**TITLE: Persistent and emerging pneumococcal carriage serotypes in a rural Gambian community after ten years of pneumococcal conjugate vaccine pressure.**

**AUTHORS**

Effua Usuf 1, Christian Bottomley 2, Rebecca Gladstone 3, Ebrima Bojang 1, Kaddijatou Jawneh 1, Isatou Cox 1, Edrissa Jallow 1, Abdoulie Bojang 1, Brian Greenwood 2, Richard A. Adegbola 4, Stephen D. Bentley 3, Philip C Hill 5, Anna Roca 1

**Affiliations**

1 Disease Control and Elimination Theme, Medical Research Council Unit The Gambia at London School Hygiene and Tropical Medicine, Fajara The Gambia

2 London School of Hygiene and Tropical Medicine, London UK

3 Wellcome Sanger Institute, Cambridge UK

4 RAMBICON, Immunisation & Global Health Consulting, Lekki, Lagos, Nigeria

5 Centre for Global Health, Otago University, Otago New Zealand

**ABSTRACT**

**Background**

The continuing impact of pneumococcal conjugate vaccines (PCVs) in regions with high pneumococcal transmission is threatened by the persistence of vaccine serotypes (VT) and the emergence of non-vaccine serotypes (NVT).

**Methods**

In 2016, we conducted a cross-sectional carriage survey (CSS5) in a community where PCV7 was first introduced in 2006 during a cluster randomised trial conducted before nationwide introduction of PCV7 (2009) and PCV13 (2011). We estimated the prevalence of PCV13 VT and NVT by age and compared these to earlier surveys before (CSS0), during (CSS1-3), and after the trial but before PCV13 (CSS4). Genomic analysis was conducted for the non-typeable pneumococci.

**Results**

The prevalence of PCV13 VT carriage decreased during the 10 years between CSS0 and CSS5 across all age groups (67·6% to 13·5%, p<0.001; 59·8% to 14·4%, p<0.001; 43·1% to 17·9%, p<0.001; and 24·0% to 5·1%, p<0.001 in <2, 2-4, 5-14 and ≥15 years respectively). However, there was no difference between CSS4 and CSS5 in children ≥2 years and adults (children < 2 years, no data). The prevalence of PCV13 NVT increased between CSS0 and CSS5 for children <2 years but decreased in older children and adults.

In CSS5, serotypes 3, 6A and 19F were the most common VT and non-typeable isolates, the most common NVT. Among non-typeable isolates, 73·0% lost the ability to express a capsule. Of these, 70·8% were from a VT background.

**Conclusions**

The decrease in PCV13 VT that has occurred since the introduction of PCV13 appears to have plateaued. Significant carriage of these serotypes remains in all age groups.

**INTRODUCTION**

The introduction of pneumococcal conjugate vaccines (PCV) has led to a dramatic decline in the burden of pneumococcal disease with the annual number of pneumococcal deaths among HIV uninfected children <5 years estimated to have decreased worldwide from about 600,000 to 294,000 between 2000 and 2015. However, the disease burden in Africa and Asia remains high and approximately 50% of all pneumococcal deaths in 2015 occurred in these two continents [1].

PCV introduction has been supported by Gavi, the Vaccine Alliance, in over fifty low- and middle-income countries (LMIC). As the Gross National Income per capita of these countries increases they are expected to transition from Gavi support to self-financing [2]. Therefore, evaluating the overall impact of vaccine introduction is crucial during the transition phase. Unfortunately, many of these countries do not have robust disease surveillance systems for invasive pneumococcal disease (IPD) and may therefore, need to rely on carriage studies to monitor the persistence of vaccine serotype (VT) [3, 4], and anticipate emerging serotypes [5] and capsular switching [6, 7] in the post vaccine era. Countries with both IPD surveillance and carriage studies have the potential to provide a better understanding of community transmission [8].

The Gambia is one of the few African countries where several carriage surveys have been conducted before and after the introduction of PCVs [6, 7, 9, 10]. Six pneumococcal cross-sectional carriage surveys (CSS) were conducted in rural villages, before any PCV, during a cluster-randomized trial (CRT) and subsequently after nationwide PCV introduction. These villages have experienced higher vaccine pressure than elsewhere in Africa and may therefore indicate long-term impact of PCV in the region.

We report data from the most recent carriage survey conducted in 2016, five years after nationwide PCV13 introduction and compare the findings with the surveys in the preceding 10 years. In addition, we provide a molecular characterisation of the non-typeable pneumococci that were isolated during the last survey.

**METHODS**

*Study area*

The pneumococcal CSSs were conducted in 21 rural villages in the Western region of the Gambia (Figure S1). The country has two seasons: a long dry season from November- May and a rainy season from June- October (annual rainfall 1200mm) [11]. The prevalence of malaria was 16% in 2012 [12] and the prevalence of HIV among adults 15- 49 years was 1·6% (1·3-2·0) in 2017 [13].

*PCV introduction– Cluster randomised trial and Expanded Program of Immunization (EPI)*

Between 2006 and 2008 a CRT was conducted in which all individuals >2.5 years received either one dose of PCV7 (11 intervention villages) or one dose of Meningococcal C vaccine (10 control villages). Children ≤ 2.5 years and children born during the trial period received 1 to 3 doses of PCV7 irrespective of trial arm [10].

In August 2009, The Gambia introduced PCV7 into the EPI. The vaccine was given at 2, 3 and 4 months of age without a catch-up campaign. In May 2011, PCV7 was replaced by PCV13 following the same schedule. National immunization coverage as reported by WHO for three doses of PCV was 99% in 2010, and 95% in 2016 in children 12-23 months of age [14].

*Cross-sectional surveys*

*CSS0 to CSS4 -* The baseline survey (CSS0) was conducted between 2003 and 2004 [9] (Figure S2). This survey was followed by three surveys (CSS1-3) conducted during the PCV7 CRT (2006-2008), and a subsequent survey (CSS4) in 2010.

*CSS5-* In 2016, we conducted an additional survey (CSS5) and collected 2,500 nasopharyngeal swabs (NPS) from all children <5 years of age, one in two children 5-14 years and one in four adults ≥15 years of age.

