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Pneumococcal vaccination: Direct and herd effect on carriage of vaccine types and antibiotic resistance in Icelandic children

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ABSTRACT

Background: Since the introduction of pneumococcal conjugate vaccines, vaccine type pneumococcal carriage and disease has decreased world-wide. The aim was to monitor changes in the nasopharyngeal carriage of pneumococci, the distribution of serotypes and antimicrobial resistance in children before and after initiation of the 10-valent pneumococcal vaccination in 2011, in a previously unvaccinated population.

Methods: Repeated cross-sectional study at 15 day-care centres in greater Reykjavik area. Nasopharyngeal swabs were collected yearly in March from 2009 to 2015. The swabs were selectively cultured for pneumococci, which were serotyped using latex agglutination and/or PCR and antimicrobial susceptibility determined. Two independent studies were conducted.

In study 1, on total impact, isolates from children aged <4 years were included. The vaccine-eligible-cohort (birth-years: 2011–2013, sampled in 2013–2015) was compared with children at the same age born in 2005–2010 and sampled in 2009–2012. In study 2 on herd effect, isolates from older non-vaccine-eligible children (3.5–6.3 years) were compared for the periods before and after the vaccination (2009–2011 vs 2013–2015. Vaccine impact was determined using 1-odds-ratio.

Results: Following vaccination, the vaccine impact on vaccine type acquisition was 94% (95% CI: 91–96%) in study 1 and 56% (95% CI: 44–65%) in study 2. The impact on serotype 6 A was 33% (95% CI: −9%; 59%) in study 1 and 42% (95% CI: 10–63%) in study 2 with minimal effect on 19A. The non-vaccine serotypes/groups 6C, 11, 15 and 23B were the most common serotypes/groups after vaccination. Isolates from the vaccine-eligible-cohort had lower penicillin MICs, less resistance to erythromycin and co-trimoxazole and less multi resistance than isolates from the control-group.

Conclusions: The efficacy of the vaccination on vaccine serotypes was high, and a milder effect on vaccine-associated-serotype 6A was observed for the vaccine-eligible-cohort. There was a significant herd effect on vaccine types in older non-vaccine-eligible children. Overall antimicrobial non-susceptibility was reduced.

1. Introduction

Streptococcus pneumoniae, is an important pathogen in respiratory tract and invasive pneumococcal infections (IPD), especially in children [1,2]. Acute otitis media (AOM) is among the most common reasons for health care visits and the most common reason for antibiotic prescriptions [3] in young children, contributing to antimicrobial resistance [4]. Major risk factors for pneumococcal carriage and antibiotic resistance include young age, crowding, recent upper respiratory tract infection, day-care centre (DCC) attendance, larger family size, passive smoking and low socioeconomic status [5–9]. In addition, recent or current antimicrobial usage temporarily reduces the risk of carriage [7,8]. Nasopharyngeal (NP) carriage of pneumococci is a prerequisite for pneumococcal infections [10]. Therefore, it is important to monitor the

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prevalence of serotypes and antibiotic-resistance in the nasopharynx of children.

Penicillin non-susceptible pneumococci (PNSP) are of worldwide concern and the main risk factors for PNSP infections are young age and recent antibiotic usage [4,5,7,8]. In recent years, serotype 19F has been the most common PNSP and multi-resistant serotype in Iceland [11].

Vaccinations with pneumococcal conjugate vaccines (PCVs) have resulted in a lower incidence of IPD [2,12–16], AOM [2,12,17–19] and pneumonia [2,12,18,20], especially in children. Decrease in nasopharyngeal carriage of vaccine types and replacement by non-vaccine types after implementation of vaccination in childhood vaccinations schedules has been widely reported both in vaccinated children [2,9,12,19,21–26] and children not vaccinated [2,27].

The 10-valent protein conjugated pneumococcal vaccine (PHiD-CV) was added to the childhood vaccination schedule in Iceland in 2011 for all children born that year without catch-up (at 3, 5 and 12 months of age). This population had previously not been routinely vaccinated against pneumococci. In this investigation, we report the results from two analyses (referred to as study 1 and 2). The aim of the first one was to compare pneumococcal carriage rate, serotype distribution and prevalence of antibiotic resistant pneumococci in the nasopharynx of healthy children attending DCC before and after the initiation of the vaccination. The second analysis evaluated the herd effect on unvaccinated children in DCC.

