

# Decline in pneumococcal vaccine serotype carriage, multiple-serotype carriage, and carriage density in Nepalese children after PCV10 introduction: A pre-post comparison study

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## ABSTRACT

**Background:** Carriage studies are an efficient means for assessing pneumococcal conjugate vaccine effect in settings where pneumococcal disease surveillance programmes are not well established. In this study the effect of 10-valent pneumococcal conjugate vaccine (PCV10) introduction on pneumococcal carriage and density among Nepalese children using a bacterial microarray and qPCR was examined.

**Methods:** PCV10 was introduced into the Nepalese infant immunisation schedule in August 2015. Nasopharyngeal swabs were collected from healthy Nepalese children in Kathmandu between April 2014 and December 2021. Samples were plated on blood agar, incubated overnight, and DNA extracted from plate sweeps. Pneumococcal serotyping was done using the Senti-SPv1.5 microarray (BUGS Bioscience, UK). DNA was extracted from swab media and qPCR performed for pneumococcal autolysin (*lytA*).

**Results:** A significant decline in prevalence of PCV10 serotypes was observed when comparing pre-PCV10 with post-PCV10 collection periods (36.5 %, 454/1244 vs 10.3 %, 243/2353,  $p < 0.0001$ ). Multiple-serotype carriage was also observed to significantly decline when comparing pre-PCV10 with post-PCV10 periods (31.4 %, 390/1244 vs 22.2 %, 522/2353,  $p < 0.0001$ ). Additionally, a significant decline in median pneumococcal density was observed when comparing pre-PCV10 with post-PCV10 periods (3.3 vs 3.25 log<sub>10</sub> GE/ml,  $p = 0.0196$ ).

**Conclusions:** PCV10 introduction was associated with reduced, prevalence of all PCV10 serotypes, multiple serotype carriage, and pneumococcal carriage density.

## 1. Introduction

*Streptococcus pneumoniae* (pneumococcus) is a common cause of pneumonia and invasive bacterial disease among children in South Asia [1,2]. In Nepal, the 10-valent pneumococcal conjugate vaccine (PCV10) was introduced into the infant immunisation schedule in August 2015 and implemented as two priming doses at 6 and 10 weeks followed by a booster at 9 months of age [3].

Assessment of PCV10 impact against invasive pneumococcal disease in Nepal is limited by the absence of a national surveillance programme. Such data are essential as it can inform decisions on schedule modifications and whether there is a rationale for using higher valence PCVs such as PCV13, PCV15 or PCV20 (Appendix Table 1). Pneumococcal carriage studies provide an approach for assessing PCV effect in such resource limited settings [4]. Carriage studies using conventional microbiological approaches have shown declines in the prevalence of

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PCV covered serotypes and an increase in some non-vaccine serotypes [5]. However, some serotypes, such as serotype 1, are infrequently detected in the context of carriage but frequently cause disease [6]. This could potentially be due to brief duration or carriage of these serotypes at a low density [7]. Low-density serotypes are more difficult to identify using conventional microbiological techniques [8]. However, newer technologies such as bacterial DNA microarrays provide a more sensitive approach for identifying low abundance and simultaneous carriage [9].

Higher prevalence of multiple serotype carriage and the higher density of carriage among children is thought to be a contributing factor to observed higher rates of disease when compared with settings with lower multiple serotype carriage and density. However, the impact of PCVs on multiple serotype carriage and density is not well understood. A study of Papua New Guinean infants which compared carriage among PCV vaccinated with unvaccinated children did not demonstrate a difference in multi-serotype carriage or density [10]. Whilst a study in Mongolian children pre and post programmatic PCV introduction demonstrated no change in multi-serotype carriage and an increase in density [11].

There have been increasing levels of pneumococcal antibiotic resistance reported in Nepal [12]. Some studies in other regions have described a decline in the prevalence of pneumococcal antibiotic resistance following programmatic PCV introduction [13,14]. It has been hypothesised that the reduction of vaccine strains which have a higher proportion of antibiotic resistance is the underlying reason for the reduction in overall pneumococcal resistance. Testing for pneumococcal antibiotic resistance across many samples can be very labour intensive. However, newer molecular approaches have now been developed, which can be used in a high throughput manner, and can accurately detect genes which confer resistance.