*Population sampling scheme*

Although the sampling scheme was designed to be similar across surveys, there were a number of differences: 1) children <2 years of age were only swabbed in CSS0 and CSS5 and not in the other surveys as all children in this age group received PCV7 during the CRT [10]; 2) fewer samples were included in CSS3 because samples collected after a national trachoma control program in the study area were excluded [15]; 3) five out of the 21 villages did not consent to participate in CSS4. As in the past, these villages were excluded from the analysis of CSS1-4, i.e. all surveys conducted during the CRT [15]; 4) CSS2 was conducted exclusively during the rainy season whereas the other surveys were conducted in the dry season (Table 1).

*Sample handling*

NPS were collected using a calcium alginate swab following WHO guidelines [16]. The samples were placed in skim milk-tryptone-glucose-glycerol medium and transported to the laboratory for storage at -70 °C.

*Isolation and serotyping of Streptococcus pneumoniae*

The stored NPS were thawed and vortexed, after which a 50µl (10µl for CSS1-4 [15]) aliquot was plated directly onto a gentamicin blood agar (CM0331 Oxoid, UK + 5% sheep blood) plate for isolation of *S. pneumoniae.* As in the previous surveys, two to three morphologically different colonies were selected and screened for optochin susceptibility, and all isolated pneumococci were serotyped using a latex agglutination test [Statens Serum Institute, Denmark] [17].

*Statistical analysis*

For each survey, we estimated the overall prevalence of pneumococcal carriage, the prevalence of PCV7 VT carriage (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F), PCV13 VT (PCV7 serotypes + 1, 3, 5, 6A, 7F and 19A), and PCV13 NVT (non-PCV13 serotypes including the non-typeable isolates). The prevalence estimates were calculated for each of the age groups used in the stratified sampling (<2, 2-4, 5-14 and ≥15 years), and the overall prevalence was estimated by weighting according to age-specific sampling proportions. We used Poisson regression with robust standard errors to estimate prevalence ratios [baseline (CSS0) versus CSS5 and, CSS4 versus CSS5] adjusted for study arm of the trial, age, use of antibiotics, gender and month the swab was taken. In a supplementary analysis to allow for temporal changes in overall carriage unrelated to vaccination, we estimated the proportion of PCV13 VT among carriers (see supplementary information). All analyses were done using Stata 14 (Statacorp 2014, College Station, TX).

*Whole Genome Sequencing of non-typeable pneumococci*

All non-typeable isolates from CSS5 phenotypically re-typed and confirmed non-typeable were sent to the Sanger Institute for sequencing. Isolates were speciated using kraken [18]. All pneumococcal isolates were assigned to the Global Pneumococcal Sequence Clusters (GPSC) [19, 20]. MLST was assigned using mlst Seeman [21]. The capsular polysaccharide synthesis locus (*cps*) was typed using SeroBA [22]. Raw data were assembled as described previously [23]. If an intact *cps* was detected the SeroBA top capsular hit was taken to be the ancestral serotype. An isolate with a non-typeable *cps* and an ST or GPSC known to belong to the classically non-typeable lineage, was classified as classically non-typeable. Otherwise, if the *cps* was un-typeable or had a classically non-typeable *cps* then the mash distance between it and all isolates in a reference genome set was determined [24]. The reference set consisted of one representative of each serotype and ST combination in the GPS dataset of Gladstone *et al* (2,807/13,454) [19]. The isolate with the smallest mash distance was used to infer the ancestral serotype. Independent loss events were defined as unique combinations of GPSC, and cps and predicted ancestral serotype, excluding the classically non-typeable lineages. Within GPSC9 there were 36 non-typeables isolates. These isolates were mapped and the 57 GPSC9 isolates in the reference genome set were mapped against the completed genome *S. pneumoniae* G54 (CP001015; a member of GPSC9). Gubbins was used to identify recombination and the resultant tree was used to determine if the 36 non-typeable isolates shared a common ancestor and to count independent capsular losses [25].

*Ethical approval*

Written informed consent was obtained from adults and parents/guardians of children ≤16 years. For children >12 and ≤16 years assent was also obtained. All surveys were approved by the joint MRCG Gambia Government ethics committee.

**RESULTS**

*Study profile*

There was a high level of compliance in the surveys [9]. In CSS5 only 166/2666 (6.2%) individuals declined to participate. A total of 7,464 NPS were collected during the six surveys (Figure S2). The proportion of individuals in the different age strata varied slightly between surveys (Table 1).

*Trends in nasopharyngeal pneumococcal carriage*

*Overall*

In CSS5, the weighted estimate of pneumococcal carriage prevalence was 46·5% (44·3%-48·7%). The highest prevalence was among children <2 years of age (88.4%) and the lowest was among individuals ≥15 years of age (30·1%). There was a decrease in overall carriage between CSS0 and CSS5 in each age group, except for the <2 years age group. Age-specific carriage prevalence remained the same between CSS4 and CSS5 (Table 2).

*PCV13 VT*

PCV13VT carriers were found in all but one of the study villages in CSS5 (Table S1), and across the study population as a whole the weightedestimate of PCV13 VT carriage was 10·2% (95% CI 9·1%-11·5%). PCV13 VT carriage was highest among children 5-14 years (17·9%) and lowest among individuals ≥15 years (5·1%) [Table 2]. Between CSS0 and CSS5, PCV13 VT carriage decreased in all age groups (Figure 1). The decrease occurred both in PCV7 serotypes and serotypes included in PCV13 but not in PCV7 (Figure 2). Furthermore, the proportion of PCV13 VT carriers among all carriers also decreased over this period (Table 3). Between CSS4 and CSS5 PCV13 VT carriage remained constant in all age groups (no data available for children <2 years), as did the proportion of PCV13 carriers.

*PCV13 NVT*

The weighted prevalence of PCV13 NVT in CSS5 was 36·8% (95% CI 34·8% - 38·9%). Between CSS0 and CSS5, PCV13 NVT prevalence increased in children <2 years of age (from 31.7% to 75.3%) A non-significant increase was seen in children 2-4 years of age (from 40.2% to 65.7%) [Table 2]. The prevalence remained constant in children 5-14 years (45·2% versus 43·8%) and decreased among individuals ≥15 years from 37·3% to 25·2% (p<0·001). Between CSS4 and CSS5 PCV13 NVT prevalence remained constant in all age groups.

*Specific PCV13 VT*

Serotype 3, 6A, and 19F were the most common PCV13 serotypes in CSS5 (2·4%, 1·9% and 1·8% respectively) representing almost 2/3 of all PCV13 VT (Table 4). Between CSS4 and CSS5 no PCV13 VT decreased, except for 9A which decreased from 2·7% to 0·7% (p<0·001).

