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**Title: Persistence of nasopharyngeal pneumococcal vaccine serotypes and increase of non-vaccine serotypes among vaccinated infants and their mothers five years after PCV13 introduction in The Gambia**

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**Running title**

Persistent pneumococcal serotypes

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## Summary

Five years post-PCV13, pneumococcal carriage has decreased among PCV13 vaccinated infants and their mothers, however some vaccine serotypes such as serotypes 3, 7F and 19A have persisted and some non-vaccine serotypes such as serotype 21 and non-typeable strains have emerged.

## Abstract

### *Background*

The widespread use of pneumococcal conjugate vaccine (PCV) has brought about a dramatic decrease in pneumococci of vaccine serotypes (VT) but non-vaccine serotypes (NVT) have emerged. Robust data on VT persistence and the extent of NVT replacement in developing countries are crucial to guide future vaccine policy.

### *Methods*

We conducted a cross sectional survey (CSS) among infants who received three doses of PCV13 and their mothers five years (CSS3) after PCV13 introduction. Nasopharyngeal swabs were collected and cultured for isolation of *Streptococcus pneumoniae*. Whole genome sequencing of the non-typeable strains was performed. Data were compared to two previous surveys conducted before PCV13 introduction (CSS1), and one year (CSS2) later.

### *Results*

Among infants, VT carriage decreased from 33.3% (113/339) in CSS1 to 11.4% (40/351) in CSS3 ( $p=0.001$ ) while NVT increased from 53.1% (180/339) in CSS1 to 74.4% (261/351) in CSS3 ( $p < 0.001$ ). Among mothers, there was a significant decrease of VT between CSS2 8.4% (29/347) and CSS3 5.6% (19/342) ( $p=0.006$ ). NVT increased from 16.6% (55/331) in CSS1 to 32.2% (110/342) in CSS3 ( $p < 0.001$ ). In CSS3, the most prevalent VT were 7F (infants) and 3 (mothers), and NVT were

serogroup 16 and non-typeables respectively. Genomic analysis showed that VT were more likely than NVT to lose their ability to express the capsule.

### *Conclusions*

Five years after PCV13, we show both direct (infants) and indirect effects (mothers) of the vaccine, while NVT replacement has occurred in both groups. Ongoing circulation of VT warrants further study of its relevance in any consideration of a reduced dose schedule.

### **Keywords**

Pneumococcal carriage serotypes herd-effect non-typeable

## Introduction

Prevention of pneumococcal disease remains a major global health priority due to the high disease burden and associated mortality, especially among children in developing countries (1). A seven-valent vaccine (PCV7) was licensed in 2009, but higher valency formulations (PCV10 and PCV13) have since been licenced (2). WHO recommends worldwide PCV introduction particularly in countries with under-5 mortality of >50/1000 live births (3). To date PCVs have been introduced in 59 low and middle income countries (4).

The impact of PCVs on pneumococcal disease results from a combination of the direct and indirect effects of the vaccine (5-7). The latter is a consequence of decreased carriage prevalence among vaccinated individuals and the resulting decrease in pneumococcal transmission within communities (8, 9). The overall vaccine impact on both disease and carriage, however, has been dampened by an increase in serotypes not included in the vaccine formulations, i.e., non-vaccine serotypes (NVT). This phenomenon is known as serotype replacement (10, 11). Fortunately, the lower virulence of NVT means there is nonetheless a net reduction in pneumococcal disease after PCV introduction (12, 13). In addition to serotype replacement, increased capsular switching (genetic recombination of the proteins on the bacterial capsule) in response to vaccine pressure has been reported (14).

In The Gambia where PCV7 was introduced in 2009 and replaced by PCV13 in 2011, we reported an 82% reduction in vaccine serotype (VT) invasive pneumococcal disease (IPD) and 47% increase in NVT IPD two years after PCV13 introduction (15). These data are consistent with data from two successive carriage surveys conducted before PCV13 introduction (two years after PCV7 and one year before PCV13) which showed a decrease in PCV13 VT carriage in infants but not their mothers (i.e. no herd effect). Despite the decrease in VT, a third of all pneumococcal isolates were of VT (16). Interestingly, an increase in the prevalence of carriage with non-typeable pneumococci, the majority of which had lost their capsule, was also observed (16).

The increase in NVT carriage and the continued presence of VT both in carriage and disease, post PCV are a cause for concern. To continue monitoring the direct and indirect effect of PCV13 among vaccinated infants and their unvaccinated mothers on overall, VT and NVT carriage, and to explore vaccine impact on non-typeable strains, we conducted a third pneumococcal carriage survey, five years after PCV13 introduction.

## Methods

### *Study setting*

The study was carried out at two government health facilities that offer maternal and child health services with regular Expanded Programme on Immunisation (EPI) clinics. PCV13 was introduced without a catch-up in a 3+0 schedule with doses given at 2, 3 and 4 months. The WHO/UNICEF immunisation data reported > 95% coverage of PCV13 dose 3 in 2016 (17).

### *Study design*

We conducted a cross sectional survey (CSS3) five years after PCV13 in 2016. The results were compared with historical data from two earlier surveys conducted before PCV13 in 2011 (CSS1) and one year after PCV13 in 2012 (CSS2) (16). The study design, recruitment sites, entry criteria, sample collection and laboratory methods were similar and all three surveys were conducted between March and June. In all three surveys, we recruited healthy infants 6-12 months of age and their mothers at the EPI clinics. Infants were recruited only if they had received three doses of PCV13, at least 1 month before recruitment and were accompanied by their biological mother. We collected information on demographics and risk factors for carriage by interviewing the mothers. Nasopharyngeal swabs (NPS) were collected from the mothers and their infants. Written informed consent was obtained from the mothers. The study was approved by the joint Medical Research Council (MRCG)-Gambia Government Ethics Committee.

### *NPS sample collection*

A calcium alginate swab was passed gently down the posterior wall of the nasopharynx. The swab was removed and placed in skim milk-tryptone-glucose-glycerol (STGG) transport medium. The STGG vials were taken to MRCG laboratories as recommended (18, 19).

### *Laboratory processing*

The samples were stored at – 70°C and processed as previously described for CSS1 and CSS2 (16). In brief, 200µl of thawed, vortexed STGG was placed into 5ml of Todd Hewitt broth containing 5% yeast extract, and 1ml of rabbit serum. This mixture was vortexed and then incubated at 37°C for 4-6 hours. Subsequently, 50µl aliquot was inoculated onto gentamycin blood agar and incubated overnight in 5% CO<sub>2</sub> at 37°C for the selective isolation of *S. pneumoniae*. The morphologically distinct alpha haemolytic colonies were screened for optochin susceptibility. Serotyping was done using latex agglutination technique (20). As in CSS1 and CSS2, serotyping was repeated for all non-typeable isolates.

#### *Whole genome sequencing for non-typeable pneumococcal serotypes*

Twenty nine non-typeable isolates from CSS1 and CSS2 were previously analysed using whole genome sequencing at the Wellcome Sanger Institute (Cambridge, UK). In this study, we analysed a further 23 non-typeables obtained from CSS3.

