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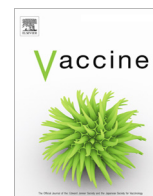This is the publisher's version. This version is defined in the NISO recommended practice RP-8-2008 http://www.niso.org/publications/rp/

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Pneumococcal vaccine impact on otitis media microbiology: A New Zealand cohort study before and after the introduction of PHiD-CV10 vaccine

Emma J. Best [a,b,*], Tony Walls [c,d], Melanie Souter [c], Michel Neeff [a], Trevor Anderson [c], Lesley Salkeld [a,e], Zahoor Ahmad [e], Murali Mahadevan [a,b], Cameron Walker [f], David Murdoch [c,d], Nikki Mills [a]

[a] Starship Children's Hospital, Auckland District Health Board, Auckland, New Zealand
[b] Department of Paediatrics, The University of Auckland, New Zealand
[c] Christchurch Hospital, Canterbury District Health Board, Christchurch, New Zealand
[d] University of Otago, Christchurch, New Zealand
[e] Manukau Superclinic, Middlemore Hospital, Counties Manukau District Health Board, Auckland, New Zealand
[f] Department of Engineering, The University of Auckland, New Zealand

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ABSTRACT

We compared the microbiology of middle ear fluid (MEF) in two cohorts of children having ventilation tube (VT) insertion; the first in the era of 7-valent *Streptococcus pneumoniae* conjugate vaccine (PCV7) and the second following introduction of the ten-valent pneumococcal vaccine (PHiD-CV10).

Methods: During 2011 (Phase 1) and again in 2014 (Phase 2) MEF and NP samples from 325 children and 319 children were taken at the time of VT insertion. A matched comparison group had NP swabs collected with 137 children (Phase 1) and 154 (Phase 2). Culture was performed on all NP and MEF samples with further molecular identification of Haemophilus species, serotyping of *S. pneumoniae*, and polymerase chain reaction (PCR) testing on all MEF samples.

Results: In Phase 2 immunisation coverage with ⩾3 doses of PHiD-CV10 was 93%. The rate and ratios of culture and molecular detection of the 3 main otopathogens was unchanged between Phase 1 and Phase 2 in both MEF and NP. *Haemophilus influenzae* was cultured in one quarter and detected by PCR in 53% of MEF samples in both time periods. *S. pneumoniae* and *Moraxella catarrhalis* were cultured in up to 13% and detected by PCR in 27% and 40% respectively of MEF samples. *H. influenzae* was the most common organism isolated from NP samples (61%) in the children undergoing VT surgery whilst *M. catarrhalis* (49%) was the most common in the non-otitis prone group. 19A was the most prominent *S. pneumoniae* serotype in both MEF and NP samples in Phase 2. Of Haemophilus isolates, 95% were confirmed to be non-typeable *H. influenzae* (NTHi) over both time periods.

Conclusion: Following implementation of PHiD-CV10 in New Zealand, there has been no significant change in the 3 major otopathogens in NP or MEF in children with established ear disease. For these children non-typeable *H. influenzae* remains the dominant otopathogen detected.

1. Introduction

Otitis media (OM) remains one of the most common disorders for which medical care is sought in childhood and a common reason for prescribing antibiotics [1]. Ventilation tube insertion (VT) is the most frequent surgical procedure performed in young children, with recurrent AOM (rAOM) and persistent otitis media with effusion (OME) being the usual indications. Worldwide, parents report that otitis media can be a significant burden on their family [2].

Prevention of otitis media by vaccination of young children may lead to reductions in antibiotic prescribing [3], have beneficial economic effects by reducing primary care visits and time off work for parents as well as reduce complications of acute otitis media such as perforations and numbers of children requiring VT [4].

The established primary bacterial otopathogens are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*, which colonise the nasopharynx prior to invasion into the middle ear [5]. *S. pneumoniae* is the only vaccine preventable pathogen of OM due to available pneumococcal conjugate vaccines. Yet introduction of a 7-valent pneumococcal conjugate vaccine (PCV7; Prevenar) to infant vaccination schedules in many developed countries had a relatively small effect on AOM. A Cochrane review of evidence concluded PCV7 to have modest benefit in reduction of AOM in healthy infants and no benefit for high-risk infants and older children with a history of AOM [6]. Post marketing surveillance has showed declines in rates of VT insertion of 6–23% in <2 year olds in Australia [7] and by up to 20% in certain states in the US [8] suggesting some impact on recurrent or persistent ear disease. These reductions may be explained by reduction in vaccine-related pneumococcal serotypes causing disease, yet the modest effects overall could be due to an increase in non-vaccine *S. pneumoniae* serotypes and/or replacement with other pathogens [9–11]. Furthermore, measuring vaccine effectiveness with otitis media as an end point is fraught with difficulty, due to the multifactorial pathogenesis of otitis media, variability in diagnosis, and the spectrum of OM disease. Other confounders such as variability in health seeking behaviour within a population, public health measures, antibiotic use and other vaccine implementations can occur simultaneously and also impact on OM outcomes creating challenges in measuring PCVs true impact [12].

