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General health, otitis media, nasopharyngeal carriage and middle ear microbiology in Northern Territory Aboriginal children vaccinated during consecutive periods of 10-valent or 13-valent pneumococcal conjugate vaccines

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ABSTRACT

Objectives: This study aims to monitor the prevalence of suppurative otitis media in remote Indigenous communities after introduction of 13-valent pneumococcal conjugate vaccine (PCV13) in October 2011. We previously reported a decline in suppurative OM following replacement of PCV7 by 10-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV10) in October 2009.
Methods: We continued regular surveillance in remote Indigenous communities between February 2010 and August 2013. This analysis reports the general health, otitis media (OM), nasopharyngeal (NP) carriage and middle ear microbiology in children less than 36 months of age who received a primary course of at least two doses of PHiD-CV10 or PCV13, and not more than one dose of another pneumococcal vaccine.
Results: Mean ages of 511 PHiD-CV10- and 140 PCV13-vaccinated children were 19 and 13 months, respectively. Most children received 3-dose non-mixed PCV schedules. At the time of assessment, general health was poor and prevalence of risk factors was high in both groups: overall, around 14% of children had scabies, 20% had impetigo, 59% had runny nose and 39% had cough. Average household size was 8 persons, and 60% of the mothers smoked. Bilaterally normal middle ears were detected in 10% and 7%, respectively. OM with effusion (OME), almost all bilateral, was diagnosed in 52% and 50%, any suppurative OM (acute OM or any tympanic membrane perforation [TMP]) in 37% and 41%, and TMP in 14% and 12%, respectively. Children in the PCV13 group had significantly less NP carriage of combined *Streptococcus pneumoniae* (Spn) and non-typeable *Haemophilus influenzae* (NTHi) (62% versus 51%) but significantly more polymicrobial (Spn and NTHi) middle ear cultures (12% versus 43%), and significantly less *Staphylococcus aureus*-positive middle ears (40% versus 7%). Although NP carriage of pneumococcal serotype 19A was low in the PCV13 group, serotypes 19F and 23F persist.
Conclusions: The general health, particularly ear health, of little children in remote Australian Indigenous communities remains in crisis. In particular, transition to PCV13 did not show substantial further improvement in ear health. Possible vaccine-related differences in microbiology, including potential beneficial effects of PHiD-CV10 on NTHi infection, need to be further evaluated in randomised trials.

Abbreviations: AOMwiP, acute otitis media with perforation; AOMwoP, acute otitis media without perforation; CI, confidence interval; CSOM, chronic suppurative otitis media; DP, dry perforation; IPD, invasive pneumococcal disease; Mcat, *Moraxella catarrhalis*; mo, month; NT, Northern Territory; NTHi, non-typeable *Haemophilus influenzae*; OM, otitis media; OME, otitis media with effusion; PCV13, 13-valent pneumococcal conjugate vaccine; PCV7, 7-valent pneumococcal conjugate vaccine; PHiD-CV10, 10-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine; PPV23, 23-valent pneumococcal polysaccharide vaccine; Prevenar® and Prevenar 13®, trademarks of Pfizer Inc; RD, risk difference; SD, standard deviation; Synflorix®, trademark of the GlaxoSmithKline group of companies; TMP, tympanic membrane perforation

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1. Background

Community based surveillance before [1] and after [2] the introduction of each pneumococcal conjugate vaccine (PCV) has highlighted the poor general health, and particularly high prevalence of otitis media, of Australian Indigenous children living in remote Northern Territory (NT) communities. Throughout this period, among children at a mean age of around 20 months, less than 10% had bilateral aerated (normal) middle ears, ~30% had skin sores or scabies, ~60% had a wet or dry cough, ~50% had a runny nose, and ~30% had abnormal haemoglobin [2]. Although the prevalence of

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Table 1
Pneumococcal vaccines in the childhood vaccination schedule for Northern Territory Indigenous children.

Date commenced	Vaccine	Age (mo)
1 July 2001	PCV7 and PPV23	2, 4, 6 18
1 October 2009[a]	PHiD-CV10	2, 4, 6, 18
1 October 2011	PCV13	2, 4, 6, 18

[a] No other Australian jurisdiction recommended that PHiD-CV10 replace PCV7.

at least one form of otitis media remained high, there has been a steady decline in the prevalence of TMP (either acute otitis media with perforation (AOMwiP), dry perforation (DP), or chronic suppurative otitis media (CSOM)), from 24% in 2001 (pre-PCV) to 17% in children vaccinated with 7-valent PCV (PCV7) to 14% in children vaccinated with 10-valent pneumococcal *Haemophilus influenzae* protein D-conjugate vaccine (PHiD-CV10) [1,2]. *Streptococcus pneumoniae* (Spn, pneumococcus) and non-typeable *H. influenzae* (NTHi) are major pathogens detected by culture [3,4] or PCR [5] in ear discharge of children with TMP. *Staphylococcus aureus* (Sa) is also a common (secondary) pathogen in ear discharge [6], whereas *Moraxella catarrhalis* is rarely cultured or detected by PCR [5]. PCV7 has greatly reduced vaccine-serotype invasive pneumococcal disease (IPD) and provides indirect protective effects via reduced vaccine type carriage [7]. Replacement by non-vaccine serotypes [8] has limited the extent and persistence of this benefit [9]. The data from a trial of 11-valent pneumococcal protein D-conjugate vaccine (11Pn-PD) [10,11] including tympanocentesis for culture of middle ear fluid showed significant protection against NTHi otitis media (OM) [10]. The re-formulated 10-valent vaccine (with serotype 3 removed) has 8 serotypes conjugated to protein-D. Immunogenicity and animal model studies comparing 11Pn-PD and PHiD-CV10 indicated that similar NTHi protection could be expected [11]. Although Australian regulatory authorities did not approve a license indication for NTHi-OM [12], the NT infant vaccination schedule was changed in 2009 from a combination PCV7/PPV23 to a 3 + 1 PHiD-CV10 schedule. This was followed in 2011 by a national shift to a 3 + 0 PCV13 schedule, plus a booster dose for Indigenous children (see Table 1). Through ongoing surveillance, we observed 12% less suppurative OM (AOMwoP, AOMwiP or CSOM) and 10% more OME in the PHiD-CV10 group compared to PCV7-vaccinated children [2]. This shift from suppurative OM to OME was associated with 27% less middle ear NTHi infection, although there was no difference in NTHi carriage [6]. Our primary hypotheses for this subsequent comparison of PHiD-CV10 with PCV13 cohorts were that in children 6–36 months of age, the prevalence of suppurative OM and NTHi-associated middle ear infection would be lower, and the prevalence of bilateral normal middle ears would be higher in PHiD-CV10 vaccinated children compared to PCV13 vaccinated children. We also report findings from general child health checks that are provided as a service by the research nurses.

