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Effect on nasopharyngeal pneumococcal carriage of replacing PCV7 with PCV13 in the Expanded Programme of Immunization in The Gambia

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ABSTRACT

Introduction: In 2011, two years after the introduction of 7-valent Pneumococcal conjugate vaccine (PCV7), the Gambian immunization programme replaced PVC7 with PCV13 (13-valent). Our objective was to assess the additional impact of PCV13 on prevalence of pneumococcal nasopharyngeal carriage.
Methods: We recruited healthy Gambian infants who had received three PCV doses. Nasopharyngeal swabs were collected from infants and their mothers during two cross-sectional surveys (CSS) conducted in infants vaccinated with PCV7 (CSS1) and vaccinated with PCV13 (CSS2). Pneumococci were isolated and serotyped following standardized methods. Whole genome sequencing was performed on non-typable pneumococcus isolated in CSS1 and CSS2.
Results: 339 and 350 infants and their mothers were recruited in CSS1 and CSS2, respectively. Overall prevalence of pneumococcal carriage was 85.4% in infants. Among infants, prevalence of vaccine type (VT) carriage was lower in CSS2 [9.4% versus 4.9% ($p = 0.025$) for PCV7-VT; 33.3% versus 18.3% ($p < 0.001$) for PCV13-VT and 23.9% versus 13.7% ($p = 0.001$) for the 6 additional serotypes included in PCV13]. At CSS2, there was a decrease of serotypes 6A (from 15.3% to 5.7%, $p < 0.001$) and 19F (from 5.6% to 1.7%, $p = 0.007$), and an increase of non-typable pneumococci (0.3–6.0%, $p < 0.001$), most of which (82.4%) were from typable serotype backgrounds that had lost the ability to express a capsule. Prevalence of overall and VT carriage in mothers was similar in CSS1 and CSS2.
Conclusions: Replacing PCV7 for PCV13 rapidly decreased prevalence of VT carriage among vaccinated Gambian infants. An indirect effect in mothers was not observed yet. Vaccine-driven selection pressure may have been responsible for the increase of non-typable isolates.

1. Background

Prevention of pneumococcal disease is a major, international public health priority due to the high burden of disease and associated mortality, especially in children in developing countries [1]. Pneumococcal conjugate vaccines (PCVs) prevent pneumococcal disease by reducing the carriage of serotypes included in the vaccine [2,3]. PCVs are currently used in routine childhood

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vaccination programmes in many developed and developing countries. Because children are the main drivers of transmission [4], vaccinating children also protects older members of the community from both nasopharyngeal carriage and invasive disease [2,5–9]. Some studies undertaken after the introduction of PCVs have shown an increase in both the incidence of invasive pneumococcal disease and in the prevalence of carriage caused by pneumococcal serotypes not included in the vaccine (non-vaccine-serotypes or NVT), [2,3,8,10–13]. This replacement of VT serotypes with NVT serotypes has also been observed in non-vaccinated older members of vaccinated communities [14]. However, the capsule is a determinant of virulence, and VT serotypes tend to be more virulent than NVT [15]. Consequently, there has been an overall reduction in invasive pneumococcal disease since the introduction of pneumococcal vaccination, despite serotype replacement [8].

Because conjugate vaccines target the pneumococcal capsule, pneumococci can evade vaccine-induced immunity by changing their serotype, or losing their capsule altogether, through genetic recombination, described as switching [16,17].

The first licensed PCV vaccine included 7 out of the more than 90 pneumococcal serotypes. This vaccine did not include the most prevalent serotypes causing disease in Africa and other developing regions. To address this challenge, PCV13 was licensed based on safety and immunogenicity data in comparison with PCV7. PCV13 contains 13 capsular pneumococcal polysaccharides that are individually conjugated to non-toxic diphtheria protein. In addition to the 7 serotypes in PCV7 (4, 6B, 9V, 14,18C, 19F and 23F), PCV13 contains serotypes 1, 3, 5, 6A, 7F and 19A. Together these are the 13 most common serotypes causing invasive pneumococcal disease globally [18]. The impact of PCVs on nasopharyngeal carriage is considered a predictor of vaccine effectiveness [19] and carriage studies can be used to predict the potential impact of different formulations of PCVs on VT among the vaccinated population (direct effect) and other age groups (indirect effect). A vaccine trial conducted in Israel, which compared the efficacy of PCV13 versus PCV7, showed a reduction in the acquisition and prevalence of nasopharyngeal colonization for the additional serotypes included in PCV13 as well as a lower prevalence of serotypes 6C and 19F [20]. The objective of this study was to compare the effectiveness of three doses of PCV13 with that of three doses of PCV7 in preventing pneumococcal nasopharyngeal colonization among infants in The Gambia and to assess the indirect effect of this formulation on their mothers. In addition, we analyzed the molecular basis for the observed increase in the prevalence of non-typable pneumococcus after PCV13 vaccination.

