Vaccine 41 (2023) 5392–5399

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Pneumococcal carriage, serotype distribution, and antimicrobial susceptibility in Papua New Guinean children vaccinated with PCV10 or PCV13 in a head-to-head trial

Tilda Orami [a], Celestine Aho [a,b], Rebecca L. Ford [a], William S. Pomat [a,b], Andrew Greenhill [c], Lea-Ann Kirkham [b], Geraldine Masiria [a], Birunu Nivio [a], Kathryn J. Britton [b,d], Peter Jacoby [b], Peter C. Richmond [b,d], Anita H.J. van den Biggelaar [b,1], Deborah Lehmann [b,1,*]

[a] Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea
[b] Wesfarmers Centre of Vaccines and Infectious Diseases, Telethon Kids Institute, University of Western Australia, Perth, Australia
[c] School of Science, Psychology and Sport, Federation University, Churchill, Australia
[d] Discipline of Paediatrics, School of Medicine, University of Western Australia, Perth, Australia

ARTICLE INFO

Keywords:
Pneumococcus
Carriage
Antimicrobial resistance
Pneumococcal conjugate vaccine
Papua New Guinea

ABSTRACT

Background: Children in Papua New Guinea (PNG) are at high risk of pneumococcal infections. We investigated pneumococcal carriage rates, serotype distribution, and antimicrobial susceptibility in PNG children after vaccination with 10-valent or 13-valent pneumococcal conjugate vaccines (PCV10; PCV13).

Methods: Infants (N = 262) were randomized to receive 3 doses of PCV10 or PCV13 at 1-2-3 months of age, followed by pneumococcal polysaccharide vaccination (PPV) or no PPV at 9 months of age. Nasopharyngeal swabs (NPS) collected at ages 1, 4, 9, 10, 23 and 24 months were cultured using standard bacteriological procedures. Morphologically distinct *Streptococcus pneumoniae* colonies were serotyped by the Quellung reaction. Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion and minimum inhibitory concentration (MIC).

Results: S. pneumoniae was isolated from 883/1063 NPS collected at 1–23 months of age, including 820 serotypeable (64 different serotypes) and 144 non-serotypeable isolates. At age 23 months, 93.6% (95%CI 86.6–97.6%) of PCV10 recipients and 88.6% (95%CI 80.1–94.4%) of PCV13 recipients were pneumococcal carriers, with higher carriage of PCV10 serotypes by PCV10 recipients (19.8%, 95%CI 12.2–29.5) than PCV13 recipients (9.3%, 95%CI 4.1–17.3) (p = 0.049). There were no other statistically significant differences between PCV10 and PCV13 recipients and children receiving PPV or no PPV. Nearly half (45.6%) of carried pneumococci were non-susceptible to penicillin based on the meningitis breakpoint (MIC ≥ 0.12 μg/mL), but resistance was rare (1.1%) using the non-meningitis cut-off (MIC ≥ 8 μg/mL). Non-susceptibility to trimethoprim-sulfamethoxazole (SXT) was common: 23.2% of isolates showed intermediate resistance (MIC 1/19–2/38 μg/mL) and 16.9% full resistance (MIC ≥ 4/76 μg/mL). PCV serotypes 14 and 19A were commonly non-susceptible to both penicillin (14, 97%; 19A, 70%) and SXT (14, 97%; 19A, 87%).

Conclusion: Even after PCV10 or PCV13 vaccination, children living in a high-risk setting such as PNG continue to experience high levels of pneumococcal colonization, including carriage of highly antimicrobial-resistant PCV serotypes.

The study is registered with ClinicalTrials.gov (CTN NCT01619462).

1. Introduction

Streptococcus pneumoniae (pneumococcus) remains a leading cause of morbidity and mortality in children under 5 years old, especially in low-income settings [1]. Invasive pneumococcal disease (IPD) is preceded by pneumococcal colonization of the upper respiratory tract (URT) [2].

* Corresponding author at: Telethon Kids Institute, 11 Hospital Avenue, Nedlands WA 6009, Australia.
E-mail address: Deborah.Lehmann@uwa.edu.au (D. Lehmann).
[1] Joint senior authors.

https://doi.org/10.1016/j.vaccine.2023.07.026
Received 15 March 2023; Received in revised form 11 July 2023; Accepted 11 July 2023
Available online 20 July 2023

Pneumococcal conjugate vaccines (PCV) are effective in preventing vaccine serotype-related IPD in children in low-risk settings and in high-risk settings albeit with lower efficacy [3–6]. PCVs also have an indirect protective effect by reducing acquisition and carriage of pneumococcal vaccine serotypes, at least in low-risk settings [7]: in high-risk, low-income settings, the effect on carriage is less evident and randomized controlled trials conducted in Papua New Guinea (PNG) and African countries have failed to show such an effect [8–13].

PCVs have also been associated with reduction in antimicrobial non-susceptible pneumococcal infections [14–17]: the overall effect of PCVs on pneumococcal antimicrobial resistance (AMR) depends on the number of pneumococcal serotypes covered by PCVs and is limited due to the emergence of infections caused by non-vaccine serotypes with high AMR prevalence [18,19].

The epidemiology of pneumococcal infections differs between populations in high- and low-risk settings, including differences in the spectrum of serotypes colonizing and causing infection, age of first acquisition and persistence of carriage [20,21]. Local surveillance and documentation of pneumococcal serotypes carried or causing disease in children and antimicrobial non-susceptibility is therefore important to understand the epidemiology and impact of PCVs on the local burden of pneumococcal disease.

Children in the highlands of Papua New Guinea, where this study was performed, have one of the highest rates of IPD in the world [20]. Compared to children in low-risk settings, children in PNG experience early and persistent pneumococcal colonization by a broad range of serotypes [8,22]. In a randomized placebo-controlled trial conducted between 2007 and 2009, 7-valent PCV (PCV7) was found to have no effect on carriage of vaccine serotypes in PNG infants [8]. Similar to findings of the last antimicrobial susceptibility carriage study that was carried out in this region before the PCV7 study in the late 1980s [23], 40% of pneumococcal isolates carried by children in the PCV7 study were non-susceptible to penicillin and 20% were non-susceptible to trimethoprim/sulfamethoxazole [24]. Antimicrobial susceptibility was most common in serogroup 15 isolates, followed by non-serotypeable pneumococci [24].

Between 2011 and 2016, a head-to-head safety and immunogenicity trial with 10-valent PCV (PCV10) and 13-valent PCV (PCV13) was conducted in the same study area as the PCV7 trial in PNG to determine the suitability of these vaccines for use under PNG's accelerated 1-2-3-month immunization schedule [25]. Results on the primary study objectives and on secondary objectives including the effects of childhood PCV10 and PCV13 vaccination (administered according to the national immunization program at 1, 2 and 3 months of age) on pneumococcal carriage and density have been reported for study infants at ages 1, 4, and 9 months [21,26]: other than lower pneumococcal carriage rates at 4 months of age in PCV13-vaccinated children, no differences compared to PCV10 were found. We now report on pneumococcal carriage rates when study children were between 10 and 23 months of age and on AMR of pneumococci isolated from nasopharyngeal swabs (NPS) obtained between 1 and 23 months of age.

