Epidemiol. Infect. (2014), **142**, 2186–2194. © Cambridge University Press 2013
doi:10.1017/S0950268813003178

Five-year prospective study of paediatric acute otitis media in Rochester, NY: modelling analysis of the risk of pneumococcal colonization in the nasopharynx and infection

V. FRIEDEL[1], S. ZILORA[2], D. BOGAARD[2], J. R. CASEY[1] AND M. E. PICHICHERO[1]*

[1] *Center for Infectious Diseases and Immunology, Rochester General Hospital Research Institute, Rochester, NY, USA*
[2] *Department of Information Sciences and Technologies, Rochester Institute of Technology, Rochester, NY, USA*

Received 19 June 2013; Final revision 25 October 2013; Accepted 23 November 2013; first published online 17 December 2013

SUMMARY

During a 5-year prospective study of nasopharyngeal (NP) colonization and acute otitis media (AOM) infections in children during the 7-valent pneumococcal conjugate vaccine (PCV) era (July 2006–June 2011) we studied risk factors for NP colonization and AOM. NP samples were collected at ages 6, 9, 12, 15, 18, 24, and 30 months during well-child visits. Additionally, NP and middle ear fluid (MEF) samples were collected at onset of every AOM episode. From 1825 visits ($n=464$ children), 5301 NP and 570 MEF samples were collected and analysed for potential otopathogens. Daycare attendance, NP colonization by *Moraxella catarrhalis*, and siblings aged <5 years increased the risk of *Streptococcus pneumoniae* NP colonization. NP colonization with *S. pneumoniae*, *M. catarrhalis*, or *Haemophilus influenzae* and a family history of OM increased the risk of AOM. Risk factors that increase the risk of pneumococcal AOM will be important to reassess as we move into a new 13-valent PCV era, especially co-colonization with other potential otopathogens.

Key words: Acute otitis media, children, *Haemophilus influenzae* (non-typable), *Moraxella catarrhalis*, pneumococcal conjugate vaccine (PCV), *Streptococcus pneumoniae*.

INTRODUCTION

Acute otitis media (AOM) is a very common childhood disease that is responsible for many paediatric office visits [1]. Previous studies have shown that by the age of 3 years 80% of children will have experienced one episode of AOM, and by the age of 7 years 40% of children will have experienced $\geqslant 6$ episodes [2]. The peak incidence of AOM occurs between the ages of 6 and 18 months [3]. AOM is associated with upper respiratory viral infections [4–10]. Children that suffer from recurrent or chronic episodes of AOM may experience temporary or permanent hearing loss, delayed language development, poor auditory processing, delayed psychosocial and cognitive development, and an adverse impact on educational progress [11, 12].

Due to the large public health burden of AOM in children, in October 2000, a 7-valent pneumococcal conjugate vaccine (PCV7) was introduced into the routine schedule of immunizations for children [13]. At the time, seven serotypes (4, 6B, 9 V, 14, 18C, 19F, 23F) accounted for 80% of pneumococcal AOM in young children; that data dictated the serotypes included in PCV7 [14]. Shortly after the

* Address for correspondence: M. E. Pichichero, MD, Rochester General Hospital Research Institute, Center for Infectious Diseases and Immunology, 1425 Portland Avenue, Rochester, NY 14621, USA.
(Email: michael.pichichero@rochestergeneral.org)

introduction of PCV7, there was a marked reduction in AOM caused by serotypes included in the vaccine [15–22].

In 2006, we began a longitudinal, multi-year, prospective study aiming to: (1) prospectively collect and study nasopharyngeal (NP) samples, middle ear fluid (MEF), and serum from young children (aged 6–30 months) who become otitis prone, who have infrequent otitis, or who remain otitis free; and (2) evaluate the role of specific immune responses to candidate vaccine bacterial proteins expressed by *S. pneumoniae* and non-typable *H. influenzae* (NT*Hi*) otopathogens. We have previously reported on microbiological aspects of the project and on the immunological results. Our study population is the only group of children in the USA currently undergoing comprehensive, ongoing monitoring for potential risk factors for NP colonization and AOM, using tympanocentesis to prove every case of AOM. We sought to understand, risk factors that may influence changes in frequency of otopathogen infections as new vaccines are introduced. Here we used a relational database and statistical modelling to assess risk factors for pneumococcal NP colonization and AOM during the PCV7 era, 2006–2011.

DESIGN AND METHODS

Study community

Study participants were recruited from private paediatric offices in Rochester, NY. Rochester is located in Monroe County in Western NY. The population of Monroe County was 744434 in 2010 (US Census Bureau: State and County Quick Facts), with <6% of the population aged <5 years. In Monroe County, 78·0% of the population identifies as White, 16·0% African American, 3·4% Asian, and 2·6% other. Of the 78% identifying as White, 72·6% identify as non-Hispanic White. The median household income for Monroe County in 2010 was $51303 and the total number of births for the year was 8466.