In New Zealand (NZ), PCV7 was introduced as a 3 + 1 schedule in 2008 and significantly decreased the burden of invasive pneumococcal disease (IPD) caused by PCV7 serotypes in young children [13]. There was also evidence of impact on non-invasive diseases such as childhood hospitalisations from pneumonia [14] and indirect (herd) effects leading to reductions in IPD due to PCV7 serotypes in older age groups [13]. Phase one of our study commenced in 2011, three years after PCV7 introduction in NZ. We collected middle ear fluid (MEF) and NP samples from children presenting for VT insertion [15]. Following completion of this study, in late 2011, the national immunisation schedule was revised with replacement of PCV7 with the 10-valent pneumococcal conjugate vaccine (PHiD-CV10) [16]. This vaccine covers 3 additional serotypes of *S. pneumoniae*, but of particular interest in otitis media prevention is use in PHiD-CV10 of protein D from *H. influenzae* as the conjugating protein for 8 of the included 10 *S. pneumoniae* serotypes. A pre-licensure randomised controlled trial using an 11-valent PCV containing a related protein D (11PnPD) demonstrated efficacy preventing AOM; both due to vaccine type *S. pneumoniae* but also due to *H. influenzae* when compared with placebo [17]. However there is little support for any impact on nasopharyngeal prevalence or density of *H. influenzae* carriage following vaccination and no post-licensure evidence for impact on recurrent AOM or AOM specifically due to *H. influenzae* [18,19].

With the opportunity provided by NZ's changing pneumococcal vaccine schedule, we aimed to describe and compare the aetiology of otitis media with specific attention to changes in the microbiology of MEF and NP carriage, including *S. pneumoniae* serotypes and *H. influenzae* prior to and following implementation of PHiD-CV10. In addition we aimed to document and compare the nasopharyngeal carriage and antibiotic susceptibility of organisms known to cause OM in children with and without a history of rAOM or OME in PCV7 and PHiD-CV10 vaccine eras.

2. Materials and methods

Children aged less than 36 months of age undergoing VT were recruited from the three major referral centres in New Zealand for children undergoing VT for rAOM or OME: two in Auckland and one in Christchurch. Recruitment occurred between May to November, 2011 (Phase 1) and May to November, 2014 (Phase 2). In these centres the surgical criteria for VT insertion is ⩾6 episodes of AOM in 12 months (recurrent AOM) or persistent bilateral middle ear effusions for >3 months (OME). Diagnosis was made by trained practitioners, together with micro-otoscopy +/− tympanometry prior to booking for surgery.

In each centre a seasonally matched comparison group of non-otitis prone children of same age and vaccination eligibility was also recruited [15]. From parental history they had no significant previous ear disease (<3 episodes of AOM in 12 months, no history of OME). These children were having a general anaesthetic for non-ear related procedures (such as radiologic imaging, general or non-ear related day surgery). NP swabs were collected from this group. Children with known immune deficiency, cystic fibrosis or craniofacial malformation were excluded from both groups.

Informed consent was obtained prior to procedures. Risk factors for ear disease and epidemiological data collected via parental/-carer questionnaire at the time of surgery. Ethnicity was assigned using a standard priority system [20].

Ethical approval was obtained from the New Zealand Northern Regional Ethics Committee (NTX/11/04/029).

All children enrolled were eligible for 3 + 1 pneumococcal conjugate vaccination (PCV7 in the first cohort, 2011; and PHiD-CV10 in the second cohort, 2014) and *H. influenzae* type b vaccinations as part of the national immunisation schedule. Receipt of PCV was recorded and confirmed for each child using the National Immunisation Register and/or primary care practice records. Children vaccinated solely with PCV13 (which was available for private purchase) were actively excluded if that information was available at enrolment.

2.1. Laboratory method for nasopharyngeal and middle ear samples

Laboratory methods were identical for Phase 1 and 2 and have been described previously [15]. Middle ear fluid (MEF) was collected by sterile suction through the myringotomy prior to VT placement, and nasopharyngeal (NP) swabs were collected from surgical and comparison groups.