The sequencing, identification of contaminants, and MLST were done as detailed previously (16). The phylogenetic tree was reconstructed from SNP-sites (21) using RAxML (22). *In-silico* serotype was determined using the k-mer based serotyping method, SeroBA (23). This identified serotypes and generated capsule loci gene assemblies. No assemblies were generated for classical non-typeable pneumococci or isolates that have lost their capsule synthesis genes. Assemblies were annotated using Prokka (24). To investigate potential reasons for the non-capsule expression, all capsule loci were compared to reference capsule loci derived from Bentley *et al* (25) using Artemis Comparison Tool [ACT] (26). Genes showing significant difference to the reference genes were aligned and visualised in SeaView to investigate for truncations and mutations that may cause loss of function.

#### *Sample size calculation*

We targeted 350 infants similar to the sample size in CSS1 and CSS2. This provided 90% power to detect a 50% reduction in the prevalence of PCV13 VT and a 25% increase in NVT compared to CSS2, i.e., a decrease of PCV13 VT prevalence from 18% to 9% among infants and 8.1% to 4.1% among



mothers, and an increase of PCV13 NVT prevalence from 66.3% to 82.9% among infants and 16.1% to 20.1% among mothers (16).

#### *Data management and analysis*

We calculated PCV7 carriage prevalence (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F), PCV13 carriage prevalence (PCV7 serotypes +1, 3, 5, 6A, 7F, 19A) and PCV13 NVT carriage prevalence (all other serotypes including non-typeables). Additionally we calculated the prevalence of the six serotypes contained in PCV13 but not in PCV7. We used Poisson regression with robust standard errors to estimate prevalence ratios comparing CSS1 & CSS3, and CSS2 & CSS3 and to adjust for potential confounders. We tested the hypothesis that the proportion of PCV13 VT was the same among 'typeables' (i.e., isolates of known serotypes) and non-typeables (16) using Fisher's exact test. All analyses were done using STATA 14 (Statacorp LP, USA).

## Results

### *Characteristics of the study participants*

We recruited 351 infants and 347 mothers in CSS3. A total of 1020 mothers and 1040 infants were swabbed over the three surveys (20 infants were twins). Across the surveys, the median (IQR) age was 25 years (21.0-29.3) for mothers and 7.9 months (6.8-9.4) for infants and 51.8% of the infants were males. The demographical and epidemiological characteristics of the women and infants were similar across the surveys except that in CSS1 mothers were less educated and infant antibiotic use was more common (Table 1).

### *Prevalence of pneumococcal carriage pre and five years post-PCV13*

#### *Vaccinated Infants*

Overall carriage was approximately 85% over the three surveys (Table 2). VT pneumococcal carriage decreased from 33.3% (113/339) in CSS1 to 11.4% (40/351) in CSS3 ( $p < 0.001$ ) while NVT carriage increased from 53.1% (180/339) in CSS1 to 74.4% (261/351) in CSS3 ( $p < 0.001$ ). VT decreased further between CSS2 and CSS3 (18.3% to 11.4%,  $p = 0.021$ ) while NVT increased (66.9% to 74.4%,  $p = 0.025$ ) (Table 2). For the six serotypes included in PCV13 but not in PCV7, there was a significant decrease in prevalence between CSS1 and CSS2 (23.9% versus 13.7%), with an additional drop to 5.4% in CSS3 ( $p < 0.001$ ).

Among vaccine-type serotypes, 19A decreased from 8.3% in CSS1 to 0.9% in CSS3 ( $p < 0.001$ ) and 19F decreased slightly from 5.6% to 2.8% ( $p = 0.078$ ). Serotype 6A decreased from 15.3% in CSS1 to 5.7% in CSS2 ( $p < 0.001$ ). The most prevalent VT in CSS3 was serotype 7F (3.4%) which had not been seen in the previous surveys ( $p < 0.001$ ). Serotype 21 was the second most prevalent NVT (7.1%) having increased significantly from CSS1 (3.2%,  $p = 0.026$ ) and there was a significant increase in the non-typeables from 0.3% in CSS1 to 3.4% in CSS3 ( $p = 0.018$ ).

#### *Mothers of vaccinated infants*

Overall pneumococcal carriage increased from 23.0% (76/331) in CSS1 to 37.7% (129/342) in CSS3 ( $p < 0.001$ ) (Table 3). Although prevalence of VT carriage was similar in all three surveys (6%), NVT carriage increased from 16.6% (55/331) in CSS1 to 32.2% (110/342) in CSS3 (Table 3).

Serotype 3 was the most prevalent VT in CSS3 (1.8%) followed by 19F (1.2%). Other VT serotypes that were prevalent in CSS1 and CSS2, decreased considerably [19A decreased from 1.8% in CSS1 to 0.3% in CSS3, and 6A from 1.8% in CSS1 to 0% in CSS3 ( $p = 0.014$ )]. Several NVT increased between CSS1 and CSS3. Notably serotype 21 increased from 0.9% in CSS1 to 2.9% in CSS3 ( $p = 0.073$ ) and non-typeables increased from 0.3% in CSS1 to 3.2% in CSS3 ( $p = 0.023$ ) (Table 3).

#### *Genotypic analysis of the non-typeable serotypes*

A total of 52 phenotypically non-typeable isolates were analysed; three from CSS1 (two from mothers and one from an infant), 26 from CSS2 (five from mothers and 21 from infants) and 23 from CSS3 (11 from mothers and 12 from infants). Three isolates were *S. pseudopneumoniae* - one from a mother in CSS1 and two isolates from infants in CSS2 (16), and one was a non-pneumococcal streptococcus from a mother in CSS3. Fifteen isolates (28.8%) were of the classical non-typeable lineage. The others (33; 63.5%) were of an encapsulated pneumococcal lineage and had lost their ability to express a capsule through three different mechanisms (Figure 1 and Table 4). Eight had a standard capsular polysaccharide synthesis (*cps*) locus but a capsule was not phenotypically detected, 15 had indels in capsule synthesis genes or complete loss of capsule loci and 10 had acquired a locus typically found in classically non-typeable isolates.

Based on combined data from CSS2 and CSS3, eight mothers and 19 infants carried non-typeables that were non classical [seven (87.5%) and eight (42.1%) were of the VT lineage respectively]. Compared to VT carriage prevalence of 22.6% (49/217) in mothers and 17.5% (104/595) in infants, this corresponds to 3.9 fold increase (95% CI 2.7, 5.6;  $p < 0.001$ ) in mothers and 2.4 fold increase (95% CI 1.4-4.2,  $p = 0.012$ ) in infants.

## Discussion

Despite the observed decline in VT pneumococcal prevalence in mothers and infants since the introduction of PCV13 in The Gambia, VT pneumococci continue to circulate in both groups. Among infants, the decrease in PCV13 VT carriage and associated increase in NVT resulted in no change in overall pneumococcal carriage. In contrast, the decrease in VT in mothers was less pronounced than the increase in NVT resulting in a net increase in overall pneumococcal carriage. Our previous observation that the increase in non-typeable pneumococcus post PCV is mainly due to serotypes that have lost their capsule was confirmed by data from CSS3 (16).