2. Materials and methods

2.1. Pneumococcal conjugate vaccination in The Gambia

The Gambia was the second country in Africa to introduce PCV7 as part of its Expanded Programme of Immunization (EPI), starting in the second half of 2009 and following three-dose schedule with vaccine given at the age of 2, 3 and 4 months of age. Although there was no formal catch-up campaign, most of children under 2 years of age received one dose of PCV7 soon after its introduction. In June 2011, PCV7 was replaced by PCV13 in the EPI programme. EPI coverage in the country is >90% for DPT3 and a similar coverage is expected for PCVs [21].

2.2. Study population

The study was conducted in a peri-urban area situated on the western margin of The Gambia, approximately 20 km from the capital Banjul. The population of the study area is ethnically mixed, and engaged in a wide range of occupations, including working for government services, some professions and trading. The climate of the area is typical of the sub-Sahel with a long dry season from November to May.

The study was carried out at two health centres, Sukuta Health Centre and Jammeh Foundation for Peace. These are government run health centres and vaccination clinics located approximately 8 km from Fajara where the main laboratories at the Medical Research Council (MRC) are based. Children and their mothers were identified and recruited in the vaccination clinics of the health centres. These health centres serve a local community of more than 25,000 inhabitants each, with an overall annual birth cohort of more than 7000 children.

2.3. Study design and samples

We conducted a study of pneumococcal nasopharyngeal carriage before and after the introduction of PCV13. The first cross-section survey (CSS1) was conducted between March and June 2011 among children who had received three doses of PCV7. The second survey (CSS2) was conducted following the introduction of PCV13 between March and June 2012 among children who had received three doses of PCV13.

Infants 6–11 months of age were recruited at EPI vaccine clinics. To be eligible for the study, children had to be healthy, to have received three documented doses of PCV (PCV7 in CSS1 or PCV13 in CSS2) with the third dose received at least 4 weeks before entering the survey. If consent was given, a nasopharyngeal swab (NPS) was taken from the infant and their mother. Demographic data and data on risk factors for carriage were obtained by interviewing the mother using a structured questionnaire.

The study was approved by the joint MRC/Gambia Government Ethics Committee and by the ethics committee of the London School of Hygiene and Tropical Medicine.

2.4. NPS collection

NPSs were collected from the posterior wall of the nasopharynx using an alginate swab and immediately inoculated into vials containing skim milk–tryptone–glucose–glycerol (STGG) transport medium, which was placed in a cold box before transfer to the Medical Research Council Laboratories at Fajara within 8 h of collection, in accordance with the WHO protocol for evaluation of pneumococcal carriage [22]. Inoculated vials were stored at −70 °C until tested in batches.

2.4.1. Microbiological methods

Frozen STGG containing nasopharyngeal swabs were initially thawed on ice and then vortexed briefly for a minimum of 20 s. 200 μl of STGG was transferred into 5 ml of Todd Hewitt broth containing 5% yeast extract, and 1 ml of rabbit serum. The mixture was vortexed briefly before incubating for 4–6 h at 37 °C. At the end of the incubation period, the broth was vortexed again and 50 μl aliquot inoculated on to gentamycin blood agar (GBA). The inoculum was streaked onto four quadrants in order to semi quantitatively determine the bacterial load (from 1 to 4) as described elsewhere [23]. After 20–24 h incubation at 37 °C with 5% CO_2, GBA plates were examined for alpha hemolytic colonies. Presumptive pneumococcal colonies were then screened for optochin susceptibility.

Pneumococcal isolates were serotyped using the latex agglutination techniques as described previously [24]. All non-typable isolates were repeated and only considered non-typables when confirmed by the second test.

2.5. Sample size calculation

We chose a sample size of 350 infants per CSS to detect a 40% reduction in the prevalence of PCV(13-7)-VT (from 24% to 14%), with 90% power and a type 1 error of 5%.

2.6. Statistical analysis

The overall prevalence of pneumococcal carriage, the prevalence of individual serotypes, and the prevalence of VT serotypes was determined in each survey. The latter were classified in three ways: (i) serotypes included in PCV7 (PCV7 VT), (ii) serotypes included in PCV13 (PCV13 VT), and (iii) serotypes included in PCV13 but not in PCV7 (PCV13-7 VT).