2. Material and methods

The study is an ongoing, repeated cross-sectional study where nasopharyngeal samples are collected annually (in March) from children attending 15 DCCs in the Reykjavik capital area, from 2009 to 2015. The DCCs were selected so they would be representative both geographically and socially for the Reykjavik capital area. The same 15 DCCs participated during the whole study period with only two exceptions, where they were changed for neighbouring DCCs due to scheduling difficulties.

The children attending the DCCs participating in this study are aged from 1.1 years (earliest time of entering DCC) to 6.3 years of age (when leaving DCC and starting primary school). All children attending the DCCs were invited to participate in this study. Signed informed consent forms were obtained and parents were asked to fill in questionnaires including questions on current and previous 30-day usage of antimicrobials and whether the child had been diagnosed with acute otitis media, sinusitis or pneumonia by a physician in the previous six months.

Single nasopharyngeal sample was obtained (COPAN transport medium swabs, Copan, Italy) from each child attending the DCCs on the day of sampling for which informed consent had been obtained. Children could participate more than once if they were still attending the DCC in the later years, at the day of sampling. Although many children participated more than once in the study, these events were considered independent in the main analysis. In an analysis of carriage of 19F, individual data was examined to search for repeated carriage of 19F. The swabs were inoculated within six hours of sampling and selectively cultured for pneumococci on blood agar containing 5 μg/mL gentamicin. The blood agar plates were incubated anaerobically at 35 °C for 18–20 h using anaerobic jars with gaspak envelopes to create a reduction in O_2 and an increase in CO_2 concentrations [28]. Pneumococci were identified by morphology and susceptibility to optochin. Normally, when all colonies appeared identical two to four colonies were selected for antimicrobial susceptibility testing and serotyping. If there were colonies with different morphology [28], additional two to four colonies were selected from each type for susceptibility testing as well as serotyping.

All pneumococcal isolates were tested for antimicrobial susceptibilities using disc diffusion and the EUCAST methods and criteria (www.eucast.org; to erythromycin, clindamycin, tetracycline and trimethoprim/sulfamethoxazole). The isolates were screened for penicillin non-susceptibility with oxacillin discs and penicillin MIC measured for all oxacillin resistant isolates using the E-test (BioMérieux, France). Isolates defined as PNSP were divided into low MIC (0.094–0.5) and high MIC (>0.5) groups. Resistant and intermediate resistant isolates were termed non-susceptible. Multi-resistance was defined as non-susceptibility to at least three different antimicrobial classes.

Serotyping was done as previously described [29] using pneumococcal Latex antisera [30] (Statens Serum Institute, Denmark) [28]. The same senior biomedical scientist did all the agglutinations. When the latex agglutination test did not detect a specific serotype, mono and multiplex PCR was used. The vaccine serotypes (VT) were defined as serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. The Vaccine associated serotypes (VaT) were defined as 6A and 19A. Non-vaccine serotypes (NVT) are those not belonging to VT or VaT.

Information on pneumococcal vaccinations was acquired from The National Vaccination Registry, and the percentage of children from each birth-cohort receiving at least two PCV doses before the age of two, were the following: For; 2003–2007: <1%, 2008: 2.4%, 2009: 7.4%, 2010: 19.2%, 2011: 97.7%, 2012: 98.4%, 2013: 98.7%.