2. Methods

2.1. Study design, setting, community recruitment and ethical approval

Since 2001 a total of thirty five remote communities in the NT [8] and one in Western Australia have participated in at least one community-based cross sectional survey of OM and NP carriage. This report includes data from 25 Top End communities participating between February 2010 and August 2013. The study was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and the Menzies School of Health Research (EC00153), and the Western Australian Aboriginal Health Information and Ethics Committee (WAAHIEC). Each community council provided written approval of the study to the ethics committee.

2.2. Participant recruitment and consent

Informed consent was sought from parents of all children less than 6 years of age (regardless of ear health status or history) for their child to have an ear examination, nasal swab, swab of ear discharge if present, and general child health check. Parents or carers were also asked for permission to access the mother's and the child's medical records and complete a lifestyle interview regarding information on likely risk or protective factors for otitis media.

2.3. Inclusion and exclusion criteria

Aboriginal children between 0 and 6 years of age, resident in participating communities, were eligible for surveillance. For this report we limit analysis as described below (Statistical analysis).

2.4. Clinical assessments

2.4.1. Ear examinations and general health assessments

All clinical assessments were made by ear health research nurses with extensive training in the diagnosis and management of OM in this population. Otoscopic findings were recorded on a standardised form. Assessments were made using a tympanometer (Grason Stadler GSI 38), a LumiView (Welch Allyn) with Siegel's speculum for pneumatic otoscopy, and a video-otoscope (Welch Allyn macroview or MedRx video-otoscopes).

2.4.2. General health measures

Common conditions of childhood were recorded at the time of ear assessment by direct observation; the child's skin (head, arms, legs and trunk) was examined for the presence of scabies, tinea, skin sores or other skin condition; presence of nasal discharge (visible at a distance of 1 meter), and any cough (spontaneous or cough on request, either wet or dry). We categorised 'not sure' as absent. Antibiotics and other treatments or referrals were provided to participants according to local guidelines.

2.4.3. Definitions of OM

We categorised middle ear states as follows: (1) normal; (2) otitis media with effusion (OME); (3) acute otitis media without perforation (AOMwoP); (4) AOM with perforation (AOMwiP); (5) dry perforation (DP); and (6) chronic suppurative otitis media (CSOM). The final middle ear diagnosis reflected the child's more severely affected ear (highest category). We based our criteria for diagnosis on recommendations for clinical practice in this population [13]: (i) OME – intact and non-bulging tympanic membrane (TM) and Type B tympanogram; (ii) AOMwoP – any bulging of the TM and Type B tympanogram; (iii) AOMwiP – middle ear discharge observed and TM perforation recently healed or present for less than six weeks or covering less than 2% of the pars tensa of the TM; (iv) dry perforation – TM perforation without any discharge observed; (v) CSOM – middle ear discharge observed and perforation present for longer than six weeks and covering at least 2% of the pars tensa of the TM. We also include combination categories of any suppurative OM (any AOM, AOMwiP or CSOM) and any TMP (any AOMwiP, dry perforation or CSOM). Where duration of discharge was not known, size of perforation was used to distinguish AOMwiP and CSOM. Where otoscopy was not successful, we used the child's tympanometry result and defined the child's status as OME if either ear had a Type B tympanogram. We asked the mother if she thought her child had ear pain that day or during the previous evening. These a priori diagnostic criteria have been applied in all our surveillance and clinical trials conducted in this population since 2001 [1].

2.4.4. Medical record review

The child's medical records were reviewed to obtain dates of vaccinations, recent clinic presentations, antibiotics prescribed within the previous 5 weeks, sex, gestational age, date of birth, birth weight, and latest haemoglobin result.

2.5. Microbiology

Nasopharyngeal (NP) swabs were collected, transported and stored as previously described [14] and in accordance with the WHO recommendations for pneumococcal NP carriage studies [15]. Swabs of ear discharge (ED) were collected after cleaning the external canal and collecting discharge from as close as possible to the TMP [14]. NP and ED swabs were cultured on selective and non-selective media and semi-quantitative colony counts were recorded as previously described [14]. For ED swabs with swarming species that precluded selection of single colonies of presumptive non-typeable *H. influenzae* (NTHi), a millipore filtration step was used [14]. At least 2 presumptive Spn colonies and 2 presumptive NTHi colonies were selected from each specimen for confirmation. Colonies of minority colony morphology were chosen if present. Single colonies of Sa and Mcat were selected on the basis of colony morphology; Sa was confirmed if coagulase positive, Mcat was confirmed if beta-lactamase positive. Pneumococci were identified by colony morphology, optochin sensitivity and positive reaction with typing sera (Statens Serum Institut, Denmark); serotype was determined by Quellung reaction. NTHi were identified by colony morphology, dependence on X and V growth factors, and Phadebact agglutination. PCR discrimination of *Haemophilus haemolyticus* was not uniformly undertaken after confirmation that less than 0.2% of presumptive NTHi isolates from NP swabs in this population is misidentified [16]. Antimicrobial susceptibility was determined by the calibrated dichotomous susceptibility (CDS) disc diffusion method [17,18]. Minimum inhibitory concentrations (MICs) were determined for macrolide and beta-lactam antibiotic resistance in Spn isolates and azithromycin resistance in NTHi isolates using Etest strips (AB bioMérieux, Sweden). Beta-lactamase production by NTHi was determined using nitrocephin (Oxoid, Australia). Resistance was defined using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (http://www.eucast.org). Penicillin non-susceptibility of Spn was defined as MIC > 0.06 mg/L and azithromycin resistance as MIC > 0.5 mg/L. Azithromycin resistance in NTHi was defined as MIC > 4 mg/L; intermediate resistance as MIC > 0.12 mg/L and ≤ 4 mg/L, and susceptibility as MIC ≤ 0.12 mg/L.