2. Materials and methods

2.1. Study design and study population

This study was part of an open randomized controlled trial of PCV10 and PCV13 given to infants at 1, 2 and 3 months of age, conducted between November 2011 and March 2016 in the Asaro Valley (which includes Goroka town) in the Eastern Highlands Province (EHP) of PNG. A detailed protocol of this study has been published previously [25]. Briefly, a total of 262 infants aged between 28 and 35 days were enrolled and randomized to receive 3 doses of PCV10 (n = 131) or PCV13 (n = 131) according to the PNG national immunization schedule at 1, 2 and 3 months of age. At 9 months of age recipients of PCV10 or PCV13 were further randomized to receive one dose of pneumococcal polysaccharide vaccine (PPV) or no PPV. NPS were collected from children at 1, 4, 9, 10, 23 and 24 months of age. Of the 262 children, 186 children (91 in the PCV10 group; 95 in the PCV13 group) completed the study at 24 months of age. A detailed flowchart, reasons for loss to follow-up and samples collected at different timepoints are reported in Lehmann *et al.* [25].

The study was conducted according to Declaration of Helsinki International Conference on Harmonisation Good Clinical Practice (ICH-GCP) and local ethical guidelines. Ethical approval was obtained from the PNG Medical Research Advisory Committee (11.03) and PNG Institute of Medical Research (PNGIMR) Institutional Review Board (1028).

2.2. Study objectives

Results on the primary trial objectives including the safety and immunogenicity of PCV10 and PCV13 [21] and the immunogenicity of a PPV booster given at 9 months of age in PCV10- or PCV13-primed infants and persistence of immune memory [27] have been published. We now report on secondary study objectives including pneumococcal (total and vaccine type) carriage rates; the relative impact of PCV10 and PCV13 on carriage; and carriage of AMR pneumococci. At 23 months of age, study children received a micro-challenge dose of PPV to study potential hypo-responsiveness to PPV given at 9 months of age (reported in [27]); pneumococcal carriage rates were assessed at 23 and 24 months, although the micro-challenge dose was not expected (and later confirmed) to affect carriage. For completeness, carriage rates at 24 months of age are included in the Supplement, but are not discussed further.

2.3. Study vaccines

PCV10 (Synflorix®, GSK, Belgium, batch numbers ASPNA0099AB and ASPNA267DD) contains 1 μg of polysaccharide for pneumococcal serotypes 1, 5, 6B, 7F, 9V, 14 and 23F conjugated to NTHi Protein D, and 3 μg of serotypes 4, 18C and 19F conjugated to NTHi Protein D, tetanus toxoid, and diphtheria toxoid, respectively. PCV13 (Prevenar13®, Pfizer, USA, batch numbers F36226 and G71540) contains 2.2 μg of polysaccharide for pneumococcal serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F and 23F, and 4.4 μg of serotype 6B, conjugated to non-toxic diphtheria CRM_{197} protein. Each 0.5 mL dose of PPV (Pneumovax 23™, Merck & Co, USA, batch numbers T0861, V1200 and K006913) contains 25 μg of polysaccharide for pneumococcal serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F in 0.25% phenol preservative.

2.4. Collection and culturing of nasopharyngeal swabs

NPS to assess pneumococcal carriage were collected as previously described [8], using a flocked swab (Copan Diagnostics, USA). NPS samples were placed in skim milk-tryptone-glucose-glycerol broth (STGGB) and stored at −80 °C within 2 h of collection. NPS were cultured using standard procedures for *S. pneumoniae* at PNGIMR as described previously [8,21,28]. Two morphologically distinct colonies of pneumococci (or more if more than two were morphologically distinct) were picked and serotyped by the Quellung reaction using antisera from Statens Serum Institut, Denmark.

2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion (OXOID, Australia) for oxacillin (OXA, 1 μg), chloramphenicol (CHL, 30 μg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μg), tetracycline (TET, 30 μg), erythromycin (ERY, 15 μg), and ceftriaxone (CRO, 30 μg). Non-susceptible isolates were subsequently tested by E-test (BioMérieux, USA) for minimum inhibitory concentrations (MIC) for penicillin (PEN), CHL, SXT and TET. Isolates were classified as

susceptible, intermediate resistant, or resistant based on the contemporary 2011 Clinical Laboratory Standard Institute (CLSI) guidelines [29].

2.6. Data analysis

Data were analyzed using SPSS 15.0. Differences in prevalence rates between groups were tested using Pearson's chi-square test. For all analyses, test outcomes were considered significantly different if the p-value was ≤ 0.05.

3. Results

3.1. Study samples

Of the 262 study children, 198 (75.6%) had ≥ 4 NPS collected between 1 and 23 months of age (77.8% of the PCV10 recipients, and 73.2% of the PCV13 recipients). In total, 1063 NPS were collected between 1 and 23 months of age (545 from PCV10, and 518 from PCV13 recipients). This included 103 NPS collected from PCV10 recipients at age 10 months (51 vaccinated and 52 not vaccinated with PPV at 9 months); 97 NPS collected from PCV13 recipients at age 10 months (51 PPV, 46 no PPV); 94 NPS collected from PCV10 recipients at age 23 months (46 PPV, 48 no PPV) and 88 NPS collected from PCV13 recipients at age 23 months (48 PPV, 40 no PPV). The number of samples collected at younger ages has been reported previously [21].

3.2. Pneumococcal isolates

From 883 of the 1063 NPS (83.1%) collected at 1, 4, 9, 10 and 23 months of age, 964 pneumococci were isolated: 504 carried by PCV10 recipients and 460 by PCV13 recipients. This included 820 serotypeable (439 PCV10; 381 PCV13) and 144 non-serotypeable isolates (65 PCV10; 79 PCV13). Multiple pneumococci were isolated from 87 NPS, including 86 swabs with two different pneumococcal serotypes or a serotypeable together with a non-serotypeable pneumococcus and one NPS with two different serotypes and a non-serotypeable pneumococcus.

3.3. Pneumococcal carriage rates

Pneumococcal carriage rates between 1 and 23 months of age are shown in Fig. 1, including previously published data for ages 1, 4, and 9 months [21] (Supplementary Table 1 including 24-month data that are comparable to 23-month data and not further discussed). Pneumococcal carriage rates remained high, with 89.0% of children at 10 months and 91.2% at 23 months of age being carriers. Carriage rates were comparable for PCV10 and PCV13 recipients, and for children who at 9 months of age had received a PPV booster vaccination or not (Supplementary Table 2). Carriage rates of non-PCV serotypes ranged from 49% to 70%. At 23 months of age, PCV10/13-shared serotypes were carried by more PCV10 recipients (19.8%, 95% CI 12.2–29.5) than PCV13 recipients (9.3%, 95% CI 4.1–17.3) ($p = 0.05$). There were no differences in carriage rates for non-PCV serotypes or non-serotypeable pneumococci between PCV10 and PCV13 recipients at any age.