2.1. Statistics

Statistics were done using the statistical software R version 3.3.2. Differences between characteristics of the children, overall carriage prevalence of pneumococci, antimicrobial resistant pneumococci and answers to questionnaires between study groups were tested using two-sided Fisher's exact test and z-test for categorical outcomes and *t*-test for continuous outcomes, using $\alpha = 0.05$ as significance level. Odds ratios and 95% confidence intervals were calculated for the risk of carrying individual and pooled serotypes in the vaccine and non-vaccine groups. The estimation of vaccine impact on acquisition (VEacq) was determined by 1 – OR as elucidated by Rinta-Kokko et al. [31]. Large sample theory was used to construct approximate confidence intervals for VEacq and a hypothesis test for the difference between two VEacq coefficients. When testing the null hypothesis of the VEacq difference being equal to zero the distribution of the test statistic is approximated with a standard normal distribution. For serotypes with zero observations in either of the groups a conservative one-sided 95% confidence interval for vaccine impact was found by replacing the observed zero with one. As some children carried two serotypes the denominator for these analyses were number of children in that group + number of children with dual carriage in that group.

Two independent studies were done (Table 1).

Study 1. For the estimation of the total impact of the vaccination on children eligible for the vaccination, the vaccine eligible cohorts (VEC, children born 2011 and later, sampled in 2013–2015) were compared to the control group (CG, children born 2010 and earlier, sampled in 2009–2012). To attain comparable age distribution, only children <4 years of age were included. To minimize the potential bias caused by the herd effect, CG children sampled in 2013 and later were not included.

Study 2. For the estimation of the herd effect on children not eligible for the vaccination, children born 2010 and earlier were compared before (sampled 2009–2011, PreVac period) and after

Table 1
Study design.

Study	Study groups	Age inclusion criteria	Birth cohort inclusion criteria	Sampling year inclusion criteria	Mean age	Median age (range)	Sample size
Study 1: total impact	CG	<4 years	2005–2010	2009–2012	2.9	2.9 (1.2–4.0)	853
	VEC	<4 years	2011–2013	2013–2015	2.8	2.8 (1.1–4.0)	371
Study 2: herd effect	PreVac	3.5–6.3 years	2003–2007	2009–2011	5.0	5.0 (3.6–6.3)	847
	PostVac	3.5–6.3 years	2007–2010	2013–2015	5.0	5.0 (3.6–6.2)	949

Two independent studies were conducted.
In Study 1 (Total impact), only children less than 4 years of age were included. Children born before the vaccine initiation (2010 and earlier, Control Group, CG), sampled in 2009–2012 were compared to children born after the vaccine initiation (2011 and later, Vaccine Eligible Cohort, VEC).
In Study 2 (Herd effect), only children between the ages 3.5 years and 6.3 years, born before the vaccination (birth-cohort 2010 and earlier) were included. Children sampled in 2009–2012 were compared to those sampled in 2013–2015.

(sampled 2013–2015, PostVac period) the vaccination. To attain comparable age distribution only children >3.5 and <6.5 years of age were included. Isolates sampled in 2012 were excluded as they belonged to the transition year. Sixteen children, born in 2007 were sampled both in 2011 and 2013, were excluded from the analysis.

In analysis of antibiotic non-susceptibility, non-typeable pneumococci were excluded, as they are not considered an important cause of disease in immunocompetent hosts.

The study was approved by The National Bioethics Committee (VSNb2013010015/03.07), The National Data Protection Authority (2013010100VEL/–), The University Hospital medical director and the appropriate directorates of the DCC's. The study is a part of a larger study on vaccinations in Iceland (The VIce study).

3. Results

3.1. Study on the impact on the vaccine eligible cohorts

The number of children was 853 in the CG and 371 in the VEC. The average age in the CG was 2.89 and 2.80 in the VEC ($p < 0.05$), the median age and ranges were 2.90 (1.15–4.00) and 2.80 (1.10–4.00) (Table 1). Males were 51.4% and 53.7% ($p = 0.45$) of children in CG and VEC respectively. Dual carriage was 7.5% in the CG and 4.3% in the VEC ($p = 0.051$). No difference was found in overall carriage prevalence of pneumococcus (69.5% vs 70.1%, $p = 0.84$). Parent-reported recent antibiotic use was lower in the VEC group than in the CG (17.8% vs 23.2%, $p < 0.05$), with no difference in parent-reported respiratory tract infections (RTIs) in the previous 6 months (43.8% vs 41.8%, for the CG and VEC respectively, $p = 0.54$).

