disease (IPD surveillance), per period and overall, from 2015 till 2018.

		Carriage monitoring					IPD surveillance				
Non-susceptibility against		Period 1: 2016 (N = 463)	Period 2: 2016–2017 (N = 752)[1]	Period 3: 2017–2018 (N = 668)[2]	Overall (N = 1883)	p-value Chi^2 for trend	Period 1: 2015–2016 (N = 110)	Period 2: 2016–2017 (N = 113)	Period 3: 2017–2018 (N = 142)	Overall (N = 365)	p-value Chi^2 for trend
Erythromycin	n	80	116	146	342	0.02	27	30	38	95	0.23
	%	17.3	15.5	22.0	18.2		24.5	26.5	26.8	26.0	
Tetracycline	n	54	86	133	273	<0.01	21	23	40	84	0.01
	%	11.7	11.5	20.0	14.5		19.1	20.4	28.2	23.0	
Penicillin	n	62	148	123	333	0.05	13	17	29	59	0.02
	%	13.4	19.8	18.5	17.7		11.8	15.0	20.4	16.2	

Non-susceptibility against levofloxacin was inexistent.
nS = non-susceptible; S = susceptible.
[1] Non-susceptibility against erythromycin, tetracycline and penicillin was determined for 747, 749 and 746 Sp-isolates respectively, [2] Non-susceptibility against erythromycin, tetracycline, and penicillin was determined for 664, 666 and 666 Sp-isolates respectively.

year study period, non-susceptibility against erythromycin and tetracycline was significantly more frequent ($p < 0.01$) in IPD (26.0% (95/365) for erythromycin and 23.0% (84/365) for tetracycline) than in carriage (18.2% (342/1883) for erythromycin and 14.5% (273/1883) for tetracycline). Penicillin non-susceptibility was similar in IPD strains (16.2%; 59/365) and carriage strains

(17.7%; 333/1883). The five pneumococcal serotypes that were most frequently non-susceptible to penicillin were also similar in carriage and IPD; 11A, 23B, 24A, 24B, and 24F. Remarkably, the non-susceptibility of serotype 19A against penicillin was three times higher (p = 0.02) in IPD (23.5%, 8/34) than in carriage (6.8%, 4/59). Analyzing trends over time (Table 4) showed that non-susceptibility for penicillin and tetracycline significantly increased over the study period in carriage and IPD. Erythromycin non-susceptibility also increased, but the increase was only significant in carriage.

4. Discussion

During and immediately after the PCV13-to-PCV10 vaccination programme switch (2015–2018), we report different predominant serotypes in IPD and carriage in healthy children. However similar trends in serotype distribution and antimicrobial non-susceptibility are noticed in IPD and carriage over this three years period. For a majority of the serotypes no significant change in serotype proportion was detected, except for serotype 19A, 15A and 6C. Interestingly, some of the serotypes with high invasive disease potential (e.g. serotype 12F and 33F) are not included in the current available PCVs, but will be included in one or both of the new upcoming PCVs (PCV15/PCV20). The information about serotype distribution in carriage and IPD is important to support the policy makers in decisions about the most appropriate pneumococcal vaccination programme, and can direct the development of new vaccines.

The sizes of both studied child population samples are large (DCC; N = 2817 and IPD; N = 365) and representative. Day-care attendance is common in Belgian children (in 2017: 52.6% of 0–30 months old children in Flanders [26]; 36.1% of 0–36 months old children in Wallonia [27]; 29.9% of 0–36 months old children in Brussels [27]) and the participating DCCs were randomly selected over the three Belgian regions (Wallonia, Flanders, Brussels). Therefore, the DCC-based sample can be considered representative of healthy children aged 6–30 months residing in daycare. The IPD surveillance is with its high and constant estimated laboratory coverage (range: 89–92%) also representative of the Belgian IPD cases over the three year study period.

Calculations on invasive disease potential are interesting in the context of pneumococcal vaccine composition and implementation of a vaccination programme. Serotypes differ in their probability to cause IPD. Hence, the inclusion of more invasive serotypes in a vaccine will determine the extent to which it can prevent IPD. As expected and in accordance with Dutch and Swedish study reports [22,24], our data (Fig. 1, Table 2) clearly indicated a higher invasive disease potential (OR > 1) for serotypes dominating in IPD (12F, 19A, 33F, 24F) and a lower invasive disease potential (OR < 1) for serotypes dominating in carriage (23B, 23A, 11A, 15B).