Poisson regression was used to estimate prevalence ratios comparing CSS1 and CSS2. For mothers and children the ratios were adjusted for maternal age, household size, health centre and maternal education. For children the ratios were additionally adjusted for age and gender, antibiotic use (of the infant) and exposure to smoke from cooking. *p*-Values and confidence intervals were calculated using robust variance estimates.

We used Fisher's exact to test the hypothesis that the proportion of PCV13 VT among non-typables that had lost their capsule was equal to the proportion in the population as a whole.

All analyses were done using Stata version 13.

Whole genome sequence analysis

Whole genome sequencing (WGS) was conducted among non-typable pneumococci isolated from mothers and infants in both study CSSs.

Samples of genomic DNA from study pneumococcal isolates were sequenced using Illumina HiSeq, 100 bp paired end with 350 bp inserts. Kraken was used to differentiate between pneumococci and closely related species and to identify any potential contamination [25]. MLST was derived from *de novo* Velvet assemblies [26]. Detection of known serotype specific sequences in assemblies and mapping against known capsular loci was performed as previously described [27,28].

Sequence reads were mapped against the reference genome for *S. pneumoniae* ATCC 700669 [29] using a pipeline developed in-house at The Sanger Institute that utilizes SMALT (http://www.sanger.ac.uk/resources/software/smalt/). In addition to the 34 non-typable genomes sequenced for this study a total of 64 isolates from independent pneumococcal sequencing projects were included in the analysis to give serological context to the phylogeny; six classically non-typable isolates [30], 52 Gambian isolates from a previous study [31] and a further 6 Gambian isolates from the Global Pneumococcal Sequencing project (http://www.pneumogen.net/gps/). The resultant alignment was reduced to variable sites and phylogeny created using RaxML [32]. Single nucleotide polymorphisms (SNPs) were reconstructed on the phylogeny using an in-house accelerated transformation parsimony method rooted on *Streptococcus pseudopneumoniae*. The phylogeny was visualized and annotated in ITOL [32]. The data were deposited in the ENA with accession numbers provided in the supplementary data.

3. Results

3.1. Study profile

Six hundred and eighty-nine infants and their mothers were recruited and sampled from the study (339 in CSS1 and 350 in CSS2). Approximately half of the recruited infants were male (50.2%) and their median age was 8 months. Because 11 children were twins, only 678 mothers were sampled [median age 25 years]. Sampling occurred between 24th March and 9th June in 2011 for CSS1; and 27th March and 7th of June 2012 for CSS2. Table 1 provides a summary of the epidemiological characteristics of individuals included in each CSS survey.

Table 1
Characteristics of the children and their mothers who participated in cross sectional surveys undertaken before (CSS1) and after (CSS2) the introduction of PCV13 into the Gambian EPI programme.

Variable	CSS1[a] (*n* = 339) *n* (%)	CSS2[a] (*n* = 350) *n* (%)
Health centre		
Jammeh Foundation	229 (67.6)	251 (71.7)
Sukuta	110 (32.4)	99 (28.3)
Total	339	350
Gender		
Female	159 (46.9)	184 (52.7)
Male	180 (53.1)	165 (47.3)
Total	339	349
Antibiotic use[b]		
No	295 (88.6)	319 (92.2)
Yes	38 (11.4)	27 (7.8)
Total	333	346
Mother's schooling		
None or <1 year	137 (41.8)	110 (31.6)
1-3 years	46 (14.0)	36 (10.3)
4-6 years	80 (24.4)	44 (12.6)
>6 years	65 (19.8)	158 (45.4)
Total	328	348
Mother cooks with child on back		
No	226 (66.9)	189 (54.3)
Yes	112 (33.1)	159 (45.7)
Child age (months)		
Median (IQR)	8.2 (7.0,9.6)	7.6 (6.7,9.2)
Household size		
Median (IQR)	5.0 (4.0,7.0)	3.0 (3.0,5.0)
Mother's age (years)		
Median (IQR)	24.5 (21.0,29.0)	25.0 (21.0,28.0)

[a] There were 11 pairs of twins (i.e., 22 twins in total), 8 pairs in the PCV-7 survey and 3 pairs in the PCV-13 survey. The number of mothers was 331 and 347 in CSS1 and CSS2 respectively.
[b] Within one month before entering the study.