Child subject recruitment

About 85% of the subjects were recruited from a single private paediatric practice (Legacy Pediatrics, Rochester NY, main contributor, two paediatricians). Four other private paediatric groups joined in the recruitment effort by referral of patients to Legacy Pediatrics.

Eligibility

Children had to be aged at least 6 months to participate in this study. Inclusion criteria were: healthy, full-term birth, no craniofacial anomalies, no known immune deficits, and no AOM events prior to enrolment at age 6 months. All participants received four doses of PCV7 at ages 2, 4, 6, and 15 months until October 2010 when PCV13 was used to complete the PCV series depending on the date of their enrolment. Parental (both parents) consent was obtained prior to any study procedures. PCV7 contained the seven most prevalent serotypes causing *invasive pneumococcal disease* in young children at that time (4, 6B, 9V, 14, 18C, 19F, 23F). PCV13 contains 13 serotypes of pneumococci (serotypes in PCV7 plus 1, 3, 5, 6A, 7F, 19A) [23]. This study was approved by the University of Rochester IRB and subsequently by the Rochester General Hospital IRB.

Sampling

At the first visit, parents were asked to complete a questionnaire regarding demographic parameters and risk factors for AOM including: date of birth, gender/sex, race/ethnicity, height, weight, number and age of siblings, daycare attendance (none, centre, or home setting), breastfeeding status, exposure to tobacco smoke (defined as any smoker in the home), family history of OM, and allergies/atopy. Parents agreed to seven scheduled visits at ages 6, 9, 12, 15, 18, 24 and 30–36 months. At each of the above visits, parents were asked if there were any changes to the participant's demographic/risk factor history and whether the child had a current or recent upper respiratory tract infection within 2 weeks of the study visit. At each visit, the following biological samples were collected from the participants: nasal wash (NW), NP swab, blood, and throat culture (TC). At an unscheduled AOM visit, the following samples were collected: MEF (both or a single tympanocentesis procedure depending on whether the infection was bilateral or unilateral), NW, NP swab, blood, and TC.

Sampling procedures

For NW samples, 2 ml saline solution was squirted into the participant's nose with a rubber bulb syringe (Bard ear syringe). For NP samples, a wire nasopharyngeal flox brush was passed to the posterior nose and

https://doi.org/10.1017/S0950268813003178 Published online by Cambridge University Press

Table 1. *Enrolment during the 5-year study (n = 464 patients with 1825 visits)*

Respiratory period	No. of enrolments	No. of visits (scheduled/unscheduled/follow-up)	No. of NP samples*	No. of MEF samples†
June 2006–June 2007	91	198 (91/65/42)	578	101
July 2007–June 2008	61	251 (174/52/25)	738	80
July 2008–June 2009	53	310 (242/50/18)	898	74
July 2009–June 2010	125	484 (339/103/42)	1427	166
July 2010–June 2011	134	582 (459/90/33)	1660	149

NP, Nasopharyngeal; MEF, Middle ear fluid.
* NP samples include throat culture, nasal wash, and NP swab.
† MEF samples include both unilateral and bilateral results in addition to broths collected for each MEF sample.

swabbed (Floq Swabs, Italy). For TC samples, sterile cotton-tipped applicators were used to swab the back of the child's throat (Select Medical Products, USA). For blood sampling, a venepuncture was performed and collection was into a heparinized tube. For MEF samples, an 8% tetracaine solution was instilled into the external auditory canal after placement of an otowick, then after ~15 min a 20-gauge 3·5″ spinal needle attached to a 3 cc syringe was used to puncture the tympanic membrane and collect MEF. All samples were collected at the paediatric office and transported to the laboratory via courier. For the remainder of this report, TC, NP, and NW samples will collectively be referred to as NP samples.

Microbiology and molecular biology

Microbiology processing, identification, and molecular testing for organism identification have been previously described [24]. The four bacteria that were analysed in this report were *Streptococcus pneumoniae*, *Haemophilus influenzae* (non-typable), *Moraxella catarrhalis*, and alpha-haemolytic streptococci (AHS).

Digital data handling

All collected data were stored and accessed via a custom-developed AOM database application that consisted of a web-based user interface, data access and manipulation software, and a MySQL database. The application was designed with the following goals: to provide a reliable and accessible repository of project data, to provide screening tools for initial analysis of the data, to maintain the data and access to the data in a secure manner, to maximize extensibility, and to provide an intuitive user-friendly interface. Due to the breadth of the study objectives, samples from one patient's visit (i.e. a patient's third visit) could be used for multiple analyses; including otopathogen colonization in multiple samples (NW, TC, and in the case of AOM, MEF samples), antibody measurements (>1 antibody per otopathogen), and patient immunology responses; the in-house relational database was created to link all of these individual test results to the originating sample and patient. The database system was constructed to be compliant with the Code of Federal Regulations, Title 21, Part 11 that states all electronic records must be trustworthy and reliable [25]. This was achieved via a set of security and data integrity processes that were incorporated in the system. Data integrity was achieved by a combination of utilizing a fully normalized database schema to eliminate any data anomalies and a tightly controlled data input process that maximized user selection of predetermined values and mitigated erroneous data entry.