Figure 1

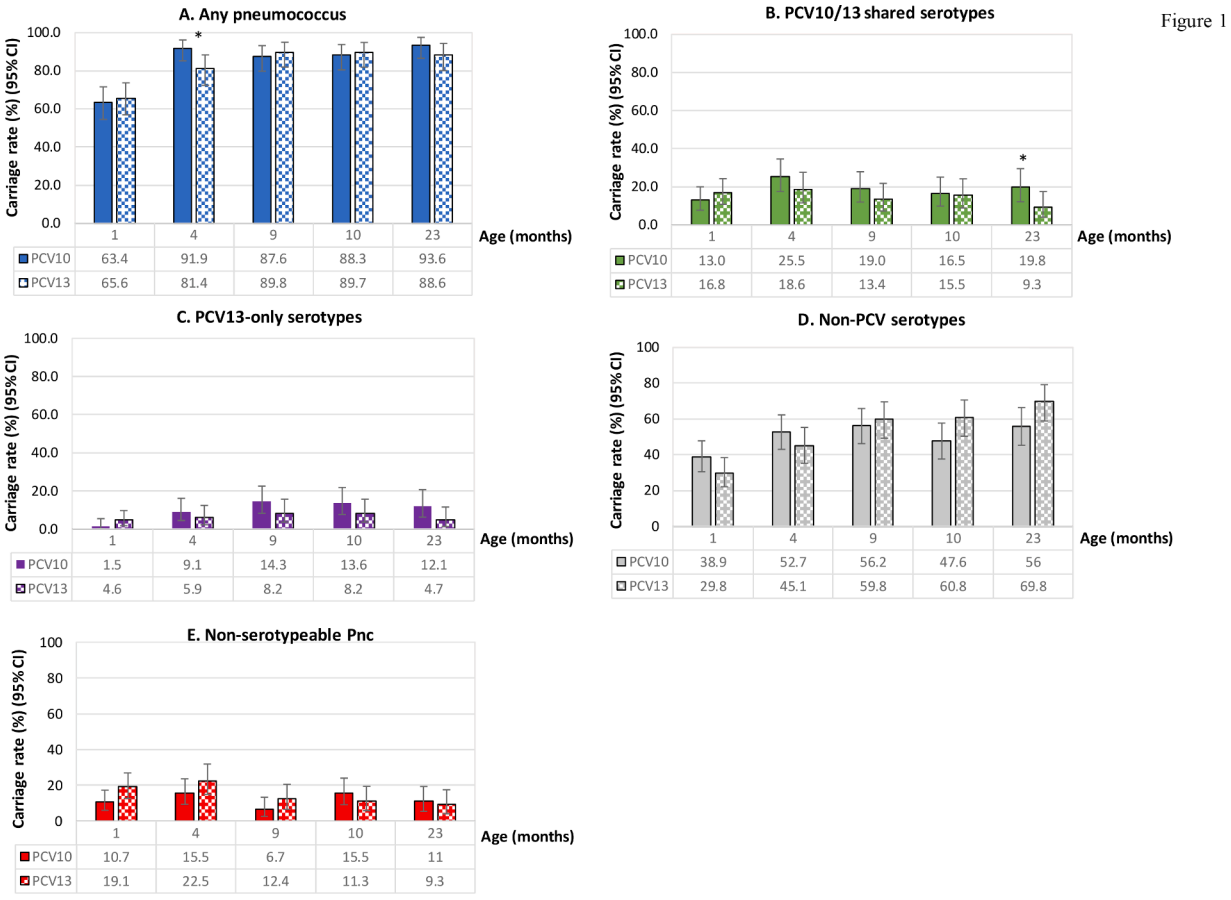

Fig. 1. Pneumococcal carriage rates in a cohort of PCV10- and PCV13-vaccinated Papua New Guinean children. Children received 3 doses of PCV10 or PCV13 at 1, 2 and 3 months of age. Pneumococcal carriage rates were assessed at 1, 4, 9, 10 and 23 months of age and are presented per vaccine group for A) Any pneumococcus; B) serotypes included in PCV10 and PCV13; C) serotypes only included in PCV13; D) serotypes not included in PCV10 or PCV13; E) non-serotypeable pneumococci. Graphs present carriage rates (%) and 95% confidence intervals at different ages for children vaccinated with either PCV10 or PCV13. * $p = 0.049$.

3.4. Pneumococcal serotype distribution

Individual pneumococcal serotypes carried between 1 and 23 months of age are presented in Supplementary Table 3. A total of 64 different pneumococcal serotypes were identified. The most frequently carried PCV serotypes at 10 months of age were: 19A (not included in PCV10) and 19F (both identified in 12 isolates; each accounting for 7.1% of serotypeable isolates), 6A (not included in PCV10) (9 isolates [6 carried by PCV10 recipients]; 5.4% of serotypeable isolates), and 14 and 23F (both 7 isolates; each accounting for 4.2% of serotypeable isolates). At 23 months of age the most frequently carried PCV serotypes were: 23F (10 isolates [9 carried by PCV10 recipients]; 6.3% of serotypeable isolates), 6A (7 isolates [all carried by PCV10 recipients]; 5.1% of serotypeable isolates), and 19A (7 isolates; 4.4% of serotypeable isolates).

The most frequently carried non-PCV serotypes at 10 months of age were: 10A (11 isolates, 6.5% of serotypeable isolates), 35B (9 isolates; 5.4% of serotypeable isolates), 29 (7 isolates; 4.2% of serotypeable isolates), and 21, 15B, and 34 (6 isolates each; each 3.6% of serotypeable isolates; 5 serotype 34 isolates were carried by PCV13 recipients). At 23 months of age the most frequently carried non-PCV serotypes included: 35B (11 isolates [7 carried by PCV13 recipients]; 7.0% of serotypeable isolates), 6C (11 isolates [9 carried by PCV10 recipients]; 7.0% of serotypeable isolates), 15B (8 isolates [6 carried by PCV13 recipients]; 5.1% of serotypeable isolates), and 16F (7 isolates [5 carried by PCV10 recipients]; 4.4% of serotypeable isolates). The five serotypes most frequently carried between 1 and 23 months of age were vaccine serotypes 19A, 19F, and 23F and non-vaccine serotypes 10A and 35B (Fig. 2).

3.5. Antimicrobial susceptibility

Antimicrobial susceptibility data by disc diffusion was available for 98% (953/974) of isolated pneumococci. Based on the disc diffusion method (Table 1), the proportion of pneumococcal isolates carried by children between 1 and 23 months of age non-susceptible to antibiotics were 51.4% for PEN, 49.4% for SXT, 13.7% for TET, 2.9% for CHL, 0.7% for ERY and 0.1% for CRO (Table 1). MIC was performed on 89% (444/490) of pneumococci non-susceptible to oxacillin by disc diffusion, 87% (408/471) of isolates that were non-susceptible to SXT and 96% (27/28) of isolates that were non-susceptible to CHL. Due to lack of reagents, MIC was only conducted on a small number of pneumococcal isolates that were non-susceptible to TET by disc diffusion (22/131, 17%), resulting in insufficient data for valid interpretation on TET non-susceptibility by MIC (hence, data are not presented). When non-susceptible isolates were tested by MIC, 45.6%, 40.2%, and 0.9% of pneumococci (based on a denominator of isolates susceptible by disc diffusion and all isolates tested by MIC) were non-susceptible to PEN, SXT, and CHL, respectively (Table 1). Considering not all isolates that were non-susceptible by disc diffusion were tested by MIC, the true proportion of non-susceptible isolates can be expected to be somewhat higher than reported here for those tested by disc diffusion and MIC but lower than the proportion based on disc diffusion alone. Based on the breakpoint for meningitis (MIC $\geq$ 0.12 μg/mL) (Table 2), resistance was higher amongst non-serotypeable pneumococci (61.7%) and PCV13-serotypes (54.0%) than non-PCV serotypes (37.9%) ($p < 0.001$). Using the non-meningitis cut-off (MIC $\geq$ 8 μg/mL), resistance to PEN was rare (1.1% of pneumococcal carriage isolates). Non-susceptibility to SXT (MIC $>$ 0.5/9.5 μg/mL) was common (40.1% of pneumococci carried)

