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Lack of effectiveness of 13-valent pneumococcal conjugate vaccination against pneumococcal carriage density in Papua New Guinean infants

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ABSTRACT

Background: Papua New Guinea (PNG) introduced the 13-valent pneumococcal conjugate vaccine (PCV13) in 2014, with administration at 1, 2, and 3 months of age. PCV13 has reduced or eliminated carriage of vaccine types in populations with low pneumococcal carriage prevalence, carriage density and serotype diversity. This study investigated PCV13 impact on serotype-specific pneumococcal carriage prevalence, density, and serotype diversity in PNG infants, who have some of the highest reported rates of pneumococcal carriage and disease in the world.

Methods: Nasopharyngeal swabs were collected at 1, 4 and 9 months of age from PCV13-vaccinated infants (n = 57) and age-/season-matched, unvaccinated infants (at approximately 1 month, n = 53; 4 months, n = 57; 9 months, n = 52). Serotype-specific pneumococcal carriage density and antimicrobial resistance genes were identified by qPCR and microarray.

Results: Pneumococci were present in 89% of swabs, with 60 different serotypes and four non-encapsulated variants detected. Multiple serotype carriage was common (47% of swabs). Vaccine type carriage prevalence was similar between PCV13-vaccinated and unvaccinated infants at 4 and 9 months of age. The prevalence of non-vaccine type carriage was also similar between cohorts, with non-vaccine types present in three-quarters of samples (from both vaccinated and unvaccinated infants) by 4 months of age. The median pneumococcal carriage density was high and similar at each age group (~7.0 $\log_{10}$ genome equivalents/mL). PCV13 had no effect on overall pneumococcal carriage density, vaccine type density, non-vaccine type density, or the prevalence of antimicrobial resistance genes.

Conclusion: PNG infants experience dense and diverse pneumococcal colonisation with concurrent

Abbreviations: PCV, pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PCV10, 10-valent pneumococcal conjugate vaccine; AMR, antimicrobial resistance; PCV7, 7-valent pneumococcal conjugate vaccine; PNG, Papua New Guinea; EHP, Eastern Highlands Province; PPV23, 23-valent pneumococcal polysaccharide vaccine; qPCR, quantitative polymerase chain reaction; GE, genome equivalents; IQR, interquartile range.

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serotypes from 1 month of age. PCV13 had no impact on pneumococcal carriage density, even for vaccine serotypes. The low prevalence of vaccine serotypes, high pneumococcal carriage density and abundance of non-vaccine serotypes likely contribute to the lack of PCV13 impact on carriage in PNG infants. Indirect effects of the infant PCV programs are likely to be limited in PNG. Alternative vaccines with broader coverage should be considered.

1. Introduction

Streptococcus pneumoniae (the pneumococcus) is a common nasopharyngeal commensal and pathogen, causing a range of major diseases including pneumonia, otitis media, meningitis and sepsis. Nasopharyngeal carriage of the pneumococcus is a precursor for disease, and children are the primary reservoir for transmission. The polysaccharide capsule of the pneumococcus is a key virulence factor, with over 100 capsular serotypes identified to date [1]. This capsule induces protective antibodies that forms the basis for pneumococcal conjugate vaccines (PCVs), including the currently licensed 10-valent (PCV10) and 13-valent (PCV13) vaccines [2].

Globally, PCVs have been highly effective in reducing carriage and disease caused by the pneumococcal serotypes they contain (vaccine serotypes) [3]. The introduction of PCVs has also been shown to reduce antimicrobial resistance (AMR) associated with pneumococcal disease, as vaccine serotypes commonly contain antibiotic resistance determinants [4]. However, as PCVs have been introduced, serotype replacement has been observed with non-vaccine serotypes replacing vaccine serotypes, and in some settings (especially high-risk) minimal impact on the overall rates of pneumococcal carriage and disease has been reported [5]. Serotype replacement is of particular concern in low-income populations as there is often higher prevalence, density and diversity of pneumococcal carriage and higher rates of pneumococcal disease [6]. The threat is even greater when the emerging non-vaccine serotypes are highly invasive and/or associated with antibiotic resistance, for example the observed global increase in serotype 19A, which is often antibiotic-resistant, following widespread introduction of the 7-valent PCV (PCV7) [7,8].

Papua New Guinea (PNG) has some of the highest reported rates of childhood pneumococcal carriage and disease in the world. PNG infants are colonised with a broad range of serotypes within weeks of birth: at least 60 different serotypes were identified in the nasopharynx of healthy children under 2 years [9)], and children under 5 years with pneumococcal pneumonia [10]. The Global Alliance for Vaccines and Immunisation (Gavi) and the World Health Organization assisted introduction of PCV13 in PNG in 2014. However, the extreme pneumococcal diversity within the nasopharynx of PNG children means that currently available PCVs are limited in their ability to provide protection against pneumococcal carriage and thus potentially disease [9]. We do not fully understand the impact of PCVs in populations such as PNG that experience early, dense and diverse pneumococcal carriage; and the impact of PCV13 on vaccine and non-vaccine serotype density and diversity in such a setting has not, to our knowledge, been assessed before. Semi-quantitative colonisation data from a PCV7 study in The Gambia, with a lower pneumococcal carriage frequency than PNG children, reported no difference in pneumococcal carriage density post-PCV7 for vaccine types and non-vaccine types [11]. In addition, a PCV7 study in Peru (also with lower carriage rates than PNG children) found no overall change in pneumococcal carriage density post-PCV7 introduction, though serotype-specific carriage densities were not measured [12]. Information on the effect of PCVs on pneumococcal carriage density in different populations is important for assessing PCV effectiveness. High pneumococcal carriage density, as observed in young children especially in lower-middle income countries and Indigenous populations throughout the world is thought to be a major contributing factor to high rates of pneumococcal disease. Vaccines that can reduce the pneumococcal load and thus transmission rate are therefore more likely to elicit herd immunity. Understanding PCV13 impact on vaccine and non-vaccine type carriage in a high-risk setting such as PNG is an important measure of PCV effectiveness, with data informing future requirements of pneumococcal vaccines and associated programs, particularly for populations with dense and diverse pneumococcal carriage.

