



Prevalence of pneumococcal nasopharyngeal colonization and serotypes circulating in Cameroonian children after the 13-valent pneumococcal conjugate vaccine introduction



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ABSTRACT

Background: *Streptococcus pneumoniae* remains a major contributor to childhood infections and deaths globally. In Cameroon, the 13-valent pneumococcal conjugate vaccine (PCV13) was introduced in July 2011, using a 3-dose Expanded programme on immunization (EPI) schedule administered to infants at 6, 10 and 14 weeks of age. To evaluate PCV13 effects, we assessed pneumococcal nasopharyngeal colonization and serotype distribution among Cameroonian children after PCV13 introduction.

Methods: Nasopharyngeal (NP) swabs were collected from eligible children aged 24–36 months in two cross-sectional surveys conducted from March to July: in 2013 (PCV13-unvaccinated), and in 2015 (PCV13-vaccinated). Using a systematic World Health Organization (WHO) cluster coverage sampling technique in 40 communities, NP swabs collected were processed following WHO recommendations. Standard bacterial culture techniques were used for the isolation of *S. pneumoniae* from gentamicin-blood agar plates and identification using optochin susceptibility testing. Serotyping was performed using sequential multiplex polymerase chain reaction, supplemented with Quellung test.

Results: Among the PCV13-vaccinated children, overall pneumococcal carriage prevalence was 61.8% (426/689) and PCV13 vaccine-type carriage prevalence was 18.0% (123/689). Eleven out of the 13 vaccine serotypes were detected in the vaccinated children. The most common serotypes were 19F (4.5%, 31/689) and 15B/C (7.3%, 50/689).

Conclusion: In Cameroon, four years after infant vaccination nearly all of the PCV13-serotypes continued to circulate in the population. This suggests that the direct and indirect effects of the vaccination programme have not resulted in expected low levels of vaccine-type transmission. Continuous monitoring is needed to assess the long term effects of the PCV13 on nasopharyngeal carriage and disease.

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Introduction

Streptococcus pneumoniae with its over ninety serotypes remains a major contributor to childhood infections and deaths globally (Neves et al., 2013; CDC, 2006). Its reported burden of morbidity and associated mortality is striking mostly in children in resource-low settings, like Cameroon, who are most often exposed to nasopharyngeal colonization (NP) shortly after birth

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(Kwambana et al., 2011). NP colonization is a prerequisite for the development of pneumococcal disease and a reservoir for pneumococcal transmission in the community (Sleeman et al., 2005). The ecological habitat of *S. pneumoniae* and other bacteria including *Haemophilus influenzae*, *Moraxella catarrhalis* and/or *Staphylococcus aureus* is the nasopharynx from where they can spread into the lower respiratory tract or the bloodstream and cause severe infections such as meningitis, sepsis or pneumonia (Bogaert et al., 2004; Simell et al., 2012).

There is a paucity of data in most low-income countries on the incidence of pneumococcal infections and other invasive diseases, and the epidemiology of circulating pneumococcal serotypes in either infant or adult populations (Kwambana et al., 2011; von Gottberg et al., 2014; Grant et al., 2016; Hammitt et al., 2014; Nzenze et al., 2013). However, a study on the epidemiologic pattern of bacterial meningitis and pneumococcal serotype distribution in Cameroon two years before vaccination reported the predominance of *S. pneumoniae* as the major pathogen among 170 hospitalized children with meningitis aged 2 months to 15 years (Gervais et al., 2012). The prevalence of pneumococcal nasopharyngeal carriage in any healthy population in Cameroon has not been documented.