Although the direct effect of PCV13 on circulating PCV13 VT among vaccinated infants was observed one year after its introduction (CSS2) (16), five years later (CSS3) VT were still circulating in infants who had received three doses of the vaccine. The PCV13 VT prevalence in this study (11.4%) is similar to VT carriage among Gambian new-borns two years after routine PCV13 (27), but two fold higher than PCV13 VT prevalence in Greenlandic under-fives (5%) three years after PCV13 (28), Belgian children (5.4%) five years after PCV13 and nine years after PCV7 (29), and Australian Aboriginal under-fives (5.8%) three years after PCV13 and ten years after PCV7 (30). We saw a significant increase in serotype 19F after PCV13 introduction which was also among the most common VT after PCV13 in the Belgian study (29). We also observed an increase in serotype 7F prevalence in CSS3. The increase is surprising since a mathematical model of pneumococcal transmission in The Gambia predicted that PCV13 would eliminate low transmission VT including 7F (31). A similar increase in 7F was observed in IPD surveillance data after introduction of PCV13 in Australia. However, elsewhere there has been a decline in 7F post-PCV13 (32).

Our results suggest a PCV13 herd effect among Gambian mothers of vaccinated infants, particularly for serotypes 6A and 19A; such an effect was not yet apparent one year after the introduction of PCV13 (CSS2). However, we have to interpret this decrease in VT with caution as the decrease between CSS1 and CSS3 was not significant, and there was an increase between CSS1 and CSS2

driven by the large increase in serotype 19A in CSS2. A cluster randomised trial that was conducted in rural Gambia demonstrated a herd effect of PCV (33), as have countries that have introduced PCV7 (34) and countries that have introduced PCV13 with a booster dose (6).

We found that the prevalence of serotype 19A decreased after PCV13 introduction and was low in CSS3 in both mothers and infants. This is in contrast to data from the UK and Alaska. In the UK, one of the first countries to switch from PCV7 to PCV13, IPD associated with 19A has begun to increase in recent years (35) and in Alaska, PCV13 protection against 19A is waning in older vaccinated children possibly due to the emergence of a new genotype. Longer surveillance in our setting is needed to better understand the trend of this serotype in carriage and disease

In mothers, serotype 3 was the most prevalent serotype in CSS3, and cases of IPD caused by this serotype are still seen in The Gambia (15). This serotype, which had already decreased before PCV13 introduction, shows secular trends in The Gambia (36). Several carriage and IPD studies have shown that PCV13 has no direct or indirect effect on serotype 3 (37, 38).

Five years post-PCV13, we have observed an increase of NVT both for vaccinated infants and their mothers. For infants, we had already observed an increase between CSS1 and CSS2. In CSS3, we detected a significant increase in serotypes 21 and 23B. These were also the most prevalent serotypes in the post-PCV13 era in studies conducted in The Gambia (27), Italy and Norway (39, 40). Unexpectedly, among mothers the increase in NVT exceeded the decrease in VT, resulting in an overall increase in carriage. Although this large increase is difficult to explain, it probably reflects an upsurge in pneumococcal transmission, rather than biased sampling since similar field and laboratory methods were used in all three surveys and recruitment took place during the same time of the year to minimise any effect of season (41).

Non-typeable strains increased in prevalence over the three surveys in both vaccinated infants and mothers which is consistent with the rise in non-typeable strains causing non-invasive and invasive disease after PCV13 introduction in Taiwan (42). Further WGS analysis of all the non-typeables

confirmed our previous hypothesis that VT are more likely than NVT to lose their ability to express the capsule after introduction of PCV. These ‘capsular switches’ may be due to the selection of variants that existed in the pre-PCV era. Capsular loss is relatively common for *S. pneumoniae*, and increased loss due to vaccine pressure has also been reported in other studies (14).

Non-typeable pneumococci are likely to be under-reported (43) as they are generally excluded from the analyses in epidemiological studies and vaccine trials, including the ongoing surveillance of the IPD in The Gambia (15). This maybe because their role in disease has been limited. For example, in South Africa over a 10 year period spanning from before to post PCV, and in the US over a three year period in the post-PCV era, non-typeables rarely caused disease (44, 45). We nonetheless advocate for the inclusion of non-typeable strains in epidemiological surveillance studies due to their increasing importance and potential for maintaining transmission.

The three surveys provide valuable data on the timeliness of vaccination. It is often assumed that vaccination in Africa is substantially delayed and more in line with a 2+1 schedule (i.e., two doses before 6 months and one “booster dose” after 9 months). However, our data suggest that in The Gambia the schedule used is close to 3+0 (the median age of PCV dose 3 vaccination was 5 months). Our study therefore represents a genuine evaluation of the 3+0 schedule and, as such, contributes to the current debate over the relative merits of the 3+0 versus the 2+1 PCV schedule (46).

The main limitations of our study were intrinsic to the study design in that we cannot exclude secular trends in individual serotypes since variability in the prevalence of serotypes were already described before vaccine introduction (47). In addition our surveys only started after PCV7 was introduced into routine immunisation and therefore our comparisons were between children vaccinated with PCV7 (CSS1) and children vaccinated with PCV13 (CSS2 and CSS3), capturing only the additional effect of PCV13 over PCV7. There may have been residual confounding since we did not adjust for number of siblings, and other studies have shown increased risk of carriage when living with other children (27, 47). However, there was no difference in maternal age between surveys, a potential proxy for

number of siblings. And though we did not collect information on household size in CSS3, there was no association between carriage and household size in CSS1 and CSS2.

## Conclusions

We have shown important effects of the introduction of PCV13 into routine immunization in The Gambia, including direct and indirect effect, and serotype replacement. Continued disease surveillance and additional carriage surveys are necessary to monitor persistence of VT, the emergence of NVT, and the role of non-typeables in transmission and disease. An alternative vaccination schedule (not necessarily reducing the number of doses) needs to be considered to halt ongoing VT transmission.



### **Author contributions**

AR & BK designed the original studies. AR, CB, PCH & EU critically reviewed the study proposal and made important contributions throughout the study. IC & AB led the microbiological isolation of *S. pneumoniae* from the swabs, and EB & RH did the WGS. EU coordinated the field work for CSS3, did the analysis and wrote the first draft of the manuscript. All authors contributed to writing the manuscript and approved the final version.