The specific objective of this study was to compare vaccine and non-vaccine serotype carriage density in PCV13-vaccinated and unvaccinated Papua New Guinean infants. We hypothesised that the carriage density of vaccine serotypes would be reduced, and the density of non-vaccine serotypes would increase in the nasopharynx of PCV13-vaccinated children compared to age-matched unvaccinated children.

2. Materials and methods

2.1. Setting

This study was conducted in the Eastern Highlands Province (EHP) of PNG. PNG is a lower-middle income country situated in the south-west Pacific, with a population of over eight million [13]. Pneumonia is the leading cause of paediatric hospital admission nationally. It is particularly prevalent in the EHP, where pneumonia accounts for 30-40% of hospitalisations [14]. Located within Goroka, the capital of the EHP, are the Eastern Highlands Provincial Hospital (the sole tertiary healthcare facility in the province) and the PNG Institute of Medical Research (PNGIMR). Living standards vary widely between urban and rural areas of the EHP, with many villages only accessible by foot or 4-wheel drive [15]. Since 2014, the national immunisation program for PNG infants has included PCV13, with recommended administration at 1, 2 and 3 months of age. However, the vaccine was not available in the study area until the following year, and by late 2015, first and third dose coverage was only around 25% and 5% respectively [16].

2.2. Study design and participant selection

Nasopharyngeal swabs were collected from participants enrolled in two studies carried out at PNGIMR: the 10v13v PCV trial, and the PNG pneumonia and meningitis aetiology study. The design and methods for these studies have been described elsewhere [15,16]. Briefly, the 10v13v PCV trial was an open randomised-controlled trial, conducted between 2011 and 2016, to determine the safety and immunogenicity of PCV10 and PCV13 and 23-valent pneumococcal polysaccharide (23vPPV) booster. Participants were randomly allocated to receive either PCV at ages 1, 2 and 3 months with or without a PPV23 booster at 9 months of age. Pneumonia episodes were identified by passive

morbidity surveillance. Nasopharyngeal swabs were collected at 1, 4, 9, 10, 23 and 24 months of age to assess pneumococcal colonisation, with 1-month swabs collected prior to the first dose of PCV13 [15]. For the present study, 1-, 4- and 9-month swabs were included.

The PNG pneumonia and meningitis aetiology study was a prospective case-control study conducted between 2013 and 2016, that enrolled children under 5 years of age to determine the aetiology of clinically defined moderate or severe pneumonia or suspected meningitis. Cases were identified following presentation for inpatient or outpatient care in Goroka town. Controls were healthy children recruited contemporaneously from cases' communities. Following enrolment, immunisation history and diagnostic specimens, including nasopharyngeal swabs, were obtained from both cases and controls [16].

The selection of participants from these two cohorts is depicted in Fig. 1. From the 10v13v PCV trial (hereafter referred to as the *PCV13 cohort*), 57 infants were identified that were vaccinated with 3 doses of PCV13 and had nasopharyngeal swabs collected at 1, 4 and 9 months of age. The resulting 171 nasopharyngeal swabs