*Specific PCV13 NVT*

Non-typeables, which accounted for 10·5% of all NVT, were the most prevalent NVT in CSS5, followed by serotypes 34 and 15B. The non-typeable pneumococci and 34 and 15B increased in prevalence between CSS4 and CSS5 (Table 4 and Figure 3).

*Genomic analysis of the non-typeable pneumococci (CSS5 only)*

Out of the 121 non-typeable isolates from CSS5, 113 (93·4%) were whole genome sequenced (Figure 4). After quality control, 89 confirmed non-typeable pneumococcal isolates were available for analysis. Twenty-four (27·9%, 24/89) were of the classical non-typeable lineage and sixty-five (73·0%, 65/89) were of an encapsulated pneumococcal lineage but had lost their ability to express a capsule. Among the latter, 18 isolates had a full-length *cps* despite having no detectable capsule, and of these 7/18 had a VT *cps*. A further 31 isolates had acquired a locus typically found in classically non-typeable isolates and 16 isolates had a *cps* that could not be typed.

The genomic analysis indicated that 46/65 (70·8%) non-typable isolates from encapsulated pneumococcal lineages originally expressed VTs. The proportion of isolates that had lost the ability to express VT capsules was more than 3-fold higher than the proportion of VTs in the phenotypically typeable isolates in CSS5 (313/1437=21·5%, p<0·001).

The 65 isolates that had lost their capsule represented 20 independent capsule losses. Thirty-six of the 65 (55·4%) were from GPSC9, a lineage that typically expresses serotype 14 and encompasses clonal complex CC63. We determined from the phylogenetic structure of the lineage that the 36non-typeable isolates represented 3 independent loss events. Among the GPSC9 isolates, 27/36 were determined to be descendants from a single capsular loss event-1, six from capsular loss event-2 and three from capsular loss event-3 (Figure 5 & <https://microreact.org/project/TPxm5z4eE> ).

**DISCUSSION**

In this study, we described pneumococcal carriage in an African population that has been under high vaccine pressure since 2006. PCV13 VT and PCV7 VT carriage rates initially declined after PCV introduction, but more recently appear to have stabilised. We also observed serotype replacement in young children, driven in part by an increase non-typeable pneumococci, particularly those that have lost VT capsule production.

The high VT prevalence in children is in keeping with data from our previous study conducted in an urban area in The Gambia which reported 11% PCV13 VT prevalence in infants five years after PCV13 introduction [7]. It is also consistent with data from other LMIC countries. For example, the prevalence of PCV13 VT was 17% in children 1-4 years in Malawi three years post-PCV13 [26], the prevalence of PCV10 VT was 23.4% and 8.8% among children <5 years in Mozambique and Kenya, three and five years post-PCV10 respectively [27, 28]. In contrast to LMICa, estimates from HIC are considerably lower. Studies in England, Belgium and France have reported prevalence of VT in children <5 years ranging between 1-5% within five years post-PCV13 [29-31]. Such difference between LMIC and HIC countries has several possible explanations. First, community and household transmission is higher in LMIC than HIC, particularly among children who are the major source of transmission [32]. Second, differences in vaccine schedule: a 2+1 schedule is used in most HIC whereas a 3+0 is used in most LMIC. A notable difference is South Africa, the only country in SSA to use 2+1 schedule, has a low prevalence of PCV13VT (<5%), despite having high rates of HIV infection [33].Finally, coverage of PCV is lower and catch up campaigns are less common in LMIC [34].

The PCV13 serotypes 3, 6A and 19F were frequently isolated in the most recent survey. These serotypes are associated with invasive disease in The Gambia in the post PCV era [35]. Serotype 19F was also common in a recent among PCV13 vaccinated children and their parents in the UK, however, 6A was not isolated in the UK survey [3]. The persistence of these serotypes in The Gambia may be due to waning of indirect protection and their continued circulation among older children.

Previously in The Gambia we showed that serotype 19A decreased in infants and mothers [7] after the introduction of PCV13. In this study we also saw its decrease in all age groups. A similar reduction was observed in the UK though, as in The Gambia, the serotype continues to circulate there [3].

As in other studies, we observed an increase in NVT among children <5 years of age [3, 6, 26, 36, 37]. Emerging serotypes in carriage would be expected to result in replacement disease, though data from Gambian IPD surveillance are inconclusive because of the small number of cases. [35].

The large increase in non-typeable pneumococci across all age groups is consistent with findings from a recent ‘peri-urban’ study in The Gambia [7]. Genomic analysis of IPD data from South Africa in 2003- 2013 showed that the non-typeable pneumococci were a rare cause of IPD [38], as in The Gambia, and they were excluded from the analysis [35]. Nonetheless we recommend surveillance programmes continue to monitor non-typeable serotypes in case they do become more invasive.

Genomic analysis showed that the classically non-typeable lineage accounted for less than a third of the non-typeable isolates. The majority of non-typeable isolates were determined to be from encapsulated lineages that had lost their ability to express a VT capsule. Isolates from encapsulated lineages that had lost the ability to express a NVT capsule were less common. However, investigation of the number of independent capsule loss events, showed that an equal number of these events removed the ability to produce a VT or a NVT capsule. It therefore appears that the descendants of VT strains that have lost their capsule are more successful than NVT strains that have lost their capsule, possibly due to escape vaccine pressure. As non-typeable isolates rarely cause disease this is a favorable outcome of serotype replacement, though it may allow antibiotic resistance associated with VT lineages to be maintained [38].

Over half of the non-typeable isolates from encapsulated lineages were from a single clonal background: GPSC9. Multiple independent VT losses were observed in GPSC9 indicating pressure for this lineage to lose the serotype 14 capsule. Descendants of capsule loss event A within GPSC9 were also observed in a previous Gambian study, [7] suggesting that this non-typeable component of GPSC9 has established itself in The Gambia. Serotype 14 losses in GPSC9 were also observed in Nepal suggesting this may not be a local phenomenon [39]. Though a subset of isolates in CSS5 and our previous study [7], had intact *cps*, such isolates may be capable of low level or variable expression of capsule. As these isolates were rare in CSS5 and more often had a NVT *cps* it suggests that undetected expression is not driving the rise in non-typeables.