The main reservoir and primary transmitters of the pneumococci are thought to be young children (Kwambana et al., 2011). To understand the epidemiology and monitor the effects of the PCV programmes, the pneumococcal nasopharyngeal carriage (NPC) in children has often been used to assess the impact of the PCV on vaccine-type carriage and replacement of vaccine-types with non-vaccine serotypes (Weinberger et al., 2011). The reduction in vaccine-type carriage decreases the transmission of the vaccine-type disease. Serotype replacement, on the other hand, can reduce the positive benefits of PCV when the non-vaccine types have increased both in carriage and disease as has been previously reported (von Gottberg et al., 2014; Weinberger et al., 2011; Mackenzie et al., 2016; Hill et al., 2008). Therefore, it is essential to evaluate the effects of PCV13 on NP carriage after vaccine introduction, especially in a low-income country like Cameroon where carriage prevalence and disease incidence are expected to be high in younger children (Cutts et al., 2005; Hill et al., 2008). Further, implementation of PCV programmes need to be monitored continuously by an appropriate surveillance system, in order to assess impact of vaccination and epidemiological shifts in disease potentially related to it (Dunne et al., 2018). Where appropriate surveillance systems are lacking as in Cameroon, carriage studies have shown to be a cheaper alternative in assessing such changes (Roca et al., 2013). We assessed NPC and serotype distribution in PCV13-unvaccinated and PCV13-vaccinated children four years after PCV13 introduction.

Methodology

Ethical consideration

The Institutional Review Boards (IRBs) of the Cameroon National Ethics Committee and the Yaoundé Gynaecology, Obstetric and Paediatric Hospital, approved the study. Signed informed consent forms were obtained from all parents who agreed for their children to participate in the study.

Pneumococcal conjugate vaccination implementation in Cameroon

In Cameroon, the 13-valent PCV was introduced in July 2011, using a 3-dose EPI schedule administered to infants at 6, 10 and 14 weeks of age. There were no catch-up schedules for older children. The PCV13 was included into the EPI based on recommendations and financial support from WHO and the Global Vaccine Alliance

initiatives (GAVI) (WHO, 2019), respectively, without any baseline data on NP carriage prevalence and invasive pneumococcal disease (IPD) incidence in Cameroon. Since 2011, EPI uptake for the third dose of diphtheria, pertussis and tetanus combination vaccine (DPT3) has been over 80% (World Health Organization, 2018); suggesting a high uptake of childhood immunization programmes in Cameroon.

Study design

Two cross-sectional surveys were conducted to collect NP swabs from children aged 24–36 months old, from March through July in 2013 (PCV13-unvaccinated) and 2015 (PCV13-vaccinated), in Yaoundé, Cameroon.

Characterization of the study population and sites

The study sites and population have been described previously (Libwea et al., 2018). Briefly, they involved localities situated within an 80 km radius from Yaoundé, Cameroon's capital city. Yaoundé and its surroundings harbour a population of over 3.5 million, out of which 18% are children aged under-five years, based on 2010 National Population Census. The sites were chosen as they constitute a group of health institutions described as the invasive disease sentinel surveillance. Sites are partitioned into 40 communities (clusters) using the health map and with each cluster hosting at least one health center/clinic, either public or private.

Inclusion and exclusion criteria

Subjects included in the study were children aged 24–36 months old; PCV-unvaccinated (for the 2013 group) and PCV-vaccinated (for the 2015 group); residing in the study area within the last 6 months prior to sampling and had a signed parental consent form. Children who did not show proof to have received at least one dose of PCV13 were excluded in the 2015 round. The proportion of children who had received 3 complete PCV13 doses was 92.5% (637/689) and for 2PCV13 doses was 7.5% (52/689). In both rounds, subjects with severe illnesses (e.g. malaria, sickle cell conditions or infectious diseases) or on antimicrobial treatment at the time of sampling were excluded.

Enrolment of subjects

In both surveys and in each study cluster, the first home to begin subject enrolment was selected randomly depending on the direction of pointer after spinning a pen at a central location e.g. church premises or market square. Subsequent subjects were enrolled through the WHO lot quality clustered sampling technique after every 10th home to provide representation within a cluster, and twenty-five children were targeted per cluster, as earlier described (Libwea et al., 2018).

Upon recruitment, parents were asked to bring their children to the study clinics where a pneumococcal nasopharyngeal sample was collected. For three successive days prior to and including the day of sampling, a community facilitator together with the study personnel with authorisation from local leaders made a public announcement to the community to inform or remind parents about the study.

Data collection procedure

Study personnel provided oral information and obtained informed consent from parents. Eligible subjects were then sampled at the study clinics and standard case report forms were

used to document demographic and clinical information following parental/caretaker interview, and a NP swab was collected from the child.