2.6. Risk factor questionnaires

The parent or guardian (usually the mother) was asked a standardised set of questions about common risk factors for OM, including the number of siblings, number of people and children (less than 5 years of age) living in the child's house, whether the mother or child's siblings had ever had TMP ("runny ears"), her highest level of education, if she smoked, and whether the child was exposed to campfire smoke within the previous week, the number of days per week that the child attended child care, whether the child washed with soap the previous day, had ever used a pacifier, or was ever breastfed.

2.7. Statistical analysis

We compared children who had received at least 2 doses of PHiD-CV10 with children who had received at least 2 doses of PCV13 as their primary course vaccines, with or without a subsequent single dose of an alternative PCV. For this analysis we included children 0–36 months of age.

Confidence intervals (CI, 95%) and risk differences (RD, 95% CI) were calculated where appropriate. Stata version 12 was used for all data analyses [19].

Univariate odds ratios and multivariate logistic regression adjusting for community were undertaken to examine risk factors for any suppurative OM. Children with dry perforation were excluded. A global test was performed for categorical risk factors.

2.7.1. Missing data

If participants declined a clinical assessment or a swab, the data were coded as missing. If parents or carers refused or were unsure of their response to an interview question, we also coded this as missing. Missing data were then excluded from the denominator for summary statistics.

2.7.2. Funding

The study was funded by GlaxoSmithKline for the years 2010–2012, and by Pfizer for 2013.

3. Results

3.1. Participant exclusions and PCV vaccination status

We enrolled 764 age-eligible children. We excluded 113 children on the basis of their vaccination status; 36 children who had not been vaccinated, 35 who had only a single dose of one or both vaccines, 10 children who had received two doses of each vaccine, and one child who had received two or more doses of more than one PCV. Of the 651 children included, 511 were in the PHiD-CV10 group and 140 in the PCV13 group. All had received two or more doses of appropriate vaccine and not more than one dose of any other PCV. Most children had received three or more PHiD-CV10 (433/511, 85%) or PCV13 (103/140, 74%) doses; 81 (16%) PHiD-CV10 children also had a booster dose compared to 14 (10%) PCV13 children (Table 2).

3.2. Region and communities

All 26 communities were in the tropical Top End region of Australia. Visits by trained research nurses were made each year between early February and mid-December, the majority being between April and December, during the dry and pre-cyclone seasons. The sampling strategy resulted in differing proportions of children from communities and regions over time. Large communities from 4 regions contributed 58% of the data in the PHiD-CV10 group and

Table 2
Number of doses of PCV7, PHiD-CV10 and PCV13 received by PHiD-CV10 children, PCV13 children and excluded children.

		Doses of PCV13						+PCV7[a]
		0	1	2	3	4	TOTAL	
Doses of PHiD-CV	0	36[b]	14[b]	**27**	**80**	**14**	**121**	
	1	17[b]	4[b]	**10**	**9**	**0**	**19**	
	2	**56**	**22**	10[b]			**78**	**23**
	3	**235**	**118**	1[b]			**353**	**16**
	4	**78**	**2**				**80**	**1**
	TOTAL	**369**	**142**	**37**	**89**	**14**	**651**	

Bold values are included schedules.
PCV7, 7-valent pneumococcal conjugate vaccine; PHiD-CV10, 10-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine.
Included mixed schedules:
[a] 40 PHiD-CV10 children had also received one dose of PCV7 (ceased in October 2010).
Excluded schedules:
[b] 113 children were excluded; 82 shown, plus 31 (not shown) that received 2 doses of PHiD-CV10 and 2 or 3 doses of PCV7.

Table 3
Participant characteristics, general health and antibiotics prescribed, by vaccination group.

	PHiD-CV10		PCV13		Absolute difference [95% CI]	p
	N	Mean (SD) or %	N	Mean (SD) or %		
Age (mo)	511	19.0	140	13.3	5.7 [4 to 7]	**<0.0001**
0 to <3	0		0			
3 to <6	10	2%	11	8%		
6 to <9	61	12%	29	21%		
9 to <12	47	9%	26	19%		
12 to <18	126	25%	46	33%		
18 to <24	109	21%	18	13%		
24 to <36	158	31%	10	7%		
Gestational age (weeks)	378	37.7 (2.8)	426	38.1(2.3)	0.4 [0 to 0.7]	**0.04**
Birth weight (kg)	489	3.06 (0.60)	133	3.01 (0.66)	0.05 [–0.07 to 0.17]	0.2
Sex (female)	69	49%	251	49%	0.1% [–10 to 9]	0.97
General health						
Skin assessed	495/511	97%	139/140	99%		
Normal	328	66%	80	58%	–9% [–18 to 0.5]	0.06
Scabies	60	12%	20	15%	2% [–4 to 9]	0.45
Impetigo	89	18%	31	22%	4% [–3 to 612]	0.24
Tinea	16	3%	4	3%	0.3% [–4 to 3]	0.84
Other	37	8%	12	9%	1% [–4 to 7]	0.60
Other						
Runny nose	232/505	46%	74/140	53%	7% [–2 to 16]	0.15
Any cough	190/506	38%	56/140	40%	2% [–7 to 12]	0.60
Any antibiotics prescribed in previous 5 weeks	208/511	41%	62/140	44%	4% [–6 to 13]	0.46
Beta-lactam	172/511	34%	49/140	35%	1.3% [–8 to 10]	0.77
Macrolide	19/511	4%	8/140	6%	2% [–2 to 6]	0.29
Topical	21/511	4%	4/140	3%	1% [–5 to 2]	0.49
Haemoglobin (<11 g/dL)	155/461	34%	47/120	39%	6% [–4 to 15]	0.26

Bold values are p-values.