We previously conducted a pneumococcal carriage study, using conventional microbiology and Quellung serotyping among Nepalese children, which spanned the introduction of PCV10 into the infant immunisation programme [5]. Using these samples, we conducted a study to further examine the relationship of PCV10 introduction to changes in pneumococcal carriage, by applying a bacterial microarray coupled with qPCR to give a more detailed understanding of vaccine effect on pneumococcal carriage, density, and antibiotic resistance amongst Nepalese children.

## 2. Methods

### 2.1. Study design

A pre and post programmatic PCV10 introduction comparison of pneumococcal serotype-specific carriage and carriage density was conducted in Nepalese children. Between 21st April 2014 and 31st December 2021, following informed consent, nasopharyngeal swabs were collected from Nepalese children aged 6–60 months who were attending the paediatric outpatient department at Patan Hospital, Kathmandu, Nepal. Patan Hospital is the main healthcare facility for the surrounding community and is a tertiary care facility for the Kathmandu Valley. Samples from 2020 were not collected due to the impact of the COVID-19 pandemic on project recruitment in Nepal. Swabs underwent culture, conventional microbiological processing, and Quellung serotyping for pneumococcus as previously described [5]. In Kathmandu, from August 2015, the 10-valent pneumococcal conjugate vaccine (manufactured by GSK) was included in the infant immunisation schedule at 6 and 10 weeks, and 9 months of age (with no catchup program for older children). Coverage rates of three doses of PCV10 in Nepal were estimated to be 94% [15]. Isolates collected before and after January 1st, 2016, were classified as pre- and post-PCV cohorts respectively.

The study was approved by the Patan Academy of Health Sciences Ethical Review Committee, the Nepal Health research Council (NHRC 39/2014), and the Oxford Tropical Research Ethics Committee (OxTREC

28–14).

### 2.2. Microarray

Specimens that had pneumococcus identified by conventional microbiology were randomly selected for microarray processing using the BUGS Senti-SP microarray (not all conventionally processed samples were analysed by microarray due to budget limitations). Initially STGG from each sample was plated onto Streptococcal selective COBA plates (Oxoid, Hampshire, United Kingdom) in neat, 1:10, and 1:100 dilutions. After overnight incubation at 37 °C, the plate with the highest density of non-confluent growth was then selected, colonies from across the entire plate were scraped into 1 ml of saline and stored at –20 °C or below until processed for DNA extraction.

Genomic DNA were extracted from pneumococcal plate sweep suspensions by BUGS bioscience, using QIAamp DNA Mini Kit protocol (Qiagen, Germany). The suspensions were centrifuged to pellet the bacterial, re-suspended in 180 µL freshly prepared lysis buffer (20 mg/mL lysozyme, 20 mM Tris-HCL, 2 mM EDTA, 1 % Triton) and incubated at 37 °C for 60 min. Proteinase K (20 µL) and Qiagen buffer AL (200 µL) were added to the mixture and incubated for another 30 min at 56 °C. 4 µL RNase A was added and incubated at room temperature for 5 min before incubation at 70 °C for 10 min. Lysates were transferred to a QIAcube HT for high-throughput DNA extraction.

Molecular serotyping was conducted using the Senti-SP microarray (BUGS Bioscience, London, United Kingdom; <https://bugsbio.org>) [16]. DNA samples were fluorescently labelled and hybridized to the Agilent 8 × 15 K format microarray according to manufacturer's instructions for the Agilent genomic DNA ULS labelling and oligo aCGH hybridisation reagent kits. Microarray data were statistically analysed using a Bayesian hierarchical model to determine the serotype, or combination of serotypes, present in the sample and assign a relative abundance to each serotype detected. Additional targets of the microarray enable detection of non-pneumococcal *Streptococcus* spp., antibiotic resistance genes, and assess genetic relatedness.

### 2.3. DNA extraction from STGG

DNA was extracted from 200 µl STGG media within which the nasopharyngeal swab had been stored using a modified protocol based around the QIAGEN DNeasy 96 kit (QIAGEN, Manchester, United Kingdom) (Appendix).

### 2.4. qPCR

Assays were carried out in a final reaction volume of 25 µl and performed in duplicate. Each assay consisted of; previously described pneumococcal autolysin primers and probe (Life Technologies Limited, Paisley, United Kingdom) (Appendix Table 2), 12.5 µl of Taqman gene expression mastermix (Thermo Fisher Scientific, Leicestershire, United Kingdom), 10 µl of template DNA, and 1 µl of molecular grade water (Thermo Fisher Scientific, Leicestershire, United Kingdom) [17].