A strength of our analysis is that it compares data from six surveys conducted using similar methodologies over a period of 10 years. However, despite using similar methodologies there are some potential sources of bias. First, CSS1-CSS3 were conducted during a CRT when there may have been greater access to antibiotics and better health care, which may have decreased carriage rates. Second, the surveys were not all conducted during the same time of year. Due to a modest impact of season on pneumococcal carriage [40], we would expect carriage to be slightly higher in CSS2 and possibly CSS4 relative to the other surveys. Third, we are unable to see any trend in carriage among children <2 years between CSS4 and CSS5, the age group that are vaccinated in routine programmes. Finally, the use of 50µl instead of 10µl as in previous surveys may have led to increased carriage in CSS5. To account for these potential confounding effects, we conducted a supplementary analysis which showed that the change in proportion of PCV13 VT carriers among all carriers (VT + NVT) were consistent with the results of overall PCV13 VT prevalence, thereby strengthening our conclusions.

In conclusion, our data suggest that PCV13 VT carriage is likely to remain high in LMIC even after significant PCV vaccine pressure. Furthermore, our genomic analysis revealed that PCV13 VT is higher than indicated by routine serotyping methods. Alternative vaccine schedules or an additional booster dose are potential strategies for reducing VT carriage, but studies are needed to determine which approach works best and is cost effective in LMICs. Further surveys are necessary to confirm the high VT carriage post PCV introduction and to identify risk factors for VT carriage among PCV vaccinated children.

**Acknowledgments**

We are grateful to, Dr M Jasseh and his team for their support to update the census in the study villages, and Dr Annick Sidebeh for help with field work and data cleaning. Sincere appreciation to the field staff led by Edrissa Sabally and the data management led by Haddy Kanyi, Alyson Lush who sketched the figure of the study design, Abdul Muhammad Khalie who sketched the map of the study area and the villagers who participated in the study.

**Author contribution**

The CRT and carriage studies were designed by RA, BG, PH and AR. EU designed and supervised the last survey and did the analysis. KJ, IC, EJ and AB were responsible for isolating and serotyping *S. pneumoniae* and bacterial DNA extraction. EU, PH, CB and AR interpreted the results. SB, RG and EB supervised the WGS and interpreted that data and contributed to the write up. EU wrote the first draft. All authors reviewed and approved the final version.

**Funding source**

This work was funded by an MRC/LSHTM West Africa post-doctoral fellowship to EU.

**Conflict of Interests**

None

**Summary**

After 10 years of high vaccine-pressure, in an African setting, PCV13 VT and PCV7 VT carriage has plateaued. These VT were still circulating among all age groups. We observed serotype replacement in young children due mainly to increase non-typeable pneumococci.

**Tables & Figures**

**Figure 1: Pneumococcal carriage of (a) any serotype, (b) PCV13 serotypes (VT) and (c) serotypes not included in PCV13 (NVT).**



Note: Children < 2 years of age were not swabbed in CSS1-4

**Figure 2: Pneumococcal carriage of serotypes included in PCV7 (panel A) and those included in PCV13 but not in PCV7 (panel B)**



Note: Children < 2 years of age were not swabbed in CSS1-4

**Figure 3: Pneumococcal carriage of the most common vaccine type serotypes (3, 6A, 19A) and non-typeable pneumococci.**

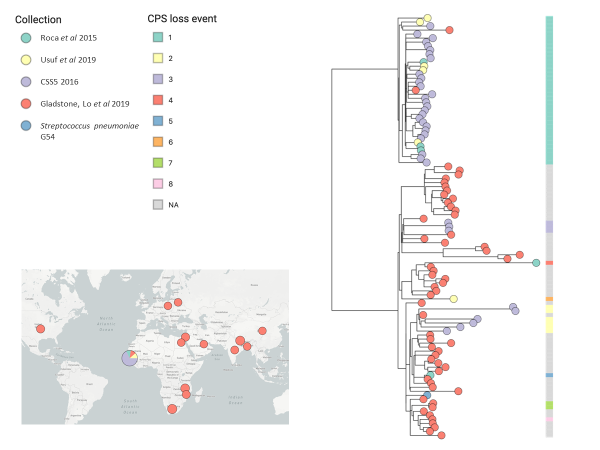


Note: Children < 2 years of age were not swabbed in CSS1-4

**Figure 4: Sample processing flow for the non-typeable pneumococci (CSS5)**

\*5 samples with known serotypes were mistakenly retyped

**Figure 5: Phylogenetic tree showing capsular polysaccharide synthesis loss event in our study compared to other studies**

****

**Table 1 Characteristics of the surveys and participants**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **CSS0** | **CSS1** | **CSS2** | **CSS3** | **CSS4** | **CSS5** | **p-value a** |
| **Year** | 2003-2004 | 2006-2007 | 2007 | 2008 | 2010 | 2016 |  |
| **Month** | Dec-May | Nov -Mar | Jul-Sep | Mar- Jun | Sep - Oct | Oct-Dec |  |
| **Season** | Dry | Early Dry | Rainy | Late Dry/  Early Rainy | Late Rainy/ Early Dry | Early Dry |  |
| **Survey part of CRT** | No | Yes | Yes | Yes | No | No |  |
| **Routine PCV**  **Introduction status** | Pre-any PCV introduction | Pre-Routine PCV | Pre-Routine PCV | Pre-Routine PCV | 1-year post  PCV7 | 5-years post  PCV13 |  |
| **PCV coverage**  **(Routine/CRT)** | 0% | 85% | 92% | 87% | 95% | 95% |  |
| **Total NPS** | 1,902 | 933 | 956 | 361 | 812 | 2500 |  |
| **Age** | n (%) | n (%) | n (%) | n (%) | n (%) |  |  |
| <2 | 278(14·6) |  |  |  |  | 275(11·0) | <0·001 **b** |
| 2-4 | 169(8·9) | 123(13·2) | 118(12·3) | 59(16·3) | 200(24·6) | 507(20·3) |  |
| 5-14 | 571(30·0) | 339(36·3) | 344(36·0) | 193(53·5) | 214(26·4) | 907(36·3) |  |
| ≥ 15 | 884(46·5) | 471(50·5) | 494(51·7) | 109(30·2) | 398(49·0) | 811(32·4) |  |
| **Gender c** |  |  |  |  |  |  |  |
| Male | 935(49·2) | 437(46·8) | 460(48·1) | 174(48·2) | 417(51·4) | 1144(45·8) | 0·081 |
| Female | 967(50·8) | 496(53·2) | 496(51·9) | 187(51·8) | 395(48·6) | 1352(54·2) |  |
| **Antibiotic d, e f** |  |  |  |  |  |  |  |
| No | 32(88·9) |  |  |  |  | 283(96·0) | 0·06 |
| Yes | 4(11·1) |  |  |  |  | 11(4·0) |  |

a Chi-square test,

b comparison excluding < 2 years of age also give p-value <0.001,

c 4 missing gender in CSS5,

d antibiotic data available for children <2 years in CSS0 [36 children only] and CCS5 [274 children],

e comparison between years limited to <2 years

CRT- cluster randomised trial- coverage in control arm < 10% during CSS1, 2 & 3.