3.2. Prevalence of pneumococcal carriage pre and post PCV13 introduction

Infants: The overall prevalence of pneumococcal carriage among infants was similar in both surveys (85.8% and 84.3%, $p = 0.594$). However, the prevalence of VT decreased between CSS1 and CSS2 [from 23.9% to 13.7% ($p < 0.001$) for PCV(13-7)VT; from 33.3% to 18.3% ($p < 0.001$) for PCV13-VT; and from 9.4 to 4.9 ($p = 0.025$) for PCV7-VT]. The decrease in VT prevalence was mainly due to a decreasing in serotype 6A (from 15.3% to 5.7% – $p < 0.001$) and 19F (from 5.6% to 1.7% – $p = 0.007$). The prevalence of non-typable serotypes increased in CSS2 (from 0.3% to 6.0% – $p < 0.001$) (Table 2). Similar results were found in the crude and adjusted analyses (Table 2). Apart from the non-typables, there was no significant increase of any NVT.

Mothers: The overall prevalence of pneumococcal carriage among mothers was lower than in infants but similar in CSS1 and CSS2 (23.0% versus 24.2%, $p = 0.718$). For the different study endpoints, differences between study CSSs were small and did not reach statistical significance in the crude or adjusted analysis (Table 3). The most prevalent serotypes among mothers were serotype 16 in CSS1 and 19A in CSS2.

3.3. Genotypic analysis of non-typable isolates

WGS was performed for all 28 phenotypically non-typable isolates; 22 from the infants (1 in CSS1 and 21 in CSS2) and 6 from the mothers (1 and 5 in CSS1 and CSS2 respectively). Three (10.7%)

Table 2
Prevalence of pneumococcal carriage in infants in surveys conducted before (CSS1) and after (CSS2) Introduction of PCV13 ito the Gambian EPI programme.

	Prevalence of carriage		RR	*p*-Value[a]	RRadj[b]	*p*-Value
	CSS1 (*N* = 339)	CSS2 (*N* = 350)				
Vaccine groups						
PCV13-VT	33.3	18.3	0.55 (0.42,0.72)	<0.001	0.52 (0.39,0.69)	<0.001
PCV7-VT	9.4	4.9	0.51 (0.29,0.91)	0.025	0.52 (0.28,0.97)	0.039
PCV(13-7)-VT	23.9	13.7	0.57 (0.41,0.79)	0.001	0.53 (0.38,0.74)	<0.001
Pneumococcus	85.8	84.3	0.98 (0.92,1.05)	0.594	1.00 (0.93,1.07)	0.897
PCV13-VT						
1	0	0	NA	NA	NA	NA
3	0	0.3	NA	1.000	NA	NA
4	0.6	0	NA	0.242	NA	NA
5	0.3	1.4	4.84 (0.57,41.30)	0.217	4.59 (0.55,38.43)	0.160
6A	15.3	5.7	0.37 (0.23,0.61)	<0.001	0.35 (0.21,0.59)	<0.001
6B	0.9	0	NA	0.119	NA	NA
7F	0	0	NA	NA	NA	NA
9V	0	0	NA	NA	NA	NA
14	0.6	0.9	1.45 (0.24,8.65)	1.000	2.12 (0.36,12.35)	0.403
18C	0.6	0	NA	0.242	NA	NA
19A	8.3	6.3	0.76 (0.44,1.30)	0.379	0.66 (0.39,1.10)	0.112
19F	5.6	1.7	0.31 (0.12,0.76)	0.007	0.29 (0.10,0.78)	0.015
23F	1.5	2.3	1.55 (0.51,4.69)	0.578	1.51 (0.49,4.64)	0.468
NVT						
10A	4.7	4	0.85 (0.42,1.71)	0.711	0.89 (0.41,1.93)	0.761
13	3.5	3.4	0.97 (0.44,2.13)	1.000	1.06 (0.45,2.52)	0.892
15B	8.3	9.1	1.11 (0.68,1.80)	0.688	1.40 (0.82,2.38)	0.220
16	5.9	6.6	1.11 (0.62,1.99)	0.755	1.00 (0.51,1.99)	0.992
19C	0.9	0.3	0.32 (0.03,3.09)	0.366	0.18 (0.03,1.06)	0.058
21	3.2	4.9	1.50 (0.71,3.15)	0.377	1.50 (0.71,3.15)	0.288
34	3.8	2.3	0.60 (0.25,1.42)	0.272	0.54 (0.22,1.36)	0.194
35B	4.7	4.9	1.03 (0.53,2.00)	1.000	0.88 (0.44,1.74)	0.713
NT	0.3	6.0	20.34 (2.75150.60)	<0.001	19.82 (2.64,148.78)	0.004

RR, risk ratios; VT, vaccine types; NVT, non-vaccine types 6; NA, not applicable.
[a] *p*-Value from Fisher's exact test.
[b] Adjusted for maternal age, household size, schooling, health centre, gender, exposure to smoke from cooking.