A plasmid containing *lytA* (GenExpress, Berlin, Germany) was used in defined concentrations (ranging from 10<sup>2</sup>–10<sup>8</sup> copies per/ml) to generate a standard curve from which the total copy number of *lytA* could be determined for each sample. All samples were run in duplicate on a StepOnePlus real time PCR platform (Thermo Fisher Scientific, Leicestershire, United Kingdom) [18]. Samples underwent a single first stage at 95 °C for 10 min followed by second stage consisting of 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

### 2.5. Statistical analyses

Prevalence data were summarised as counts and percentages and comparisons made using a Fisher's Exact test. Vaccine efficacy against carriage was calculated using the formula, 1-(proportion of children

who had received a dose/doses of PCV10 who were carrying a PCV10 serotype/proportion of children who had not had any doses of PCV10 who were carrying a PCV10 serotype). Pneumococcal density data averaged across the duplicate runs and were log10 transformed before medians and interquartile ranges were calculated and expressed at genome equivalents/ml (GE/ml). If the quantity was undetermined and CT > 35 a value of 0.01 GE/ml was arbitrarily assigned. Mann-Whitney U test was applied when comparing density between groups. A p-value < 0.05 was considered significant. Statistical analyses were conducted in RStudio V2022.12.0 [19].

### 3. Results

Between 21st April 2014 and 31st December 2021, 6360 children were enrolled into the carriage study with pneumococcus identified by conventional microbiology on 3820 swabs (Appendix Table 3). Of these 3820 pneumococcal samples, 3597 (94.2 %) underwent analysis by microarray. Comparison of serotyping results by Quellung with microarray revealed 86.2 % (3102/3597) concordance for the detection of the same serotype by both methods. For the 13.8 % (495/3597) of samples with discordant results, 21.2 % (105/495) were non-typeable by Quellung but typed by microarray as either pneumococcal serotypes (45/105), related *Streptococcus* spp. (50/105) or a mixture of these (10/105). The subsequent analyses will focus on the cohort of samples which underwent microarray analyses (Table 1). This study cohort consisted of 1244 pre-PCV10 and 2353 post-PCV10 samples.

#### 3.1. Effect of PCV10 on pneumococcal carriage

The 1244 pre-PCV10 swabs yielded 1719 pneumococcal serotypes (average of 1.4 serotypes/swab) whilst in comparison 2353 post-PCV10 swabs had 2833 serotypes identified (average of 1.2 serotypes/swab). Prior to PCV10 introduction the most prevalent typeable serotypes were 19F, 23F, and 6A (Appendix Fig. 1). Post-PCV10 introduction the most prevalent typeable serotypes were 6A, 34, and 19A. A significant decline in detection of any PCV10 serotype on a swab was noted when comparing pre- with post-PCV10 collection periods (454/1244, 36.5 % vs 243/2353, 10.3 %, p < 0.0001). Significant declines in the detection of all PCV10-specific serotypes were noted when comparing pre-PCV10 with post-PCV10 collection periods (Table 2). The vaccine effectiveness against carriage of any PCV10 serotype at the time of sampling was 31.4 %, 58.2 %, and 61.9 % for one dose, two doses, and three doses of PCV10 respectively. From the 3597 swabs, microarray detected significantly more PCV10 serotypes compared with conventional processing and Quellung serotyping (823 versus 710, p = 0.0013).

The proportion of non-vaccine serotypes identified increased when comparing pre-PCV10 with post-PCV10 periods (pre-PCV10 = 1210/1719, 70.4 % versus post-PCV10 = 2494/2833, 88 %, p < 0.0001) (Appendix Table 4). For the additional serotypes covered by PCV13,

there was no significant change in the prevalence of serotype 3 and a significant increase in prevalence of serotypes 6A and 19A when comparing pre-PCV10 with post-PCV10 periods (Table 3). For the additional serotypes covered by PCV15 and PCV20 there were no significant changes in prevalence when comparing pre-PCV10 with post-PCV10 periods. Additionally, there was no change in the proportion of non-pneumococcal streptococci detected on swabs when comparing pre-PCV10 with post-PCV10 periods (276/1244 versus 561/2352, p = 0.2624).