MEF was cultured for bacterial pathogens by standard methods including extended culture to detect *Alloiococcus otitidis* [21]. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* isolates were tested for susceptibility to standard antimicrobials by agar disc diffusion. Pneumococcal capsular serotyping was performed via Quellung reaction at the national reference laboratory. From 2014, factor sera were available to identify serotype 35A and serotypes 15 and 16. All *S. pneumoniae* isolates from both Phase 1 and 2 had serotyping repeated to classify these.

For nucleic acid extraction and polymerase chain reactions 200 μL of MEF and 5.0 μL of internal control DNA were extracted with EasyMag (BioMerieux, Auckland, NZ) generic 2.0.1 protocol. Nucleic acid was recovered in 60 μL of elution buffer. PCR reactions were based on the Real-time TaqMan PCR format with *S. pneumoniae* [22] performed as a duplex with an internal control (un-published assay) while *H. influenzae* [23] and *M. catarrhalis* [24] were detected using single-plex PCR assay (Supplemental Table 1).

Differentiation between *H. influenzae* and *Haemophilus haemolyticus* was performed using absence or presence of the hpd#3 gene by PCR [25]. Samples identified as *H. influenzae* by

Table 1
Demographic data from children aged less than 3 years undergoing ventilation tube insertion and comparison group: Phase 1, 2011 and Phase 2, 2014.

	Children undergoing ventilation tube insertion		Non-otitis prone comparison		[a]P values
	Phase 1	Phase 2	Phase 1	Phase 2	
Total number	325	319	137	154	
Median age: months (interquartile range)	21.6 (16.9–27.4)	22.6 (16.9–27.3)	21.1 (15.1–29.9)	17.6 (10.8–27.3)	**<0.001**
Male (%)	203 (62)	218 (68)	98 (72)	101 (66)	0.56
Median birth weight: kg (interquartile range)	3.45 (3.09–3.85)	3.55 (3.10–3.85)	3.40 (2.85–3.83)	3.44 (2.91–3.76)	0.15
Gestational age: number less than 38 weeks (%)	52 (16)	56/315 (18)	32 (23)	29/144 (20)	0.06
Child has history of atopy (%)	61/323 (19)	58/318 (18)	19 (14)	20/145 (14)	0.20
Family has history of ear disease (%)	155 (48)	169 (53)	29 (12)	33/145 (23)	**<0.01**
Ethnicity					
European	58%	54%	45%	42%	**<0.05**
Maori	22%	24%	18%	26%	0.51
Pacific Island	12%	12%	15%	10%	0.68
Other	8%	10%	23%	22%	**<0.01**
Vaccination status (%)					
3 or more doses of PCV at enrolment	315 (96)	301 (94)	129 (93.5)	145 (94)	0.93
Received only PCV10	–	289 (91)	–	141 (92)	0.73
Up to date[b]	299 (92)	304 (95)	123 (89)	144 (94)	0.42
Environmental factors (%)					
Ever breastfed	281/324 (87)	279/319 (87)	116/137 (85)	129/143 (90)	0.56
Exposure to household smoke	101/324 (31)	91/317 (29)	43/136 (31)	39/143 (27)	0.93
Day care attendance (>4 h/week)	205/322 (64)	235/318 (73)	54/134 (40)	40/146 (26)	**<0.01**
Child has ⩾1 sibling	226/324 (70)	229/311 (74)	88/136 (65)	97/129 (75)	0.29
>5 people in home	59/325 (18)	53/319 (17)	23/137 (17)	37/144 (26)	0.76
Antibiotic use (month prior to surgery)	198/321 (62)	159/313 (51)	51/133 (38)	40/146 (27)	**<0.01**

Significant *P* values in bold text.

[a] *P* values represent difference between children undergoing ventilation tube insertion compared with non-otitis prone group within Phase 2 (Phase 1 data published previously).

[b] Up to date – received age-appropriate number of doses of pneumococcal vaccine by date of surgery (3 doses if aged over 5 months and up to end of 15th month; and 4 doses if age over 16 months).

culture or PCR were also tested for presence of capsule using bexB gene PCR [26].

2.2. Sample size calculation

Sample size calculations were performed prior to Phase 1 and were based on potential change in the proportion of children with *H. influenzae* MEF infection pre and post introduction of PHiD-CV10. We hypothesized there would be a reduction in proportions of *H. influenzae* isolated from MEF samples between Phase 1 (PCV 7 vaccinated cohort) and Phase 2 (PHiD-CV10 vaccinated cohort). We elected to use a conservative estimate of vaccine efficacy at 20% for prevention of otitis media based on reported vaccine efficacy against *H. influenzae* AOM of 35% [17].