Pneumococcal nasopharyngeal samples and laboratory procedures

In accordance with WHO guidelines (Satzke et al., 2013), trained study personnel collected deep nasopharyngeal swabs by means of a sterile, flexible aluminum shaft and a dry cotton-wool tip inserted through the nasal cavity reaching the posterior nasopharyngeal area until some resistance was felt. The swab stayed fixed for a couple of seconds before being slowly withdrawn. NP samples were then inoculated immediately into vials containing 1.0 ml of skim milk–tryptone–glucose–glycerol (STGG) transport medium and placed in a cold box prior to transfer to the Mother and Child Hospital (MCH) bacteriology laboratories (a radius-distance of about 80 km from the study sites), within 8 h of collection according to WHO recommendations (Satzke et al., 2013). Inoculated vials were stored at -70°C until they were transported in dry ice containing boxes. The 2013 samples were transported from Cameroon first to the National Institute for Health and Welfare (THL), Oulu, Finland and the 2015 samples to THL, Helsinki, Finland before they were all transported to the bacteriology laboratories at the Institute of Biomedicine, Research Center for Cancer, Infections and Immunity, University of Turku, Finland, for bacterial isolation. However, during shipment from Cameroon to Finland, the 2013 specimens were delayed for over two months at a port of entry in Germany and were reportedly stored at -20°C .

Isolation and identification of pneumococci from nasopharyngeal samples

As previously reported (Kaijalainen and Palmu, 2015), in 2017 nasopharyngeal samples were cultured for the isolation of *S. pneumoniae*. After thawing and passage through a vortex rotator for thorough mixing, 10 μl of each specimen was pipetted and inoculated on 5% sheep blood agar plate (for semi-quantitative evaluation of the growth) and 5% sheep blood agar + 2.5 $\mu\text{l}/\text{ml}$ of gentamicin for isolation of *S. pneumoniae*. The plates were incubated in 5% carbon dioxide atmospheric conditions at 35°C for 18–20 h. If no colonies appeared, the incubation was continued

for up to 48 h. Pneumococci were identified from both plates by their morphological α -hemolytic characteristics and by optochin sensitivity testing. From each sample, if several suspected pneumococcal colonies appeared, up to four colonies were confirmed and stored. The isolates were stored in 10% skimmed milk-glycerol and sent in batches on dry ice for serotyping at the bacteriology laboratory of THL, in Helsinki, Finland.

Serotyping of pneumococcal isolates

In accordance with a previously validated typing scheme (Siira et al., 2012), based on sequential multiplex polymerase chain reaction (mPCR) supplemented with Quellung test, when needed, pneumococcal isolates were serotyped. In mPCRs, a primer pair targeting the pneumococcal specific *cpsA* locus was used as an internal control. The species of *cpsA* negative, Omni serum negative suspected non-encapsulated (NC) pneumococci were verified by *lytA* PCR. Serotypes were categorized as vaccine types (VT: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F), vaccine-related serotypes (VRT: 6C, 7A, 7B, 7C, 9A, 9N, 9L, 18A, 18B, 18F, 19B, 23A, and 23B), non-vaccine types (NVT) and NC pneumococci. Serotypes 15B and 15C were reported together as 15B/C because of the reversible capsule switching between these serotypes (Van Selm et al., 2003). To assess for co-colonization by multiple pneumococcal serotypes, randomly selected duplicate pneumococcal isolates obtained from 13.5% ($n = 70$) of the swabs (15/108 isolates obtained from 2013 swabs and 55/406 isolates obtained from 2015 swabs) were also serotyped. Although more colonies were available for testing, we could only swab for 70 due to limited resources.

Statistical analyses

The intention of the study was to compare 2013 and 2015 NP samples but this was hampered by transport delays and subsequent high temperature exposure which might have compromised the 2013 samples. Based on literature, we assumed the estimated carriage prevalence of any pneumococcus circulating in the population to be around 60% (Gervais et al., 2012); the proportion of vaccine-type pneumococci out of all: 62%, i.e. 37.2% out of all subjects, expected reduction of carriage of vaccine-type is

Table 1

Comparison of baseline characteristics of 887 children 2–3 years old sampled in 2013 (PCV13-unvaccinated, $n = 198$) and in 2015 (PCV13-vaccinated, $n = 689$) for nasopharyngeal specimens collected in Yaoundé, Cameroon.