Serotype 12F contributed most to IPD over the three year study period, except for period 3 in which serotype 19A became the most frequent IPD-type. Serotype 12F was also rare in carriage (15/1883; 0.8%), and therefore had the highest invasive disease potential (OR = 23.5, 95%CI = 13.2–42.0). It is important to know that the upcoming higher-valent PCV20 will target serotype 12F and some other serotypes dominating IPD in Belgium (10A, 11A, 15B, 33F, but only 33F with OR > 1)) [28]. The PCV20-non-PCV13 serotypes are responsible for 38.6% and 24.8% of strains in respectively IPD and carriage in Belgian children, whereas PCV15-non-PCV13 (22F and 33F) serotypes represent 8.8% of IPD and 2.9% of carriage. This emphasizes the importance of dual surveillance of serotypes, both in terms of carriage and IPD.

Among PCV13 vaccine types, OR could only be estimated for serotypes 1, 3, and 19A. All three had a significant OR > 1 and are thus considered to present a high risk of causing IPD. The OR we calculated for serotypes 1 (OR = 15.6, 95%CI = 1.6–150.4) and 3 (OR = 9.9, 95%CI = 3.9–25.0) were within the range described by other reports (range OR for serotype 1: 8.8–33.4; range OR for serotype 3: 0.9–18.8), but for 19A, the OR we calculated (OR = 3.1, 95%CI = 2.0–4.8) was higher than reported elsewhere (range OR: 1.0–2.4) [22,23,24,29]. Similar to a Swedish report, we found a borderline non–significant OR for serotype 10A (OR = 1.5, 95%CI = 1.0–2.2), whereas a Dutch study reported a significant OR of 1.7 (95%CI = 1.1–2.7) for this serotype [22,24].

Similar serotypes with high invasive disease potential were reported in a meta-analysis of 13 carriage/IPD settings in Europe, North-America, Latin-America, and Africa, which adopted serotype 19A as the reference type for invasive disease potential [30]. Their results among children up to twenty-three months of age showed that, serotypes 7F, 12F, 1, and 3 had a higher invasive disease potential than 19A, corroborating our findings except that serotype 7F was absent in our study population. Other PCV13 vaccine serotypes with a significant OR identified in the meta-analysis were 6A, 23F, 6B, and 19F, with an OR < 1. In our study only 19F had an OR estimate lower than 19A. This difference may be due to the different period of the studies post PCV-introduction, or the use of a different PCV. In Belgium, after PCV13-introduction in 2011, serotypes 6A, 6B, 23F, and 19F almost completely disappeared as paediatric IPD causing serotypes, and only 19F is still carried at low level in healthy children.

Even though it was expected that a PCV13-to-PCV10 vaccination programme switch would have a more immediate impact on serotype distribution in childhood carriage than IPD, the current study suggests a simultaneous change. Indeed, the proportion of 19A significantly increased in parallel in carriage and in IPD from 2015 to 2016 onward (Table 3). This increase was accompanied by a simultaneous increase of the non-vaccine serotype 6C proportion, though at much lower level, suggesting absence of PCV10-induced cross-protection against serotype 6C. Additionally, a simultaneous decrease of the non-PCV13 serotype 15A was seen, also only significant in carriage. Any hypothesis explaining this coincidence remains to be found, and it needs to be confirmed in future follow-up. Also unexplained, the proportion of PCV10 vaccine serotypes significantly decreased over the study period in carriage but not in IPD. However, it is difficult to make conclusions on evolution of PCV10 serotypes over the study period, as numbers in carriage and IPD were already low at the beginning of the study.

In a Dutch study investigating pneumococcal carriage in PCV10-vaccinated 24-month-old children and in children with IPD who were up to five years old [24], serotypes 6C, 23B, 11A, 15B, and 23A were the most frequently reported serotypes in carriage. These are with exception of 6C, the same frequent serotypes as in our study. The most frequently identified IPD serotypes reported in this Dutch study were serotypes 19A, 3, 8, 6C, 27, and 33F, whereas in our study the dominating serotypes in 2017–2018 were 19A, 12F, 24F, 23B, 33F, and 10A.