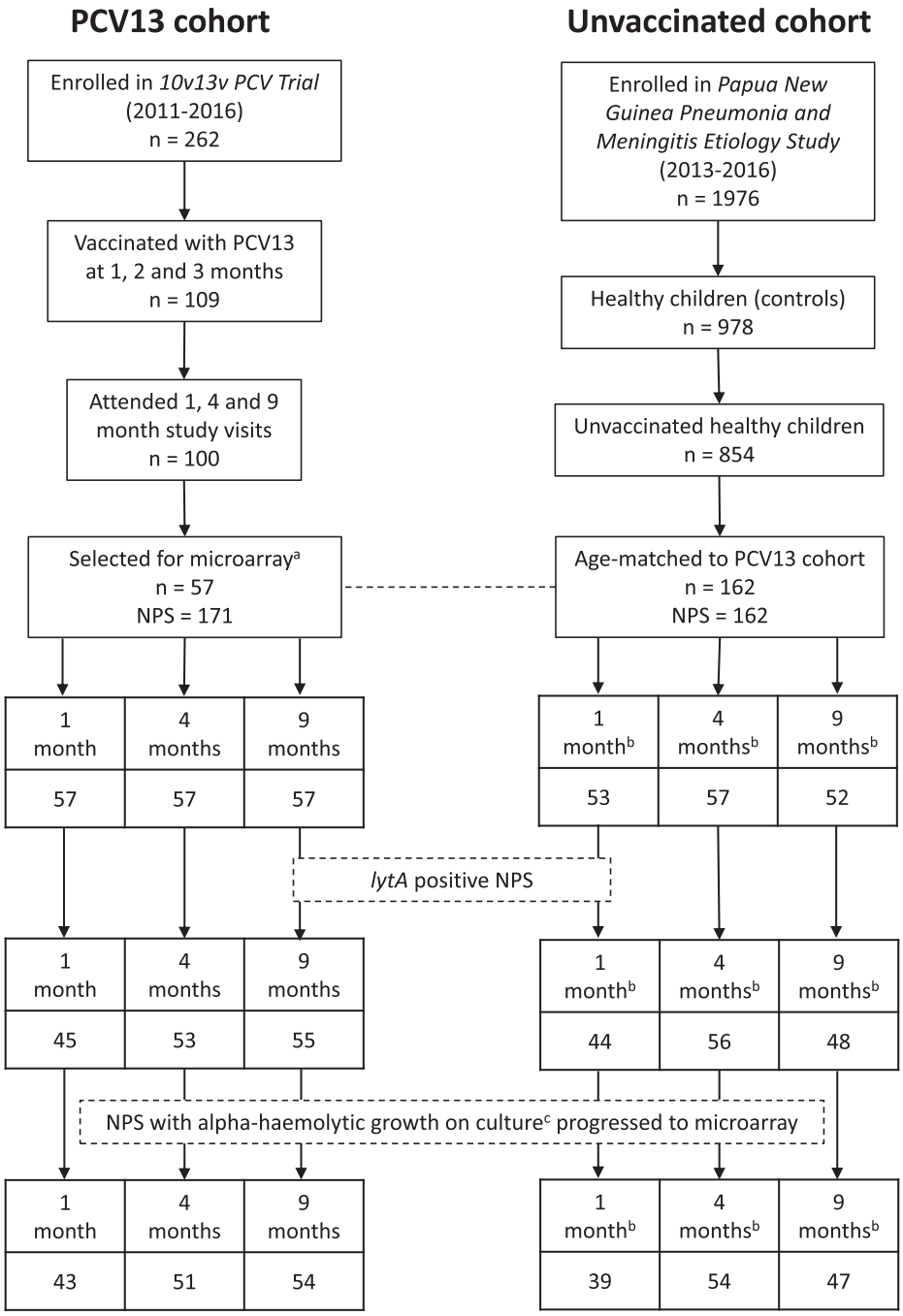

Fig. 1. Development of the analytical cohort. NPS, number of nasopharyngeal swabs collected; PCV13, 13-valent pneumococcal conjugate vaccine; [a] number of specimens selected for microarray was based on funding limitations, selection was at random; [b] in the unvaccinated cohort, 1- and 4-month nasopharyngeal swabs were age-matched ± 1 month, 9-month nasopharyngeal swabs were age-matched ± 2 months; [c] dilutions of swab sample aliquots were placed onto horse blood agar plates with gentamicin and incubated for 18–24 h (4). Four nasopharyngeal swabs were *lytA* positive and culture-positive, however no pneumococcus was detected by microarray, thus these samples were not included in prevalence and density analyses. The 10v13v PCV trial was a longitudinal study, participants were swabbed at multiple timepoints.

were then matched according to age and season with nasopharyngeal swabs from 162 unvaccinated children that were controls in the cross-sectional PNG pneumonia and meningitis aetiology study (hereafter referred to as the *unvaccinated cohort*), with one swab collected per participant at ages 0-2, 3-5, or 7-11 months (n = 53, 57 and 52, respectively). This resulted in a total of 333 nasopharyngeal swabs for analysis.

2.3. Specimen collection and processing

Nasopharyngeal swabs were collected, transported and stored at PNGIMR in accordance with the WHO guidelines [15,17], and then cryogenically shipped to the Telethon Kids Institute in Perth, Australia for pneumococcal quantification.

2.4. Pneumococcal detection and microarray analysis

Real-time quantitative-polymerase chain reaction (qPCR) targeting the *lytA* gene was undertaken on all 333 nasopharyngeal swabs [18]. A cycle threshold value of <30 was considered positive. Pneumococcal positive nasopharyngeal swabs (n = 301) were transported to the Murdoch Children's Research Institute in Melbourne, Australia, for pneumococcal enrichment and molecular serotyping by microarray as described previously [4]. In brief, nasopharyngeal swabs were cultured on horse blood agar containing 5 μg/mL of gentamicin, and DNA extracted from the bacterial growth with the QIAcube HT instrument (Qiagen). Molecular serotyping was conducted using Senti-SP v1.5 microarray (BUGS Bioscience), which reports the identity and relative abundance (%) of all pneumococcal serotypes detected within a sample. The microarray can also detect 10 AMR genes associated with mobile genetic elements (*tetM*, *tetK*, *tetO*, *tetL, cat*, *aphA3*, *sat4*, *mefA*, *ermB* and *ermC*). Pneumococcal carriage was defined as detection of any pneumococci, including non-encapsulated lineages, in the nasopharyngeal swabs.