The vaccine impact for VT carriage was 94% (95% CI: 91–96%), with reduction of individual serotypes ranging from 84% to 100%. The combined impact against the VaT (6A and 19A) was 33% (95% CI: 1–55%), with non-significant individual impact being 33% (95% CI: −9%; 59%) and 29% (95% CI: −31%; 61%) for 6A and 19A respectively (Tables 2 and 5). Serotype replacement was noted with non-vaccine serotypes being more common in the VEC than CG (53% vs 18%, respectively). Serogroup 15 was the most prevalent isolate in the VEC, 9.6% compared to 3.9% in the CG. The greatest difference was noted for Serotype 23B (0.8% vs 7.8% for CG and VEC, respectively) (Table 2).

The prevalence of PNSP isolates in CG and VEC was similar. When comparing these groups after stratifying the MIC into low vs high MIC a significant difference was found between the CG and VEC, where the isolates from the VEC were mainly in the low MIC range and the CG mainly in the high MIC range (Table 3).

The prevalence of co-trimoxazole and erythromycin resistance was higher in the CG than VEC, 22.1% and 13.1% vs 12.1% ($p < 0.001$) and 9.0% ($p < 0.05$) respectively. The prevalence of clindamycin, tetracycline, erythromycin and penicillin non-susceptible strains did not differ between the two cohorts. Resistance to ≥ 3 antibiotic classes were more common in the CG than the VEC 9.4% vs 1.6% respectively ($p = 0.003$). Isolates that showed non-susceptibility to all antibiotics tested (penicillin, erythromycin, co-trimoxazole, tetracycline and clindamycin) were also more common in the CG, 4.4% vs 0.3% ($p < 0.001$) respectively (Table 3). Serotypes/groups non-susceptible to ≥ 3 antibiotic classes belonged to serotypes 19F (n = 73), 6B (n = 6), 14 (n = 2) and 6C (n = 1) in the CG and 15 (n = 8), 19F (n = 5) and 6C (n = 3) in the VEC. Isolates non-susceptible to all five antibiotics tested belonged to serotypes 19F (n = 30), 6B (n = 6) and 14 (n = 2) in the CG and 19F (n = 1) in the VEC.

3.2. Study on the herd effect

There were 831 children in the pre-vaccination (PreVac) period and 933 in the post vaccination (PostVac) period. The average age in the PreVac period was 4.97 and 4.98 in the PostVac period ($p = 0.83$) and median ages (with ranges) were 4.96 (3.59–6.33) and 5.00 (3.59–6.22) respectively (Table 1). Males were 49.4% and 51.2% of participants in PreVac and PostVac respectively. Dual carriage was seen in 5.8% and 5.0% respectively ($p = 0.56$). No difference was found in overall carriage (62.6% vs 64.4%, $p = 0.42$ for PreVac period and PostVac period, respectively).

For the PostVac the vaccine impact against acquisition of VT was 56% (95% CI: 44–65%) and 33% (95% CI: 7–51%) for 6A and 19A. A significant VEacq was found for 6A and VTs 6B, 9V, 14, 18C and 23F. (Tables 4 and 5). Further analysis of the 19F carriage prevalence showed stable prevalence over the study-period of study 2 with 4.2%, 2.7%, 4.7%, 4.2%, 4.5% and 3.1% of isolates being 19F for the study years 2009, 2010, 2011, 2013, 2014 and 2015 respectively. Of the 39 isolates of serotype 19F identified in the PostVac period, seven were sampled from children that carried 19F more than once, and up to 3 years in-a-row. Vaccine serotypes 1, 5 and 7F were not isolated. Serotype replacement was noted, but no change in total NP carriage. Non-vaccine serotypes increased from 24.5% of all isolates in the PreVac to 42.6% in the PostVac. Serotypes 6C, 10, 21, 22, 23A, 23B, 35B and 35F/47F all differed significantly between the periods and were more common in the PostVac period. Serotype 23B differed the most between the periods, 0% vs 4.5% in the PreVac and PostVac, respectively (Table 4).