Available reported rates of MEF culture for *H. influenzae* were 16% in a cohort of New Zealand children with OME [27] and PCR detection rates of 29% in another OME cohort [28]. For *H. influenzae* detection rate of 25% a sample of 300 pre-vaccine and 300 post-vaccine would be required to detect a 20% reduction in *H. influenzae* infections at the 5% level of confidence with 90% power.

2.3. Statistical analyses

Demographic and response data was compared between study cohorts using odds ratios (comparison of proportions between the two groups), *t*-tests (for comparison of continuous variables between the two groups) and chi-squared tests (for comparing distributions of the two groups). Detection of MEF organisms was analysed with respect to NP organisms using McNemar change test with continuity correction (to allow for matched nature of data). Confidence intervals generated were at 95% level, with *p*-value of 0.05 as cut-off. Analysis used R software package version 2.15.2 [29].

3. Results

3.1. Study population

In Phase I 462 children were recruited (325 in the VT group and 137 in non-otitis prone group) representing 78% of all VT surgery performed in this age group in the 3 centres. In Phase 2, 473 children were recruited (319 in the VT surgical group and 154 in the non-otitis prone comparison group) representing 77% of all children presenting to these centres for VT surgery. Non-participation was predominantly due to missed recruitment (patients not captured at the time of surgery) and fewer than 2% declined to participate.

Baseline demographic and clinical data between phases were not significantly different. There was a male predominance (over 60%) in both VT and comparison groups in both phases, with an average age of around 21 months. The significant differences noted in Phase 1 between children undergoing VT surgery and non-otitis prone comparison group were also present in Phase 2 (Table 1) with children undergoing VT surgery being more likely to attend day-care, have a family history of ear disease, and have had antibiotics in the past month. Information on antibiotic usage was available for 98% of all participants. Recent antibiotic use was high in both groups, with amoxicillin being the most commonly used antibiotic (in both time periods). In both phases the otitis-prone children were more likely to have received antibiotics at a doctor's (parental report) on >5 occasions in the prior 12 months (46% and 51%) compared to children in the comparison groups (1% and 0%).

3.2. Vaccination status

The vast majority of the children had received 3 or more doses of PCV at study entry in both phases (Table 1). A small proportion of

Table 2
Comparison of surgical indication and findings at surgery between two cohorts of children aged less than 3 years undergoing ventilation tube insertion: Phase 1, 2011 & Phase 2, 2014.

Ventilation tube patients		Phase 1 (2011) N = 325	Phase 2 (2014) N = 319	p value: Phase 1 vs 2
Surgical indication at time of referral	Otitis media with effusion	32%	29%	0.39
	Recurrent AOM	29%	45%	**<0.05**
	Acute symptoms and OME	37%	26%	**<0.05**
	Other (tympanic membrane retraction)	2%	<1%	0.10
Middle ear findings at surgery (%)				
Bilateral dry tap		70 (21.5)	87 (27)	0.09
Unilateral dry tap		71 (22)	63 (20)	0.51
Bilateral effusion		184 (57)	169 (53)	0.35
Fluid in one or both ears		255 (78.5)	232 (73)	0.09
	Number of MEF examined	N = 441	N = 401	
Gross description of surgical findings when fluid identified (%)	Mucoid/thick	265 (60)	266 (66)	0.06
	Mucopus	86 (19.5)	54 (13.5)	**<0.05**
	Serous/thin	88 (20)	81 (20)	0.93

Significant *P* values in bold text.

Table 3
Culture and polymerase chain reaction detection of otopathogens from middle ear fluid of two cohorts of children aged less than 3 years undergoing ventilation tube insertion: Phase 1, 2011 & Phase 2, 2014.

		Phase 1: 2011 N = 255 (428)[a]	Phase 2: 2014 N = 232 (388)[a]	p value: Phase 1 vs 2
Culture positive (% of patients)	*Streptococcus pneumoniae*	26 (10)	24 (10)	0.96
	Haemophilus influenzae	63 (25)	65 (28)	0.41
	Moraxella catarrhalis	26 (10)	31 (13)	0.28
PCR detection (% samples)	*Streptococcus pneumoniae*	104 (24)	104 (27)	0.43
	Haemophilus influenzae	227 (53)	205 (53)	0.92
	Moraxella catarrhalis	182 (43)	156 (40)	0.48

[a] *N* = number of children with unilateral or bilateral positive MEF samples (number of samples collected).

children in each group and phase were completely unimmunised in surgical groups (Phase 1: 3% and Phase 2: 1%) and comparison groups (3% both phases) which did not significantly change over time periods. In Phase II over 93% of children had received at least 3 doses of PHiD-CV10, and 15 of the 319 children (5%) having VT insertion had received less than 3 doses (1 received no PHiDCV10 doses, 7 received one dose and 7 received 2 doses). Amongst the comparison group, 5 children received less than 3 doses of PHiD-CV10 with 4 having had only 1 dose of PHiD-CV10 and 1 received 2 doses. No children received PCV13 in Phase 1 and in Phase 2, 12 children (2.5%) received 1 or more doses of PCV13.