Characteristics	PCV13-unvaccinated Cohort (2013) N (%)	PCV13-vaccinated cohort (2015) N (%)	p-Value*
Gender			
Male	117 (59.1)	372 (54.0)	0.204
Female	81 (40.9)	317 (46.0)	
No. of children <18years old in household			
One	66 (33.3)	101 (14.7)	<0.0001
Two	56 (28.3)	175 (25.4)	
≥Three	76 (38.4)	413 (59.9)	
Respiratory symptoms within last 30 days prior to sampling			
No	18 (9.6)	186 (27.0)	<0.0001
Yes, but the child has been symptomless for 1–7 days	155 (78.3)	433 (62.8)	
Yes, but the child has been symptomless for 8–14 days	8 (4.1)	19 (2.8)	
Yes, but the child has been symptomless for 15–28 days	13 (6.6)	9 (1.3)	
Unknown	3 (1.5)	42 (6.1)	
Antimicrobial treatment within last 3 months prior to sampling			
No	168 (84.8)	563 (81.7)	0.59
Yes but treatment stopped 1–2 months ago	8 (4.0)	35 (5.1)	
Yes but treatment stopped 2–3 months ago	22 (11.1)	91 (13.2)	
Source of cooking fuel (wood)			
Yes	147 (74.2)	511 (74.2)	0.983
No	51 (25.8)	178 (25.8)	

* p-Value estimates reflecting the difference in pneumococcal carriage status were computed using the Pearson Chi-square Test.

at least 50% i.e. from 37.2% to 18.6% after vaccination. Using the online tool <http://statpages.org/proppowr.html>, the minimum sample size for this study was 101 subjects per group to achieve 80% power. However, to increase the study statistical power, more subjects were sampled in 2015. We assessed pneumococci serotype distribution after PCV13 implementation and used the χ^2 -test to compare differences between the PCV13-vaccinated and PCV13-unvaccinated groups (Tables 1 and 2). Statistical significance level was set at 5%. The statistical software package SPSS version 25.0 was used for analyses.

Results

Demographic and risk factors associated with pneumococcal carriage in 2015 compared with 2013

Of the over 1250 potentially eligible subjects contacted in both surveys, 887 were enrolled (71%). Altogether, 94 subjects were excluded in the final analyses either because they were not within the age range of 24–36 months or not residing in the study areas. The remainder were excluded because of lack of signed parental consent (n = 183) or due to severe illnesses (n = 54) or because they were on antimicrobial treatment at the time of sampling (n = 32). Nasopharyngeal swabs were collected from a total of 887 children

Table 2
Prevalence of pneumococcal serotypes in the unvaccinated (2013) and vaccinated (2015) children 2–3 years old in Yaoundé, Cameroon.

Serotype	Unvaccinated group		Vaccinated group	
	N = 198 (% ^a)		N = 689 (%)	
	N	%	n	%
Vaccine-serotypes				
1	–	–	–	–
3	1	0.5	9	1.3
4	–	–	2	0.3
5	–	–	1	0.2
6A	7	3.5	16	2.3
6B	5	2.5	19	2.7
7F	–	–	–	–
9V	1	0.5	3	0.4
14	2	1.0	22	2.8
18C	1	0.5	1	0.2
19A	1	0.5	6	0.9
19F	14	7.1	31	4.5
23F	10	5.1	15	2.2
Sub-total	42	21.2	124	18.0
Vaccine-related serotypes				
7C	1	0.5	3	0.4
9L	1	0.5	1	0.2
18A	–	–	2	0.3
19B	4	2.0	13	1.9
23A	–	–	3	0.4
23B	1	0.5	15	2.2
Sub-total	7	3.5	37	5.4
Non-vaccine serotypes				
15A	3	1.5	22	3.0
15B/C	18	9.1	50	7.3
21	6	3.0	9	1.3
34	6	3.0	18	2.6
38	4	2.0	23	3.3
OTHERS ^b	18	11.6	93	14.2
Sub-total	55	27.8	215	31.2
NC	10	5.1	50	7.3
Total	114	57.6	426	61.9

^a Prevalence of serotype-specific carriage (Number of serotype detected divided by total number of samples); NC = non-encapsulated pneumococci; % = percent; n = number.