The prevalence of PNSP was 5.0% in both study periods ($p > 0.99$). The prevalence of erythromycin, clindamycin, tetracycline, combined penicillin and erythromycin and multi resistance did not differ significantly between the PreVac and PostVac periods (6.2% vs 4.4%, $p = 0.12$, 3.6% vs 2.5%, $p = 0.26$, 5.2% vs 5.1%, $p = 0.97$, 4.2% vs 3.7%, $p = 0.70$ and 5.0% vs 3.3%, $p = 0.09$, respectively). The prevalence of co-trimoxazole non-susceptibility was significantly higher in the PreVac than in the PostVac (13.4% vs 8.3%, $p < 0.001$).

19F was the dominant serotype causing penicillin non-susceptibility and multi-resistance in both periods, causing 66.7%

Table 2
PHiD-CV vaccine impact estimates by serotype, for vaccine eligible cohort compared to control cohort.

Serotype		Number of CG isolates (%)	Number of VEC isolates (%)	VEacq (95% CI)	p-value
VT	VT total	322 (35.1)	12 (3.1)	0.94 (0.91; 0.96)	<0.001
	6B	77 (8.4)	2 (0.5)	0.94 (0.84; 0.98)	<0.001
	9V	3 (0.3)	0 (0)	1.00 (−6.57; 1.00)λ	0.26
	14	57 (6.2)	0 (0)	1.00 (0.85; 1.00)λ	<0.001
	18C	6 (0.7)	0 (0)	1.00 (−2.04; 1.00)λ	0.11
	19F	84 (9.2)	6 (1.6)	0.84 (0.67; 0.93)	<0.001
	23F	95 (10.4)	4 (1)	0.91 (0.80; 0.96)	<0.001
VAT	VAT total	122 (13.3)	36 (9.3)	0.33 (0.01; 0.55)	0.04
	6A	76 (8.3)	22 (5.7)	0.33 (−0.09; 0.59)	0.1
	19A	46 (5)	14 (3.6)	0.29 (−0.31; 0.61)	0.27
NVT	NVT total	169 (18.4)	207 (53.5)	−4.09 (−5.53; −2.97)	<0.001
	3	14 (1.5)	11 (2.8)	−0.89 (−3.14; 0.14)	0.11
	6C	8 (0.9)	25 (6.5)	−6.85 (−14.6; −2.94)	<0.001
	10	0 (0)	8 (2.1)	Not calculated	
	11	32 (3.5)	32 (8.3)	−1.49 (−3.07; −0.53)	<0.001
	15	36 (3.9)	37 (9.6)	−1.59 (−3.10; −0.63)	<0.001
	16F	9 (1)	2 (0.5)	0.48 (−1.37; 0.88)	0.4
	21	4 (0.4)	10 (2.6)	−5.05 (−15.9; −1.17)	<0.001
	22	2 (0.2)	5 (1.3)	−4.99 (−24.5; −0.41)	0.02
	23A	19 (2.1)	18 (4.7)	−1.31 (−3.37; −0.22)	0.01
	23B	7 (0.8)	30 (7.8)	−9.92 (−20.5; −4.56)	<0.001
	29/35B	5 (0.6)	9 (2.3)	−3.34 (−10.9; −0.58)	0.004
	33	13 (1.4)	2 (0.5)	0.64 (−0.51; 0.91)	0.16
	35F/47F	0 (0)	10 (2.6)	Not calculated	
	38	8 (0.9)	1 (0.3)	0.71 (−1.09; 0.96)	0.22
	Other	7 (0.8)	6 (1.6)	−1.05 (−5.00; 0.30)	0.19
	NT	47 (5.1)	22 (5.7)	−0.12 (−0.88; 0.34)	0.68
	NONE	261 (28.5)	111 (28.7)	−0.01 (−0.32; 0.22)	0.94
Total		917 (100)	387 (100)		

Direct Vaccine impact on carriage prevalence. NVEC: Non-Vaccine eligible cohort, CG: Control group. Children in the CG sampled 2013 and later were excluded to prevent possible bias caused by herd immunity. λ Conservative one-sided 95% confidence interval for vaccine impact. The total number is higher than the number of children because some children carried more than one serotype.

Table 3
Antibiotic non-susceptibility for vaccine eligible cohort compared to non-vaccine eligible cohort.