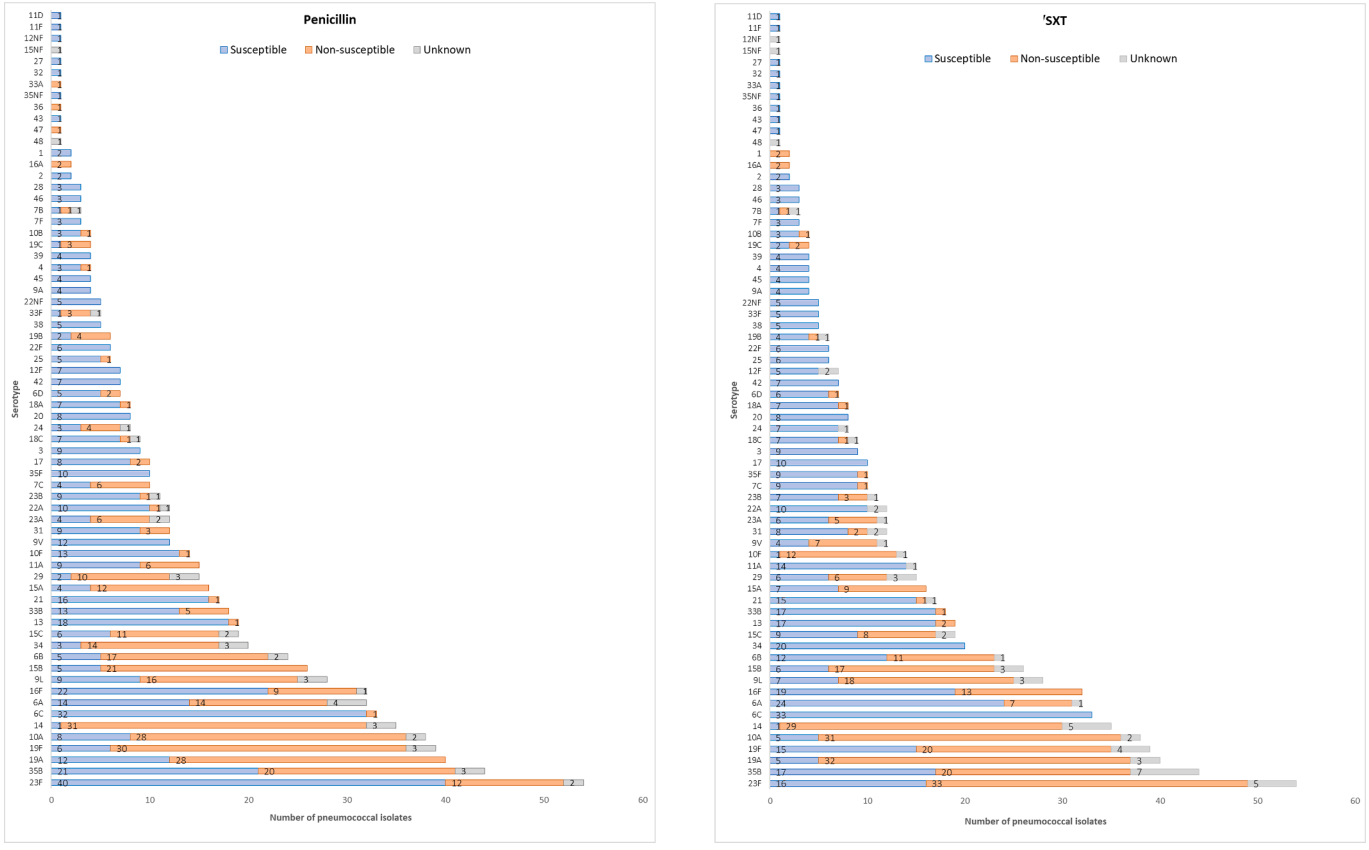

Fig. 2. Penicillin and trimethoprim-sulfamethoxazole susceptibility and non-susceptibility of individual pneumococcal serotypes carried by study children. The graph presents individual pneumococcal serotypes (and non-serotypeable pneumococci) isolated between 1 and 23 months of age, and the number of isolates susceptible (blue) or non-susceptible (orange) to penicillin and/or trimethoprim-sulfamethoxazole (SXT) based on disc diffusion and MIC. Isolates that were non-susceptible in disc diffusion but not tested by MIC are indicated as unknown (grey). Serotypes are ranked based on frequency of carriage. Some pneumococci were serotyped but not factor-typed (NF). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Antimicrobial non-susceptibility of *Streptococcus pneumoniae* (Pnc) carried by PNG infants between 1 and 23 months of age, based on disc diffusion or disc diffusion and minimum inhibitory concentration.

			Non-Susceptible Isolates, N (%)				
Antibiotics	**Test method**	**Isolates** N	**Any Pnc** n (%)	**PCV10/13-shared** n (%)	**PCV13-only** n (%)	**Non-PCV** n (%)	**Non-serotypeable** n (%)
OXA/PEN	Disc	953	490 (51.4)	106 (58.2)	49 (60.5)	242 (43.7)	92 (69.2)
	Disc + MIC	906	413 (45.6)	92 (53.8)[#]	42 (54.5) [#]	200 (37.8)[#]	79 (61.7) [#]
SXT	Disc	953	471 (49.4)	134 (73.6)	47 (58.0)	223 (40.3)	66 (49.6)
	Disc + MIC	889	357 (40.2)	103 (62.4)[$]	39 (50.6)[$]	159 (30.8)[$]	56 (43.8)[$]
CHL	Disc	953	28 (2.9)	3 (1.6)	3 (3.7)	18 (3.4)	3 (2.3)
	Disc + MIC	951	9 (0.9)	2 (1.1)	0 (0)	7 (1.3)	0 (0)
TET	Disc	953	131 (13.7)	47 (25.8)	2 (2.4)	70 (12.6)	10 (7.6)
ERY	Disc	953	7 (0.7)	2 (1.1)	0 (0)	3 (0.5)	2 (1.5)
CRO	Disc	953	1 (0.1)	0 (0)	0 (0)	1 (0.2)	0 (0)