^b In the NVT category, only the most prevalent serotypes have been presented individually. The rest for analytical purposes were grouped as "OTHERS". However, all individual serotypes are outlined in Figure 1.

aged 24–36 months, 198 in 2013 (PCV13-unvaccinated) and 689 in 2015 (PCV13-vaccinated), from March through June of each year. The demographic and risk factor variables are shown in Table 1. The vaccine uptake in 2015 was high (88%) for the complete 3 PCV13 doses received (Table 3).

Pneumococcal carriage and serotype prevalence

A total of 540 primary pneumococcal isolates were initially isolated from the 887 NP samples. The overall prevalence of *S. pneumoniae* in the PCV13-unvaccinated group was 57.6% (114/198); VT, VRT and NVT pneumococci prevalence were 21.2%, 3.5% and 27.8%, respectively. The overall nasopharyngeal *S. pneumoniae* carriage prevalence for the PCV13-vaccinated children was 61.8% (426/689). The prevalence was 18.0%, 5.4% and 31.2% for VT, VRT and NVT pneumococci, respectively. Overall, 39 different serotypes (Figure 1) were identified from the 540 pneumococcal isolates and there were no major shifts in the serotype distribution between the vaccinated and unvaccinated groups. Additionally, 6.8% (60/887) were non-encapsulated (5.1% (10/198) in the PCV13-unvaccinated and 7.3% (50/689) in the survey). In the unvaccinated, the most frequent serotypes were 19F (7.1%, 14/198), 23F (5.1%, 10/198) and 15B/C (9.1%, 18/198). In the vaccinated, the most common serotypes were 19F (4.5%, 31/689) and 15B/C (7.3%, 50/689); and 11 of 13 vaccine serotypes were detected (Table 2). Serotypes 4 and 5 were only detected post-PCV13 introduction (0.5% and 0.2%, respectively) and this was probably due to differences in number of samples obtained between the two periods and/or storage of baseline samples under temperatures not suitable for pneumococci viability, whereas vaccine serotypes 1 and 7F or the cross-reactive 6C were not detected in either groups. Further, other microbes including *H. influenzae*, *S. aureus*, *M. catarrhalis* and beta-hemolytic Streptococci were detected (Figure 2).

Detection of multiple-serotype colonization

Multiple serotypes were detected in 11.4% (8/70) of the swabs tested and a single serotype in the rest of the 62/70 (88.6%) swabs. In three cases, the first isolate was serotype 3 and the second isolate non-encapsulated (NC) pneumococcus. The other double serotype findings were 21/19B, 19F/9N, 14/6B, 19F/33F and 15C/33B. However, due to limited resources only 13.5% (70/514) of the second isolates could be randomly screened for co-colonization.

Discussion

PCV13 infant vaccination in Cameroon using a 3 + 0-dose EPI-schedule at 6, 10 and 14 weeks of age resulted in an 18% prevalence for vaccine-type pneumococci and the overall pneumococcal carriage prevalence among the vaccinated children was 61.8%. Further, eleven of the thirteen vaccine serotypes were detected among vaccinated children in 2015, four years after vaccine introduction.

Reductions in vaccine-type pneumococci carriage have previously been demonstrated elsewhere among the vaccinated and the unvaccinated populations following PCV vaccination (Grant et al., 2016; Hammit et al., 2014; Nzenze et al., 2013; Palmu et al., 2017; de Cunto Brandileone et al., 2016; Collins et al., 2017; Roca et al., 2011; Flasche et al., 2011). In comparison to other studies conducted in Africa (Table 3), four years after PCV7 implementation in The Gambia, a 13.3% reduction in VT-pneumococci proportion was observed among children in the 2–5 year age group, with an over 90% vaccine uptake (Roca et al., 2013). In Kenya where the impact of PCV10 was assessed two years after its introduction, a 19.1% reduction in VT-pneumococci proportion in children younger than five years was reported (Hammit et al.,

Table 3
Studies evaluating the impact of pneumococcal conjugate vaccine (PCV) immunization on nasopharyngeal carriage in Cameroon and other African countries.