50% in the PCV13 group. One region contributed 24% of the data in the PCV13 group compared to 8% in the PHiD-CV10 group.

3.3. Participant characteristics, general health and OM risk factor prevalence

3.3.1. Age, gestational age, birth weight and gender

The mean ages were 19.0 and 13.3 months for PHiD-CV10 and PCV13 groups, respectively. Mean gestational age and mean birth weight were 37.7 and 38.1 weeks and 3.06 and 3.01 kg, respectively. Overall, 51% were female (Table 3).

3.3.2. General health

Many children had health problems in addition to their ear disease. There were no significant differences in general health between vaccine groups. Overall, 36% of children had a skin problem, mainly impetigo (19%) or scabies (13%), 47% had visible runny nose, and 38% of children were diagnosed with cough, predominantly wet cough (30%). Abnormal haemoglobin (<11 g/dL) was recorded for 35% of children tested. Antibiotics, predominantly beta-lactams, had been prescribed within 5 weeks of clinical assessment for 41% of PHiD-CV10 children and 44% of PCV13 children (Table 3).

3.3.3. Risk factor questionnaire respondents

In the PHiD-CV10 group, 355 of 501 (71%) parents or carers who were approached consented compared to 125 of 139 (90%) in the PCV13 group (Supplementary Table S1).

3.3.4. Comparison of risk factor prevalence among PHiD-CV10 and PCV13 respondent subgroups

Total household occupancy (8 persons per household) and the mean number of children less than 5 years of age per household (2 children) were not significantly different between the PCV groups. Other measures of household crowding (proportion with >2 additional children < 5 years of age, 22% overall) and child care attendance (17% overall) were not significantly different between PHiD-CV10 and PCV13 groups (Supplementary Table S1). For both vaccine groups, almost all children (~90%) washed with soap the previous day, around 30% of children had a sibling with a history of CSOM ('runny ears') and most mothers (~60%) smoked cigarettes. Significantly more children in the PCV13 group (53% versus 34%, p = 0.0004) had been exposed to campfire smoke. Mean maternal age was ~26 years, 60% of the mothers completed school years 11 or 12, and one in four had a short course certificate as their highest level of formal education. Very few babies were never breastfed (~6%), and pacifier use (~12%) was relatively uncommon (Supplementary Table S1).

3.4. Primary outcome: otitis media prevalence, by vaccine group and age

At least ninety six per cent of children in each group had at least one ear successfully assessed (Table 4). One child had a diagnosis based on tympanometry alone. In both groups, less than 10% had bilateral normal middle ears. A diagnosis of OME was made for 52% of PHiD-CV10 children and 50% of PCV13 children; AOMwoP was diagnosed for 24% and 31%, AOMwiP for 5% and 3%, dry perforation for 2% and 3%, and CSOM for 7% and 6%, respectively. Of the 476 PHiD-CV10 children and 130 PCV13 children with bilateral otoscopic assessments, 249 (52%) and 65 (50%) had worse ear diagnosis of OME. Of these, almost all had bilateral OME (86% and 94%, respectively).

For combination diagnostic categories, any suppurative OM was diagnosed in 36% of the PHiD-CV10 group and 40% of the PCV13 group (RD 3% [95% CI –6 to 13] p = 0.47). The PCV groups had similar rates of TMP (14% versus 12%. RD –3% [95% CI –9 to 2] p = 0.39). Overall, very few of the 524 interviewed mothers (7%) reported that their child had had ear pain on the day or during the night prior to the ear assessment (Table 4). The proportion of assessments associated with ear pain reported by the mother was higher for children with AOMwiP (6/23, 26%) than for children with OME (11/260, 4%) or bilateral normal ears (2/52, 4%).

Table 4
Prevalence of otitis media including combination categories of any suppurative OM, any TMP, and any pain, by vaccination groups.

	PHiD-CV10 511		PCV13 140		Absolute difference [95% CI]	P value
	n	%	n	%		
No. children without assessment	17	3%	1	1%		
No. children with diagnosis based on tympanometry alone	0	0%	1	1%		
No. children with unilateral otoscopy	18	4%	8	6%		
No. children with bilateral otoscopy	476	93%	130	93%		
No. children with at least one ear successfully assessed	494	96%	139	97%		
Clinical diagnosis	**PHiD-CV10 494**		**PCV13 139**			
Normal	49	10%	10	7%	–3% [–8 to 2]	0.33
OME	257	52%	70	50%	–2% [–11 to 8]	0.73
AOMwoP	117	24%	43	31%	7% [–1 to 16]	0.08
AOMwiP	25	5%	4	3%	–2% [–6 to 1]	0.28
Dry Perforation	9	2%	4	3%	1% [–2 to 4]	0.44
CSOM	37	7%	8	6%	–2% [–6 to 3]	0.48
Bilateral OME[a]	213	45%	61	47%	2% [–12 to 7]	0.66
Unilateral OME[a]	36	8%	4	3%	–4% [0.6 to 8]	0.07
Combination OM categories						
Any suppurative OM (AOMwoP, AOMwiP, CSOM)	179	36%	55	40%	3% [–6 to 13]	0.47
Any TMP (AOMwiP, DP, CSOM)	71	14%	16	12%	–3% [–9 to 3]	0.39
Any ear pain (today or last night)	27/396	7%	11/128	9%	2% [–4 to 7]	0.50
Non-mixed PCV schedules	**PHiD-CV10-only 329**		**PCV13-only 121**			
No. children with at least one ear successfully assessed	318	97%	120	99%		
Normal	31	10%	9	8%	–2% [–8 to 3]	0.47
OME	158	50%	60	50%	0.3% [–10 to 11]	0.95
Any suppurative OM (AOMwoP, AOMwiP, CSOM)	124	40%	48	41%	1% [–9 to 12]	0.79
Any TMP (AOMwiP, DP, CSOM)	49	15%	11	9%	–6% [–13 to 0.2]	0.09