Detection of multiple pneumococcal serotypes on swabs significantly declined when comparing pre-PCV10 with post-PCV10 periods (390/1244 versus 522/2353, p < 0.0001, Appendix Table 5). Multiple serotype carriage rates each year showed variation however, with a significant decline noted when comparing 2014 with 2015 (334/908 versus 56/336, p < 0.0001) whilst a significant increase was observed when comparing 2015 with 2017 (56/336 versus 186/743, p = 0.0021). No significant change in multiple serotype carriage was noted when comparing children who had not received a dose of PCV10 with those who had received one dose (414/1324 vs 15/45, p = 0.7464). A significant decline in multiple serotype carriage was noted when comparing children who had not received a dose of PCV10 to children who had received either two (414/910 vs 22/114, p = 0.0077) or three (414/1324 vs 461/2114, p < 0.0001) doses.

#### 3.2. Effect of PCV10 on density

There was a significant decline in median pneumococcal carriage density when comparing pre-PCV10 with post-PCV10 periods (3.3 vs 3.25 log10 GE/ml, p = 0.0196, Appendix Table 6). When examining density by collection year, the median pneumococcal density increased when comparing 2014 with 2015 (3.1 log10 GE/ml vs 3.6 log10 GE/ml, p < 0.0001, Fig. 1). Whilst, no difference in median density was observed when comparing 2015 with 2017 (3.6 log10 GE/ml vs 3.8 log10 GE/ml, p = 0.0635) however, median densities declined when comparing 2015 with 2018 (3.6 log10 GE/ml vs 3.2 log10 GE/ml, p < 0.0001), 2019 (3.6 log10 GE/ml vs 3 log10 GE/ml, p < 0.0001), and 2021 (3.6 log10 GE/ml vs 2.8 log10 GE/ml, p < 0.0001). PCV10 covered serotype densities are described in Appendix Fig. 2. When examining density by number of PCV10 doses received, the median density was similar when comparing children who had not received any doses with those who had received two (3.3 log10 GE/ml vs 3.5 log10 GE/ml, p = 0.2178) or three doses (3.3 log10 GE/ml vs 3.2 log10 GE/ml, p = 0.3442). There was a significant increase in density noted when comparing children who had not received any doses with those who had received a single dose (3.3 log10 GE/ml vs 3.7 log10 GE/ml, p = 0.0011).

**Table 1**  
Cohort characteristics for samples analysed by microarray.

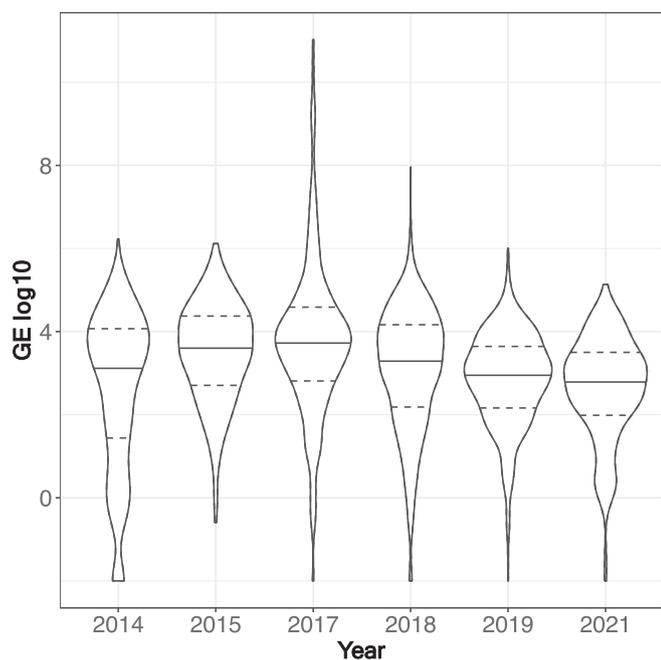
Collection year	Number of participants	Number of swabs with pneumococcus detected by conventional microbiology (%)	Number of swabs analysed by microarray	Male %	Age, years median (IQR)	0 PCV doses	1 PCV dose	2 PCV doses	3 PCV doses
2014	1305	908 (69.6)	908	56.6	1.2 (0.8)	908 (100)	0 (0)	0 (0)	0 (0)
2015	600	336 (56)	336	53.6	1.1 (0.5)	336 (100)	0 (0)	0 (0)	0 (0)
2017	1301	866 (66.6)	734	55	1.2 (0.6)	43 (5.9)	32 (4.4)	54 (7.4)	605 (82.4)
2018	1303	797 (61.2)	710	52.8	1.1 (0.6)	31 (4.4)	9 (1.3)	43 (6.1)	627 (88.3)
2019	1303	724 (55.6)	720	57.4	1.1 (0.6)	6 (0.8)	4 (0.6)	14 (1.9)	696 (96.7)
2021	548	189 (34.5)	189	57.1	1.1 (0.7)	0 (0)	0 (0)	3 (1.6)	186 (98.4)