Non-susceptibility to:	Number CG isolates (%)	Number VEC isolates (%)	OR (95% CI)	p value
PNSP (all isolates)	97 (11.2%)	38 (10.4%)	0.92 (0.62; 1.38)	0.76
Penicillin (Low MIC)	9 (1.0%)	33 (9.0%)	9.48 (4.54; 20.3)	<0.001
Penicillin (High MIC)	88 (10.1%)	5 (1.4%)	0.12 (0.047; 0.31)	<0.001
Erythromycin	114 (13.1%)	33 (9.0%)	0.66 (0.44; 1.00)	0.05
Tetracycline	95 (10.9%)	31 (8.5%)	0.76 (0.48; 1.16)	0.22
Clindamycin	54 (6.2%)	26 (7.1%)	1.15 (0.70; 1.16)	0.61
Co-trimoxazole	192 (22.1%)	44 (12.1%)	0.48 (0.34; 0.69)	<0.001
Penicillin and Erythromycin	82 (9.4%)	30 (8.2%)	0.86 (0.55; 1.33)	0.59
≥3 antibiotic classes	82 (9.4%)	16 (4.4%)	0.44 (0.25; 0.77)	0.003
All 5 tested antibiotics	38 (4.4%)	1 (0.27%)	0.06 (0.003; 0.37)	<0.001
Number of isolates	869	365		

Antimicrobial non-susceptibility in the CG and the VEC groups. For isolates with penicillin non-susceptibility non-typeable pneumococci were excluded. *PNSP penicillin non-susceptible pneumococci. The denominator signifies the number of isolates (number of children sampled + number of children with dual carriage). CG: Control Group. VEC: Vaccine Eligible Cohort.

(28/42) of penicillin non-susceptibility and 64.3% (27/42) of multi-resistance in the PreVac period and 45.8% (22/48) of PNSP and 71.0% (22/31) of multi-resistance in the PostVac period.

The PreVac period reported 6.2% higher RTIs in the last 6 months (27.6 vs 21.4%, p = 0.003) and 3.3% more antibiotic usage in the last 3 months than the PostVac (12.2 vs 8.9%, p = 0.02).

4. Discussion

The vaccine impact, determined as a reduction of VT pneumococci in the nasopharynx was very high and some VT serotypes were not detected in the VEC and thus arguably eliminated from the nasopharynx by the vaccination. This was also seen for unvaccinated children after the initiation of the vaccination – although with lower impact, indicating an important herd effect. This is in line with other studies on the PCVs which have showed reduction in VT pneumococci [9,15,19,21–24,26,32]. Interestingly, despite strong direct effect on carriage of 19F, no obvious herd effect was observed. As 19F is the most antibiotic resistant serotype in this study its persistence in older, unvaccinated children is of concern. Of the 40 children carrying 19F in the PostVac period, seven carried the serotype, at one to three sampling-occasions. Persistent, continuous low level carriage of 19F has been described in older children after initiation of PCV [33].Waning immunity in older children [34] and longer duration of carriage may be important factors for this persistent or repeated carriage of 19F [35–37].

Carriage was similar as in earlier studies conducted in Iceland [5,7] but higher than most other studies [9,21,22]. This high carriage can partly be due to the study design, as all isolates were plated within six hours and cultured on selective medium under

Table 4
Indirect PHiD-CV vaccine impact estimates by serotype for PreVac compared to PostVac.