Overall children in Phase 2 were more likely to be up to date with pneumococcal vaccination compared with Phase 1 (odds ratio 0.57, 95% CI 0.34, 0.96).

All recruited children were included in analysis regardless of vaccination status.

3.3. Surgical indication

The proportion of children requiring ventilation tubes for persistent middle ear effusions (OME) remained the same between time periods. The primary indication for VT surgery changed between time periods with more children identified as rAOM (29% Phase 1 compared with 45% Phase 2) rather than acute symptoms with persistent middle ear fluid (37% Phase 1 versus 26% Phase 2) (Table 2). Macroscopic findings at time of surgery showed less children observed to have MEF described as 'mucopus' in the later cohort (19.5% Phase 1 versus 13.5% Phase 2; *p* =< 0.05).

3.4. Microbiology: middle ear fluid

In Phase 2, amongst the 319 children having ventilation tubes, 87 had no MEF bilaterally (27%), giving 232 children with MEF in one or both ears and 401 MEF samples collected. Thirteen samples (3%) were unprocessed thus 388 samples from 232 patients with bilateral or unilateral MEF were cultured and had PCR in Phase 2. This compared with 441 samples being collected from 255 children in Phase 1, with 428 of these available to process (Table 3).

Comparison between phases of the three main otopathogens in MEF by culture and PCR is shown in Table 3. There were no significant differences in culture proportions or PCR detection of 3 main otopathogens between phases, with *H. influenzae* the dominant pathogen in MEF in both. In Phase 2, 18 MEF specimens (5%) were culture positive for ⩾2 organisms with 12/18 having *H. influenzae* as one of the organisms present. In Phase 1, 11 MEF (4%) were positive for ⩾2 organisms with all having *H. influenzae* as one of the organisms.

In Phase 2, 13 children (6%) grew *Alloiococcus otitidis* (6 bilaterally) compared with 33 children (10% and 11 bilaterally) in Phase 1.

3.5. Microbiology: nasopharyngeal carriage

Culture results for otopathogens isolated from NP specimens are shown in Table 4. A small proportion of NP samples from children undergoing VT surgery were not available in both phases due to failed collection or loss of specimen (3% Phase 1 and 1% Phase 2). The overall proportion of pathogens carried remained constant.

Table 4
Nasopharyngeal carriage of bacteria in children undergoing ventilation tube surgery compared with non-otitis prone group; differences between time periods Phase 1, 2011 & Phase 2, 2014.

	Ventilation tube surgical group		*p*-value	Non-otitis prone comparisons		*p*-value
	Phase 1: 2011	Phase 2: 2014	VT group	Phase 1: 2011	Phase 2: 2014	Comparison group
NP cultured from children recruited *n/N*	316/325	316/319		137/137	154/154	
NP culture positive for ⩾1 pathogens	275 (87%)	281 (88%)	NS	105 (77%)	116 (75%)	NS
H. influenzae	195 (62%)	195 (61%)	NS	58 (42%)	65 (42%)	NS
S. pneumoniae	135 (43%)	156 (49%)	NS	40 (29%)	49 (32%)	NS
Vaccine serotype[a]	36 (11%)	5 (1%)	*P* =< 0.001	11 (8%)	0 (0%)	*P* =< 0.001
Non vacc serotype[b]	98 (31%)	151 (48%)	*P* =< 0.001	29(21%)	49 (32%)	*P* =< 0.05
M. catarrhalis	181 (57%)	170 (53%)	NS	68 (50%)	76 (49%)	NS
Single pathogen only	102 (32%)	105 (33%)	NS	58 (42%)	58 (38%)	NS
H. inf	57 (18%)	51 (16%)		21 (15%)	23 (15%)	
S. pn	13 (4%)	30 (9.5%)		5 (4%)	9 (5.8%)	
M. cat	32 (10%)	24 (7.6%)		32 (23%)	26 (17%)	
Two pathogens	128 (41%)	112 (35%)	NS	33 (24%)	43 (28%)	NS
S. pn + H. inf	30 (9%)	30 (10%)		11 (8%)	8 (5%)	
S. pn + M. cat	41 (13%)	32 (10%)		10 (7%)	16 (10%)	
M. cat + H. inf	57 (18%)	50 (16%)		12 (9%)	19 (12%)	

NP = nasopharyngeal sample.