Table 3
Prevalence of pneumococcal carriage in mothers before (CSS1) and after (CSS2) introduction of PCV13 into the Gambian EPI programme.

	Prevalence of carriage		RR	*p*-Value[a]	RRadj[b]	*p*-Value
	CSS1 (*N* = 331)	CSS2 (*N* = 347)				
Vaccine groups						
PCV13	6.6	8.4	1.26 (0.74,2.14)	0.467	1.14 (0.65,2.01)	0.651
PCV7	2.7	2.6	0.95 (0.38,2.38)	1.000	0.85 (0.31,2.30)	0.744
PCV(13-7)VT	3.9	6.1	1.54 (0.78,3.03)	0.222	1.39 (0.67,2.86)	0.377
Pneumococcus	23.0	24.2	1.05 (0.80,1.38)	0.718	1.19 (0.88,1.60)	0.264
PCV13-VT						
1	0.3	0	NA	0.488	NA	NA
3	0	0.3	NA	1.000	NA	NA
4	0.6	0.6	0.95 (0.13,6.74)	1.000	1.09 (0.09,13.70)	0.945
5	0	0.9	NA	0.249	NA	NA
6A	1.8	2.0	1.11 (0.38,3.28)	1.000	1.25 (0.42,3.72)	0.685
6B	0	0	NA	NA	NA	NA
7F	0	0	NA	NA	NA	NA
9V	0	0	NA	NA	NA	NA
14	0.6	0.3	0.48 (0.04,5.24)	0.616	0.36 (0.06,2.16)	0.266
18C	0.3	0.6	1.91 (0.17,20.98)	1.000	1.72 (0.05,60.93)	0.266
19A	1.8	3.2	1.75 (0.65,4.68)	0.328	1.36 (0.44,4.21)	0.589
19F	0.6	0.3	0.48 (0.04,5.24)	0.616	0.24 (0.02,3.07)	0.274
23F	0.6	0.9	1.43 (0.24,8.52)	1.000	1.23 (0.24,6.37)	0.805
NVT						
10A	0.6	0.3	0.48 (0.04,5.24)	0.616	0.53 (0.05,5.12)	0.580
13	0.6	1.7	2.86 (0.58,14.09)	0.287	3.07 (0.60,15.62)	0.177
15B	0.6	1.7	2.86 (0.58,14.09)	0.287	2.72 (0.71,19.50)	0.120
16	2.4	0.6	0.24(0.05,1.12)	0.058	0.15 (0.03,0.72)	0.018
19C	1.2	0	NA	0.056	NA	NA
21	0.9	0.9	0.95 (0.19,4.70)	1.000	0.52 (0.07,4.05)	0.535
34	1.2	1.2	0.95 (0.24,3.79)	1.000	0.75 (0.11,4.93)	0.761
35B	1.8	0.6	0.32 (0.06,1.57)	0.168	0.39 (0.08,1.87)	0.240
NT	0.3	1.4	4.77 (0.56,40.67)	0.217	3.93 (0.42,37.03)	0.231

RR, risk ratios; VT, vaccine types; NVT, non-vaccine types 6; NA, not applicable.
[a] *p*-Value from Fisher's exact test.
[b] Adjusted for maternal age, household size, schooling, health centre.

Table 4
Results of whole genome sequencing of non-typable isolates.

