Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

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

ARTICLE INFO

Article history:
Received 5 February 2016
Received in revised form 4 May 2016
Accepted 23 May 2016
Available online xxxx

Keywords:
Otitis media
Pneumococcal conjugate vaccine
Nasopharyngeal carriage
Child

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 $\geqslant$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].

Abbreviations: PCV, pneumococcal conjugate vaccine; NP, nasopharyngeal; MEF, middle ear fluid; NTHi, non-typeable *Haemophilus influenzae*; PHiD-CV10, 10 valent pneumococcal conjugate vaccine *Synflorix*; VT, ventilation tube insertion (grommet); NZ, New Zealand; OM, otitis media; OME, otitis media with effusion; AOM, acute otitis media; rAOM, recurrent acute otitis media; IPD, invasive pneumococcal disease; PCR, polymerase chain reaction.

* Corresponding author at: Department of Paediatrics, Child and Youth Health, Faculty of Medical and Health Sciences, The University of Auckland, Level 12, Support Building, Auckland District Health Board, Park Road Grafton, Auckland 1001, New Zealand. Tel.: + 64 9 3074949x22559, Mobile: +64 212417719; fax: +64 9 307 8977.

E-mail address: e.best@auckland.ac.nz (E.J. Best).

http://dx.doi.org/10.1016/j.vaccine.2016.05.041
0264-410X/

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 $\geqslant$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

Please cite this article in press as: Best EJ et al. Pneumococcal vaccine impact on otitis media microbiology: A New Zealand cohort study before and after the introduction of PHiD-CV10 vaccine. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.05.041