**Table 2**  
Effect of PCV10 on vaccine covered serotypes.

Serotype	2014, n (%) N = 908	2015, n (%) N = 336	2017, n (%) N = 734	2018, n (%) N = 710	2019, n (%) N = 720	2021, n (%) N = 189	Pre, n (%) N = 1244	Post, n (%) N = 2353	P-value Pre vs Post
1	12 (1.3)	2 (0.6)	6 (0.8)	0 (0)	1 (0.1)	0 (0)	14 (1.1)	7 (0.3)	0.0042
4	15 (1.7)	2 (0.6)	5 (0.7)	7 (1)	1 (0.1)	0 (0)	17 (1.4)	13 (0.6)	0.0189
5	7 (0.8)	0 (0)	1 (0.1)	1 (0.1)	0 (0)	0 (0)	7 (0.6)	2 (0.1)	0.0103
6B	68 (7.5)	28 (8.3)	28 (3.8)	22 (3.1)	8 (1.1)	2 (1.1)	96 (7.7)	60 (2.5)	<0.0001
7F	8 (0.9)	4 (0.5)	4 (0.5)	1 (0.1)	3 (0.4)	1 (0.5)	12 (1)	9 (0.4)	0.0375
9 V	14 (1.5)	13 (3.9)	14 (1.9)	7 (1)	6 (0.8)	0 (0)	27 (2.2)	27 (1.1)	0.0206
14	72 (7.9)	15 (4.5)	26 (3.5)	21 (3)	15 (2.1)	1 (0.5)	87 (7)	63 (2.7)	<0.0001
18C	32 (3.5)	7 (2.1)	10 (1.4)	9 (1.3)	3 (0.4)	2 (1.1)	39 (3.1)	24 (1)	<0.0001
19F	82 (9)	26 (7.7)	20 (2.7)	19 (2.7)	15 (2.1)	0 (0)	108 (8.7)	54 (2.3)	<0.0001
23F	79 (8.7)	23 (6.8)	33 (4.5)	22 (3.1)	22 (3.1)	3 (1.6)	102 (8.2)	80 (3.4)	<0.0001

**Table 3**  
Variations in prevalence following PCV10 introduction for pneumococcal serotypes covered by higher valence PCVs.

Serotype (Vaccine)	2014, n (%) N = 908	2015, n (%) N = 336	2017, n (%) N = 734	2018, n (%) N = 710	2019, n (%) N = 720	2021, n (%) N = 189	Pre, n (%) N = 1244	Post, n (%) N = 2353	P-value Pre vs Post
3 (PCV13)	19 (2.1)	4 (1.2)	21 (2.9)	13 (1.8)	22 (3.1)	5 (2.6)	23 (1.8)	61 (2.6)	0.2012
6A (PCV13)	79 (8.7)	19 (5.6)	49 (6.7)	51 (7.2)	44 (6.1)	18 (9.5)	98 (7.9)	260 (11)	0.0023
19A (PCV13)	26 (2.9)	8 (2.4)	40 (5.4)	29 (4.1)	65 (9)	11 (5.8)	34 (2.7)	145 (6.2)	<0.0001
22F (PCV15)	8 (0.9)	3 (0.9)	5 (0.7)	11 (1.5)	10 (1.4)	2 (1.1)	11 (0.9)	39 (1.7)	0.0716
33F (PCV15)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	–
8 (PCV20)	12 (1.3)	1 (0.3)	6 (0.8)	3 (0.4)	10 (1.4)	1 (0.5)	13 (1)	20 (0.8)	0.5835
10A (PCV20)	24 (2.6)	13 (3.9)	27 (3.7)	17 (2.4)	31 (4.3)	3 (1.6)	37 (3)	78 (3.3)	0.6194
11A (PCV20)	43 (4.7)	15 (4.5)	51 (6.9)	22 (3.1)	26 (3.6)	13 (6.9)	58 (4.7)	112 (4.8)	0.9343
12F (PCV20)	2 (0.2)	0 (0)	4 (0.5)	3 (0.4)	3 (0.3)	0 (0)	2 (0.2)	10 (0.4)	0.2374
15B (PCV20)	67 (7.4)	14 (4.2)	52 (7.1)	46 (6.5)	33 (4.6)	13 (6.9)	81 (6.5)	144 (6.2)	0.6642