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### **Conflict of Interests**

None

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## Tables

Table 1 Characteristics of the study participants

	CSS1* <sup>^</sup> N=339	CSS2* <sup>^</sup> N=350	CSS3 <sup>^</sup> N=351	P-value <sup>-</sup>
Variable	n(%)	n(%)	n(%)	
<b>Era**</b>	Before PCV13	1 year after	5 years after	
<b>Health centre</b>				
Jammeh Foundation	229(67.6)	251(71.7)	250(71.2)	0.428
Sukuta	110(32.4)	99(28.3)	101(28.8)	
<b>Gender<sup>a</sup></b>				
Male	180(53.1)	165(47.3)	195(55.6)	0.069
Female	159(46.9)	184(52.7)	156(44.4)	
<b>Infant's age (months)</b>				
Median (IQR)	8.2 (7.0,9.6)	7.6 (6.7,9.2)	8.0 (6.9,9.3)	
<b>Infant PCV age (weeks)</b>				
Median (IQR)				
Dose 1	10.3 (9.3,11.9)	10.6 (9.3, 12.3)	10.0 (9.0, 11.6)	
Dose 2	15.9 (14.1, 18.3)	16.6 (14.6, 18.7)	15.4 (14.0-17.7)	
Dose 3 <sup>b</sup>	21.9 (19.4, 24.9)	22.4 (20.1, 25.6)	20.7 (18.9, 23.4)	

**Infant antibiotic use <sup>c</sup>**

No	295(88.6)	319(92.2)	333(94.9)	0.010
Yes	38(11.4)	27(7.8)	18(5.1)	

**Mother's schooling <sup>d</sup>**

None or < 1 year	134(41.7)	108(31.3)	135(39.5)	<0.001
1-3 years	44(13.7)	35(10.1)	8(2.3)	
4-6 years	78(24.3)	44(12.8)	26(7.6)	
>6 years	65(20.3)	158(45.8)	173(50.6)	

**Mother's age (years)**

Median(IQR)	24.5 (21.0,29.0)	25.0 (21.0,28.0)	25.0 (21.0, 30.0)	
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**Household size**

Median (IQR)	5.0 (4.0,7.0)	3.0 (3.0,5.0)	Na	
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\*Effect on nasopharyngeal pneumococcal carriage of replacing PCV7 with PCV13 in the Expanded Programme of Immunization in The Gambia Roca et al, Vaccine 33 (2015) 7144–7151

^ There were 20 twins; CSS1 8, CSS2 3 & CSS3 9. The number of mothers was 331, 341 and 347 in CSS1, 2 and 3 respectively.

\*\*PCV 7 intro in 2009, two years before the start of this study

– p-value obtained from chi-square test.

<sup>a</sup> one missing in CSS2

<sup>b</sup> one missing in CSS3

b six missing CSS1 and 2 missing CSS2

<sup>c</sup> antibiotic use within 4 months of survey

<sup>d</sup> 10 missing in CSS1 and 2 missing in CCS2

Na                      Not            available            as            data            were            not            collected



Table 2

Prevalence of pneumococcal carriage in Infants before (CSS1) and one (CSS2) and five years (CSS3) after introduction of PCV13 into the Gambian EPI.

	Prevalence of Carriage (%)			CSS2 versus CSS3		CSS1 versus CSS3	
	CSS1(N=339)	CSS2(N=350)	CSS3(N=351)	RRadj	p-value	RRadj	p-value
<b>Vaccine groups</b>							
<b>PCV 13 VT</b>	33.3	18.3	11.4	0.64(0.44,0.93)	0.021	0.60(0.50,0.71)	<0.001
<b>PCV 13 NVT</b>	53.1	66.9	74.4	1.12(1.01,1.23)	0.025	1.19(1.11,1.26)	<0.001
<b>PCV13-7 VT</b>	23.9	13.7	5.4	0.40(0.24,0.67)	0.001	0.23(0.14,0.37)	<0.001
<b>PCV 7 VT</b>	9.4	4.9	6.0	1.30(0.67,2.53)	0.441	0.67(0.31,1.00)	0.051
<b>All serotypes</b>	85.8	84.3	85.5	1.02(0.96,1.09)	0.459	1.00(0.97,1.04)	0.885
<b>PCV 13 VT</b>							
<b>1</b>	0	0	0	NA	NA	NA	NA
<b>3</b>	0	0.3	1.1	3.99(0.45,35.56)	0.215	NA	NA
<b>4</b>	0.6	0	0.9	NA	NA	1.20(0.49,2.94)	0.684
<b>5</b>	0.3	1.4	0	NA	NA	NA	NA
<b>6A</b>	15.3	5.7	0	NA	NA	NA	NA
<b>6B</b>	0.9	0	0	NA	NA	NA	NA

<b>7F</b>	0	0	3.4	NA	NA	NA	NA
<b>9V</b>	0	0	0.6	NA	NA	NA	NA
<b>14</b>	0.6	0.9	0.6	0.66(0.11,3.96)	0.654	0.98(0.37,2.61)	0.972
<b>18C</b>	0.6	0	0	NA	NA	NA	NA
<b>19A</b>	8.3	6.3	0.9	0.14(0.04,0.45)	0.001	0.32(0.18,0.58)	<0.001
<b>19F</b>	5.6	1.7	2.8	1.66(0.61,4.53)	0.32	0.71(0.49,1.04)	0.078
<b>23F</b>	1.5	2.3	1.1	0.50(0.15,1.64)	0.252	0.88(0.46,1.69)	0.699
<b>PCV 13 NVT</b>							
<b>10A</b>	4.7	4	6	1.50(0.77,2.89)	0.232	1.13(0.82,1.55)	0.463
<b>13</b>	3.5	3.4	3.4	1.00(0.45,2.19)	0.994	0.98(0.66,1.46)	0.931
<b>15B</b>	8.3	9.1	6.8	0.75(0.45,1.24)	0.263	0.91(0.70,1.18)	0.48
<b>16</b>	5.9	6.6	9.7	1.47(0.89,2.45)	0.135	1.28(0.98,1.67)	0.068
<b>19C</b>	0.9	0.3	0	NA	NA	NA	NA
<b>21</b>	3.2	4.9	7.1	1.47(0.81,2.67)	0.21	1.48(1.05,2.10)	0.026
<b>23B</b>	1.5	3.1	4.6	1.45(0.68,3.08)	0.334	1.76(1.07,2.89)	0.026
<b>34</b>	3.8	2.3	4.6	1.99(0.86,4.60)	0.106	1.09(0.76,1.56)	0.637
<b>35B</b>	4.7	4.9	4.6	0.94(0.48,1.83)	0.852	0.98(0.70,1.38)	0.92
<b>NT</b>	0.3	6.0	3.4	0.57(0.28,1.14)	0.112	3.40(1.23,9.42)	0.018

RR risk ratio, RRadj risk ratio adjusted for health centre, maternal age, and maternal education infant's age, gender, and antibiotic intake within four weeks of the survey

Table 3

Prevalence of pneumococcal carriage in Mothers before (CSS1) and one (CSS2) and five years (CSS3) after introduction of PCV13 into the Gambian EPI.