[a] Laterality of OME could only be determined where bilateral assessments were successful.
Adjustment for age made no substantial difference to these findings.

Adjustment for age made no substantial difference to these findings (e.g. for any suppurative OM, odds ratio unadjusted 1.2 [95% CI 0.8–1.7], OR age adjusted 0.9 [95% CI 0.6–1.4]).

Comparisons of OM prevalence between recipients of non-mixed PCV schedules (318 PHiD-CV10 recipients and 120 PCV13 recipients who had at least one ear assessed) showed almost identical findings. Prevalence of TMP was 9% in the PCV13 group, compared to 15% in the PHiD-CV10 group (Table 4), but this did not reach statistical significance (p = 0.09), including after adjustment for age (p = 0.12).

Among the 80 PHiD-CV10 recipients (mean age 26 months [95% CI 9–35]) who also had a PHiD-CV10 booster dose, 14% had bilateral normal ears, 54% had OME, 14% had AOMwoP and 13% had tympanic membrane perforation (6% were not seen). Among the 14 PCV13 recipients (mean age 20 months [95% CI 12–23]) who also had a PCV13 booster dose, these figures were 21%, 50%, 14% and 14%, respectively (data not shown).

3.4.1. Age and any suppurative OM, by vaccine group

No significant difference between PHiD-CV10 and PCV13 groups in the prevalence of suppurative OM was seen for any age group; 50% versus 47%, respectively in children less than 12 months of age, 38% versus 37% in children 1 to <2 years of age, and 24% versus 20% in children 2 to <3 years of age (data not shown).

3.5. Univariable and multivariable analyses of risk factors for any suppurative OM

In a univariate analysis adjusting for community, only older age was protective of suppurative OM (odds ratio 0.31 [95% CI 0.19–0.51] for 2–3 year olds compared to infants less than 12 months of age, p < 0.0001). None of the other measured predictors was significant, including PCV group (Supplementary Table S2). Multivariate analyses did not change these findings.

3.6. Nasopharyngeal carriage

NP swabs were collected from 499 PHiD-CV10 children and 136 PCV13 children. No differences in the prevalence of NP carriage were found for NTHi, Spn, *M. catarrhalis* or *Staphylococcus aureus* (Sa) (Table 5). Fewer PCV13 children were co-colonised by both NTHi and Spn (62% and 51%, RD –10% [95% CI –20 to –1] p = 0.03]; 89% of each group had one or other of NTHi or Spn.

Serotype 16F was predominant in PHiD-CV10 children (14% isolates 11% NP swabs) and in PCV13 children (23% isolates 16% NP swabs) (Fig. 1, Table 5). Serotype 19A was the second most common serotype in the PHiD-CV10 group and was still present in PCV13 children, whereas 19F was only detected in the top ten serotypes of PCV13 children. PCV13-serotypes colonised the NP of 21% of PHiD-CV10 children compared to 15% of PCV13-vaccinated children (RD –6% [95% CI –14 to 2] p = 0.18) (Fig. 2).

In the subgroup of children receiving non-mixed 3-dose PCV schedules, the PCV13 group had significantly less NTHi carriage (73% versus 58% RD –15% [95% CI –25 to –5] p = 0.003; OR = 0.5 [95% CI 0.33–0.79]). Adjustment for age did not change this finding (OR age adjusted = 0.5 [95% CI 0.33–0.84]). Small differences in NP carriage of other otopathogens did not reach statistical significance.

3.7. NP carriage associations

To explore potential interactions among NP pathogens, we compared co-colonisation associations independent of the PCV group. Children colonised by Spn, compared to children not Spn-colonised, were more likely to also have NTHi (75% versus 48% OR age adjusted = 3.3 [95% CI 2.2–5.0]) and more likely to be colonised with Mcat (49% versus 25% OR age adjusted = 2.9 [95% CI 1.8–4.4]). Conversely, children colonised by Spn were less likely to also have Sa than those not Spn-colonised (18% versus 26% OR age adjusted = 0.62

Table 5
Prevalence of **NP carriage** of OM pathogens in NP-swabbed children, by vaccination group.

	PHiD-CV10 511		PCV13 140		Absolute difference [95% CI]	P value
	n/N	%	n/N	%		
NP swabs	**499 (98%)**		**136 (97%)**			
NP carriage						
NTHi	356	71%	86	63%	–8% [–17 to 0.9]	0.07
Spn	402	81%	105	77%	–3% [–11 to 5]	0.39
Mcat	218	44%	61	45%	1% [–8 to 10]	0.84
Sa	101	20%	22	16%	–4% [–11 to 3]	0.29
Combination NP carriage categories						
Spn and NTHi	311	62%	70	51%	–10% [–20 to –1]	**0.03**
Spn or NTHi	477	89%	121	89%	0% [–6 to 6]	0.98
PCV7 types	36/414	9%	12/102	12%	3% [–4 to 10]	0.34
PCV13 types	85/414	21%	15/102	15%	–6% [–14 to 2]	0.18
Top 7 serotypes(%)[a]	16F[c](14%) 19A[c,d](8%) 11A[c,d](7%) 10A[c](6%) 6C[b](5%) 15A[c,d](5%) 23F[b,c,d](5%)		16F[c](23%) 15A[c,d](10%) 23F[b,c,d](6%) 11A[c,d](6%) 35B[c](6%) 19F[c,d](5%) 15B[c](5%)			
Non-mixed PCV schedules						
NP carriage	**324**		**118**			
NTHi	237	73%	69	58%	–15% [–25 to –5]	**0.003**
Spn	266	82%	90	76%	–6% [–15 to 3]	0.15
Mcat	132	41%	50	42%	2% [–8 to 13]	0.67
Sa	71	22%	20	17%	–5% [–13 to 3]	0.25