**Fig. 1.** Pneumococcal carriage density amongst Nepalese children by year of collection. This violin plot demonstrates the median density (bold line) and interquartile ranges (dashed lines). PCV10 was introduced into the infant immunisation programme in Kathmandu, Nepal in August 2015.

**3.3. Effect of PCV10 on antibiotic resistance gene prevalence**

The prevalence of genes, *mefA* and *ermB* that confer resistance to macrolides, and *tetM* that confers resistance to tetracyclines were highly prevalent throughout the study period and each of these genes significantly increased in prevalence when comparing the pre-PCV10 with post-PCV10 periods (Appendix Table 7). The prevalence of genes, *tetK*

that confers resistance to tetracyclines, and *sat4* and *aphA3* that confer resistance to aminoglycosides significantly decreased when comparing pre-PCV10 with post-PCV10 periods. The proportion of swabs with more than one antibiotic resistance gene significantly increased when comparing pre- with post-PCV10 periods (561/1244 vs 1442/2353,  $p < 0.0001$ ).

**4. Discussion**

This is the first study to assess the effect of PCV10 on multi-serotype pneumococcal carriage and carriage density among Nepalese children. We demonstrate at a population level, that following programmatic PCV10 introduction there was a decline in vaccine serotype carriage, prevalence of multiple serotype carriage, and pneumococcal carriage density. The data also indicate that serotypes frequently associated with invasive pneumococcal disease (such as serotypes 3, 6A, and 19A) and covered by higher valency PCVs continue to circulate in this setting. Additionally, we observed that the most prevalent antibiotic resistance genes in the first two years of the study (*mefA*, *ermB*, and *tetM*), significantly increased in later time periods.

The declines in carriage of vaccine serotypes following PCV10 introduction are in keeping with those described in the parent study which used conventional microbiological processing and Quellung serotyping [5]. This present study however, was able to detect 15.9 % more vaccine serotypes using the microarray platform compared with conventional processing of the same swabs. This is largely due to the superior capability of the microarray to detect minor serotypes of lower abundance when compared with conventional processing [20]. As such there is improved ability to discriminate changes in carriage prevalence at a serotype-specific level using the same sample size. This present study analysed samples which were positive for pneumococcus by conventional microbiological processing however, it has been shown that use of a molecular assay, such as PCR, to screen samples for pneumococcus prior to microarray analysis, yields a greater number of positive samples [20].

The prevalence of serotypes in the post-PCV10 period of this study

provide an insight into the utility of higher valent PCVs in this region. The study shows that an additional 19.8 %, 21.5 %, and 37 % of serotypes in the post-PCV10 period would be covered by PCV13, PCV15, and PCV20 respectively. These data suggest that there is likely to be a benefit in using higher valency PCVs, although the benefit of PCV15 over PCV13 appears to be marginal.

Multiple serotype carriage has been frequently described, with the wider use of high throughput molecular technologies making it more readily identified when compared with conventional microbiological approaches [7,9,20]. Carriage of multiple serotypes implies a greater capacity to transmit and for disease to become established, with one study showing that invasive pneumococcal disease was associated with the presence of multiple serotype carriage [21]. Studies in other regions have reported variable effects of PCV on multiple serotype carriage with some studies reporting a decline and others reporting no detectable change [22–24]. Notably no studies reported an increase in multiple serotype carriage. In this present study the decline in multiple serotype carriage is associated with PCV10 introduction and the decline in vaccine serotype prevalence. Notably an increase in single serotype carriage prevalence was also observed, when comparing pre- with post-PCV10 periods, further supporting the implication multiple serotype carriage declined secondary to the decline in PCV10 serotypes.