PCV10 serotypes were defined as those serotypes contained in PCV10 (1, 4, 5, 6B, 7F, 9 V, 14, 18C, 19F and 23F). PCV13 serotypes include those serotypes contained in PCV10, plus serotypes 3, 6A and 19A. Non-encapsulated pneumococci (NT2, NT4a, NT4b, NT2/NT3b) were categorised based upon previously described genetic variants [2]. All remaining serotypes were defined as non-PCV13 serotypes (excluding non-encapsulated pneumococci). For example, a swab containing both a PCV13 serotype and a non-PCV13 serotype, was considered positive for both PCV13 and non-PCV13 serotype carriage. Serotypes 15B and 15C were reported as 15B/C as these serotypes are known to interconvert [19]. Pneumococci that typed as "-like" by microarray were tested phenotypically (latex agglutination and/or Quellung). Specifically, 23B-like serotyped as 23B, 19A-like serotyped as 19F [20], and 11F-like serotyped as 11A [21]. Genetic variants that could not be resolved phenotypically were reported as per the microarray result.

Overall pneumococcal density (from *lytA* qPCR) was determined for pneumococcal positive samples and reported in genome equivalents/mL (GE/mL). Serotype-specific density was calculated by multiplying overall pneumococcal density by the serotype relative abundance (determined by microarray) [22].

2.5. Statistical analyses

Categorical data were summarised as counts and percentages, and compared using the Pearson Chi-Square test, or Fisher's Exact test. Continuous data were summarised as medians and interquartile ranges (IQR) and compared using the Mann-Whitney *U* test for non-parametric data, except where indicated. A p value of ≤ 0.05 was considered significant. Pneumococcal density data were $\log_{10}$ transformed prior to analysis. Samples that were pneumococcal positive with unknown serotype (*lytA* positive, culture negative samples were not able to proceed to microarray) were excluded from analyses of serotype-specific pneumococcal carriage prevalence. Analyses of overall pneumococcal density were restricted to samples that were positive for pneumococcal carriage, and analyses of serotype-specific pneumococcal density and AMR genes were restricted to samples that were pneumococcal positive by microarray. Serotype diversity was assessed by calculating Shannon's diversity index for the PCV13 and unvaccinated cohorts at each timepoint. Data were cleaned and analysed in SPSS version 26 (IBM Corp, New York, NY, US). Graphs were created using GraphPad Prism version 8 (GraphPad Software, La Jolla, CA, USA).

2.6. Ethics statement

Ethical approvals for this study were obtained from the PNGIMR Institutional Review Board (IRB no. 1028) and the Government of PNG Medical Research Advisory Committee (MRAC no. 11.03). Samples from the PNG pneumonia and meningitis aetiology study were collected under the following ethics approvals: IRB no. 1204, MRAC no. 11.29 and The University of Western Australia RA/4/1/7960. Samples from the 10v13v PCV trial were collected under the following ethics approvals: IRB no. 1028 and MRAC no. 11.03. The 10v13v PCV trial is registered with ClinicalTrials.gov (CTN NCT01619462).

3. Results

3.1. Participant characteristics

Characteristics are shown in Table 1. There were 219 participants included in this study: 57 infants in the PCV13 cohort (with swabs collected at 1, 4, and 9 months of age; 171 swabs), and 162 infants in the cross-sectional unvaccinated cohort (53, 57 and 52 children for each age group; 162 swabs). The median age at swab collection for the 1-, 4-, and 9-month sample time-points was different between the cohorts, with the unvaccinated cohort ~2 weeks older at the 1-month and 4-month time-point and 3 weeks older at the 9-month timepoint. This was because the range for age at sample collection was relaxed in the unvaccinated cohort to allow for season matching of samples to compensate for the differences in year of sample collection. While the years over which swabs were collected overlap between the cohorts, they are not entirely the same (2011 to 2014 for the PCV13 cohort vs 2013 to 2016 for the unvaccinated cohort); therefore, specimens from the cross-sectional unvaccinated cohort were season-matched with the PCV13 swab collection to ensure even collection across seasons (by quarters) between the two groups ($p = 0.893$). Of the 57 infants in the PCV13 cohort, 28.1% (n = 16) had at least one episode of clinically-defined moderate or severe pneumonia in their first year of life [23]. Preceding clinical data was not collected for children recruited to the cross-sectional unvaccinated cohort.

3.2. Carriage prevalence

The overall pneumococcal carriage prevalence at 1 month of age was 80% (with 78.9% pneumococcus-positive in the PCV13 cohort and 81.1% in the unvaccinated cohort, Table 2a). Pneumococcal carriage prevalence continued to increase by 4 months and 9 months of age, irrespective of PCV13-vaccination status. By 9 months of age, pneumococcal carriage was detected in 94.7% of children in the PCV13 cohort and 92.3% in the unvaccinated cohort. Table 2b shows the carriage prevalence of vaccine serotypes, non-vaccine serotypes, and non-encapsulated pneumococci, by cohort and age. PCV13 serotype carriage prevalence was similar between

Table 1
Characteristics of the study cohorts.