Coverage data source (routine - <https://www.who.int/immunization/monitoring_surveillance/data/gmb.pdf> & CRT -<https://doi.org/10.1371/journal.pmed.1001107.s002>)

**Table 2 Prevalence of pneumococcal carriage by age group**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **CSS0\***  **(%)** | **CSS4**  **(%)** | **CSS5\***  **(%)** | **RR1**  **CSS0 vs CSS5** | **p-value** | **RR1adj** | **p-value** | **RR2**  **CSS4 vs CSS5** | **p-value** | **RR2adj** | **p-value** |
| **All ages\*** | | | | | | | | | | | |
| Any | 74·1 |  | 46·5 | 0·68 (0·65, 0·73) | <0·001 | 0·76 (0·68, 0·86) | <0·001 |  |  |  |  |
| PCV7 | 22.3 |  | 5·1 | 0·27 (0·22, 0·33) | <0·001 | 0·41 (0·28, 0·59) | <0·001 |  |  |  |  |
| PCV13 | 37.2 |  | 10·2 | 0·32 (0·28, 0·37) | <0·001 | 0·40 (0·31, 0·51) | <0·001 |  |  |  |  |
| PCV13-7 | 17·1 |  | 5·3 | 0·36 (0·29, 0·44) | <0·001 | 0·37 (0·26, 0·52) | <0·001 |  |  |  |  |
| PCV13 NVT | 40.4 |  | 36·8 | 0·95 (0·87, 1·03) | <0·001 | 0·99 (0·83, 1·19) | 0·954 |  |  |  |  |
| NT | 2·7 |  | 4·6 | 1·61 (1·12, 2·30) | <0·001 | 1·61 (1·12, 2·30) | 0.009 |  |  |  |  |
| **< 2 years¶** | | | | | | | | | | | |
| Any | 93·5 |  | 88·4 | 0·94 (0·90,1·00) | 0·038 | 0·98 (0·88, 1·10) | 0·784 |  |  |  |  |
| PCV7 | 44·2 |  | 8·7 | 0·20 (0·13,0·30) | <0·001 | 0·29 (0·17, 0·51) | <0·001 |  |  |  |  |
| PCV13 | 67·6 |  | 13·5 | 0·20 (0·15,0·27) | <0·001 | 0·26 (0·18, 0·39) | <0·001 |  |  |  |  |
| PCV13-7 | 27·7 |  | 4·7 | 0·17 (0·10,0·30) | <0·001 | 0·22 (0·10, 0·48) | <0·001 |  |  |  |  |
| PCV13 NVT | 31·7 |  | 75·3 | 2·38 (1·97, 2·86) | <0·001 | 1·99 (1·43, 2·77) | <0·001 |  |  |  |  |
| NT | 0·7 |  | 3·6 | 5·05 (1·12, 22·89) | 0·021 | 5·05 (1·12, 22·89) | 0·036 |  |  |  |  |
| **2-4 years** | | | | | | | | | | | |
| Any | 89·3 | 67·0 | 79·3 | 0·89 (0·83, 0·95) | 0·003 | 0·85 (0·74, 0·96) | 0·012 | 1·18 (1·06, 1·32) | 0·001 | 1·09 (0·91, 1·30) | 0·350 |
| PCV7 | 42·0 | 6·5 | 8·9 | 0·21 (0·15, 0·29) | <0·001 | 0·27 (0·15, 0·48) | <0·001 | 1·37 (0·75, 2·48) | 0·362 | 1·30 (0·46, 3·74) | 0·620 |
| PCV13 | 59·8 | 17·5 | 14·4 | 0·24 (0·19, 0·31) | <0·001 | 0·29 (0·19, 0·45) | <0·001 | 0·82 (0·57, 1·19) | 0·299 | 0·84 (0·42, 1·67) | 0·623 |
| PCV13-7 | 23·1 | 11·5 | 5·5 | 0·24 (0·15, 0·38) | <0·001 | 0·27 (0·12, 0·58) | 0·001 | 0·48 (0·28, 0·81) | 0·009 | 0·50 (0·18, 1·36) | 0·173 |
| PCV13 NVT | 40·2 | 52·0 | 65·7 | 1·63 (1·34,1·98) | <0·001 | 1·39 (0·94, 2·05) | 0·094 | 1·26 (1·09 ,1·46) | 0·001 | 1·13 (0·89, 1·43) | 0·311 |
| NT | 0·6 | 3·5 | 5·7 | 9·67 (1·32,70·53) | 0·002 | 9·67 (1·32, 70·53) | 0·025 | 1·63 (0·73, 3·67) | 0·26 | 1·63 (0·73, 3·67) | 0·234 |
| **5-14 years** | | | | | | | | | | | |
| Any | 84·1 | 39·3 | 60·4 | 0·72 (0·67,0·77) | <0·001 | 0·75 (0·64, 0·88) | <0·001 | 1·54(1·29,1·83) | <0·001 | 1·35 (1·05, 1·75) | 0·071 |
| PCV7 | 25·7 | 3·7 | 8·0 | 0·31 (0·24,0·41) | <0·001 | 0·50 (0·32, 0·85) | <0·001 | 2·15(1·05,4·40) | 0·027 | 1·99 (0·71, 5·56) | 0·188 |
| PCV13 | 43·1 | 15·0 | 17·9 | 0·41 (0·35,0·49) | <0·001 | 0·55 (0·37 ,0·80) | 0·002 | 1·19(0·84,1·69) | 0·366 | 1·31 (0·74, 2·32) | 0·357 |
| PCV13-7 | 20·5 | 11·2 | 10·1 | 0·50 (0·38,0·64) | <0·001 | 0·55 (0·30, 1·00) | 0·049 | 0·90(0·59,1·38) | 0·619 | 1·03 (0·48, 2·24) | 0·931 |
| PCV13 NVT | 45·2 | 25·2 | 43·8 | 0·97 (0·86, 1·09) | 0·628 | 0·88 (0·68, 1·15) | 0·353 | 1·73 (1·36, 2·21) | <0·001 | 1·40 (0·99, 1·98) | 0·057 |
| NT | 2·8 | 2·3 | 5·4 | 1·93 (1·11, 3·36) | 0·019 | 1·93 (1·11, 3·36) | 0·020 | 2·31 (0·93, 5·74) | 0·074 | 2·31 (0·93, 5·74) | 0·071 |
| **≥15 years#** | | | | | | | | | | | |
| Any | 61.5 | 17·6 | 30·1 | 0·50 (0·45, 0·57) | <0·001 | 0·61 (0·50, 0·76) | <0·001 | 1·71 (1·35, 2·17) | <0·001 | 1·36 (0·91, 2·04) | 0·139 |
| PCV7 | 13·5 | 0·3 | 2·5 | 0·18 (0·11, 0·29) | <0·001 | 0·31 (0·14, 0·67) | 0·003 | 9·82 (1·32, 72·93) | 0·004 | 7·52 (0·48, 117·30) | 0·150 |
| PCV13 | 24·6 | 2·5 | 5·1 | 0·21 (0·15, 0·29) | <0·001 | 0·27 (0·17, 0·44) | <0·001 | 2·01 (1·02, 3·98) | 0·047 | 2·99 (0·94, 9·57) | 0·064 |
| PCV13-7 | 11·9 | 2·3 | 2·6 | 0·23 (0·14, 0·36) | <0·001 | 0·22 (0·12, 0·43) | <0·001 | 1·15 (0·53, 2·48) | 0·845 | 2·84 (0·62, 13·09) | 0·180 |
| PCV13 NVT | 38.5 | 15·3 | 25·2 | 0·67 (0·58, 0·78) | <0·001 | 0·78 (0·59, 1·03) | 0·078 | 1·64 (1·27, 2·13) | <0·001 | 1·15 (0·73, 1·81) | 0·543 |
| NT | 3·2 | 3·0 | 4·1 | 1·28 (0·78, 2·11) | 0·361 | 1·28 (0·78, 2·11) | 0·321 | 1·35 (0·70, 2·59) | 0·421 | 1·35 (0·70, 2·59) | 0·366 |