In addition, contrasting our study results, the Dutch study reported a decline in carriage of serotype 19A as soon as PCV10 was implemented, however, it remained one of the serotypes contributing most to IPD (besides serotypes 3 and 8). Whether the increase of serotype 19A in Belgian IPD and carriage resulted from the serotypes' natural evolution or from a causal relationship with vaccine switch remains to be further investigated. As from mid-2019, decision was made to switch back from PCV10 to PCV13 in Belgium, further follow up of IPD and carriage serotype distribution will be very important.

The penicillin non-susceptibility in serotype 19A IPD strains (23.5%, 8/34) was lower than in the pre-PCV13 period (in 2011: 38.6% of Belgian serotype 19A IPD strains in children were non-susceptible, data NRC). Interestingly, penicillin non-susceptibility

was less frequent among serotype 19A carriage strains (6.8%, 4/59) than in 19A IPD-strains which may suggest that other serotype 19A clones are involved in IPD than in carriage. By means of whole-genome sequencing of serotype 19A strains of both carriage and IPD, we will further investigate this hypothesis. The micro-epidemiology of these 19A strains will be important to better understand the recent evolutions. For serogroup 24 (also highly invasive), penicillin non-susceptibility was more frequent than for serotype 19A, ranging from 53.8% (7/13, 24A) to 70.0% (14/20, 24F) in carriage and from 40.0% (4/10, 24B) to 76.9% (10/13, 24A) in IPD. In France, shortly after PCV13-implementation, other penicillin non-susceptible serotypes were reported compared to our study; 11A, 15A, 15B/C, 19A, and 35B [32,32]. However, this was in children with acute otitis media. Similar to our results, in Italy serotypes 15A, 19A, 23B, and 24F were reported to be frequently penicillin non-susceptible in IPD isolates [33]. We found non-susceptibility against erythromycin and tetracycline significantly more frequent in IPD than in carriage strains (Table 4). This is peculiar as erythromycin and tetracycline are not frequently used in children in Belgium. Therefore, this result might be the consequence of antibiotic treatment in adults and consequent transmission of resistant strains to children.

The main limitations of our study are the following. First, the detection of *S. pneumoniae* was performed by culture. Culture is less sensitive than PCR and therefore we might have missed pneumococcal strains that were carried at low density or together with other serotypes. We previously reported PCR-based overall carriage for the first 2 years of carriage monitoring [16,17] and indeed, it was higher (80.1%) than culture-based carriage (60.8%). Therefore, we could have missed changes in individual serotypes and non-susceptibility of strains present at low density. Second, IPD surveillance is performed year-round, whereas carriage monitoring was performed during spring in the first period and winter/spring (five-month period) in the second and third period. Since carriage samples were collected in the period of maximal transmission, we do not expect this to impact our results [34]. Third, age distribution of children with IPD was not exactly the same as in carriage, as no children aged<6 months were included in the carriage study. The low number of children younger than 6 months with IPD did not allow to detect or correct for any eventual age-related differences in serotype distribution. As children younger than 6 months old account for only 20% of all IPD strains and the serotype distribution of IPD strains from children younger than 6 months is comparable to the serotype distribution in children aged 6–30 months (data not shown), we do not expect this to have an impact on our conclusions.

In conclusion, we found that different serotypes dominated in IPD and carriage in Belgian children. The circulating serotypes with highest invasive disease potential (12F, 1, 3, 24B, 24F and 24A) were not frequently carried (<1.5%), and only a minority are included in the currently available PCVs. Nevertheless, some of them (serotype 12F, 33F) will be included in the future PCV15 and/or PCV20 [35]. Furthermore, we have found that after a PCV13-to-PCV10 vaccination programme switch, serotype 19A increased simultaneously in childhood IPD and carriage, but the antimicrobial non-susceptibility of this serotype against penicillin was lower in carriage than in IPD. Hence, our results reinforce the need for continued monitoring of carriage and IPD in order to implement the most suitable PCV in the national immunisation programme and in order to optimise the composition of future vaccines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.11.044.

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