Characteristic	Cohort		*p* value[a]
	PCV13[b]	Unvaccinated	
Number of individuals sampled	57	162	
Female sex, n (%)	31 (54.4)	80 (49.4)	0.541
Number of NPS collected	171	162	
Median age in months at NPS collection (IQR)			
1 month	1.02 (0.9–1.0)	1.45 (1.0–2.2)	<0.001
4 months	4.04 (3.9–4.1)	4.47 (3.5–5.3)	0.041
9 months	9.03 (9.0–9.1)	9.75 (8.5–11.2)	0.003
Year of NPS collection: n (%)			
2011	4 (2.3)	–	
2012	83 (48.5)	–	
2013	75 (43.9)	63 (38.9)	
2014	9 (5.3)	67 (41.4)	
2015	–	32 (19.8)	
Quarter of NPS collection: n (%)			0.893
January - March	43 (25.1)	37 (22.8)	
April - June	38 (22.2)	37 (22.8)	
July - September	44 (25.7)	39 (24.1)	
October - December	46 (26.9)	49 (30.2)	

PCV13, 13-valent pneumococcal conjugate vaccine; NPS, nasopharyngeal swab; IQR, interquartile range; [a]*p* values compare between cohorts using Pearson chi-square test for categorical data and Mann-Whitney *U* test for continuous data; [b]PCV13 cohort received three doses of PCV13 at 1, 2 and 3 months of age.

the PCV13 cohort and unvaccinated cohort at 4 months and 9 months of age (35.1% vs 49.1%, $p = 0.129$ and 38.6% vs 44.2%, $p = 0.551$, respectively). Non-vaccine type carriage prevalence was also similar between the PCV13 cohort and the unvaccinated cohort at 4 months and 9 months of age (75.4% vs 66.7%, $p = 0.302$ and 73.7% vs 71.2%, $p = 0.768$, respectively).

Of the 301 *lytA*-positive samples, 4.3% (n = 13) were culture-negative and therefore not serotyped. The non-encapsulated lineage NT2, and serotypes 15B/C, 6A, 6B, 19F and 23F were the most common. A complete list of all identified pneumococcal serotypes is shown in Supplementary Table 1, where 60 capsular serotypes and four non-encapsulated lineages were detected.

3.3. Pneumococcal carriage density

Pneumococcal carriage density detected in the nasopharyngeal swabs ranged from 3.76 to 9.73 $\log_{10}$ GE/mL, with a median of 6.98 $\log_{10}$ GE/mL. The median pneumococcal carriage density did not change with age or PCV13 vaccination status (Fig. 2, Table 3a). There was also no difference in the carriage density of vaccine types and non-vaccine types between the PCV13 cohort and the unvaccinated cohort for each age (Table 3b). Carriage densities for each serotype are shown in Supplementary Table 1, where the carriage density of individual serotypes was similar between cohorts. The high diversity of serotypes present in the nasopharynx of these children means that numbers were too small to conduct further statistical analyses.

3.4. Concurrent carriage of pneumococcal serotypes

Carriage of multiple serotypes (including non-encapsulated) was common, with $\geq$ 2 types identified in 47.2% (n = 133) of swabs: 69.2% with two types, 27.1% with three, 3.0% with four and 0.8% with five different types identified in a single swab. Overall, there were no significant differences in the prevalence of multiple serotype carriage observed between cohorts. By timepoint, co-colonisation was observed in 21.4% of the PCV13 cohort and 32.4% of the unvaccinated cohort at 1 month of age ($p = 0.269$), 63.3% of the PCV13 cohort and 44.4% of the unvaccinated cohort at 4 months ($p = 0.056$), and 56.6% of the PCV13 cohort and 57.4% of the unvaccinated cohort at 9 months of age ($p = 0.932$). There was a small increase in median overall pneumococcal density as the number of serotypes detected in a sample increased, however the Kruskal Wallis test showed this did not reach statistical significance (6.93 $\log_{10}$ GE/mL, 7.01 $\log_{10}$ GE/mL, 7.24 $\log_{10}$ GE/mL, 7.57 $\log_{10}$ GE/mL and 7.63 $\log_{10}$ GE/mL in samples with one, two, three, four and five serotypes, respectively; $p = 0.240$).

3.5. Diversity of pneumococcal serotypes

Serotype diversity was assessed by calculating Shannon's diversity index for the PCV13 and unvaccinated cohorts at each timepoint, whereby 0 indicates low diversity (dominated by one serotype) and 1 indicates high diversity. We found the range of serotypes detected to be highly and equally diverse between cohorts and timepoints: 0.94, 0.93 and 0.94 at 1, 4 and 9 months respectively in the PCV13 cohort; and 0.96, 0.94, and 0.94 at 1, 4 and 9 months respectively in the unvaccinated cohort. Serotype carriage within individuals was also found to be highly variable and unstable over time. Of the 44 infants from the PCV13 cohort that carried pneumococci at all three timepoints, 56.8% (n = 25) carried the same serotype on a subsequent sample, and only 6.8% (n = 3) carried the same serotype on all three samples.

Table 2a
Comparing pneumococcal nasopharyngeal carriage prevalence by cohort at age 1, 4 and 9 months.

	1-month old infants			4-month old infants			9-month old infants		
	PCV13 cohort[a] (N = 57) n (%)	Unvaccinated cohort (N = 53) n (%)	*p* value[b]	PCV13 cohort (N = 57) n (%)	Unvaccinated cohort (N = 57) n (%)	*p* value	PCV13 cohort (N = 57) n (%)	Unvaccinated cohort (N = 52) n (%)	*p* value
Pneumococcal carriage (any)	45 (78.9)	43 (81.1)	0.775	51 (89.5)	56 (98.2)	0.051	54 (94.7)	48 (92.3)	0.605

Table 2b
Comparing pneumococcal vaccine and non-vaccine type nasopharyngeal carriage prevalence by cohort at age 1, 4 and 9 months.