	Prevalence of Carriage (%)			CSS2 versus CSS3		CSS1 versus CSS3	
	CSS1(N=331)	CSS2(N=347)	CSS3(N=342)	RRadj	pvalue	RRadj	p-value
<b>Vaccine group</b>							
<b>PCV 13 VT</b>	6.6	8.4	5.6	0.58(0.33,1.02)	0.06	0.92(0.67,1.27)	0.627
<b>PCV 13 NVT</b>	16.6	16.1	32.2	2.01(1.51,2.67)	<0.001	1.40(1.20,1.63)	<0.001
<b>PCV 13-7 VT</b>	3.9	6.1	2.3	0.34(0.15,0.74)	0.007	0.65(0.28,,1.52)	0.319
<b>PCV 7 VT</b>	2.7	2.6	3.2	1.14(0.46,2.79)	0.782	0.99(0.40-2.48)	0.989
<b>All serotypes</b>	23.0	24.2	37.7	1.51(1.20,1.91)	<0.001	1.29(1.14,1.47)	<0.001
<b>PCV13 VT</b>							
<b>1</b>	0.3	0	0	NA	NA	NA	NA
<b>3</b>	0	0.3	1.8	6.09(0.74,50.38)	0.094	NA	NA
<b>4</b>	0.6	0.6	0.6	1.01(0.14,7.17)	0.988	0.98(0.37,2.62)	0.974
<b>5</b>	0	0.9	0	NA	NA	NA	NA

<b>6A</b>	1.8	2	0	NA	NA	NA	NA
<b>6B</b>	0	0	0	NA	NA	NA	NA
<b>7F</b>	0	0	0.3	NA	NA	NA	NA
<b>9V</b>	0	0	0.3	NA	NA	NA	Na
<b>14</b>	0.6	0.3	0.6	2.03(0.18,22.31)	0.563	0.98(0.37,2.62)	0.974
<b>18C</b>	0.3	0.6	0.3	0.51(0.05,5.58)	0.579	0.98(0.25,3.93)	0.982
<b>19A</b>	1.8	3.2	0.3	0.09(0.01,0.71)	0.022	0.40(0.14,1.16)	0.091
<b>19F</b>	0.6	0.3	1.2	4.06(0.46,36.19)	0.21	1.39(0.60,3.24)	0.444
<b>23F</b>	0.6	0.9	0.3	0.34(0.04,3.24)	0.347	0.70(0.21,2.31)	0.553
<b>PCV13 NVT</b>							
<b>10A</b>	0.6	0.3	2.3	8.12(1.02,64.65)	0.048	1.97(0.91,4.26)	0.086
<b>13</b>	0.6	1.7	0.6	0.34(0.07,1.67)	0.183	0.98(0.37,2.62)	0.974
<b>15B</b>	0.6	1.7	1.2	0.68(0.19,2.38)	0.542	1.39(0.60,3.24)	0.444
<b>16</b>	2.4	0.6	2.3	4.06(0.87,19.00)	0.075	0.98(0.61,1.60)	0.947
<b>19C</b>	1.2	0	0	NA		NA	NA
<b>21</b>	0.9	0.9	2.9	3.38(0.94,12.19)	0.063	1.80(0.95,3.41)	0.073
<b>23B</b>	0	0.9	2.0	2.37(0.62,9.09)	0.209	NA	NA
<b>34</b>	1.2	1.2	1.8	1.52(0.43,5.35)	0.513	1.20(0.64,2.26)	0.561
<b>35B</b>	1.8	0.6	1.8	3.04(0.62,14.99)	0.171	0.98(0.56,1.72)	0.954

NT	0.3	1.4	3.2	2.23(0.78,6.36)	0.133	3.26(1.17,9.06)	0.023
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RR risk ratio, RRadj-risk ratio adjusted for health centre, maternal age, and maternal education

Table 4 Results of whole genome sequencing of non-typeable isolates<sup>a</sup>

Isolate N	Mother /Infant	S.pn % reads	S.pseudopn % reads	ST	Nearest ST	Capsular locus top hit	Serotype specific sequence detected	Ancestral capsular type from phylogeny	Conclusion	VT /NVT
<b>CSS1</b>										
108887	Infant	78.08	0.71	3407		16F	16F	16F	Wzd gene loss and truncated wzh	NVT
105098	Mother	80.51	0.14	1778		34	34	34	Intact capsule synthesis genes- No genetic explanation	NVT
104550	Mother	19.47	40.9	Unknown		-			<i>S.pseudopneumoniae</i>	---
<b>CSS2</b>										
201376	Infant	80.46	0.25	5521		10A	10A	10A	Intact capsule synthesis genes- No genetic	NVT

*explanation*

207381	Infant	82.09	0.4	Novel ST D	2052	20	20	Long branch, inconclusive	Truncated whaF and wzx genes	NVT
206628	Infant	81.38	0.08	989		12F	12F/A/46	12F/A/46	Intact capsule synthesis genes- No genetic explanation	NVT
210240	Infant	80.96	0.08	989		12F	12F/A/46	12F/A/46	Intact capsule synthesis genes- No genetic explanation	NVT
210201	Infant	83.51	0.08	2447		14	14	14	Truncated transferase genes, wciY and Irp	VT
201867 <sup>b</sup>	Infant	46.64	0.57	Novel ST B	4040	Classical NT		14	Capsule switch from 14 to classically NT locus	VT
200954	Mother	81.22	0.25	Novel ST G	975	15B/C	15B/C	15B/C	12 nucleotide insertion at the 5' end of the initial transferase gene, wchA	NVT

206379	Infant	81.51	0.16	4033		15B/C	15B/C	15B/C	12 nucleotide insertion at the 5' end of the initial transferase gene, wchA	NVT
207139	Infant	76.55	0.57	3407		16F	16F	16F	Wzd gene loss and truncated wzh	NVT
210300	Infant	78.96	0.62	Novel ST F	3407	16F	16F	16F	Wzd gene loss and truncated wzh	NVT
210092	Infant	80.13	0.28	Novel ST E	847	19A	19A	19A	3 nucleotide deletion at the 3' end of the rmlC gene	VT
210344	Infant	79.93	0.19	Unknown	7661	19B		19B	Intact capsule synthesis genes- <i>No genetic explanation</i>	NVT
206438	Infant	81.8	0.1	202		19A	19A	Long branch, inconclusive	3 nucleotide deletion at the 3' end of the rmlC gene	VT
208478	Infant	81.95	0.17	4033		19F	19F	19F/15B/C	Intact capsule synthesis genes- <i>No genetic explanation</i>	VT

202731	Infant	81.26	0.08	Novel ST C	6712	28A/F	28A/F	6 nucleotide insertion in the <i>wze</i> gene	NVT	
201297	Mother	81.95	0.34	5734		6A/B/C/D	6A/B/C/D	6A	12 nucleotide insertion in 3' end of the pseudogene HG262	VT
201794	Infant	78.82	0.8	Novel ST A	71	38 (low match 35%)	Long branch, inconclusive	Loss of <i>cps</i> locus	---	
201398	Mother	76.17	1.3	Novel ST H	3582	Classical NT	Long branch, inconclusive	Capsule switch to classically NT locus	---	
201843	Infant	77.28	1.11	4040		Classical NT	14	Capsule switch from 14 to classically NT locus	VT	
205597	Infant	75.37	0.96	Novel ST B	4040	Classical NT	14	Capsule switch from 14 to classically NT locus	VT	
206158	Mother	78.28	0.89	Novel ST B	4040	Classical NT	14	Capsule switch from 14 to classically NT locus	VT	
201133	Infant	64.27	3.02	344		Classical NT	Classical NT	Classical NT	---	
202601	Mother	65.69	2.64	448		Classical NT	Classical NT	Classical NT	---	
208136	Infant	66.12	2.67	448		Classical NT	Classical NT	Classical NT	---	