Serotype		PreVac period (%)	PostVac period (%)	VEacq (95% CI)	p value
VT	VT total	219 (24.91)	125 (12.76)	0.56 (0.44; 0.65)	<0.001
	4	2 (0.23)	0 (0)	1.00 (−3.65; 1.00)λ	0.14
	6B	51 (5.8)	27 (2.76)	0.54 (0.27; 0.71)	0.001
	9V	13 (1.48)	0 (0)	1.00 (0.68; 1.00)λ	<0.001
	14	26 (2.96)	14 (1.43)	0.53 (0.10; 0.75)	0.02
	18C	28 (3.19)	10 (1.02)	0.69 (0.38; 0.84)	<0.001
	19F	34 (3.87)	39 (3.98)	−0.03 (−0.65; 0.36)	0.90
	23F	65 (7.39)	35 (3.57)	0.54 (0.30; 0.69)	<0.001
VAT	VaT total	95 (10.81)	74 (7.55)	0.33 (0.07; 0.51)	0.01
	6A	50 (5.69)	32 (3.26)	0.44 (0.12; 0.64)	0.01
	19A	45 (5.12)	42 (4.29)	0.17 (−0.28; 0.46)	0.40
NVT	NVT total	215 (24.46)	417 (42.55)	−1.29 (−1.79; −0.88)	<0.001
	3	58 (6.60)	46 (4.69)	0.30(−0.04; 0.53)	0.07
	6C	4 (0.46)	18 (1.84)	−3.09 (−10.17; −0.50)	0.006
	9A/N/L	9 (1.02)	13 (1.33)	−0.30 (−2.05; 0.45)	0.55
	10	4 (0.46)	13 (1.33)	−1.94 (−7.60; −0.01)	0.05
	11	42 (4.78)	42 (4.28)	0.11 (−0.38; 0.42)	0.61
	15	27 (3.07)	45 (4.60)	−0.52 (−1.46; 0.06)	0.09
	16F	14 (1.59)	8 (0.82)	0.49 (−0.20; 0.78)	0.12
	21	5 (0.57)	32 (3.27)	−4.90 (−12.6; −1.56)	<0.001
	22	5 (0.57)	39 (3.98)	−6.25 (−15.2; −2.25)	<0.001
	23A	16 (1.82)	33 (3.37)	−0.88 (−2.41; −0.04)	0.04
	23B	0 (0)	44 (4.49)	Not calculated	
	33	5 (0.57)	10 (1.02)	−0.80 (−4.21; 0.38)	0.28
	35B	3 (0.34)	26 (2.65)	−6.96 (−20.1; −1.89)	<0.001
	35F/47F	0 (0)	31 (3.16)	Not calculated	
	38	12 (1.37)	5 (0.51)	0.63 (−0.01; 0.87)	0.05
	Other	11 (1.25)	12 (1.2)	0.02 (−1.23; 0.57)	0.96
	NT	38 (4.32)	33 (3.37)	0.23 (−0.24; 0.52)	0.28
	NONE	312 (35.50)	331 (33.78)	0.07 (−0.12; 0.24)	0.44
Total		879 (100)	980 (100)		

Indirect impact of the vaccination. PreVac period vs the PostVac period (study years: 2009–2011 vs 2013–2015). Only isolates from children born 2010 and earlier & >3.5 years of age were included. λ Conservative one-sided 95% confidence interval for vaccine impact. The total number is higher than the number of children due to some children carrying more than one serotype.

Table 5
Vaccine impact of study 1 and study 2.

Study	Impact on overall pneumococcal carriage (95% CI)	Impact against vaccine types (95% CI)	Impact against vaccine associated types (95% CI)		
			Overall	6A	19A
Study 1	1% (−32; 22%)	94% (91; 96%)	33% (1; 55%)	33% (−9; 59%)	29% (−31; 61%)
Study 2	7% (−12; 24%)	56% (44; 65%)	33% (8; 51%)	44% (12; 64%)	17% (−28; 46%)

Impact on carriage prevalence in study 1 and in study 2. In study 1 Vaccine Eligible Children (birth-year 2011 and later) were compared to the Control Group (birth-year 2010 and earlier) and in study 2 older, Non-Vaccine-Eligible children (born 2010 and earlier) were compared before (calendar year: 2009–2011) and after the vaccination (calendar year: 2013–2015).

anaerobic conditions. In addition, the isolates were sampled in March every year when carriage may be higher during and shortly after the frequent winter infections in this age group [8,38].