	1-month old infants			4-month old infants			9-month old infants		
	PCV13 cohort[a] (N = 55) n (%)	Unvaccinated cohort (N = 48) n (%)	p value[b]	PCV13 cohort (N = 55) n (%)	Unvaccinated cohort (N = 55) n (%)	p value	PCV13 cohort (N = 56) n (%)	Unvaccinated cohort (N = 51) n (%)	p value
VACCINE TYPES									
Any PCV13-type	17 (30.9)	14 (29.2)	0.847	20 (36.4)	28 (50.9)	0.124	22 (39.3)	23 (45.1)	0.543
Any PCV10-type	13 (23.6)	10 (20.8)	0.733	16 (29.1)	19 (34.5)	0.539	13 (23.2)	16 (31.4)	0.343
1	-	1 (2.1)		-	-		1 (1.8)	-	
4	2 (3.6)	2 (4.2)		–	1 (1.8)		–	1 (2.0)	
5	–	–		–	–		–	1 (2.0)	
6B	1 (1.8)	2 (4.2)		3 (5.5)	5 (9.1)		4 (7.1)	5 (9.8)	
7F	1 (1.8)	–		–	–		–	–	
9V	–	–		–	1 (1.8)		–	–	
14	2 (3.6)	1 (2.1)		5 (9.1)	3 (5.5)		5 (8.9)	3 (5.9)	
18C	–	1 (2.1)		2 (3.6)	1 (1.8)		–	2 (3.9)	
19F	3 (5.5)	–		4 (7.3)	5 (9.1)		4 (7.1)	4 (7.8)	
23F	4 (7.3)	3 (6.3)		4 (7.3)	4 (7.3)		2 (3.6)	3 (5.9)	
Extra PCV13 types	5 (9.1)	5 (10.4)	1.000	6 (10.9)	11 (20.0)	0.187	10 (17.9)	9 (17.6)	0.977
3	2 (3.6)	1 (2.1)		–	–		1 (1.8)	1 (2.0)	
6A	2 (3.6)	3 (6.3)		5 (9.1)	6 (10.9)		2 (3.6)	5 (9.8)	
19A	1 (1.8)	2 (4.2)		1 (1.8)	5 (9.1)		7 (12.5)	3 (5.9)	
NON-VACCINE TYPES									
Any non-vaccine type[c]	30 (54.5)	31 (64.6)	0.301	43 (78.2)	38 (69.1)	0.279	42 (75.0)	37 (72.5)	0.773
Non-PCV13 serotypes	21 (38.2)	28 (58.3)	0.041	39 (70.9)	34 (61.8)	0.313	40 (71.4)	34 (66.7)	0.594
Non-encapsulated	9 (16.4)	7 (14.6)	0.803	12 (21.8)	9 (16.4)	0.467	6 (10.7)	8 (15.7)	0.446

PCV13, 13-valent pneumococcal conjugate vaccine; [a]PCV13 cohort received three doses of PCV13 at 1, 2 and 3 months of age, 1-month nasopharyngeal swabs were collected prior to the first dose of vaccine; [b]Pearson chi-square test was used to compare PCV13 and unvaccinated cohorts; [c]includes non-encapsulated and non-PCV13 serotypes. Samples that were pneumococcal positive with unknown serotype (*lytA* positive, culture negative samples were not able to proceed to microarray) were excluded from analyses of serotype-specific pneumococcal carriage prevalence.

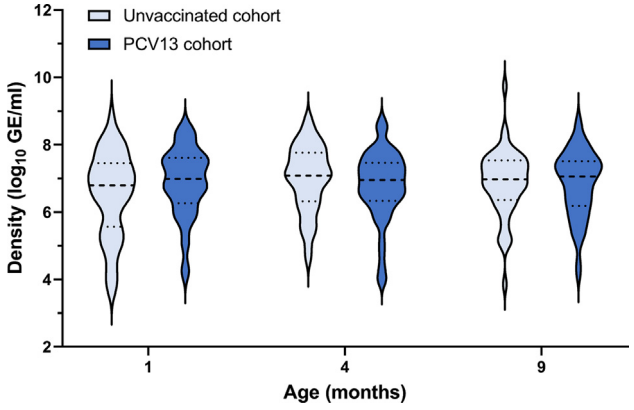

Fig. 2. Pneumococcal carriage densities at 1, 4 and 9 months of age. Violin plots of overall pneumococcal carriage densities (in $\log_{10}$ genome equivalents (GE)/mL) by cohort in infants aged 1, 4 and 9 months. Dashed lines indicate median densities, dotted lines indicate quartiles, and the density plot width indicates the frequency of observations. The PCV13 cohort received three doses of PCV13 at 1, 2 and 3 months of age; 1-month nasopharyngeal swabs were collected prior to the first dose of vaccine.

3.6. Antimicrobial resistance

AMR genes were identified in 19% (n = 54) of pneumococcal positive samples: 18.4% from the PCV13 cohort versus 16.7% from the unvaccinated cohort at 4 months (p = 0.820); and 11.3% in the PCV13 cohort versus 12.8% in the unvaccinated cohort at 9 months (p = 0.824; Supplementary Table 2). The most frequently detected AMR gene was *tetM* which encodes resistance to tetracycline. There were no significance differences in the prevalence of the ten AMR genes assessed, between cohorts at any age.