H. inf = *Haemophilus influenzae*; S. pn = *Streptococcus pneumoniae*; M. cat = *Moraxella catarrhalis*.

  [a] Vaccine serotype = *S. pneumoniae* serotypes included in PCV7 (4, 6B, 9V, 14, 18C, 19F, 23F) for Phase 1 & PCV 10 (PCV7 plus 1,5,7F) for Phase 2.

  [b] Non vaccine serotype = *S. pneumoniae* serotypes not included in PCV7 for Phase 1 & not included in PCV10 for Phase 2.

There was a significant decline in carriage of vaccine type *S. pneumoniae* serotypes in both groups in the second phase and an increase in non-vaccine serotypes between time periods.

Higher rates of NP carriage of *H. influenzae* or *S. pneumoniae* occurred in children undergoing VT insertion compared with non-otitis prone children in both phases.

3.6. Molecular differentiation of Haemophilus species

In both phases, isolates of Haemophilus (281 in Phase 1 and 375 in Phase 2) were further characterised by molecular methods to show 96% were *H. influenzae* and 4% were *H. haemolyticus*. Of the *H. influenzae* 99% were non-typeable in Phase 1 and 97% in Phase 2. Capsulated isolates from Phase 2 were further typed as type e (7 isolates) and type f (4 isolates). In Phase 2, 14 children had *H. haemolyticus* identified in the NP alone, with 1 child having the organism concurrently identified in MEF.

3.7. Streptococcus pneumoniae serotyping

Thirty-two isolates of *S. pneumoniae* from MEF from 25 children were available for serotyping in Phase 1 and 29 isolates from 25 children in Phase 2 (Fig. 1). Serotype 19A was the dominant serotype detected in MEF in Phase 2. Amongst serotypes isolated from

NP (Fig. 2), changes were seen in the VT group and non-otitis prone children between phases. In Phase 1 serotype 19F was the most common serotype followed by 19A in both groups. In Phase 2 19A was the most common serotype identified in NP followed by 6C and 35B. There were differences in serotypes carried between children undergoing VT surgery and those in the comparison group. In the VT group 19A was most common followed by 35B and 6C whereas in the comparison group 6C was most common followed by 19A.

3.8. Antibiotic susceptibilities

Overall there was no change in the antibiotic susceptibility profile of the 3 otopathogens between time periods. *H. influenzae* isolates from MEF and NP were commonly amoxycillin sensitive (Phase 1: 80% and Phase 2: 84%) and infrequently had detectable β-lactamase (10% and 8%). There was no significant change for *M. catarrhalis* susceptibility between phases with the majority having detectable β-lactamase present conferring resistance to amoxycillin (Phase 1: 87%; Phase 2: 95%) with resistance to other antibiotics uncommon.

For *S. pneumoniae* reduced susceptibility to penicillin (MIC > 0.06) was found in almost 1/3 of isolates in both time periods (64/206 (31%) Phase 1 and 75/228 (33%), Phase 2). Amongst
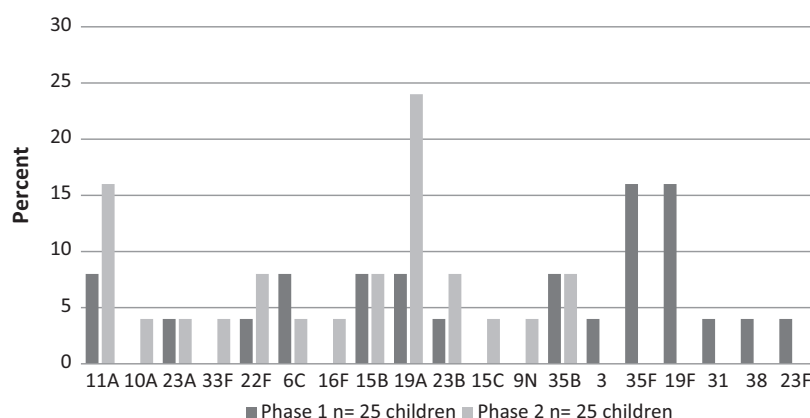