RR Risk Ratio,

RR1adj (CSS0 vs CSS5) and RR2adj (CSS4 vs CSS5) adjusted for study arm of the cluster randomised trial, gender, and month of swabbing,

NT non-typeable pneumococci,

PCV13-7 serotypes present in PCV13 but not in PCV7,

\* weighted prevalence calculated for CSS0 and CSS5 using age-specific sampling probabilities,

# weighted prevalence calculated for CSS0 but not CSS5,

**¶** children <2 years were only surveyed in CSS0 and CSS5.

**Table 3 Prevalence of PCV13 VT among pneumococcal carriers**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Age** | **CSS0** | **CSS4** | **CSS5** | **RR1** | **p-value** | **RR1adj** | **p-value** | **RR2** | **p-value** | **RR2adj** | **p-value** |
| **(years)** | **(%)** | **(%)** | **(%)** | **CSS0 vs CSS5** |  |  |  | **CSS4 vs CSS5** |  |  |  |
| <2**¶** | 72.3 |  | 15.2 | 0.21 (0.16, 0.29) | <0.001 | 0.26 (0.18, 0.39) | <0.001 |  |  |  |  |
| 2-4 | 66.9 | 26.1 | 18.2 | 0.27 (0.21, 0.34) | <0.001 | 0.34 (0.22, 0.51) | <0.001 | 0.70 (0.49, 0.99) | 0.062 | 1.10 (0.81, 2.38 | 0.811 |
| 5-14 | 51.2 | 38.1 | 29.4 | 0.57 (0.49, 0.67) | <0.001 | 0.71 (0.49, 1.04) | 0.082 | 0.77 (0.57, 1.04) | 0.126 | 0.85 (0.45, 1.61) | 0.626 |
| ≥15 | 40.2 | 14.3 | 16.8 | 0.42 (0.31, 0.56) | <0.001 | 0.41 (0.26, 0.64) | <0.001 | 1.18 (0.62, 2.23) | 0.715 | 3.28 (0.75, 14.4) | 0.115 |

RR Risk Ratio,

RR1adj (CSS0 vs CSS5) and RR2adj (CSS4 vs CSS5) adjusted for study arm of the cluster randomised trial, gender, and month of swabbing,