The table presents the number and proportion of isolates testing non-susceptible based on disc diffusion (Disc), and disc diffusion followed by testing of non-susceptible isolates for MIC (Disc + MIC). Antibiotics tested: OXA/PEN, oxacillin/penicillin; SXT, Trimethoprim-sulfamethoxazole; CHL, chloramphenicol; TET, tetracycline; ERY, erythromycin; CRO, ceftriaxone. Non-susceptibility to erythromycin and chloramphenicol was tested by disc diffusion only. Zone cut-off values for non-susceptibility by disc diffusion: OXA $\leq$ 20 mm, SXT $\leq$ 15 mm, CHL $\leq$ 20 mm, TET $\leq$ 24 mm, ERY $\leq$ 15 mm. Cut-off values for non-susceptibility by MIC: PEN $>$ 0.06 μg/mL, SXT $>$ 0.5/9.5 μg/mL, CHL $\geq$ 8.0 μg/mL. Due to unavailability of E-strips MIC results are not available for the following number of isolates that were non-susceptible by Disc: OXA/PEN, any Pnc n = 46; PCV10/13-shared n = 11, PCV13-only n = 4, non-PCV n = 26, non-serotypeable n = 5; TMP/SXT, any Pnc n = 63; PCV10/13-shared n = 17, PCV13-only n = 4, non-PCV n = 37, non-serotypeable n = 5; CHL, any Pnc n = 2; non-serotypeable n = 2. The proportion of non-susceptible isolates in Disc + MIC is not taking into account the Disc non-susceptible isolates that were not tested for MIC. Other abbreviations: MIC, minimum inhibitory concentration; PCV, pneumococcal conjugate vaccine. [#] Comparison of proportion of penicillin non-susceptibility: non-serotypeable isolates $>$ PCV serotypes (PCV10/13-shared and PCV13-only) $>$ non-PCV serotypes, Chi squared = 33.19, 2df, $p < 0.0001$. [$] Comparison of proportion of SXT non-susceptibility: PCV serotypes (PCV10/13-shared and PCV13-only) $>$ non-serotypeable isolates $>$ non-PCV serotypes, Chi-squared = 78.88, 2df, $p < 0.0001$.

Table 2
Antimicrobial susceptibility of *Streptococcus pneumoniae* (Pnc) carried by PNG infants between 1 and 23 months of age, for penicillin and trimethoprim-sulfamethoxazole based on combined disc diffusion and minimum inhibitory concentration.

	Breakpoints (μg/mL)	**Any Pnc** n (%)	**PCV10/13-shared** n (%)	**PCV13-only** n (%)	**Non-PCV** n (%)	**Non-serotypeable** n (%)
PEN, non-meningitis		*N = 904*	*N = 171*	*N = 77*	*N = 528*	*N = 128*
Susceptible	$\leq$ 2	881 (97.5)	161 (94.2)	77 (100)	521 (98.7)	122 (95.3)
Intermediate resistant	4	13 (1.4)	7 (4.1)	0 (0)	2 (0.4)	4 (3.1)
Resistant	$\geq$ 8	10 (1.1)	3 (1.8)	0 (0)	5 (0.9)	2 (1.6)
PEN, meningitis						
Susceptible	$\leq$ 0.06	491 (54.3)	79 (46.2)	35 (45.5)	328 (62.1)	49 (38.3)
Resistant	$\geq$ 0.12	413 (45.7)	92 (53.8)[#]	42 (54.5)[#]	200 (37.9)[#]	79 (61.7)[#]
SXT		*N = 889*	*N = 165*	*N = 67*	*N = 517*	*N = 128*
Susceptible	$\leq$ 0.5/9.5	532 (59.8)	62 (37.6)	38 (49.4)	358 (69.2)	72 (56.3)
Intermediate resistant	1/19-2/38	207 (23.2)	57 (34.5)	28 (36.4)	94 (18.2)	28 (21.9)
Resistant	$\geq$ 4/76	150 (16.9)	46 (27.9)[$]	11 (14.3)[$]	65 (12.6)[$]	28 (21.9)[$]

For penicillin different breakpoints (in line with the CLSI guidelines [ref 29]) are used to reflect what resistance would be if the isolates were from patients with meningitis or non-meningitis IPD. PEN resistance is undecided for 46 isolates with an oxacillin zone $\geq$ 20 mm but for which no penicillin MIC test was performed (11 PCV10/13-shared serotypes; 4 PCV13-only; 26 non-PCV serotypes; 5 non-serotypeable). SXT resistance is undecided for 63 isolates with a disc diffusion zone $\geq$ 19 mm but for which a MIC test was not performed (17 PCV10/13-shared serotypes; 4 PCV13-only; 37 non-PCV serotypes; 5 non-serotypeable).

[#] Based on the breakpoint for meningitis proportion of PEN resistance: (MIC $\geq$ 0.12 μg/mL), resistance was higher amongst non-serotypeable pneumococci $>$ PCV13-serotypes (PCV10/13-shared and PCV13-only serotypes) $>$ non-PCV serotypes, $p < 0.001$.

[$] Comparison of proportion of SXT resistance: PCV10/13-shared serotypes $>$ non-serotypeable $>$ PCV13-only serotypes $>$ non-PCV serotypes, Chi-squared = 59.67, 3df, $p < 0.0001$.

and 16.9% of isolates were fully resistant (MIC ≥ 4/76 μg/mL). SXT resistance was highest amongst PCV10/13 serotypes (27.9%) and lowest amongst non-PCV serotypes (12.6%). Non-susceptibility to TET (based on disc diffusion, Table 1) was highest amongst PCV10/13-shared serotypes (25.8%) compared to other serotype groups. For all pneumococci, non-susceptibility to CHL (0.9% by disc diffusion and MIC), CRO (0.1%) and ERY (0.7%) was low (Table 1).

Susceptibility and non-susceptibility of individual pneumococcal serotypes to PEN or SXT are shown in Fig. 2. Amongst frequently carried serotypes (≥20 isolates) non-susceptibility to PEN (MIC > 0.06 μg/mL) was high in serotypes 14 (31/32, 96.9%), 19F (30/36, 83.3%), 15B (21/26, 80.8%), 10A (28/36, 77.8%) and 19A (28/40, 70.0%). Non-susceptibility to SXT was high in frequently carried serotypes 14 (29/30, 96.7%), 19A (32/37, 86.5%), 10A (31/36, 86.1%), 15B (17/23, 73.9%), and 9L (18/25, 72.0%). There were no significant differences in non-susceptibility rates between PCV10 and PCV13 recipients for individual serotypes (data not shown).

4. Discussion

Considering the epidemiology of pneumococcal infections differs with the level of endemicity, studies in highly endemic settings are important to understand the impact and possible limitations of PCVs in these environments. As reported previously [21], this head-to-head study in a highly endemic setting in PNG has demonstrated that both PCV10 and PCV13 are immunogenic and well tolerated when given at the accelerated national schedule of 1, 2, and 3 months of age. A main finding of this report on pneumococcal carriage and antimicrobial susceptibility over the course of the head-to-head trial, is that, even after vaccination with PCV10 or PCV13, children living in a high-risk setting such as PNG experience persistent pneumococcal colonization by a broad range of serotypes. Sixty-four different pneumococcal serotypes were identified in this cohort, including PCV10 and PCV13 serotypes and serotypes associated with antimicrobial resistance. At every screening time point after receiving 3 doses of PCV10 or PCV13, approximately 90% of children were pneumococcal carriers. One-fifth of the carried serotypeable pneumococci belonged to serotypes covered by PCV10 and PCV13. The most common serotypes were 23F 35B, 19F, and 19A. Antimicrobial resistance continues to be a concern in PNG, with half of pneumococci carried by children being non-susceptible to PEN and half non-susceptible to SXT. Non-susceptibility was higher amongst PCV10/13 serotypes than non-PCV serotypes. Of all pneumococci carried by study children, 15% were non-serotypeable and approximately two-thirds of these pneumococci were non-susceptible, which contributes to maintaining high rates of AMR.