Isolate N	CSS	Mother/infant	*S. pneumoniae* % reads	*S. pseudopneumoniae* % reads	ST	Nearest ST	Capsular locus top hit	Serotype specific sequence detected	Ancestral capsular type from phylogeny	Conclusion	VT or NVT
108887	1	Infant	78.08	0.71	3407		16F	16F	16F	16F not expressed	NVT
105098	1	Mother	80.51	0.14	1778		34	34	34	34 not expressed	NVT
104550	1	Mother	19.47	40.9	Unknown					*S. pseudopneumoniae*	—
201376	2	Infant	80.46	0.25	5521		10A	10A	10A	10A not expressed	NVT
207381	2	Infant	82.09	0.4	Novel ST D	2052	20	20	Long branch, inconclusive	20 not expressed	NVT
206628	2	Infant	81.38	0.08	989		12F	12F/A/46	12F/A/46	12F/A/46 not expressed	NVT
210240	2	Infant	80.96	0.08	989		12F	12F/A/46	12F/A/46	12F/A 46 not expressed	NVT
210201	2	Infant	83.51	0.08	2447		14	14	14	14 not expressed	VT
201867[a]	2	Infant	46.64	0.57	Novel ST B	4040	Classical NT		14	Capsule switch from 14 to classically NT locus	VT
200954	2	Mother	81.22	0.25	Novel ST G	975	15B/C	15B/C	15B/C	15B/C not expressed	NVT
206379	2	Infant	81.51	0.16	4033		15B/C	15B/C	15B/C	15B/C not expressed	NVT
207139	2	Infant	76.55	0.57	3407		16F	16F	16F	16F not expressed	NVT
210300	2	Infant	78.96	0.62	Novel ST F	3407	16F	16F	16F	16F not expressed	NVT
210092	2	Infant	80.13	0.28	Novel ST E	847	19A	19A	19A	19A not expressed	VT
210344	2	Infant	79.93	0.19	Unknown	7661	19B		19B	19B not expressed	NVT
206438	2	Infant	81.8	0.1	202		19A	19A	Long branch, inconclusive	19A not expressed	VT
208478	2	Infant	81.95	0.17	4033		19F	19F	19F/15B/C	19F not expressed	VT
202731	2	Infant	81.26	0.08	Novel ST C	6712	28A/F		28A/F	28A/F not expressed	NVT
201297	2	Mother	81.95	0.34	5734		6A/B/C/D	6A/B/C/D	6A	6A not expressed	VT
201794	2	Infant	78.82	0.8	Novel ST A	71	38 (low match 35%)		Long branch, inconclusive	*cps* locus lost	—
201398	2	Mother	76.17	1.3	Novel ST H	3582	Classical NT		Long branch, inconclusive	Capsule switch to classically NT locus	—
201843	2	Infant	77.28	1.11	4040		Classical NT		14	Capsule switch from 14 to classically NT locus	VT
205597	2	Infant	75.37	0.96	Novel ST B	4040	Classical NT		14	Capsule switch from 14 to classically NT locus	VT
206158	2	Mother	78.28	0.89	Novel ST B	4040	Classical NT		14	Capsule switch from 14 to classically NT locus	VT
201133	2	Infant	64.27	3.02	344		Classical NT		Classical NT	Classical NT	–
202601	2	Mother	65.69	2.64	448		Classical NT		Classical NT	Classical NT	–
208136	2	Infant	66.12	2.67	448		Classical NT		Classical NT	Classical NT	–
201017	2	Infant	17.54	43.18	Unknown					*S. pseudopneumoniae*	–
209574	2	Infant	20.05	40.36	Unknown					*S. pseudopneumoniae*	–

CSS, cross-sectional; VT, vaccine-types; NVT, non-vaccine types; NT, non-typable; ST, sequence type.

[a] Sample contaminated with unclassified organism, pneumococcal coverage sufficient for analysis and conclusions.