**¶** Children < 2 years were only surveyed in CSS0 and CSS5.

**Table 4 Nasopharyngeal carriage of specific serotypes (all ages)**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Serotype** | **CSS0\***  **(%)** | **CSS4**  **(%)** | **CSS5\***  **(%)** | **RR1**  **CSS0 vs CSS5** | **p-value** | **RR1adj** | **p-value** | **RR2**  **CSS4 vs CSS5** | **p-value** | **RR2adj** | **p-value** |
| **PCV7 VT** | | | | | | | | | | | |
| 4 | 3·3 | 0·1 | 0·4 | 0·12 (0·05, 0·26) | <0·001 | 0·12 (0·05, 0·26) | <0·001 | 2·92 (0·37, 23·05) | 0·467 | 2·92 (0·37, 23·05) | 0·309 |
| 6B | 4·1 | 0·1 | 0·1 | 0·04 (0·01, 0·13) | <0·001 | 0·04 (0·01, 0·13) | <0·001 | 0·97 (0·10, 9·36) | 1·000 | 0·97 (0·10, 9·36) | 0·982 |
| 9V | 3·7 | 0 | 0·8 | 0·28 (0·17, 0·48) | <0·001 | 0·28 (0·17, 0·48) | <0·001 | na | 0·002 | na | <0·001 |
| 14 | 1.9 | 1·0 | 0·7 | 0·47 (0·27, 0·82) | 0·001 | 0·47 (0·27, 0·82) | 0·008 | 1·02 (0·46, 2·24) | 1·000 | 1·02 (0·46, 2·24) | 0·971 |
| 18C | 2·6 | 0·1 | 0·4 | 0·18 (0·08, 0·39) | <0·001 | 0·18 (0·08, 0·39) | <0·001 | 3·25 (0·42, 25·34) | 0·313 | 3·25 (0·42, 25·34) | 0·261 |
| 19F | 3.3 | 0·9 | 1·8 | 0·66 (0·46, 0·96) | 0·008 | 0·66 (0·46, 0·96) | 0·030 | 3·02 (1·39, 6·55) | 0·002 | 3·02 (1·39, 6·55) | 0·005 |
| 23F | 4·4 | 0·5 | 0·8 | 0·23 (0·15, 0·38) | <0·001 | 0·23 (0·15, 0·38) | <0·001 | 2·27 (0·80, 6·46) | 0·147 | 2·27 (0·80, 6·46) | 0·123 |
| **PCV13 VT (not included in PCV7 VT)** | | | | | | | | | | | |
| 1 | 0·5 | 0·1 | 0·1 | 0·19 (0·05, 0·76) | 0·038 | 0·19 (0·05, 0·76) | 0·019 | 0·97 (0·10, 9·36) | 1·000 | 0·97 (0·10, 9·36) | 0·982 |
| 3 | 8.7 | 1·5 | 2·4 | 0·30 (0·22, 0·42) | <0·001 | 0·30 (0·22, 0·42) | <0·001 | 1·62 (0·88, 3·00) | 0·129 | 1·62 (0·88, 3·00) | 0·122 |
| 5 | 0·4 | 0 | 0·1 | 0·27 (0·05, 1·38) | 0·083 | 0·27 (0·05, 1·38) | 0·116 | na | 1·000 | na | ,0·001 |
| 6A | 4.7 | 2·5 | 1·9 | 0·47 (0·33, 0·67) | <0·001 | 0·47 (0·33, 0·67) | <0·001 | 0·97 (0·59, 1·61) | 0·896 | 0·97 (0·59, 1·61) | 0·919 |
| 7F | 0·5 | 0·4 | 0·4 | 1·15 (0·42, 3.17) | <0.001 | 1·15 (0·42, 3.17) | 0·784 | 1·08 (0·30, 3·93) | 1·000 | 1·08 (0·30, 3·93) | 0·904 |
| 19A | 2.6 | 2·7 | 0·7 | 0·37 (0·22, 0·64) | <0·001 | 0·37 (0·22, 0·64) | <0·001 | 0·37 (0·21, 0·65) | 0·001 | 0·37 (0·21, 0·65) | 0·001 |
| **PCV13 NVT** | | | | | | | | | | | |
| 10A | 1·1 | 1·4 | 1·4 | 1·35 (0·76, 2·41) | 0·118 | 1·35 (0·76, 2·41) | 0·311 | 1·21 (0·63, 2·34) | 0·630 | 1·21 (0·63, 2·34) | 0·571 |
| 13 | 1·1 | 0·9 | 2·7 | 2·42 (1·41, 4·15) | <0·001 | 2·42 (1·41, 4·15) | 0·001 | 3·25 (1·50, 7·04) | 0·001 | 3·25 (1·50, 7·04) | 0·003 |
| 15B | 1·6 | 1·2 | 2·0 | 1·47 (0.94, 2·30) | 0·007 | 1·47 (0.94, 2·30) | 0·091 | 2·70 (1·41, 5·17) | 0·001 | 2·70 (1·41, 5·17) | 0·003 |
| 16 | 2·4 | 0 | 1·8 | 0·89 (0·57, 1·41) | 0·395 | 0·89 (0·57, 1·41) | 0·632 | na | <0·001 | na | na |
| 19C | 0·3 | 0·5 | 0·0 | na | 0·035 | na | na | na | 0·004 | na | na |
| 21 | 1·8 | 2·2 | 1·2 | 0·70 (0·42, 1·16) | <0.001 | 0·70 (0·42, 1·16) | 0·170 | 0·78 (0·45, 1·34) | 0·368 | 0·78 (0·45, 1·34) | 0·361 |
| 34 | 2.1 | 1·2 | 3·3 | 1.76 (1·17, 2.64) | <0·001 | 1.76 (1·17, 2.64) | 0.007 | 3·80 (2·00, 7·21) | <0·001 | 3·80 (2·00, 7·21) | <0·001 |
| 35B | 2·3 | 2·1 | 2·2 | 1·26 (0·81, 1·94) | 0·559 | 1·26 (0·81, 1·94) | 0·304 | 1·26 (0·74, 2·14) | 0·44 | 1·26 (0·74, 2·14) | 0·389 |
| NT | 2·7 | 3·0 | 4·6 | 1·61 (1·12, 2·30) | <0·001 | 1·16 (1·12, 2·30) | 0.009 | 1·64 (1·06, 2·52) | 0·023 | 1·64 (1·06, 2·52) | 0·025 |

RR Risk Ratio,

RR1adj (CSS0 vs CSS5) and RR2adj (CSS4 vs CSS5) adjusted for study arm of the cluster randomised trial, antibiotic use one month prior to swabbing, age, gender, and month of swabbing,

NT non-typeable pneumococci,

na - not applicable,

\* weighted prevalence in CSS0 and CSS5 calculated using age-specific sampling probabilities

Children < 2 years were only surveyed in CSS0 and CSS5.

**References**

1. Wahl, B., et al., *Burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000-15.* Lancet Glob Health, 2018. **6**(7): p. e744-e757.

2. WHO. *Vaccine Pricing: Gavi Transitioning Countries*. 11/03/2019]; Available from: <https://lnct.global/wp-content/uploads/2018/02/Vaccine-Pricing-for-GAVI-Transitioning-Countries-1.pdf>.

3. Kandasamy, R., et al., *Persistent circulation of vaccine serotypes and serotype replacement after five years of UK infant immunisation with PCV13.* J Infect Dis, 2019.

4. Kwambana-Adams, B., et al., *Rapid replacement by non-vaccine pneumococcal serotypes may mitigate the impact of the pneumococcal conjugate vaccine on nasopharyngeal bacterial ecology.* Sci Rep, 2017. **7**(1): p. 8127.

5. Vissers, M., et al., *Increased carriage of non-vaccine serotypes with low invasive disease potential four years after switching to the 10-valent pneumococcal conjugate vaccine in The Netherlands.* PLoS One, 2018. **13**(3): p. e0194823.

6. Roca, A., et al., *Effect on nasopharyngeal pneumococcal carriage of replacing PCV7 with PCV13 in the Expanded Programme of Immunization in The Gambia.* Vaccine, 2015. **33**(51): p. 7144-51.

7. Usuf, E., et al., *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.* Clin Infect Dis, 2018.

8. Nzenze, S.A., et al., *Imputing the Direct and Indirect Effectiveness of Childhood Pneumococcal Conjugate Vaccine Against Invasive Pneumococcal Disease by Surveying Temporal Changes in Nasopharyngeal Pneumococcal Colonization.* Am J Epidemiol, 2017. **186**(4): p. 435-444.