4. Discussion

This study found that PCV13 had no impact on pneumococcal carriage prevalence, carriage density or serotype diversity in Papua New Guinean infants. By 1 month of age, 80% of infants were colonised with the pneumococcus, prior to eligibility for their first dose of PCV13. Following three doses of PCV13 at 1-2-3-months of age, pneumococcal carriage prevalence increased to 89–95%, showing no effect of vaccination on overall carriage in this study population. The carriage rates are the highest reported in the literature, where studies in endemic settings such as Kenya, The Gambia, and a refugee camp in Thailand, report a maximum prevalence of pneumococcal carriage of 63–80% at 3 months of age [11,24,25]. Our study also demonstrates higher carriage prevalence, and at an earlier age, than a previous study conducted in 2005 to 2009 in the same area of PNG, which reported 59% (n = 152) of infants carrying *S. pneumoniae* at 1 month of age, although this was only determined using culture-based techniques [9].

The non-encapsulated lineage NT2, and serotypes 15B/C, 6A, 6B, 19F and 23F were the most common types detected in our study. At least one of these six types were detected in 46% (n = 155) of all samples. Serotypes 6B, 19F, 23F, 15B/C and non-encapsulated lineage NT2 were also amongst the most frequently reported carriage serotypes in infants in Indonesia and Fiji prior to PCV introduction [22,26].

The persistent carriage of PCV13 serotypes in PCV13-vaccinated infants (36% and 39% prevalence at one and six months post-vaccination, respectively) is an important observation from this study. There was some evidence of a reduction in carriage prevalence of PCV13 vaccine types in the vaccinated cohort (compared to the unvaccinated cohort) at 4 months of age, however this was not sustained to 9 months. This finding suggests that PCV13 does not have the same effect on prevention of vaccine type carriage as observed in other populations with lower densities and

Table 3a
Overall pneumococcal carriage density (in log_{10} genome equivalents/mL) by cohort in infants aged 1, 4 and 9 months.

	1-month old infants			4-month old infants			9-month old infants		
	PCV13 cohort[a] (N = 45) Median density (IQR)	Unvaccinated cohort (N = 43) Median density (IQR)	*p* value[b]	PCV13 cohort (N = 51) Median density (IQR)	Unvaccinated cohort (N = 56) Median density (IQR)	*p* value	PCV13 cohort (N = 54) Median density (IQR)	Unvaccinated cohort (N = 48) Median density (IQR)	*p* value
All pneumococci	6.98 (6.26–7.61)	6.79 (5.56–7.45)	0.238	6.95 (6.33–7.46)	7.07 (6.32–7.76)	0.272	7.06 (6.18–7.51)	6.96 (6.36–7.53)	0.979

Table 3b
Serotype-specific pneumococcal carriage densities (in log_{10} genome equivalents/mL) by cohort in infants aged 1, 4 and 9 months.

	1-month old infants			4-month old infants			9-month old infants		
	PCV13 cohort[a] (N = 43) Median density (IQR)	Unvaccinated cohort (N = 38) Median density (IQR)	*p* value[b]	PCV13 cohort (N = 49) Median density (IQR)	Unvaccinated cohort (N = 54) Median density (IQR)	*p* value	PCV13 cohort (N = 53) Median density (IQR)	Unvaccinated cohort (N = 47) Median density (IQR)	*p* value
PCV13 serotypes	7.36 (5.86–7.72)	6.73 (5.18–7.28)	0.164	6.83 (6.22–7.10)	7.01 (6.02–7.64)	0.374	6.63 (6.13–7.32)	6.78 (5.94–7.30)	0.624
Non-PCV13 serotypes	6.76 (6.42–7.50)	6.52 (5.58–7.27)	0.151	6.70 (5.77–7.23)	6.46 (6.08–7.12)	0.732	6.74 (6.52–7.01)	6.80 (5.85–7.08)	0.786
Non-encapsulated pneumococci	6.18 (5.53–7.07)	6.85 (5.73–7.52)	0.408	6.42 (5.72–6.98)	7.09 (5.96–7.40)	0.345	6.03 (5.50–6.56)	5.57 (4.51–6.03)	0.345

PCV13, 13-valent pneumococcal conjugate vaccine; IQR, interquartile range; [a]PCV13 cohort received three doses of PCV13 at 1, 2 and 3 months of age, 1-month nasopharyngeal swab collected prior to first dose of vaccine; [b]Mann-Whitney *U* test used to compare unvaccinated and PCV13 cohorts. Analyses of overall pneumococcal density were restricted to samples that were positive for pneumococcal carriage, and analyses of serotype-specific pneumococcal density were restricted to samples that were pneumococcal positive by microarray.

diversity of pneumococcal carriage. With continued carriage of PCV13 serotypes in a PCV13-vaccinated population it is unlikely that pneumococcal transmission will be prevented, and thus vaccinating infants will have limited impact on pneumococcal disease in unvaccinated children and adults. Of further concern, non-vaccine types were present in three-quarters of samples (from both vaccinated and unvaccinated infants) by 4 months of age. Considering the wide diversity of pneumococci detected in this population, and the propensity for infants to carry up to five serotypes at one time, it is unlikely that limited valency PCVs will be able to offer the desired protection against overall pneumococcal disease. For example, the recently FDA-approved 20-valent PCV (containing PCV13 components plus CRM197-conjugated polysaccharide from serotypes 8, 10A, 11A, 12F, 15B, 22F and 33F) would cover 44.3% of all pneumococci identified within this study, compared with the 30.9% of detected pneumococci that are covered by PCV13. Of note is the considerable proportion of non-encapsulated pneumococci (11%) that would not be covered by any PCV.