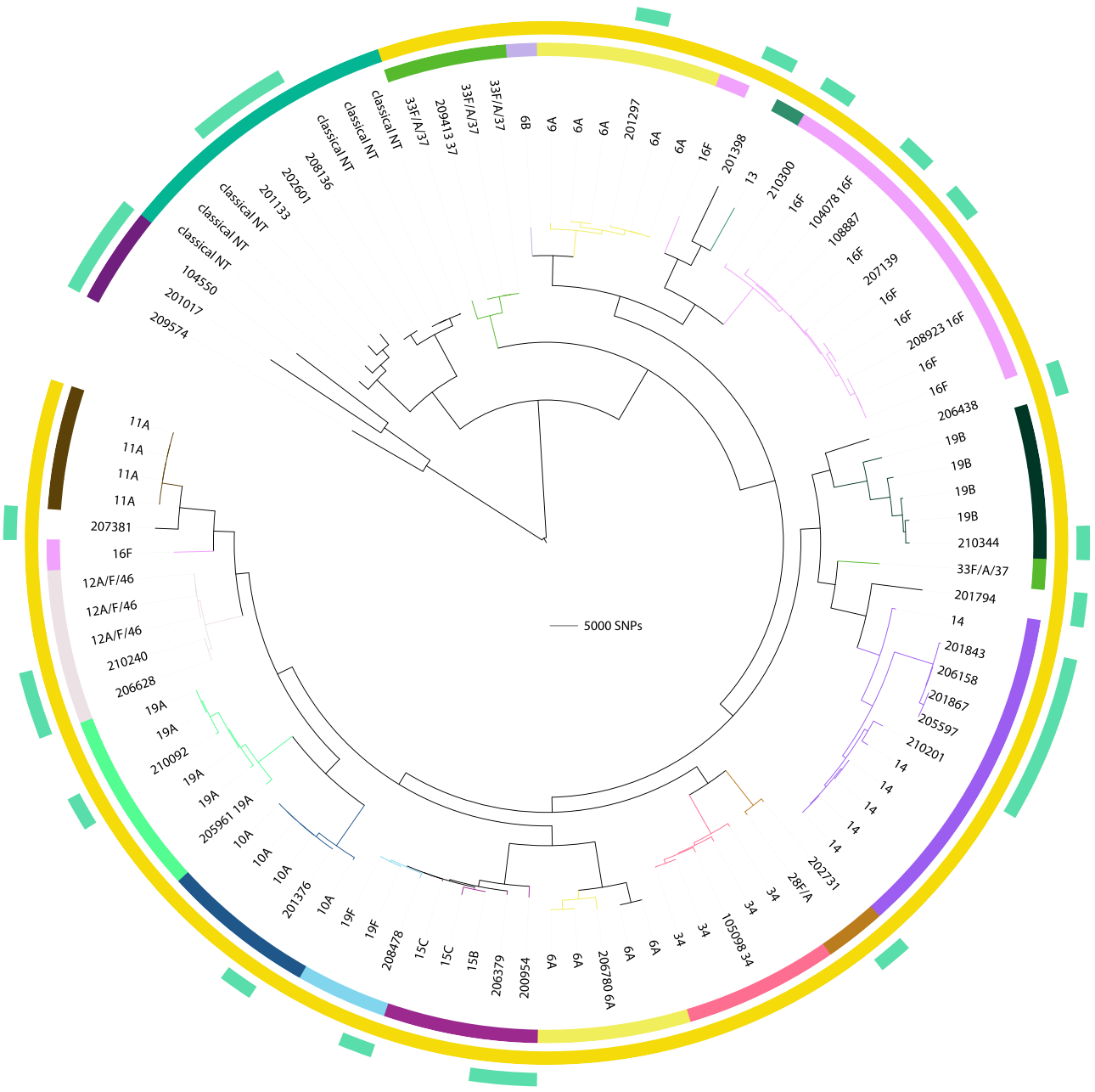

Fig. 1. Phylogenetic context of non-typable isolates. (i) Outer ring: non-typable isolates from this study highlighted (light green). (ii) Middle ring: major lineages, *S. pseudopneumoniae* (purple), Classical non-typable (green), Typically encapsulated (yellow). (iii) Inner ring and branch colouring: clusters of serotypes giving context to study isolates. (iv) Leaf labels: Serotype of isolate giving context to study isolates or study ID of non-typable study isolates.

of these isolates were *S. pseudopneumoniae*, 3 were of the classical non-typable lineage (10.7%) and the others [22 out of 28 (78.6%)] were of the typically encapsulated pneumococcal lineage and had lost their ability to express capsule through one of three different mechanisms (Fig. 1). Sixteen of these appear to have a standard *cps* locus but a capsule was not phenotypically detected, one isolate only had remnants of the *cps* locus whilst the remaining 5 had acquired a locus typically found in classically non-typable isolates. These latter isolates were closely related and cluster in the phylogeny predominantly within a serotype 14 lineage (Fig. 1 and Table 4), accounting for 4 out of the 5 NT isolates from a serotype 14 background.

Among the 16 infants carrying non-typable isolates that were not classical, 7 (43.7%) were of VT lineage. This is almost double the proportion of VT carriers among infant carriers in CSS2 (23.4%) ($p = 0.076$).

4. Discussion

The Gambia was the second African country to introduce PCV7 into the EPI. For two years children received PCV7, but soon after PCV13 was licensed, the earlier vaccine was replaced by the higher valency formulation. To the best of our knowledge, the impact of replacing PCV7 with PCV13 within EPI programmes has not yet been reported in the African continent. Our study showed that overall prevalence of pneumococcal nasopharyngeal carriage among infants was comparable between PCV7 and PCV13 vaccinated children but that the distribution of serotypes differed between groups,

with a significant decrease of the additional serotypes included in PCV13 [PCV(13-7)-VT]. We also observed an increase on non-typables isolates in CSS2, mainly of serotypes that had lost their capsule, most likely in response to vaccine pressure. No significant increase of NVT between CSS was observed.