The total nasopharyngeal carriage remained high throughout our study despite the reduction of VT. This can be attributed to serotype replacement with NVTs. Serotype replacement has been widely recognized after introduction of the pneumococcal vaccines [9,12,21–26]. In the current study, non-vaccine serotypes/groups most prevalent in the post vaccination years were 15, 11, 23B, 23A, 6C all with large increases in prevalence. This is similar to other studies on PHiD-CV and PCV-13 [9,19,21,25,32], although studies on PCV-13 generally show no difference or reduction in the prevalence of 6C. This indicates that replacing serotypes in Iceland are similar to others using the higher valent vaccines.

For the VEC, a non-significant reduction was found for serotypes 6A and 19A, when considering each serotype individually, but with a significant reduction in the combined prevalence. This is interesting primarily due to the magnitude of serotype replacement witnessed with other serotypes. Similar results were seen for the PostVac period, which had significant herd effect on the carriage of 6A but not 19A. Various studies have shown an increase in the prevalence of 19A after the implementation of the PCV-7 vaccine, due to serotype replacement [15,21,23,24,32,39]. The PHiD-CV direct effectiveness against 19A IPD has been reported in recent years [2], with data suggesting similar direct effectiveness as the PCV-13 vaccine [40]. Similar findings, albeit milder have been reported for 6A IPD [13,19] and carriage of both 6A and 19A [2,19], although other studies show less or no effectiveness against acquisition [39,41,42]. This apparent discrepancy, where more consistent data on 19A IPD effectiveness is available than for carriage, could be explained by the fact that the protection against IPD is more complicated than can be explained by acquisition alone [10].

This study shows replacement of serotypes non-susceptible to penicillin, with the replacing serotypes exhibiting lower MIC. This has been reported after the introduction of PCV-7 [23].

Pneumococcal isolates cultured from the VEC had less erythromycin and co-trimoxazole resistance and were less often multi-resistant as compared to CG. The reduction noted for the VEC was mainly driven by the reduction in serotype 19F which was the main PNSP and multi-resistant serotype in the pre-vaccination period.

Due to a lack of herd effect on serotype 19F in the PostVac cohort, as discussed above, no change in PNSP or multi resistance was noted, but there was a reduction in co-trimoxazole resistance.

The resistant and multi-resistant NVTs, mainly of serogroup 15, but also serotypes 6C and 23B, are emerging in the post vaccination period. Reports on serogroup 15 have shown it to be one of the most prevalent resistant serogroups in many parts of the world [32,43]. Other studies have shown a decrease in non-susceptible pneumococci after vaccination [9,22,44] or even no effect on antibiotic resistance [45]. Many factors may influence this, the most important being the level of antibiotic resistance in both the vaccine serotypes and the replacing serotypes, the selective pressure from antibiotic usage, the level of day-care attendance, vaccine uptake in the study population in addition to study size and design [5–9].

Different capsular types have varying invasive potential [10,46]. Replacing invasive [46], antibiotic resistant vaccine types with more benign ones may result in a reduction in pneumococcal infections and treatment failures. Parent-reported antibiotic usage by children in the VEC was lower than by children in the CG, yet with no difference in RTIs. In the herd effect analysis, children in the PostVac period had less antibiotic consumption and fewer recent RTIs than those in the PreVac period. As this is parent-reported and thus prone to recall bias, conclusions on vaccine impact on RTI must be drawn with caution. The reduction noted here is however in line with other studies that show fewer infections after PCVs [12–18,20].

The strength of this study is its size, its well-defined groups and high vaccine uptake. All culturing and serotyping was done at the same laboratory, using exactly the same methods and protocols throughout the study period. The weakness is that it is a pre-post analysis where the groups compared are sampled in different years, and subject to different external factors, such as fluctuations in serotype prevalence and incidence of viral infections that influence both pneumococcal carriage and disease. This cannot be easily controlled in a study such as this and must be acknowledged as a source of potential bias. However, the study periods compared are directly adjacent to each other minimizing such differences.

Studies like this one, with monitoring of serotype prevalence, serotype replacement and antibiotic resistance are important for public health policies and treatment guidelines. Moreover, they add to the important knowledge base needed for deciding which serotypes should be included in the next generation of pneumococcal vaccines. Importantly, it shows that the vaccination has great impact against all VT in vaccinated children in addition to herd effect on unvaccinated children contributing to lowering antimicrobial resistance rates.

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