Country	References/study design	Schedule/age	PCV formulation/inclusion	Age group at swabbing	Years after vaccination/catch-up	VT detected post-vaccination	% of VT-carriers before vaccination	% of VT-carriers after vaccination	Vaccine uptake (3PCV doses)	VT-pneumococci proportion after vaccination
Cameroon	Current study/cross-sectional	3 + 0 schedule given at 6, 10, 14 weeks of age	PCV13; 2011	24–36 months	4 years; no catch-up campaigns	11 out of 13	21.2%	18%	23% in 2011, 84% in 2012, 88% in 2013, 87% in 2014, 88% in 2015	29.1% (18/61.8)
Kenya	Hammit et al. (2014)/cross-sectional	3 + 0 Schedule given at 6,10 and 14 weeks of age	PCV10; 2011	<5 years old	2 years; catch-up in children <5years old	6 out of 10	34%	13%	32% in 2009, 94% in 2010, 93% in 2011, 98% in 2012, 96% in 2013	19.1% (13/68)
Gambia	Roca et al. (2011)/randomized control trial	3 + 0 schedule given at 2, 3 and 4 months of age	PCV7; 2009	2.5 to <5 years	2 years; 1 dose of PCV given to subjects >30 months old	3 out of 7	50%	13.3%	94% in 2010, 93% in 2011, 98% in 2012, 96% in 2013	17.3% (13.3/76.7)
Gambia	Roca et al. (2013)/randomized control trial	3 + 0 schedule given at 2, 3 and 4 months of age	PCV7; 2009	2.5 to <5 years	4 years; 1 dose of PCV given to subjects >30 months old	Not specified, but the cross-reactive 6A prevalence decreased	50%	8.9%	94% in 2010, 93% in 2011, 98% in 2012, 96% in 2013	13.3% (8.9/67)
South Africa	Nzenze et al. (2013)/cross-sectional	2 + 1 schedule given as two primarily doses at 6, 14 and a booster at 40weeks	PCV7; 2009	<2 years	2 years; no catch-up campaign (switched in May 2011 to PCV13).	7 out of 7; and after the switch to PCV13 it was 11 out of 13	45.1%	23.5%	12% in 2009, 86% in 2010, 51% in 2011	32.0% (23.5/73.4)

VT = vaccine type; PCV7, PCV10 or PCV13 = 7, 10 or 13-valent pneumococcal conjugate vaccines; VT-pneumococci proportion after vaccination = VT-pneumococci prevalence divided by overall-pneumococci prevalence after vaccination; % = percent.

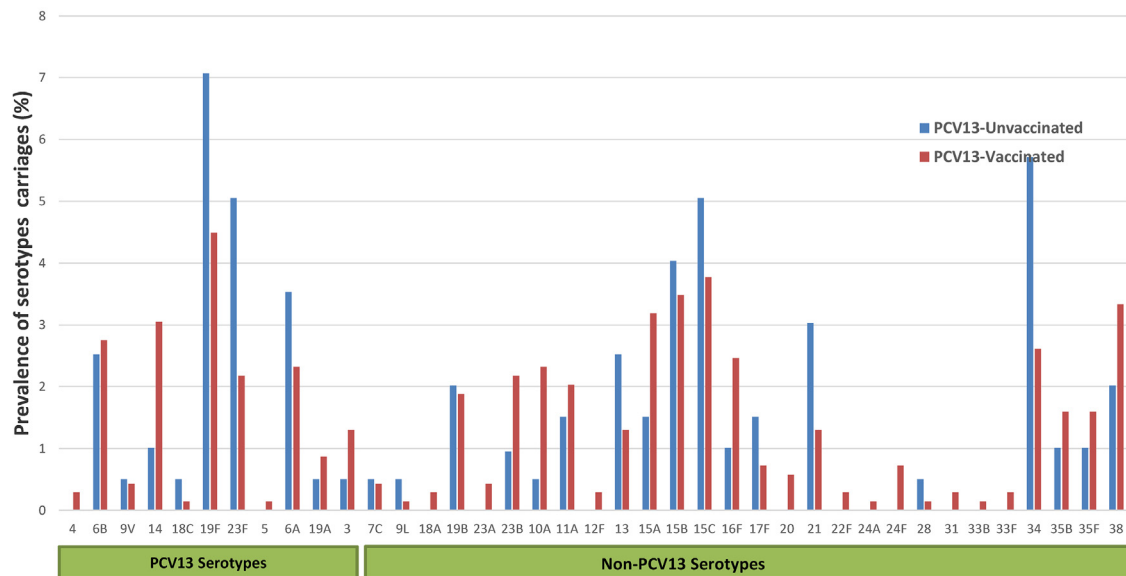


Figure 1. Serotype-specific prevalence among pneumococci carriage isolates in 2013 (PCV13-unvaccinated, n = 198) and in 2015 (PCV13-vaccinated, n = 689) cohorts of 2–3 years old children in Yaounde, Cameroon.