High nasopharyngeal pneumococcal density has been associated with acute respiratory infections and increased pneumococcal transmission [27]. The PERCH study found that a pneumococcal carriage density > 6.9 log_{10} copies/mL was strongly associated with microbiologically confirmed pneumococcal pneumonia [28]. In the current study, we found a median density of 6.98 log_{10} GE/mL in a population of infants without pneumonia. This figure is one log higher than the median density noted in other high carriage density settings including The Gambia (5.85 log_{10} GE/mL) and Bangladesh (5.99 log_{10} GE/mL) [28]. In contrast with cross-sectional carriage surveys conducted in Fiji that found lower pneumococcal carriage densities in PCV10-vaccinated 12–23 month old children than in unvaccinated children in the same age group [22], we did not observe any reduction in pneumococcal carriage density (either overall, vaccine types or non-vaccine types) in PCV13-vaccinated infants compared with unvaccinated children.

In contrast with the rates of AMR reported by microarray studies in other lower-middle income countries, including Laos PDR (70%) and Mongolia (82%) [29,30], the number of pneumococcal positive samples containing at least one AMR gene was relatively low within our study (under 20%). While this finding is promising, the antibiotics used to treat pneumonia in PNG (including amoxicillin, penicillin and gentamicin [23]) were not assessed. Due to the high prevalence of multiple serotype carriage in our study it was not practical to examine whether PCV13 serotypes were more likely to possess resistance genes than non-PCV13 serotypes using microarray. However, there was no difference in the prevalence of AMR genes between the PCV13 and unvaccinated cohorts.

There are some limitations to this study, in particular the limited sample size. Population-based data are required to assess whether the introduction of PCV13 in PNG has an impact on pneumococcal carriage and more importantly disease at the community level, including elimination of vaccine serotypes and serotype replacement. Surveillance of pneumococcal carriage following the introduction of PCV13 has been undertaken in the EHP, with results anticipated in 2022. Testing for respiratory viruses would enhance our results and may provide an explanation for the high carriage density observed in these infants, as several studies have shown a positive correlation between respiratory virus detection and pneumococcal density [31,32]. Given that one-third of vaccinated children experienced at least one episode of moderate or severe pneumonia in their first year of life (and this information is unknown in the unvaccinated children), sampling at more frequent intervals would allow for assessment of colonisation dynamics surrounding periods of respiratory infection.

Despite these limitations, our results clearly show that PCV13 administration at 1-2-3-months of age had minimal to no impact on pneumococcal carriage rates, carriage density and diversity in PNG infants. It is likely that the limited impact of PCV13 on vaccine type carriage is due to the low prevalence of vaccine serotypes in

this population and high prevalence of non-vaccine serotypes, as well as the high rate of pneumococcal acquisition and force of infection. Further investigation into the social, environmental and genetic factors predisposing this population to such high pneumococcal carriage is warranted, as is exploration of alternate immunisation schedules and serotype-independent pneumococcal vaccines.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lea-Ann S. Kirkham reports financial support was provided by Pfizer. William S. Pomat reports a relationship with Pfizer Australia that includes: funding grants and travel reimbursement. Deborah Lehmann reports a relationship with Pfizer Australia that includes: funding grants and travel reimbursement. Deborah Lehmann reports a relationship with Merck Vaccines that includes: speaking and lecture fees and travel reimbursement. Lea-Ann S. Kirkham reports a relationship with Pfizer Australia that includes: funding grants and travel reimbursement. Lea-Ann S. Kirkham reports a relationship with GlaxoSmithKline that includes: funding grants and travel reimbursement. Lea-Ann S. Kirkham reports a relationship with Merck Sharpe & Dohme that includes: funding grants and travel reimbursement. Christopher C. Blyth reports a relationship with Pfizer Australia that includes: funding grants. Peter C. Richmond reports a relationship with Pfizer Australia that includes: funding grants and non-financial support. Peter C. Richmond reports a relationship with GlaxoSmithKline that includes: funding grants and non-financial support. Catherine Satzke reports a relationship with Pfizer that includes: funding grants. Papua New Guinea Institute of Medical Research reports a relationship with Pfizer Australia that includes: funding grants. Lea-Ann S. Kirkham has patent 'Mutant pneumolysin proteins' licensed to Pfizer.

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Author contributions

K.J.B., J.L.P. and L-A.S.K. compiled and analysed the datasets. K.J.B. wrote the first draft of this manuscript under guidance from L-A.S.K. C.dG. and C.M.G. conducted qPCR analysis and contributed to data interpretation. M.L.N. and C.L.P. conducted the microarray analysis and assisted in interpretation of results under the guidance of C.S. and J.H. R.L.F. was responsible for biobanking and shipment of samples as well as data interpretation. W.S.P., V.S., A.G., P.C.R., C.C.B. and D.L. oversaw recruitment and provided guidance on interpretation of the clinical datasets as lead investigators on these trials. H.C.M., C.C.B., P.C.R. and L-A.S.K. supervise K.J.B. and contributed to data interpretation. All authors contributed to the final draft of the manuscript.

Appendix A

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Appendix B. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.07.085.

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