Fig. 1. Comparison of *Streptococcus pneumoniae* serotypes isolated from middle ear fluid in children undergoing grommets between time periods.
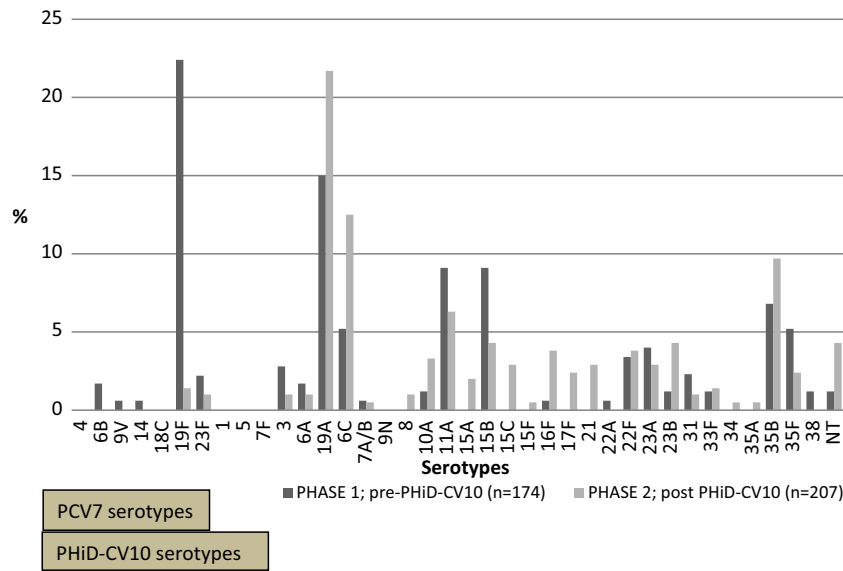
Fig. 2. Comparison of nasopharyngeal *Streptococcus pneumoniae* serotypes from children aged less than 3 years over 2 time periods.

S. pneumoniae with penicillin resistance (MIC ⩾ 2 µg/mL), 15/16 (94%) were multidrug-resistant (resistant to ⩾3 additional antibiotic classes) which was also unchanged compared with Phase 1 (19/21 (90%) of *S. pneumoniae* with penicillin resistance were also multidrug-resistant).

In Phase 1 the majority of penicillin resistant and multidrug-resistant isolates were serotype 19F (18/19 isolates) with the remaining one being serotype 19A. In Phase 2 where 19F was an uncommon serotype, the majority of the penicillin and multidrug-resistant isolates were serotype 19A (12/15; 80%).

4. Discussion

Our study provides the most comprehensive evaluation of the microbiology of otitis media to date New Zealand children. We have been unable to demonstrate changes in the microbiology of OM and NP carriage in otitis-prone children, between a cohort vaccinated with PCV7 and a later cohort vaccinated with PHiD-CV10. The persisting dominance of NTHi in middle ear fluid and NP carriage in our otitis prone children, without change in the rates of detection or ratios when compared with other otopathogens, suggests that the introduction of the PHiD-CV10 vaccine has not impacted on NTHi in established ear disease in this age group. This has implications for vaccine development in combating NTHi as an upper respiratory pathogen.

Strengths of our study include the high enrolment through both phases of the study, with over 75% recruitment of all children undergoing ventilation tube procedure captured from NZ's two major cities. Our cohort time periods were recruited with identical inclusion criteria and seasonally matched, yet separated by 3 years with a full 2 year period following PHiD-CV10 introduction to the national schedule. This enabled the Phase 2 cohort to be >90% exclusively PHiD-CV10 exposed. Both demographic data and risk factors examined in our cohorts were consistent over the two time periods.

Increasing experience with PHiD-CV10 has shown effectiveness against invasive pneumococcal disease (IPD) in children in Finland [30], Brazil [31], Netherlands [32], and Quebec, Canada [33,34]. A randomised controlled trial in South America involving over 20,000 children demonstrated efficacy of PHiD-CV10 in reducing radiologic pneumonia in vaccine recipients by over 20% and vaccine efficacy of 16% against clinical AOM. However the small

numbers of presentations of OM and very small numbers of MEF cultures limited assessment of impact on OM of the *H. influenzae* component of AOM in that study [35]. More recently a reduction in incidence of both AOM presentations and pneumonia at a large children's hospital in Iceland has been observed after introduction of PHiD-CV10 to their national schedule [36].

The lack of change on rate of carriage of NTHi in our study following PHiD-CV10 introduction reflect findings in other studies [18,37]. Lack of effect of PHiD-CV10 to prevent carriage of *H. influenzae* implies also that there will be no indirect (herd) protection from *H. influenzae* carriage. Our data does show near elimination in carriage of PHiD-CV10 vaccine-type *S. pneumoniae* serotypes in 3 years following PHiD-CV10 introduction.