9. Hill, P.C., et al., *Nasopharyngeal carriage of Streptococcus pneumoniae in Gambian villagers.* Clinical Infectious Diseases, 2006. **43**(6): p. 673-679.

10. Roca, A., et al., *Effects of community-wide vaccination with PCV-7 on pneumococcal nasopharyngeal carriage in the Gambia: a cluster-randomized trial.* PLoS Med, 2011. **8**(10): p. e1001107.

11. Woolfson, A., et al., *Nasopharyngeal carriage of community-acquired, antibiotic-resistant Streptococcus pneumoniae in a Zambian paediatric population.* 1997.

12. Mwesigwa, J., et al., *On-going malaria transmission in The Gambia despite high coverage of control interventions: a nationwide cross-sectional survey.* Malar J, 2015. **14**: p. 314.

13. UNAIDS. *Country factsheets Gambia 2017* 13/05/19].

14. WHO, *WHO vaccine-preventable diseases: monitoring system. 2019 global summary. WHO UNICEF estimates time series for Gambia (GMB).* 2019

15. Roca, A., et al., *Nasopharyngeal carriage of pneumococci four years after community-wide vaccination with PCV-7 in The Gambia: long-term evaluation of a cluster randomized trial.* PLoS One, 2013. **8**(9): p. e72198.

16. Satzke, C., et al., *Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group.* Vaccine, 2013. **32**(1): p. 165-79.

17. Brueggemann, A.B., et al., *Clonal relationships between invasive and carriage Streptococcus pneumoniae and serotype- and clone-specific differences in invasive disease potential.* J Infect Dis, 2003. **187**(9): p. 1424-32.

18. Wood, D.E. and S.L. Salzberg, *Kraken: ultrafast metagenomic sequence classification using exact alignments.* Genome Biol, 2014. **15**(3): p. R46.

19. Gladstone, R.A., et al., *International genomic definition of pneumococcal lineages, to contextualise disease, antibiotic resistance and vaccine impact.* EBioMedicine, 2019.

20. Lees, J.A., et al., *Fast and flexible bacterial genomic epidemiology with PopPUNK.* Genome Res, 2019. **29**(2): p. 304-316.

21. Seemann, T., *mlst. Github* [*https://github.com/tseemann/mlst*](https://github.com/tseemann/mlst).

22. Epping, L., et al., *SeroBA: rapid high-throughput serotyping of Streptococcus pneumoniae from whole genome sequence data.* Microb Genom, 2018. **4**(7).

23. Page, A.J., et al., *Robust high-throughput prokaryote de novo assembly and improvement pipeline for Illumina data.* Microb Genom, 2016. **2**(8): p. e000083.

24. Ondov, B.D., et al., *Mash: fast genome and metagenome distance estimation using MinHash.* Genome Biol, 2016. **17**(1): p. 132.

25. Croucher, N.J., et al., *Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins.* Nucleic Acids Res, 2015. **43**(3): p. e15.

26. Heinsbroek, E., et al., *Pneumococcal carriage in households in Karonga District, Malawi, before and after introduction of 13-valent pneumococcal conjugate vaccination.* Vaccine, 2018. **36**(48): p. 7369-7376.

27. Adebanjo, T., et al., *Pneumococcal carriage and serotype distribution among children with and without pneumonia in Mozambique, 2014-2016.* PLoS One, 2018. **13**(6): p. e0199363.

28. Hammitt, L.L., et al., *Effect of ten-valent pneumococcal conjugate vaccine on invasive pneumococcal disease and nasopharyngeal carriage in Kenya: a longitudinal surveillance study.* Lancet, 2019. **393**(10186): p. 2146-2154.

29. Southern, J., et al., *Pneumococcal carriage in children and their household contacts six years after introduction of the 13-valent pneumococcal conjugate vaccine in England.* PLoS One, 2018. **13**(5): p. e0195799.

30. Wouters, I., et al., *Nasopharyngeal s. pneumoniae carriage and density in Belgian infants after 9years of pneumococcal conjugate vaccine programme.* Vaccine, 2018. **36**(1): p. 15-22.

31. Dunais, B., et al., *Impact of the 13-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of Streptococcus pneumoniae among children attending group daycare in southeastern France.* Pediatr Infect Dis J, 2015. **34**(3): p. 286-8.

32. Lourenco, J., et al., *Determinants of high residual post-PCV13 pneumococcal vaccine-type carriage in Blantyre, Malawi: a modelling study.* BMC Med, 2019. **17**(1): p. 219.

33. Nzenze, S.A., et al., *Temporal Changes in Pneumococcal Colonization in HIV-infected and HIV-uninfected Mother-Child Pairs Following Transitioning From 7-valent to 13-valent Pneumococcal Conjugate Vaccine, Soweto, South Africa.* J Infect Dis, 2015. **212**(7): p. 1082-92.

34. WHO. *Subnational immunization coverage data*. 2018 26/02/2020]; Available from: <https://www.who.int/immunization/monitoring_surveillance/data/subnational/en/>.

35. Mackenzie, G.A., et al., *Effect of the introduction of pneumococcal conjugate vaccination on invasive pneumococcal disease in The Gambia: a population-based surveillance study.* Lancet Infect Dis, 2016. **16**(6): p. 703-11.

36. Dunne, E.M., et al., *Effect of ten-valent pneumococcal conjugate vaccine introduction on pneumococcal carriage in Fiji: results from four annual cross-sectional carriage surveys.* Lancet Glob Health, 2018. **6**(12): p. e1375-e1385.

37. Ladhani, S.N., et al., *Rapid increase in non-vaccine serotypes causing invasive pneumococcal disease in England and Wales, 2000-17: a prospective national observational cohort study.* Lancet Infect Dis, 2018. **18**(4): p. 441-451.

38. Mohale, T., et al., *Genomic analysis of nontypeable pneumococci causing invasive pneumococcal disease in South Africa, 2003-2013.* BMC Genomics, 2016. **17**: p. 470.

39. Gladstone, R.A., et al., *International genomic definition of pneumococcal lineages, to contextualise disease, antibiotic resistance and vaccine impact.* EBioMedicine, 2019. **43**: p. 338-346.

40. Bojang, A., et al., *Seasonality of Pneumococcal Nasopharyngeal Carriage in Rural Gambia Determined within the Context of a Cluster Randomized Pneumococcal Vaccine Trial.* PLoS One, 2015. **10**(7): p. e0129649.