In contrast to a randomized-controlled PCV7 trial we conducted in the same study area between 2005 and 2009 [20], the PCV10-PCV13 trial did not include a non-vaccinated control group. In the PCV7 trial, vaccination did not reduce overall and vaccine-specific pneumococcal carriage rates in this population [8]. In a separate analysis, we have recently shown that PCV13-serotype carriage rates and carriage density were similar among children given PCV13 as part of the current trial compared to age-matched unvaccinated community controls [26]. This indicates that PCV13 has little impact on overall and vaccine-serotype-specific pneumococcal carriage rates and carriage density in this high-risk population. Nevertheless, despite the lack of impact on carriage, PCV13 vaccination has been demonstrated to effectively protect children against hypoxic pneumonia (29% reduction) and pneumonia hospitalisation (57% reduction) in the same geographic area as where this study was performed [6]. This is of vital importance considering the high burden of childhood morbidity and mortality due to pneumonia in this population and suggests that carriage studies alone are not sufficient to study and monitor the impact of PCV vaccinations in this high-risk setting. Surveillance of IPD and pneumonia-related hospitalizations of children and adults is lacking but essential to estimate the impact of routine childhood PCV vaccination in PNG, albeit the uptake has remained low (with no more than 40% of eligible children receiving 3 doses[30]) since PCV13 was introduced nationally in 2014.

As the PCV7 [8] and PCV10-PCV13 trials were performed a few years apart in the same study area among children of the same age, we may consider similarities and differences in serotype-specific carriage rates to assess temporal trends (no statistics were performed). For certain serotypes, carriage rates were comparably high, including PCV serotypes 23F, 19A, and 14. In contrast, PCV serotype 19F was the most frequently carried serotype in the PCV7 trial (10.2% of serotypeable isolates) compared to 4.9% of serotypeable pneumococci in the current study, whilst non-PCV serotypes 10A and 35B were rarely carried by children in the PCV7 study (0.3–0.4% of serotypeable pneumococci) but were more common in the current trial (10A: 4.6% of serotypeable and 3.9% of all pneumococci; 35B: 5.4% of serotypeable and 4.6% of all carried pneumococci). Serotype 35B has been described in the literature as a 'replacement' serotype that has become increasingly prevalent as a cause of invasive disease after the introduction of PCVs. For example, in the United States, IPD caused by serotype 35B increased after implementation of routine PCV7 vaccination, and this increased further after implementation of PCV13 [31,32]. This change has been attributed to the emergence of the 35B/sequence type (ST) 558 lineage, which is highly resistant to antibiotics and is currently commonly found in disease and asymptomatic pneumococcal carriage in many countries [31,32]. As there is no ongoing hospital surveillance, the role of serotype 35B in causing invasive disease in PNG children is unknown. However, the rising prevalence of serotype 35B carriage and its non-susceptibility to antibiotics warrants further research into the epidemiology of serotype 35B causing disease in PNG children. Finally, carriage of non-serotypeable pneumococci remains consistent but substantial in PNG children (12% and 15% of isolates in the PCV7 and current study, respectively). Non-serotypeable pneumococci are associated with relatively high non-susceptibility to PEN and SXT and cannot be prevented by PCVs. To facilitate surveillance studies, high-throughput microarray assays could be considered to capture prevalence trends of serotypeable as well as non-serotypeable pneumococci: the culture-based approach used here validates the microarray study conducted on NPS from PCV13-vaccinated children in this cohort, where 11% of pneumococci collected at 1, 4, 9 months of age were non-encapsulated [26].

Since routine childhood PCV (PCV13) vaccination was not implemented in the study area until 2015 and uptake was low, the relative emergence versus disappearance of certain serotypes is unlikely explained by PCV13 uptake in the wider community. There are not many studies reporting on temporal trends of pneumococcal carriage in high-risk populations. A study of pneumococcal carriage in healthy Icelandic children between 1992 and 1999, before PCV introduction, reported that serotype prevalence fluctuated from year to year [33]. Notably, a report on carriage studies conducted in Israel over a decade preceding PCV introduction concluded that carriage rates were stable and that a single survey may be sufficient to characterize pneumococcal carriage pre-PCV [34]. However, this Israeli study did not report on temporal trends of individual serotype prevalence. Our findings and that of the Icelandic study demonstrate that, even without vaccination, serotype prevalence is not static and needs to be considered when the effects of widespread pneumococcal vaccination programs on serotype prevalence are being evaluated, and factors other than pneumococcal vaccination may need to be considered (for example introduction of other vaccines, and changes in living conditions).

Antibiotic resistance can provide pneumococcal serotypes a 'survival' advantage to infect and spread in populations with high pneumococcal transmission rates and high antibiotic usage. In the early 1970s, when penicillin-resistant pneumococci were occasionally documented in Australia and were rare in other countries, infections with penicillin-non-susceptible strains were already frequent in PNG [35]. No less than 75% of pneumococci isolated from nasopharyngeal swabs and 44% of pneumococci isolated from blood cultures from children in PNG between 1985 and 1987 were non-susceptible to penicillin [36]. Of 180

clinical pneumococcal isolates obtained from patients hospitalized with meningitis in Goroka Hospital between August 1996 and June 2005, more than 1/5th was non-susceptible to penicillin [37], which, despite penicillin no longer being used to treat meningitis, is a concerning number. While current standard treatment of meningitis and severe sepsis is with ceftriaxone, amoxicillin (or benzyl penicillin) and gentamicin IV (or chloramphenicol if these are not available) are used to treat moderate/severe pneumonia [38]. Of all pneumococci that were isolated from NPS in this study, 46% were non-susceptible to penicillin, which is comparable to the 40% found during the earlier 2006–2007 PCV7 study [24]. In contrast, in the current study 40% of isolates were intermediate or fully resistant to SXT compared to 20% in 2006–2007. Antibiotic non-susceptibility was higher amongst PCV serotypes and non-serotypeable pneumococci than among non-PCV serotypes.

Regular surveys of the pneumococcal serotypes and antimicrobial susceptibility of pneumococci carried by children but also adults in high-risk settings like PNG are useful to understand the local epidemiology of pneumococcal disease and associated antibiotic resistance. It is, however, important to recognize that the antimicrobial non-susceptibility of pneumococci isolated from the upper respiratory tract may be higher than those causing invasive disease [36]. Decision-making on antibiotic treatment guidelines and changes in clinical management should therefore be supported by local surveillance data derived from CSF and blood cultures in children with suspected IPD. Of further consideration are the methods used to assess antimicrobial non-susceptibility, with use of the disc diffusion method alone likely leading to overestimating levels of non-susceptibility. Although access to and costs of supplies may restrict feasibility of MIC testing in low-income countries, complementary MIC testing of isolates non-susceptible by disc diffusion is encouraged and important to understand AMR.