H. haemolyticus is a non-pathogenic commensal organism which is difficult to distinguish from *H. influenzae* by conventional microbiology [25,38]. *H. haemolyticus* appears to be infrequently carried in the NP of our population and was only detected in 2 MEF samples. Our data demonstrates that *H. influenzae* remains the most common otopathogen carried in the nasopharynx in otitis-prone children; and in both cohorts over 95% of *H. influenzae* isolates were truly NTHi. We have also demonstrated the persisting NP carriage of Haemophilus is not due to *H. haemolyticus* replacement. However the complexity of NTHi and its evolution under selective pressure continues to be elucidated and increasingly other variants are recognised [39]. Ongoing monitoring of the molecular diversity within NTHi carriage isolates in the era of new generation pneumococcal conjugate vaccines continues to be important for future understanding of NTHi carriage and disease [40].

S. pneumoniae was least commonly detected otopathogen by culture or PCR in MEF or by culture of NP, with the overall detection rate not changed between Phases. However both the nasopharyngeal and MEF isolates demonstrated a change in serotype prevalence in the PHiD-CV10 era with increased proportions of serotype 19A, 6C and 35B. In Phase 1, the PCV-7 vaccinated cohort, 19F (a PCV7 vaccine serotype) remained frequently detected in NP carriage for both otitis-prone and comparison groups despite PCV7 introduction 3 years prior and high vaccine coverage (95% of Phase 1 cohort received at least 3 doses of PCV). Infrequent detection of 19F in both NP and in MEF in Phase 2 suggests effectiveness of PHiD-CV10 against this serotype. However serotype 19A is considered a cross-reactive serotype with 19F and

PHiD-CV10 licensing studies showing improved immunogenicity in infants receiving PHiD-CV10 who produced cross reactive antibodies to 19A when compared with PCV7 vaccinated infants [41,42]. Post licensure effectiveness analyses from both Brazil and Finland support some cross protection against IPD from serotype 19A [30,31]. Our data does not support PHiD-CV10 preventing carriage of non-vaccine serotype 19A. In New Zealand IPD is notifiable and national data demonstrates decline of IPD due to 19F in children aged <2 years since PCV introduction. Although IPD has declined, serotype 19A is now the commonest serotype causing IPD in children aged <2 years (7/23 IPD cases in under 2 year olds in 2013 and 12/24 in 2014) [13]. Subsequently PHiD-CV10 has been replaced by PCV13 in the NZ national immunisation schedule 2014.

Our data demonstrates a shift from 19F to 19A as the serotype currently associated with multidrug-resistance and penicillin resistance [43,44]. Both children undergoing VT surgery and those in non-otitis prone groups had high rates of antibiotic pre-treatment (>50% had received antibiotics in last month) which was consistent between time periods. High antibiotic exposure seen in our cohort and recognised in New Zealand [45] is known to impact on pneumococcal population genomics and capsular switching [46]. Therefore both high antibiotic exposure along with apparent lack of effectiveness of PHiD-CV10 in preventing carriage of serotype 19A, may be responsible for our observed increase in serotype 19A.

A small number of completely unvaccinated children and children who received an incomplete course of PHiD-CV10 or had received one or more doses or PCV13 were included in the study. However the vast majority of the Phase 2 cohort received at least 3 doses of PHiD-CV10. In addition our cohort was collected at least 2 years after implementation of PHiD-CV10 which means indirect (herd) effects are likely on those non-vaccinated or partly vaccinated children.

Our data was not designed to look at incidence of rAOM nor OME. As others have suggested using ventilation tube insertion as an indirect measure of vaccine impact is fraught with difficulty, as it is prone to influence from local guidelines, surgical referral patterns, parent or cultural acceptance of surgical procedure and inequity in socioeconomic status [47,48].

Our study was powered to detect a decrease of 20% in NTHi infection; it is possible a smaller reduction may have occurred below the detection limits of our study. However the frequency of NTHi detection in MEF in our study was greater than initially accounted for in our sample size calculations.

As NTHi is clearly an important pathogen in established ear disease, there is need for head to head comparison through randomised controlled trial to determine impact on otitis media with PHiD-CV10 and other pneumococcal conjugate vaccines [49]. For NZ assessment of AOM and OME presentations both to hospital and family practitioners are also warranted to look at pneumococcal vaccination impact on young children, acknowledging children presenting for VT insertion are a small proportion of overall children suffering from ear disease.

5. Conclusion

This large multicentre multiphase study represents the most comprehensive national data collection on the microbiology of middle ear disease and nasopharyngeal carriage in New Zealand children. We were unable to identify any significant differences between the two cohorts in the rates of identification of NTHi carriage or presence in middle ear fluid collected during VT insertion. The introduction of PHiD-CV10 vaccine appears to have had no significant impact on the microbiology of MEF in children receiving VT or nasopharyngeal carriage in New Zealand children.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.05.041.

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