This study shows a persisting decline in carriage density each year following PCV10 introduction. This may reflect not only the increased PCV10 coverage among the study cohort each year but also the broader effect of programmatic use. In other settings where PCV effect on density has been measured there have been heterogeneous observations, which may reflect the wide variation in study designs, variations in the use of catchup schedules, and the relatively smaller sample sizes [25]. This study also showed that the decline in density appeared to be independent of the number of doses of PCV10 received. This suggests that the observed decline may be related to ongoing programmatic PCV10 use rather than direct protective effects. In keeping with reports from settings with more mature PCV programmes [26], it is anticipated there will be further serotype replacement in Nepal and studies exploring changes in density as this occurs will be needed to determine if the observations from this study are transient or not.

There is an increasing trend in multi-drug resistant invasive bacterial disease in Kathmandu, Nepal [27]. However, beta-lactams continue to be the first line antibiotic choice for community-acquired pneumonia even though growing levels of pneumococcal resistance to this class have been observed in this setting [28]. Variations in pneumococcal penicillin binding proteins which results in beta-lactam resistance are not detected by the microarray used in this study [29]. This present study does however show that macrolide resistance genes are becoming more prevalent, which may be due to relatively easy access and widespread use of azithromycin for suspected enteric fever in this setting. The implementation of the typhoid conjugate vaccine in Nepal in 2022 [30], could lead to a reduction in macrolide use in the community and consequently dampen the prevalence of resistance to this drug class. Macrolides are often recommended as an adjunct to first line therapy for childhood pneumonia as well [31], however the high levels of resistance would suggest more judicious use both in the hospital and community settings should be considered.

The strengths of this study include the large sample size and the duration of collection beyond initial PCV10 introduction. These factors result in the study being well powered to detect differences at a serotype-specific level when comparing pre- and post-PCV10 periods, whilst also providing an insight to the medium-term effects of PCV10 use on carriage. Limitations include the study design which makes it possible to only associate changes in carriage with PCV10 use, rather to directly link causation. It should also be noted that only a small portion of the cohort had received either one or two doses of PCV10 and as such these analyses should be interpreted with this small cohort size in mind. A further limitation is the impact of public health measures against COVID-19 from 2020 onwards and the limited number of samples collected

following this period.

This study demonstrates that PCV10 introduction is associated with reduced vaccine serotype circulation which has consequently reduced the prevalence of multiple serotype carriage and carriage density. Continued observation in this setting is required to determine whether PCV10 continues to suppress multiple serotype carriage and density or if non-vaccine serotypes subsequently replace the vaccine serotypes in these measures. Further, the ongoing detection of serotype 3 and the increase in serotypes 6A and 19A among carriage samples from this study would support the use of a vaccine which covers these serotypes. Finally, the observed increases, following PCV10 introduction, in genes which confer antibiotic resistance, even though vaccine serotypes had significantly reduced, indicates further policies directed at improved antimicrobial regulation may be beneficial.

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### Potential conflicts of interest

R.K. receives a National Health and Medical Research Council (NHMRC) Emerging Leader Fellowship (GNT1174010) and is an investigator on industry sponsored studies. AJP is chair of the UK Joint Committee on Vaccination and Immunisation and was a member of WHO's SAGE while this study was being conducted.

### Credit authorship contribution statement

**Rama Kandasamy:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Meeru Gurung:** Writing – review & editing, Project administration. **Sonu Shrestha:** Writing – review & editing, Methodology, Data curation. **Madhav C. Gautam:** Writing – review & editing, Methodology, Data curation. **Sarah Kelly:** Writing – review & editing, Project administration. **Imran Ansari:** Writing – review & editing, Supervision. **Katherine Gould:** Writing – review & editing, Methodology, Data curation. **Jason Hinds:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dominic F. Kelly:** Writing – review & editing, Investigation, Conceptualization. **David R Murdoch:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Andrew J. Pollard:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Shrijana Shrestha:** Writing – review & editing, Supervision, Investigation, Conceptualization.

### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Andrew J Pollard reports financial support was provided by GAVI Alliance. Rama Kandasamy reports a relationship with National Health and Medical Research Council that includes: funding grants. Rama Kandasamy reports a relationship with Sanofi that includes: funding grants. AJP is chair of the UK Joint Committee on Vaccination and Immunisation and was a member of WHO's SAGE while this study was being conducted. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2024.05.018>.

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