201017	Infant	17.54	43.18	Unknown	-			<i>S.pseudopneumoniae</i>	---
209574	Infant	20.05	40.36	Unknown	-			<i>S.pseudopneumoniae</i>	---
<b>CSS3</b>									
258M		89.95	1.43	10967		Classical NT	14	Capsule switch from 14 to classically NT locus	VT
141B		78.34	3.66	448		Classical NT	Classical NT	Classical NT	---
219B		76.67	4.01	344		Classical NT	Classical NT	Classical NT	---
034B		77.2	4.86	11729		Classical NT	Classical NT	Classical NT	---
153M		83.33	1.86	Novel ST	4040	Classical NT	14	Capsule switch from 14 to classically NT locus	VT
114B		87.33	1.67	4040		Classical NT	14	Capsule switch from 14 to classically NT locus	VT
223M		73.83	4.19	344		Classical NT	Classical NT	Classical NT	---
028B		77.13	4.1	344		Classical NT	Classical NT	Classical NT	---
284M		81.26	0.97	Unknown		14	14	14	Truncated wzg and wchA genes.
092B		88.08	1.84	Novel ST	3582	Classical NT	Long branch, inconclusive	Capsule switch to classically NT locus	---

113M	86.71	1.79	10967		Classical NT	14	Capsule switch from 14 to classically NT locus	VT
201B	78.23	4.84	11729		Classical NT	Classical NT	Classical NT	---
239B	93.26	0.12	989		12F	12F	12F	<i>No genetic explanation</i> NVT
248M	29.05	18.34	Unknown		alternative_ai B_NT	Long branch, inconclusive	<i>Non-Pneumococcal streptococci</i>	---
236M	87.4	1.48	Unknown	~908	25F_or_25A (Low match 29.1%)	14	---	---
073B	77.4	4.11	344		Classical NT	Classical NT	Classical NT	---
268M	76.62	4.11	344		Classical NT	Classical NT	Classical NT	---
268B	76.49	4.14	344		Classical NT	Classical NT	Classical NT	---
171B	76.89	3.59	448		Classical NT	Classical NT	Classical NT	---
003M	89.41	0.37	847		19A	19A	19A	<i>4bp insertion on the wchA. 3bp deletion in the rmlC gene</i> VT
314M	77.8	4.16	344		Classical NT	Classical NT	Classical NT	---
243M	75.91	3.63	448		Classical NT	Classical NT	Classical NT	---

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258B	93.29	0.1	989	12F	12F	12F	<i>No genetic explanation</i>	NVT
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S.pn *S. pneumoniae* S. pseudopn *S. pseudopneumoniae*

<sup>a</sup>CSS1 & 2 updated mechanisms for lack of capsular expression

<sup>b</sup> Sample contaminated with unclassified organism, pneumococcal coverage sufficient for analysis and conclusion

## Figure legend

Figure 1. Phylogenetic context of non-typable isolates. (i) Outer ring: Non-typable isolates from this study (green). (ii) Middle ring: This is the SeroBA serotype results of all the samples, the brown colour represents all isolates without a capsule locus. The leaves are labelled with either the isolate's serotype (for context samples) or the isolate's ID (study samples). (iii) The innermost ring depicts the major lineages, classical non-typables (purple), *S. pseudopneumoniae* (red) and typically encapsulated lineages (orange).

## Tables

Table 1 Characteristics of the study participants

	CSS1* <sup>^</sup> N=339	CSS2* <sup>^</sup> N=350	CSS3 <sup>^</sup> N=351	P-value <sup>-</sup>
Variable	n(%)	n(%)	n(%)	
<b>Era**</b>	Before PCV13	1 year after	5 years after	
<b>Health centre</b>				
Jammeh Foundation	229(67.6)	251(71.7)	250(71.2)	0.428
Sukuta	110(32.4)	99(28.3)	101(28.8)	
<b>Gender<sup>a</sup></b>				
Male	180(53.1)	165(47.3)	195(55.6)	0.069
Female	159(46.9)	184(52.7)	156(44.4)	
<b>Infant's age (months)</b>				
Median (IQR)	8.2 (7.0,9.6)	7.6 (6.7,9.2)	8.0 (6.9,9.3)	
<b>Infant PCV age (weeks)</b>				
Median (IQR)				
Dose 1	10.3 (9.3,11.9)	10.6 (9.3, 12.3)	10.0 (9.0, 11.6)	
Dose 2	15.9 (14.1, 18.3)	16.6 (14.6, 18.7)	15.4 (14.0-17.7)	

Dose 3 <sup>b</sup>	21.9 (19.4, 24.9)	22.4 (20.1, 25.6)	20.7 (18.9, 23.4)	
<b>Infant antibiotic use<sup>c</sup></b>				
No	295(88.6)	319(92.2)	333(94.9)	0.010
Yes	38(11.4)	27(7.8)	18(5.1)	
<b>Mother's schooling<sup>d</sup></b>				
None or < 1 year	134(41.7)	108(31.3)	135(39.5)	<0.001
1-3 years	44(13.7)	35(10.1)	8(2.3)	
4-6 years	78(24.3)	44(12.8)	26(7.6)	
>6 years	65(20.3)	158(45.8)	173(50.6)	
<b>Mother's age (years)</b>				
Median(IQR)	24.5 (21.0,29.0)	25.0 (21.0,28.0)	25.0 (21.0, 30.0)	
<b>Household size</b>				
Median (IQR)	5.0 (4.0,7.0)	3.0 (3.0,5.0)	Na	

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\*Effect on nasopharyngeal pneumococcal carriage of replacing PCV7 with PCV13 in the Expanded Programme of Immunization in The Gambia Roca et al, Vaccine 33 (2015) 7144–7151

^ There were 20 twins; CSS1 8, CSS2 3 & CSS3 9. The number of mothers was 331, 341 and 347 in CSS1, 2 and 3 respectively.

\*\*PCV 7 intro in 2009, two years before the start of this study

– p-value obtained from chi-square test.

<sup>a</sup> one missing in CSS2

<sup>b</sup> one missing in CSS3

<sup>b</sup> six missing CSS1 and 2 missing CSS2

<sup>c</sup> antibiotic use within 4 months of survey

<sup>d</sup> 10 missing in CSS1 and 2 missing in CCS2

Na                      Not            available            as            data            were            not            collected

Table 2

Prevalence of pneumococcal carriage in Infants before (CSS1) and one (CSS2) and five years (CSS3) after introduction of PCV13 into the Gambian EPI.