Bold values are p-values.
[a] Strains within serotypes that are:
[b] Azithromycin non-susceptible (MIC > 0.5 mg/L).
[c] Penicillin non-susceptible (MIC > 0.06 mg/L).
[d] Non-susceptible to both.

[95% CI 0.39–0.98] p = 0.039]). We also observed a negative association between NTHi and Sa (OR age adjusted = 0.42 [95% CI 0.28–0.63]) and to some degree between Mcat and Sa (OR age adjusted = 0.63 [95% CI 0.42–0.96]). Serotype 10A (n = 25) was positively associated with Sa (OR age adjusted = 2.56 [95% CI 1.08–6.06], p = 0.032), whereas serotype 19A (n = 36) was negatively associated (OR age adjusted = 0.38 [95% CI 0.11–1.27], p = 0.12) and carriage of serotypes 15A (n = 32), 11A (n = 37) and 16F (n = 79) were not different in Sa carriers and Sa non-carriers.

3.8. Microbiology of middle ear discharge

Seventy one children in the PHiD-CV10 group and 16 children in the PCV13 group had a worse ear diagnosis of any TMP (Table 4). We obtained at least one specimen of ear discharge (ED) from 51 (72%) and 11 (69%) children, respectively, providing a total of 63 and 14 ED swabs, respectively.

Analysis of ED swabs showed significantly higher recovery of NTHi from PCV13 specimens compared to PHiD-CV10 (9/14, 64%

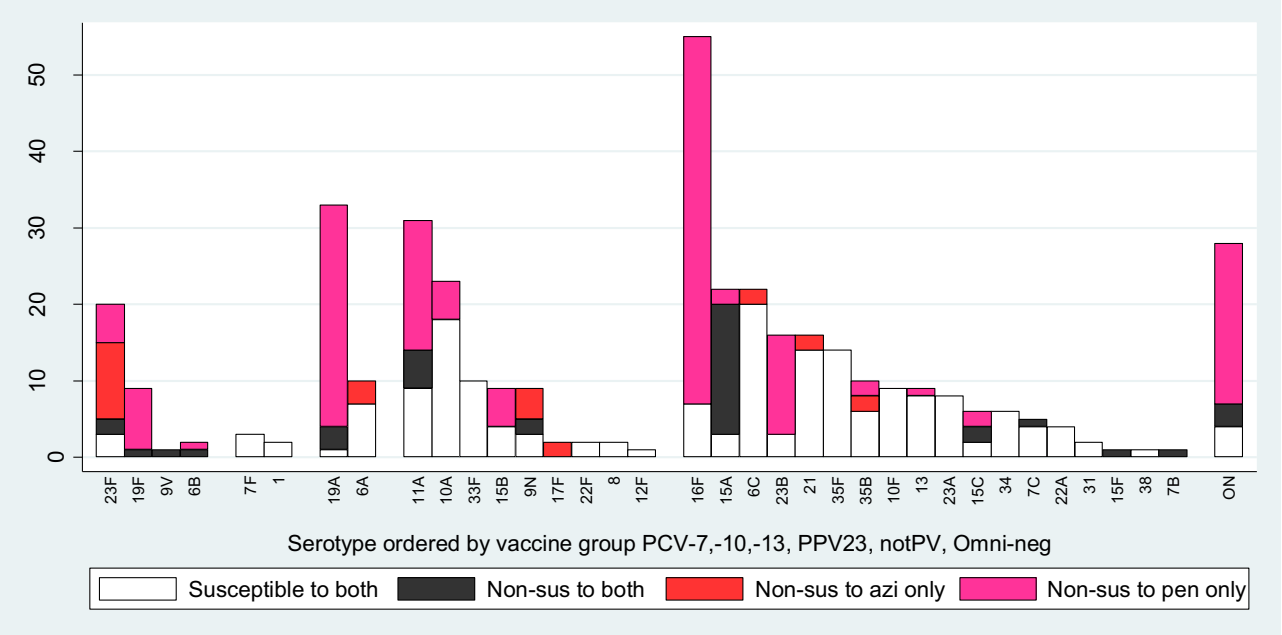

Fig. 1. Pneumococcal serotypes and antimicrobial susceptibility of isolates colonising the nasopharynx of the PHiD-CV10 group. The first isolate selected per NP swab is included. Serotype is ordered by pneumococcal vaccine group: PCV7, PHiD-CV10, PCV13, 23PPV and non-vaccine types (notPV and Omniserum-negative). Penicillin non-susceptibility was defined as MIC > 0.06 mg/L. Azithromycin resistance was defined as MIC > 0.5 mg/L.