A 20-valent pneumococcal conjugate vaccine (PCV20) that contains capsular polysaccharides derived from pneumococcal serotypes 8, 10A, 11A, 12F, 15B, 22F, and 33F in addition to the PCV13 serotypes, was licensed in the United States for use in adults aged $\geq$ 18 years in June 2021, and for infants and children aged 6 weeks and older in April 2023. Considering nasopharyngeal carriage in PNG infants as reported here, PCV20 would, in comparison to PCV13, cover an additional 11% of pneumococci carried by PNG infants between 1 and 23 months of age (increase from 32 to 43%). Although PCV does not prevent colonization in PNG infants, colonization with S. pneumoniae is a pre-requisite for invasive disease and therefore can within limits give an indication of potential vaccine coverage. There are no recent data on pneumococcal serotypes associated with invasive disease in PNG populations. A study of invasive pneumococcal serotypes before the introduction of PCV13 in PNG showed that 54% of invasive pneumococcal isolates were PCV13-related serotypes, and 58% PCV20-related [39]. A clinical trial studying the effectiveness and additional benefit of PCV20 compared to PCV13 in the PNG setting that is epidemiologically so different from high-income countries would be needed to help guide decision makers on its potential implementation.

In summary, this study demonstrates ongoing high pneumococcal carriage rates and antimicrobial non-susceptibility of *S. pneumoniae* carried by young PCV10- and PCV13-vaccinated children prior to the national introduction of routine childhood PCV13. Together with data collected between 2007 and 2009 from same-age children, this study presents baseline information for future post-vaccine implementation studies. Pneumococcal carriage rates remain high in PNG children, even after PCV vaccination, due to the broad range and high carriage density of pneumococcal serotypes. In addition to continued efforts to reduce modifiable risk factors such as malnutrition, overcrowding and indoor air pollution, alternative pneumococcal vaccines and vaccination strategies that provide broader coverage such as the newly licensed PCV20 are required to reduce pneumococcal colonization and disease in Papua New Guinea.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: WP has received funding from Pfizer Australia to attend a conference. LAK has received educational grants and travel support from Pfizer and GSK to attend conferences, is an investigator on investigator-initiated research grants funded by Pfizer and MSD that are not related to this study and is an inventor on patents for a pneumococcal protein vaccine antigen. PR has received non-financial support from Pfizer, grants from GlaxoSmithKline, grants from Pfizer, and non-financial support from GlaxoSmithKline for work outside the submitted work. AvdB works a consultant for vaccine companies, and in the past has worked on projects on pneumococcal conjugate vaccines that were not related to this study. She is currently an employee of Leyden Laboratories BV. AG and DL were investigators on an investigator-initiated research grant that was funded by Pfizer Australia. The Papua New Guinea Institute of Medical Research (PNGIMR) received sponsorship from Pfizer Australia to host a national Medical Symposium in 2014. The other authors 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.

Acknowledgement

We thank all families that took part in this study. We thank Professor John Vince (Chair) and Dr Christopher Blyth for their support as members of the Data Safety Monitoring Board, and the Safety Monitors Drs Harry Poka and Paul Wari. This work was supported and conducted by the institutions and investigators in alphabetical order comprising the **10v13v PCV Trial team**: *Papua New Guinea Institute of Medical Research:* L Bele, M Dreyam, A Elizah, R Ford, J Francis, A Gihigupa, A Greenhill, S Javati, J Kave, W Kirarock, M Lai, B Martin, G Masiria, A Michael (deceased), L Moliki, B Nagepu, M Nenikuro, B Nivio, C Opa, T Orami, WS Pomat, G Saleu, P Siba, V Solomon, S Wana, L Wawae, M Yoannes· *Eastern Highlands Provincial Hospital:* I Hwaihwanje, T Korowi, C Mond, P Wari. *Telethon Kids Institute:* P Jacoby, D Lehmann, A van den Biggelaar· *University of Western Australia:* K Corscadden, C de Gier, L-A Kirkham, T Rahman, P Richmond, R Thornton. *The University of Sydney:* M Passey.

Funding

This work was supported by the Australian National Health and Medical Research Council (grant numbers APP 1087200, APP 1061428 to LAK); the Papua New Guinea Institute of Medical Research Internal Competitive Research Award Scheme; the Papua New Guinea Liquefied Natural Gas Project (a joint venture of Exxon Mobil Corporation, Oil Search, Santos, Nippon Oil, Mineral Resource Development Company and Eda Oil).

Author contributions

Conception and design of the study: DL, PR, WP, LAK; Acquisition of data: GM, GS, BN, JK, TO, CA, RF, AG; Data analysis and interpretation of data: AvdB, DL, PJ, KB; Drafting of the article: TO, AvdB, DL; Reviewing and final approval of the article version to be submitted: all authors.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2023.07.026.

References

[1] GBD 2016 Lower Respiratory Infections Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis 2018;18:1191–210.

[2] Bogaert D, De Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. Lancet Infect Dis 2004;4:144–54.

[3] Mackenzie GA, Hill PC, Jeffries DJ, Hossain I, Uchendu U, Ameh D, 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;16:703–11.

[4] Hammitt LL, Etyang AO, Morpeth SC, Ojal J, Mutuku A, Mturi N, et al. Effect of ten-valent pneumococcal conjugate vaccine on invasive pneumococcal disease and nasopharyngeal carriage in Kenya: a longitudinal surveillance study. Lancet 2019;393:2146–54.

[5] Berman-Rosa M, O'Donnell S, Barker M, Quach C. Efficacy and effectiveness of the PCV-10 and PCV-13 vaccines against invasive pneumococcal disease. Pediatrics 2020;145.

[6] Blyth CC, Britton KJ, Nguyen CD, Sapura J, Kave J, Nivio B, et al. Effectiveness of 13-valent pneumococcal conjugate vaccine against hypoxic pneumonia and hospitalisation in Eastern Highlands Province, Papua New Guinea: An observational cohort study. Lancet Reg Health West Pac 2022;22:100432.

[7] Fleming-Dutra KE, Conklin L, Loo JD, Knoll MD, Park DE, Kirk J, et al. Systematic review of the effect of pneumococcal conjugate vaccine dosing schedules on vaccine-type nasopharyngeal carriage. Pediatr Infect Dis J 2014;33(Suppl 2):S152–60.

[8] Aho C, Michael A, Yoannes M, Greenhill AR, Jacoby P, Reeder J, et al. Limited impact of neonatal or early infant schedules of 7-valent pneumooccal conjugate vaccination on nasopharyngeal carriage of *Streptococcus pneumoniae* in Papua New Guinean children: A randomized controlled trial. Vaccine Reports 2016;6:36–43.

[9] Scott JA, Ojal J, Ashton L, Muhoro A, Burbidge P, Goldblatt D. Pneumococcal conjugate vaccine given shortly after birth stimulates effective antibody concentrations and primes immunological memory for sustained infant protection. Clin Infect Dis 2011;53:663–70.

[10] Njuma Libwea J, Gröndahl-Yli-Hannuksela K, Kobela M, Toropainen M, Nyholm O, Ndombo PK, et al. Prevalence of pneumococcal nasopharyngeal colonization and serotypes circulating in Cameroonian children after the 13-valent pneumococcal conjugate vaccine introduction. Int J Infect Dis 2020;98:113–20.

[11] Swarthout TD, Fronterre C, Lourenço J, Obolski U, Gori A, Bar-Zeev N, et al. High residual carriage of vaccine-serotype *Streptococcus pneumoniae* after introduction of pneumococcal conjugate vaccine in Malawi. Nat Commun 2020;11:2222.

[12] Kaboré L, Adebanjo T, Njanpop-Lafourcade BM, Ouangraoua S, Tarbangdo FT, Meda B, et al. Pneumococcal carriage in Burkina Faso after 13-valent pneumococcal conjugate vaccine introduction: Results from 2 cross-sectional population-based surveys. J Infect Dis 2021;224:S258–66.