	Prevalence of Carriage (%)			CSS2 versus CSS3		CSS1 versus CSS3	
	CSS1(N=339)	CSS2(N=350)	CSS3(N=351)	RRadj	p-value	RRadj	p-value
<b>Vaccine groups</b>							
<b>PCV 13 VT</b>	33.3	18.3	11.4	0.64(0.44,0.93)	0.021	0.60(0.50,0.71)	<0.001
<b>PCV 13 NVT</b>	53.1	66.9	74.4	1.12(1.01,1.23)	0.025	1.19(1.11,1.26)	<0.001
<b>PCV13-7 VT</b>	23.9	13.7	5.4	0.40(0.24,0.67)	0.001	0.23(0.14,0.37)	<0.001
<b>PCV 7 VT</b>	9.4	4.9	6.0	1.30(0.67,2.53)	0.441	0.67(0.31,1.00)	0.051
<b>All serotypes</b>	85.8	84.3	85.5	1.02(0.96,1.09)	0.459	1.00(0.97,1.04)	0.885
<b>PCV 13 VT</b>							
<b>1</b>	0	0	0	NA	NA	NA	NA
<b>3</b>	0	0.3	1.1	3.99(0.45,35.56)	0.215	NA	NA
<b>4</b>	0.6	0	0.9	NA	NA	1.20(0.49,2.94)	0.684
<b>5</b>	0.3	1.4	0	NA	NA	NA	NA
<b>6A</b>	15.3	5.7	0	NA	NA	NA	NA
<b>6B</b>	0.9	0	0	NA	NA	NA	NA



<b>7F</b>	0	0	3.4	NA	NA	NA	NA
<b>9V</b>	0	0	0.6	NA	NA	NA	NA
<b>14</b>	0.6	0.9	0.6	0.66(0.11,3.96)	0.654	0.98(0.37,2.61)	0.972
<b>18C</b>	0.6	0	0	NA	NA	NA	NA
<b>19A</b>	8.3	6.3	0.9	0.14(0.04,0.45)	0.001	0.32(0.18,0.58)	<0.001
<b>19F</b>	5.6	1.7	2.8	1.66(0.61,4.53)	0.32	0.71(0.49,1.04)	0.078
<b>23F</b>	1.5	2.3	1.1	0.50(0.15,1.64)	0.252	0.88(0.46,1.69)	0.699
<b>PCV 13 NVT</b>							
<b>10A</b>	4.7	4	6	1.50(0.77,2.89)	0.232	1.13(0.82,1.55)	0.463
<b>13</b>	3.5	3.4	3.4	1.00(0.45,2.19)	0.994	0.98(0.66,1.46)	0.931
<b>15B</b>	8.3	9.1	6.8	0.75(0.45,1.24)	0.263	0.91(0.70,1.18)	0.48
<b>16</b>	5.9	6.6	9.7	1.47(0.89,2.45)	0.135	1.28(0.98,1.67)	0.068
<b>19C</b>	0.9	0.3	0	NA	NA	NA	NA
<b>21</b>	3.2	4.9	7.1	1.47(0.81,2.67)	0.21	1.48(1.05,2.10)	0.026
<b>23B</b>	1.5	3.1	4.6	1.45(0.68,3.08)	0.334	1.76(1.07,2.89)	0.026
<b>34</b>	3.8	2.3	4.6	1.99(0.86,4.60)	0.106	1.09(0.76,1.56)	0.637
<b>35B</b>	4.7	4.9	4.6	0.94(0.48,1.83)	0.852	0.98(0.70,1.38)	0.92
<b>NT</b>	0.3	6.0	3.4	0.57(0.28,1.14)	0.112	3.40(1.23,9.42)	0.018

RR risk ratio, RRadj risk ratio adjusted for health centre, maternal age, and maternal education infant's age, gender, and antibiotic intake within four weeks of the survey

Table 3

Prevalence of pneumococcal carriage in Mothers before (CSS1) and one (CSS2) and five years (CSS3) after introduction of PCV13 into the Gambian EPI.

	Prevalence of Carriage (%)			CSS2 versus CSS3		CSS1 versus CSS3	
	CSS1(N=331)	CSS2(N=347)	CSS3(N=342)	RRadj	pvalue	RRadj	p-value
<b>Vaccine group</b>							
<b>PCV 13 VT</b>	6.6	8.4	5.6	0.58(0.33,1.02)	0.06	0.92(0.67,1.27)	0.627
<b>PCV 13 NVT</b>	16.6	16.1	32.2	2.01(1.51,2.67)	<0.001	1.40(1.20,1.63)	<0.001
<b>PCV 13-7 VT</b>	3.9	6.1	2.3	0.34(0.15,0.74)	0.007	0.65(0.28,,1.52)	0.319
<b>PCV 7 VT</b>	2.7	2.6	3.2	1.14(0.46,2.79)	0.782	0.99(0.40-2.48)	0.989
<b>All serotypes</b>	23.0	24.2	37.7	1.51(1.20,1.91)	<0.001	1.29(1.14,1.47)	<0.001
<b>PCV13 VT</b>							
<b>1</b>	0.3	0	0	NA	NA	NA	NA
<b>3</b>	0	0.3	1.8	6.09(0.74,50.38)	0.094	NA	NA
<b>4</b>	0.6	0.6	0.6	1.01(0.14,7.17)	0.988	0.98(0.37,2.62)	0.974
<b>5</b>	0	0.9	0	NA	NA	NA	NA

<b>6A</b>	1.8	2	0	NA	NA	NA	NA
<b>6B</b>	0	0	0	NA	NA	NA	NA
<b>7F</b>	0	0	0.3	NA	NA	NA	NA
<b>9V</b>	0	0	0.3	NA	NA	NA	Na
<b>14</b>	0.6	0.3	0.6	2.03(0.18,22.31)	0.563	0.98(0.37,2.62)	0.974
<b>18C</b>	0.3	0.6	0.3	0.51(0.05,5.58)	0.579	0.98(0.25,3.93)	0.982
<b>19A</b>	1.8	3.2	0.3	0.09(0.01,0.71)	0.022	0.40(0.14,1.16)	0.091
<b>19F</b>	0.6	0.3	1.2	4.06(0.46,36.19)	0.21	1.39(0.60,3.24)	0.444
<b>23F</b>	0.6	0.9	0.3	0.34(0.04,3.24)	0.347	0.70(0.21,2.31)	0.553
<b>PCV13 NVT</b>							
<b>10A</b>	0.6	0.3	2.3	8.12(1.02,64.65)	0.048	1.97(0.91,4.26)	0.086
<b>13</b>	0.6	1.7	0.6	0.34(0.07,1.67)	0.183	0.98(0.37,2.62)	0.974
<b>15B</b>	0.6	1.7	1.2	0.68(0.19,2.38)	0.542	1.39(0.60,3.24)	0.444
<b>16</b>	2.4	0.6	2.3	4.06(0.87,19.00)	0.075	0.98(0.61,1.60)	0.947
<b>19C</b>	1.2	0	0	NA		NA	NA
<b>21</b>	0.9	0.9	2.9	3.38(0.94,12.19)	0.063	1.80(0.95,3.41)	0.073
<b>23B</b>	0	0.9	2.0	2.37(0.62,9.09)	0.209	NA	NA
<b>34</b>	1.2	1.2	1.8	1.52(0.43,5.35)	0.513	1.20(0.64,2.26)	0.561
<b>35B</b>	1.8	0.6	1.8	3.04(0.62,14.99)	0.171	0.98(0.56,1.72)	0.954

<b>NT</b>	0.3	1.4	3.2	2.23(0.78,6.36)	0.133	3.26(1.17,9.06)	0.023
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RR risk ratio, RRadj risk ratio adjusted for health centre, maternal age, and maternal education