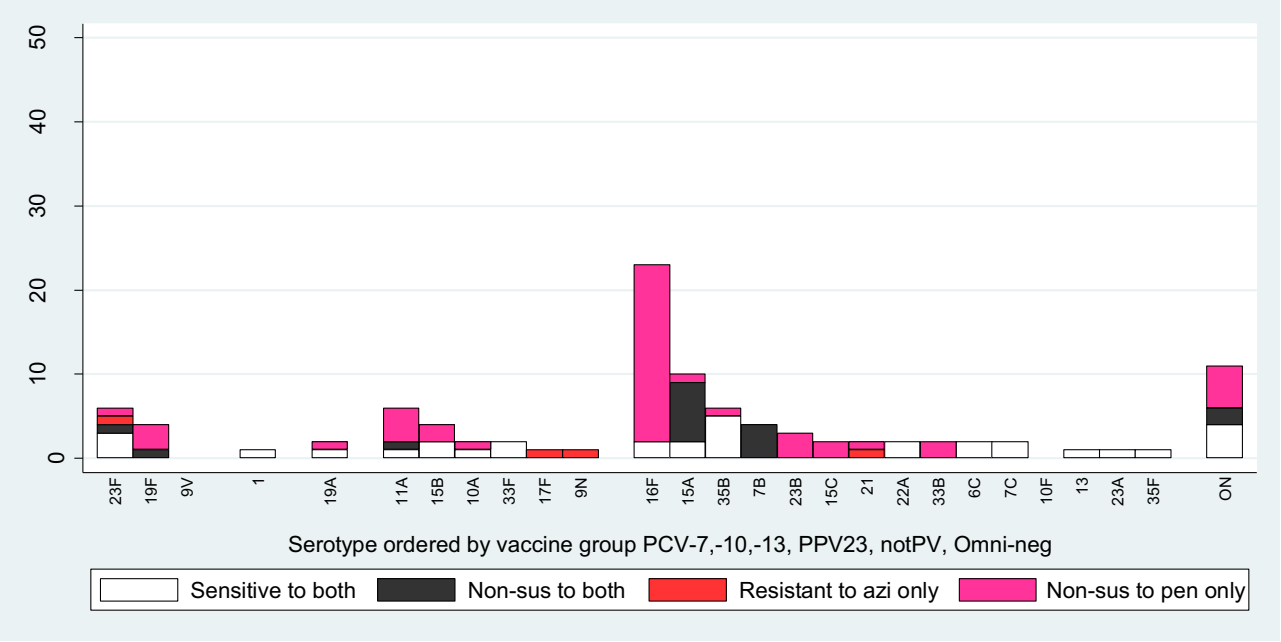

Fig. 2. Pneumococcal serotypes and antimicrobial susceptibility of isolates colonising the nasopharynx of the PCV13 group. The first isolate selected per NP swab is included. Serotype is ordered by pneumococcal vaccine group: PCV7, PHiD-CV10, PCV13, 23PPV and non-vaccine types (notPV and Omniserum-negative). Penicillin non-susceptibility was defined as MIC > 0.06 mg/L. Azithromycin resistance was defined as MIC > 0.5 mg/L.

versus 21/59, 36%). There was also a higher recovery of Spn (6/14, 43% versus 10/69, 17%) from PCV13 specimens, but significantly lower prevalence of Sa middle ear infections (1/14, 7% versus 24/60, 40%). No difference in Mcat (1/14, 7% versus 5/59, 8%) was detected (Table 6).

In a comparison of data by child (data combined if bilateral), differences were smaller and no longer statistically significant, with the exception of Sa (1/11, 9% versus 22/49, 45%) (Table 6).

Pneumococcal serotypes were 11A(3 isolates), 15A(2) and one isolate each of serotypes 16F, 19F, 21, 22A and 35F in the PHiD-CV10 group, and 33F(2), 9N(2), 1(1), and 35B(1) in the PCV13 group.

4. Discussion

Following a prior comparison of PHiD-CV10 with PCV7-vaccinated children, in which a significant reduction in suppurative OM was

Table 6
Prevalence of OM pathogens in 75 ear discharge (ED) swabs from 62 children with AOMwiP or CSOM, by vaccination groups.

	PHiD-CV10		PCV13		Absolute difference [95% CI]	P value
	N, Mean (SD)	%	N, Mean (SD)	%		
ED swabs	63		14			
Children with ED	51		11			
Mean age (mo)	18.6 (5.8)		15.3 (8.5)			
Bilateral ED swabs	26		6			
Bilateral CSOM	16		6			
Bilateral AOMwiP	6		0			
AOMwiP and CSOM	4		0			
All ED swabs						
NTHi	21/59	36%	9/14	64%	29% [0.8 to 57]	**0.05**
Spn	10/60	17%	6/14	43%	26% [–1 to 54]	**0.03**
NTHi and Spn	7/59	12%	6/14	43%	31% [4 to 58]	**0.006**
Mcat	5/59	8%	1/14	7%	–1% [–17 to 14]	0.87
Sa	24/60	40%	1/14	7%	–33% [–51 to –15]	**0.02**
Spn serotypes (n)[a]	11A[d](3) 15A[d](2) 16F[c] 19F[d] 21[b] 22A 35F		33F(2) 1(1) 9N(2) 35B[c](1)			
Children with ED (data combined if bilateral)						
NTHi	17/48	35%	6/11	55%	19% [–13 to 52]	0.24
Spn	10/49	20%	4/11	36%	16% [–15 to 47]	0.26
NTHi and Spn	7/49	14%	4/11	36%	22% [–8 to 52]	0.09
Mcat	4/48	8%	1/11	9%	1% [–18 to 19]	0.94
Sa	22/49	45%	1/11	9%	–36% [–58 to –14]	**0.02**

Bold values are p-values.
[a] Strains within this serotype are:
[b] Azithromycin non-susceptible (MIC > 0.5 mg/L).
[c] Penicillin non-susceptible (MIC > 0.06 mg/L).
[d] Non-susceptible to both.