[13] Tvedskov ESF, Hovmand N, Benfield T, Tinggaard M. Pneumococcal carriage among children in low and lower-middle-income countries: A systematic review. Int J Infect Dis 2022;115:1–7.

[14] Dagan R, Klugman KP. Impact of conjugate pneumococcal vaccines on antibiotic resistance. Lancet Infect Dis 2008;8:785–95.

[15] Liñares J, Ardanuy C, Pallares R, Fenoll A. Changes in antimicrobial resistance, serotypes and genotypes in *Streptococcus pneumoniae* over a 30-year period. Clin Microbiol Infect 2010;16:402–10.

[16] Tin Tin Htar M, van den Biggelaar AHJ, Sings H, Ferreira G, Moffatt M, Hall-Murray C, et al. The impact of routine childhood immunisation with higher-valent pneumococcal conjugate vaccines on antimicrobial-resistant pneumococcal diseases and carriage: a systematic literature review. Expert Rev Vaccines 2019;18:1069–89.

[17] Wang LM, Cravo Oliveira Hashiguchi T, Cecchini M. Impact of vaccination on carriage of and infection by antibiotic-resistant bacteria: a systematic review and meta-analysis. Clin Exp Vaccine Res 2021;10:81–92.

[18] Kim L, McGee L, Tomczyk S, Beall B. Biological and epidemiological features of antibiotic-resistant *Streptococcus pneumoniae* in pre- and post-conjugate vaccine eras: a United States perspective. Clin Microbiol Rev 2016;29:525–52.

[19] Camacho Moreno G, Imbachi LF, Leal AL, Moreno VM, Patiño JA, Gutiérrez IF, et al. Emergence of *Streptococcus pneumoniae* serotype 19A (Spn19A) in the pediatric population in Bogotá, Colombia as the main cause of invasive pneumococcal disease after the introduction of PCV10. Hum Vaccin Immunother 2020;16:2300–6.

[20] Pomat WS, van den Biggelaar AH, Phuanukoonnon S, Francis J, Jacoby P, Siba PM, et al. Safety and immunogenicity of neonatal pneumococcal conjugate vaccination in Papua New Guinean children: a randomised controlled trial. PLoS One 2013;8:e56698.

[21] Pomat WS, van den Biggelaar AHJ, Wana S, Greenhill AR, Ford R, Orami T, et al. Safety and immunogenicity of pneumococcal conjugate vaccines in a high-risk population: a randomised controlled trial of 10-valent and 13-valent PCV in Papua New Guinean infants. Clin Infect Dis 2018;68:1472–81.

[22] Francis JP, Richmond PC, Pomat WS, Michael A, Keno H, Phuanukoonnon S, et al. Maternal antibodies to pneumolysin but not to pneumococcal surface protein A delay early pneumococcal carriage in high-risk Papua New Guinean infants. Clin Vaccine Immunol 2009;16:1633–8.

[23] Montgomery JM, Lehmann D, Smith T, Michael A, Joseph B, Lupiwa T, et al. Bacterial colonization of the upper respiratory tract and its association with acute lower respiratory tract infections in Highland children of Papua New Guinea. Rev Infect Dis 1990;12(Suppl 8):S1006–16.

[24] Aho C, Binks M, Michael A, Yoannes M, Greenhill A, Pomat W, et al. Pneumococcal antibiotic susceptibility patterns and nasopharyngeal bacterial carriage in Papua New Guinean infants vaccinated with a 7-valent pneumococcal conjugate vaccine (7vPCV). In: 11th International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD-11). Melbourne, Australia: Kenes; 2018. p. 419.

[25] Lehmann D, Kirarock W, van den Biggelaar AHJ, Passey M, Jacoby P, Saleu G, et al. Rationale and methods of a randomized controlled trial of immunogenicity, safety and impact on carriage of pneumococcal conjugate and polysaccharide vaccines in infants in Papua New Guinea. Pneumonia 2017;9:20.

[26] Britton KJ, Pickering JL, Pomat WS, de Gier C, Nation ML, Pell CL, et al. Lack of effectiveness of 13-valent pneumococcal conjugate vaccination against pneumococcal carriage density in Papua New Guinean infants. Vaccine 2021;39:5401–9.

[27] van den Biggelaar AHJ, Pomat WS, Masiria G, Wana S, Nivio B, Francis J, et al. Immunogenicity and immune memory after a pneumococcal polysaccharide vaccine booster in a high-risk population primed with 10-valent or 13-valent pneumococcal conjugate vaccine: a randomized controlled trial in Papua New Guinean Children. Vaccines 2019;7:17.

[28] Satzke C, Turner P, Virolainen-Julkunen A, Adrian PV, Antonio M, Hare KM, 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;32:165–79.

[29] CLSI. Performance Standards for Antimicrobial Susceptibility Testing. CLSI Document M100-S21. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

[30] Papua New Guinea: WHO and UNICEF estimates of immunization coverage: 2021 revision. 2021. Available from: https://www.who.int/publications/m/item/immunization-png-2022-country profile.

[31] Chochua S, Metcalf BJ, Li Z, Walker H, Tran T, McGee L, et al. Invasive serotype 35B pneumococci including an expanding serotype switch lineage, United States, 2015–2016. Emerg Infect Dis 2017;23:922–30.

[32] Fuji N, Pichichero M, Ehrlich RL, Mell JC, Ehrlich GD, Kaur R. Transition of serotype 35B pneumococci from commensal to prevalent virulent strain in children. Front Cell Infect Microbiol 2021;11:744742.

[33] Tomasson G, Gudnason T, Kristonsson KG. Dynamics of pneumococcal carriage among healthy Icelandic children attending day-care centres. Scand J Infect Dis 2005;37:422–48.

[34] Wagner BG, Althouse BM, Givon-Lavi N, Hu H, Dagan R. Stable dynamics of pneumococcal carriage over a decade in the pre-PCV era. Vaccine 2019;37:5625–9.

[35] Hansman D, Devitt L, Riley I. Pneumococci with increased resistance to penicillin. BMJ 1973;3:405.

[36] Lehmann D, Gratten M, Montgomery J. Susceptibility of pneumococcal carriage isolates to penicillin provides a conservative estimate of susceptibility of invasive pneumococci. Pediatr Infect Dis J 1997;16:297–305.

[37] Greenhill AR, Phuanukoonnon S, Michael A, Yoannes M, Orami T, Smith H, et al. *Streptococcus pneumoniae* and *Haemophilus influenzae* in paediatric meningitis patients at Goroka General Hospital, Papua New Guinea: serotype distribution and antimicrobial susceptibility in the pre-vaccine era. BMC Infect Dis 2015;15:485.

[38] Standard Treatment for Common Illnesses of Children in Papua New Guinea. A manual for nurses, community health workers, health extension officers, and doctors. Port Moresby; 2016. Available from: http://pngpaediatricsociety.org/wp-content/uploads/2016/11/PNG-Standard-Treatment-Book-10th-edition-2016.pdf.

[39] Mellor KC, Lo S, Yoannes M, Michael A, Orami T, Greenhill AR, et al. Distinct *Streptococcus pneumoniae* cause invasive disease in Papua New Guinea. Microb Genom 2022;8(7):mgen000835.