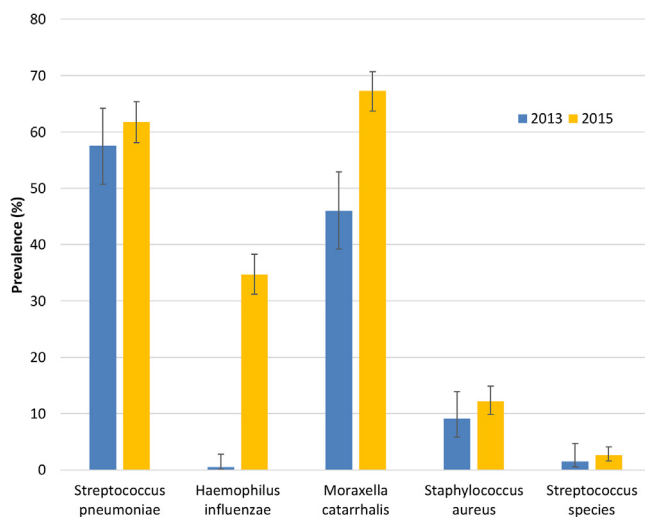


Figure 2. Prevalence of bacterial species isolated from NP swabs in 2013 (PCV13-unvaccinated) and 2015 (PCV13-vaccinated) cohorts of 2–3 years old children in Yaounde, Cameroon.

2014), with about 80% vaccine uptake. Additionally, results on the impact of PCV have been similar in studies which have either involved catch-up programmes for older populations or not. In both the Kenyan and Gambian studies, PCV implementation was rolled-on along with a catch-up plan. Whereas, in South Africa, without a catch-up campaign a 32% reduction in VT-pneumococci proportion was noted in all study age groups, including unvaccinated children aged 2–5 years. This was achieved within two years following PCV7 introduction and when only about 51% of the targeted infant population were fully immunized with a primary 2 dose series at 6 and 14 weeks after birth, followed by a booster dose at age 9 months (Nzenze et al., 2013).

The significant reductions in PCV7/PCV10/PCV13-serotypes observed in studies from the Gambia (Roca et al., 2011; Roca et al., 2015), South Africa (Nzenze et al., 2013) and Kenya (Hammit et al., 2014) in both vaccinated and unvaccinated children are reported to have resulted from the direct and indirect effects of the PCV following a reduced transmission and acquisition of vaccine

serotypes, which made children less vulnerable to subsequent carriage of vaccine-type pneumococci (Hammit et al., 2014; Madhi, 2013). This finding was not clearly observed in our vaccinated cohort four years after PCV13 implementation. It suggests that the direct and indirect effects of the PCV13 have not resulted in expected low levels of vaccine-type transmission in our setting. This suggests that the Cameroon EPI schedule has not been able to block the transmission of vaccine type circulation. Absence of a booster PCV13 dose and waning effects may have contributed to the lower impact on carriage in this age group, as the vaccine is given on a 3 + 0 schedule early in infancy (6, 10 and 14 weeks of age). Recent data from Burkina Faso and Kenya, in addition to earlier reports from South Africa, suggest a switch to a 2 + 1 schedule in which the middle dose is given at 9–12 months along with the measles vaccine could lead to a longer duration of protection (Hammit et al., 2019; Kambire et al., 2016; Madhi and Nunes, 2016).

Pneumococcal carriage is most prevalent in children, and its progression to disease has been reportedly influenced by predisposing risk factors such as overcrowding and upper respiratory tract symptoms (Auranen et al., 2016). Further, the risk of carriage acquisition has been demonstrated to be almost three times higher in association with the onset respiratory infection compared to health (Auranen et al., 2016). There were significant differences in the number of household children and in the different classes of respiratory symptoms among the children vaccinated and those not vaccinated (Table 1). These suggest that the unchanged overall pneumococcal carriage prevalence obtained in our study may have been influenced by children living in homes with one or more children under 18 years, and/or with respiratory symptoms, who were more likely to carry and transmit pneumococci than those without.