Table 4 Results of whole genome sequencing of non-typeable isolates<sup>a</sup>

Isolate N	Mother /Infant	S.pn % reads	S.pseudopn % reads	ST	Nearest ST	Capsular locus top hit	Serotype specific sequence detected	Ancestral capsular type from phylogeny	Conclusion	VT /NVT
<b>CSS1</b>										
108887	Infant	78.08	0.71	3407		16F	16F	16F	Wzd gene loss and truncated wzh	NVT
105098	Mother	80.51	0.14	1778		34	34	34	Intact capsule synthesis genes- <i>No genetic explanation</i>	NVT
104550	Mother	19.47	40.9	Unknown		-			<i>S.pseudopneumoniae</i>	---
<b>CSS2</b>										
201376	Infant	80.46	0.25	5521		10A	10A	10A	Intact capsule synthesis genes- <i>No genetic explanation</i>	NVT
207381	Infant	82.09	0.4	Novel ST D	2052	20	20	Long branch,	Truncated whaF and wzx	NVT

								inconclusive	genes	
206628	Infant	81.38	0.08	989		12F	12F/A/46	12F/A/46	Intact capsule synthesis NVT	
									genes- <i>No genetic explanation</i>	
210240	Infant	80.96	0.08	989		12F	12F/A/46	12F/A/46	Intact capsule synthesis NVT	
									genes- <i>No genetic explanation</i>	
210201	Infant	83.51	0.08	2447		14	14	14	Truncated glycosyl VT	
									transferase genes, wciY and Irp	
201867 <sup>b</sup>	Infant	46.64	0.57	Novel ST B	4040	Classical NT		14	Capsule switch from 14 to classically NT locus	VT
200954	Mother	81.22	0.25	Novel ST G	975	15B/C	15B/C	15B/C	12 nucleotide insertion at the 5' end of the initial transferase gene, wchA	NVT
206379	Infant	81.51	0.16	4033		15B/C	15B/C	15B/C	12 nucleotide insertion at the 5' end of the initial transferase gene, wchA	NVT

207139	Infant	76.55	0.57	3407		16F	16F	16F	Wzd gene loss and NVT truncated wzh
210300	Infant	78.96	0.62	Novel ST F	3407	16F	16F	16F	Wzd gene loss and NVT truncated wzh
210092	Infant	80.13	0.28	Novel ST E	847	19A	19A	19A	3 nucleotide deletion at the 3" end of the rmlC gene VT
210344	Infant	79.93	0.19	Unknown	7661	19B		19B	Intact capsule synthesis NVT genes- <i>No genetic explanation</i>
206438	Infant	81.8	0.1	202		19A	19A	Long branch, inconclusive	3 nucleotide deletion at the 3" end of the rmlC gene VT
208478	Infant	81.95	0.17	4033		19F	19F	19F/15B/C	Intact capsule synthesis NVT genes- <i>No genetic explanation</i>
202731	Infant	81.26	0.08	Novel ST C	6712	28A/F		28A/F	6 nucleotide insertion in the wze gene NVT

201297	Mother	81.95	0.34	5734		6A/B/C/D	6A/B/C/D	6A	12 nucleotide insertion in 3' end of the pseudogene HG262	VT
201794	Infant	78.82	0.8	Novel ST A	71	38 (low match 35%)		Long branch, inconclusive	Loss of <i>cps</i> locus	---
201398	Mother	76.17	1.3	Novel ST H	3582	Classical NT		Long branch, inconclusive	Capsule switch to classically NT locus	---
201843	Infant	77.28	1.11	4040		Classical NT		14	Capsule switch from 14 to classically NT locus	VT
205597	Infant	75.37	0.96	Novel ST B	4040	Classical NT		14	Capsule switch from 14 to classically NT locus	VT
206158	Mother	78.28	0.89	Novel ST B	4040	Classical NT		14	Capsule switch from 14 to classically NT locus	VT
201133	Infant	64.27	3.02	344		Classical NT		Classical NT	Classical NT	---
202601	Mother	65.69	2.64	448		Classical NT		Classical NT	Classical NT	---
208136	Infant	66.12	2.67	448		Classical NT		Classical NT	Classical NT	---
201017	Infant	17.54	43.18	Unknown		-			<i>S.pseudopneumoniae</i>	---
209574	Infant	20.05	40.36	Unknown		-			<i>S.pseudopneumoniae</i>	---



CSS3									
258M		89.95	1.43	10967		Classical NT	14	Capsule switch from 14 to classically NT locus	VT
141B		78.34	3.66	448		Classical NT	Classical NT	Classical NT	---
219B		76.67	4.01	344		Classical NT	Classical NT	Classical NT	---
034B		77.2	4.86	11729		Classical NT	Classical NT	Classical NT	---
153M		83.33	1.86	Novel ST	4040	Classical NT	14	Capsule switch from 14 to classically NT locus	VT
114B		87.33	1.67	4040		Classical NT	14	Capsule switch from 14 to classically NT locus	VT
223M		73.83	4.19	344		Classical NT	Classical NT	Classical NT	---
028B		77.13	4.1	344		Classical NT	Classical NT	Classical NT	---
284M		81.26	0.97	Unknown		14	14	14	Truncated wzg and wchA genes.
092B		88.08	1.84	Novel ST	3582	Classical NT	Long branch, inconclusive	Capsule switch to classically NT locus	---
113M		86.71	1.79	10967		Classical NT	14	Capsule switch from 14 to classically NT locus	VT

201B	78.23	4.84	11729		Classical NT		Classical NT	Classical NT	---
239B	93.26	0.12	989		12F	12F	12F	<i>No genetic explanation</i>	NVT
248M	29.05	18.34	Unknown		alternative_ai		Long branch,	<i>Non-Pneumococcal</i>	---
					B_NT		inconclusive	<i>streptococci</i>	
236M	87.4	1.48	Unknown	~908	25F_or_25A		14	---	---
					(Low match				
					29.1%)				
073B	77.4	4.11	344		Classical NT		Classical NT	Classical NT	---
268M	76.62	4.11	344		Classical NT		Classical NT	Classical NT	---
268B	76.49	4.14	344		Classical NT		Classical NT	Classical NT	---
171B	76.89	3.59	448		Classical NT		Classical NT	Classical NT	---
003M	89.41	0.37	847		19A	19A	19A	<i>4bp insertion on the wchA.</i>	VT
								<i>3bp deletion in the rmlC</i>	
								<i>gene</i>	
314M	77.8	4.16	344		Classical NT		Classical NT	Classical NT	---
243M	75.91	3.63	448		Classical NT		Classical NT	Classical NT	---
258B	93.29	0.1	989		12F	12F	12F	<i>No genetic explanation</i>	NVT

S.pn *S. pneumoniae* S. pseudopn *S. pseudopneumoniae*

<sup>a</sup>CSS1 & 2 updated mechanisms for lack of capsular expression

<sup>b</sup> Sample contaminated with unclassified organism, pneumococcal coverage sufficient for analysis and conclusion

Tree scale: 0.1

- Major lineages**
- Classical NT
  - Typically encapsulated lineages
  - *S. pseudopneumoniae*
  - Non-pneumococcus

- Serotypes**
- Untypeable
  - 19A
  - 14
  - 12F
  - 16F
  - 6A/6B
  - 19F
  - 11A
  - 15B/C
  - 20/21
  - 19B
  - 34
  - 37
  - 28F
  - 10A
  - Non-pneumococcus

- Study isolates**
- ★ Study isolates