reported in association with PHiD-CV10 vaccination [2], and for whom less NTHi was cultured from middle ear discharge [6], we anticipated that PCV13-vaccinated children, who would not have vaccine-induced anti-HiD antibody protection from NTHi, would have an increased prevalence of suppurative OM, increased NTHi middle ear infection, and possibly increased NP carriage of NTHi. What we did find was that compared to the PHiD-CV10 group, PCV13-vaccinated children had a similar prevalence of suppurative OM (around 40%), a higher prevalence of middle ear co-infection with NTHi and Spn (significant for comparison by ear) and less NTHi NP carriage (significant when 3-dose schedules compared). Whilst not statistically significant, the PCV13 group had a lower TMP prevalence (particularly for the 3-dose comparison) of 9% compared to 15% in the PHiD-CV10 group. Compared with our earliest (2001) pre-PCV7 OM surveillance, when the prevalence of TMP was 24% among children of this age [1], the findings are encouraging. These somewhat positive clinical findings are not entirely consistent with the microbiology of the middle ear discharge, since the PCV13 group had significantly more Spn and NTHi mixed middle ear infection. It is possible that the lower prevalence of TMP in the PCV13 group was related to the significantly lower prevalence of *S. aureus* middle ear infection.

As mentioned we continue to find that almost every child has some form of OM and that less than 10% of children have bilaterally normal middle ear status. Unilateral OME, where the contralateral ear is normal, would not meet criteria for hearing assessment and could be considered normal in audiological terms. However, of the 50% of children with a diagnosis of OME in their worse ear, almost all (90%) had bilateral OME. Of the 21 infants under the age of 6 months, and a further 90 infants between the ages of 6 and 9 months, half had a diagnosis of suppurative OM. This early onset remains a huge challenge and requires new strategies for prevention. It is intriguing that OM is so rarely associated with pain as reported by the child's mother. Asymptomatic AOM in this population has been documented over many years with translation to national OM guidelines [13], health care provider training and community awareness campaigns [20] that promote ear assessments for all high risk children regardless of the primary reason for presentation. Failure to recognise and treat asymptomatic AOM may explain in part the progression to tympanic membrane perforation and CSOM.

In addition to these ongoing ear health problems, one in four or five children had impetigo, one in six had scabies, half had runny nose and two in five had a cough. Antibiotics had been recently prescribed for almost half the children. These children were generally not considered unwell by their carer. Children were in their homes and were not presenting for health services [13].

As previously discussed [2], limitations of our study are that surveillance is dependent on annual community participation approval processes, so whilst we have adjusted some group comparisons in consideration of this diversity, it would be possible that over- or under-representation of higher- or lower-risk communities could influence our findings. Also, our non-random selection of children for assessment could contribute to bias. Our a priori analysis plan was to compare children who received predominantly one PCV formulation or another in their primary 3-dose course. Comparisons of OM prevalence between recipients of non-mixed 3-dose PCV schedules showed very similar findings.

Failure to detect significant vaccine group differences in clinical or NP carriage was not influenced by group differences in classical risk or protective factors such as crowding, smoke exposure or breastfeeding. Younger children had the highest risk of suppurative OM, but adjustment for age made no substantial differences to odds ratios. As with our previous comparison of PCV7 and PHiD-CV10 vaccine groups, this comparison of cross sectional data from PHiD-CV10 and PCV13 groups is made complex by the lack of concurrent data. Factors not measured may also have changed during this period.

Several studies have reported invasive pneumococcal disease, OM and NP carriage outcomes of PCV RCTs [21–23] and pre- versus post-changes in vaccination schedules [24–26]. In a RCT conducted in the Netherlands [21], PHiD-CV10 had no impact on nasopharyngeal (NP) carriage prevalence or density of NTHi, whereas the Kilifi NP carriage cross sectional comparisons showed a significant reduction in NTHi carriage after PHiD-CV10 was introduced to the national immunisations program [24]. Paradoxically, we found that NP carriage of NTHi was significantly lower in the 3-dose PCV13 group, whereas middle ear infection and co-infection (NTHi and Spn) rates were substantially higher in the PCV13 group than the PHiD-CV10 group, as anticipated. In Israel, where PCV13 replaced PCV7, rates of PCV13-serotype OM (confirmed by tympanocentesis and middle ear fluid microbiology) declined with a small increase in non-PCV13-serotype OM [25]. All-cause OM also declined substantially in PCV13 compared to PCV7 children, which the authors presumed to be due to reductions in co-infection with non-pneumococcal otopathogens (non-pneumococcal infections were not reported) [25]. Our findings from a small number of samples would also suggest that reductions in *S. aureus* middle ear infection could be an unanticipated outcome of PCV13 vaccination, despite a lack of substantial differences in NP carriage. Our failure to detect a change in *S. aureus* NP carriage contrasts with data suggesting replacement NP carriage by *S. aureus* following PCV introduction and reduced VT carriage (summarised in Ref. 27). Our analysis of all NP carriage data, independent of PCV formulation, indicates positive associations among the otopathogens Spn, NTHi and Mcat, contrasting with negative associations between each of these otopathogens and *S. aureus*. Potential for pneumococcal serotype-specific positive and negative associations needs to be confirmed.

Increased NTHi-specific immune responses to PHiD-CV10 in the lungs of Indigenous children with chronic suppurative lung disease (elevated IFNγ, IL13 and IL 5) [28] offer some insight as to the possible mechanisms of protection in the middle ear. Randomised controlled vaccine trials with OM outcomes and concomitant studies of both NP and middle ear microbiology are needed to clarify these findings and substantiate the potential mechanisms involved in the host response to HiD carrier protein of PHiD-CV10, and other putative NTHi and pneumococcal vaccine candidates as well as indirect effects of vaccines on polymicrobial OM [29].

5. Conclusion

Our report of the general health and comparison of ear health of children receiving PHiD-CV10 or PCV13 according to the NT childhood vaccination schedules shows no substantial change in the prevalence of OM or TMP, and some differential effects on middle ear microbiology. Multivariate logistic regression did not change these findings. Clinical trials of vaccines that offer broader coverage of OM pathogens from an earlier age are needed in this high risk population.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijporl.2016.05.011.

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