In summary, a considerable effect on VT-pneumococci colonization with vaccine uptake in the range of 50 to >90%, 1.5–4 years post-PCV introduction has generally been observed in these African studies, with or without catch-up campaigns. The Kenyan and Gambian randomized control trials and follow-up ecological studies reported VT-pneumococci proportions of 19.1%, 17.3% and 13.3% after the vaccine introduction, respectively (Hammit et al., 2014; Roca et al., 2013; Roca et al., 2011); compared to the 29.1% obtained in our study. However, in a South African study (Nzenze

et al., 2013), VT-pneumococci proportion was higher (32%), but this may be as a result of only 51% of eligible subjects having completed the schedule of 3 PCV doses. Our findings are surprising and possible explanations for the differences in VT-pneumococci proportion may include lower vaccine impact, age at sampling, variations in the nasopharyngeal microbiome and/or secular trends. For instance, changes in HIV treatment regimens in South Africa and a community-wide azithromycin campaign in the Gambia are thought to have influenced the high vaccine effects in these countries (Hammitt et al., 2014; Roca et al., 2013; Roca et al., 2011).

The main strength of our study was the application of the WHO cluster sampling design which involved a systematic recruitment of all subjects from the same communities following the same procedures including sampling period/time and age range.

A major caveat in this study stems from the storage of NPS specimens (2013 collection) in a European port of entry not within the recommended laboratory conditions of -70°C for over two months (Satzke et al., 2013; Kajjalainen and Palmu, 2015; Hill et al., 2016). This may have affected the viability of pneumococci in these samples (Satzke et al., 2013; Kajjalainen and Palmu, 2015; Hill et al., 2016). However, the overall pneumococcal carriage prevalences were nearly identical during both periods, yet variations in the prevalence for other bacteria, especially non-typeable *H. influenzae* (Figure 2) were observed. This suggests that our baseline carriage prevalence was probably under-estimated and therefore, no comparison between the vaccinated and non-vaccinated was performed. Moreover, our study used a different methodology in which PCV impact was assessed from a baseline of PCV13-unvaccinated 24–36 months old children, when routine vaccination had already been rolling for nearly two years. Therefore, the unvaccinated group was exposed to early indirect effects following routine infant vaccination (Roca et al., 2011). However, the indirect effects develop gradually, and the PCV13 uptake was low during the first 2 years of implementation (mean coverage for 2011 and 2012 was <55%) (World Health Organization, 2018).

In conclusion, the reported reductions in VT-pneumococci proportion are lower in our study compared to expected levels based on previous reports in similar settings. These suggest that either the vaccination programme with the 3 + 0-dose schedule is not eliciting sufficient protection against transmission of vaccine serotypes or there are programmatic challenges with the programme (Roca et al., 2015; Madhi and Nunes, 2016; Nzenze et al., 2015). More so, pneumococcal carriage is a precursor to invasive pneumococcal disease (IPD) (Sleeman et al., 2005) and as postulated by Boula et al., the prevalence of non-PCV13 serotypes among paediatric bacterial meningitis cases in Cameroon may play an increasing role in disease aetiology in the PCV13 era (Boula et al., 2019). Such findings as obtained in our study are important to identify which serotypes show increased propensity for IPD as well as in clinical practice and regulations on antibiotic prescription.

Authors' contributions

Conceived, planned and designed the study: JNL, HN, JPP and AAP; participated and supervised local data collection: JNL, PKN, MK and SKS; performed laboratory analyses: JV, KGH, MT, ON and JNL; contributed reagents and other logistics: AAP, MT and JV; drafted the manuscript: JNL and AAP; significantly contributed to the revised and final versions of the manuscript: JNL, KGH, MK, MT, PKN, ON, SKS, JV, HN, JPP and AAP

Conflicts of interest

AAP, JNL, TM, HN and ON are employed by the National Institute for Health and Welfare (THL), Finland which has

received research funding from GlaxoSmithKline and Pfizer, Inc. "This does not alter our adherence to THE-IJID policies on sharing data and materials".

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