In PCV13 vaccinated infants, the prevalence of PCV(13-7)-VT almost halved. Most of the difference was attributable to a decrease in the prevalence of serotype 6A, from 15.3% in CSS1 to 5.7% in CSS2. Even with this decrease, the prevalence of serotype 6A was still 6% in CSS2 and represented 42% of all PCV(13-7)-VT serotypes. These results, along with previous results in The Gambia, suggest that cross-protection between 6A and 6B was probably very low, if any [7]. The observed decrease of serotype 19A was not statistically significant; it was the most prevalent PCV(13-7)-VT in CSS2, and accounted for 47% of all PCV(13-7)-VT serotypes.

A notable difference between CSS1 and CSS2 was the increase in non-typable isolates in CSS2 (adjusted RR among infants 19.82). Prevalence of non-typable isolates in CSS2 (6% in infants) was higher than in CSS1 and also higher than in previous studies conducted in The Gambia [7,33]. The WGS analysis of these non-typables pneumococci showed that most of them were not among the classical non-typable lineage, but instead, represented serotypable isolates that did not express a capsule. Capsule loss is not a rare event in the pneumococcus [34]. However, because the prevalence of VT lineages among isolates with loss of capsule expression was higher than the overall prevalence of VT isolates among carriers, this loss of capsule expression may be, at least partly, due to a response of the pneumococcal population to vaccine selection pressure. Although this analysis was not statistically significant the study was not powered for this endpoint. If our findings are true, we may expect an increase in carriage of non-typable pneumococcus. Further monitoring is warranted to determine whether non-typable serotypes carried in the nasopharynx are associated with increase in disease.

Our results differ slightly from similar studies recently conducted in other continents. In Israel, investigators observed a moderate decrease of overall carriage [35] contrasting with our results where no effect on overall pneumococcal carriage was observed in either vaccinated infants or their mothers. In the US, the prevalence of carriage remained stable after PCV13 introduction but a moderate increase of NVT, mainly due to serotype 35B, was observed [36]. In Italy, a greater reduction of serotype 19A was observed in children vaccinated with PCV13 [37].

As young children are the major reservoir of *S. pneumoniae*, the impact of the vaccine on carriage in this group plays a significant role in determining the impact of the vaccine on the community as a whole [7]. We included mothers in our analysis as, among adults, they are likely to benefit soonest from the indirect effect of the vaccine. However, one year after vaccine introduction we did not find a reduction in PCV13 serotypes among mothers whose children had been vaccinated with PCV13. This emphasises the fact that the indirect protective effect of PCVs may take several years to reach their maximum impact, and is consistent with the prediction of a recent mathematical model that population-wide serotype replacement in The Gambia will occur over a five year period [38].

Unfortunately, because of the study design, it is impossible to account for increasing herd effect after PCV7 introduction. Some of the differences among infants in CSS1 and CSS2, such as the decrease in PCV7-VT, could be related to the time since PCV introduction rather than the effect of the new vaccine. However, the decrease of PCV(13-7)VT (which includes only the serotypes from PCV13 that are not included in PCV7) in CSS2 cannot be explained by the longer time since the introduction of PCV7 and therefore the most likely explanation is the introduction of the new formulation. Our findings are in line with data from a RCT conducted in Israel comparing PCV7 versus PCV13 [20]. Secular trends, however, can occur in a before and after design.

Despite the challenges considered above, our data show that PCV13 resulted in lower carriage of PCV(13-7)VT with the strongest effect on an important serotype, 6A. These findings represent the first evidence of the impact of PCV13 in nasopharyngeal colonization in Africa, which may serve as a predictor of vaccine effectiveness through both direct and indirect effects. In addition, our results suggest that non-typable isolates will probably become more common in Africa with increasing deployment of PCV13.

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Authors' contribution

AR, BG and BK designed the study. AR drafted the manuscript. CB, RAG, BG, SB and BK revised the manuscript critically with important conceptual contributions. AB, IC, EFN performed the microbiological analysis with supervision from SJ and MA. JA, UE, CO and BI conducted the clinical work. BAKA, SB and MA donated data. RAG and SB conducted the whole genome sequencing analysis. All authors approved the final version of the manuscript.

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Conflicts of interest: All authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.11.012.

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