Statistical analyses

For the modelling analysis, the main outcomes of interest were the risk of AOM (AOM visits *vs.* non-AOM visits) and the risk of *S. pneumoniae* colonization in the NP of children (presence in the NP *vs.* no presence in the NP). All statistical analyses were conducted by using Stata version 12.0 (StataCorp., USA). Repeated-measures logistic regression with an unstructured correlation structure using the command XTMELOGIT was used for the analysis of the two models (the unit of study was the samples collected during a paediatric office visit). For both models, a univariate analysis was conducted first (a significance level of $P = 0{\cdot}10$ was used for the univariate analysis), then all variables that were significant in the univariate analysis, were included in the

https://doi.org/10.1017/S0950268813003178 Published online by Cambridge University Press

full model. A backwards stepwise procedure was used to find the most parsimonious final model. A likelihood-ratio test was used to compare models as variables were dropped from previous to current models. If the two models changed significantly with the removal of a variable, then that variable was kept in the full model. Host factors that were included in this analysis were breastfeeding (<6 months *vs.* ⩾6 months), daycare attendance (centre/home setting *vs.* none), age (months), family history of OM (yes/no), siblings in the home (<5 years old, ⩾5 years old, both <5 and ⩾5 years old), sex, exposure to tobacco smoke (yes/no), and history of AOM episodes (⩾1) (yes/no). Results for the model were expressed as odd ratios (ORs) with 95% confidence intervals (CIs).

RESULTS

Recruitment results and demographics

During the 5-year period (June 2006–June 2011), 464 children were enrolled in the study. There were 5301 NP samples and 570 MEF samples collected (Table 1). In total, 159 patients completed the study by 30 June 2011, the end of the study period of this paper. Of the 159 patients, 21·4% completed all seven visits, another 31·4% completed 6/7 visits and the remaining 47·2% completed 5/7 visits. At the end of the 5-year time-frame for this report, the remaining 305 children enrolled were still participating in the study, and therefore had only completed a portion of their scheduled seven visits. Table 2 shows the demographic data for the study cohort. About 40% of the 464 patients experienced ⩽2 AOM events during their participation in the study up to 30 June 2011.

Table 2. *Demographics of children enrolled during the study period (n = 464)*

	n	%
Age (months)		
Mean (S.D.)	8·6	5·0
Range	5·3–39·7	—
Ethnicity		
White	335	72·2
African American	72	15·5
Asian	8	1·7
Hispanic/Latino	21	4·5
Other/mixed	28	6·1
Sex		
Male	240	51·7
Female	224	48·3
Smokers in the home*		
Yes	68	14·7
No	383	82·5
Familial history of OM*		
No	244	52·3
Yes	206	44·4
Daycare*		
None	304	65·5
Home setting or centre	152	32·8
Breastfeeding		
⩾6 months	131	28·2
<6 months	257	55·4
Unknown†	76	16·4
Sibling status		
No siblings	174	37·5
<5 years old	184	39·7
⩾5 years old	50	10·8
Both <5 and ⩾5 years old	49	10·6
No. of AOM episodes during study participation		
0	248	53·5
1–2	183	39·4
3–5	27	5·8
⩾6	6	1·3

AOM, Acute otitis media.
* Missing data by variable: smoking status (*n* = 13); family history (*n* = 14); daycare (*n* = 8); sibling status (*n* = 7).
† There were 76 patients missing breastfeeding data, questionnaire captures data at the time of the visit.

Colonization

During the 5-year study there were a total of 1825 visits; 1305 (71·5%) were scheduled healthy visits, 360 (19·8%) were during episodes of AOM, and 160 (8·8%) were follow-up visits (3 weeks after an AOM episode) (Table 3). The predominant otopathogen during episodes of AOM was *S. pneumoniae* followed by NT*Hi*. The predominant otopathogen during health visits was *M. catarrhalis* followed closely by *S. pneumoniae*. Compliance with a follow-up visit after an AOM episode was 44·3%; the predominant otopathogen in the NP at the follow-up visit was NT*Hi*.

We observed 31 different serotypes of *S. pneumoniae* during the 5 years of this study. The most common serotypes/serogroups cultured during health visits were 19A (89 isolates), 15 (66 isolates), and 23B (40 isolates) and the most common during AOM visits were 19A (39 isolates), 15 (23 isolates), and 11 (nine isolates) in NP samples and 19A (25 isolates), 15 (seven isolates), and 11 (three isolates) in MEF samples (Fig. 1).

https://doi.org/10.1017/S0950268813003178 Published online by Cambridge University Press

Table 3. *Children enrolled during the 5-year study period (n = 1825 visits for 464 patients)*

	Total	*S. pneumoniae*	NT*Hi*	*M. catarrhalis*
Scheduled visits (healthy)	1305	404	168	476
Acute otitis media visits	360	188	171	153
Follow-up visits	160	47	64	48
Total	1825	639	403	677

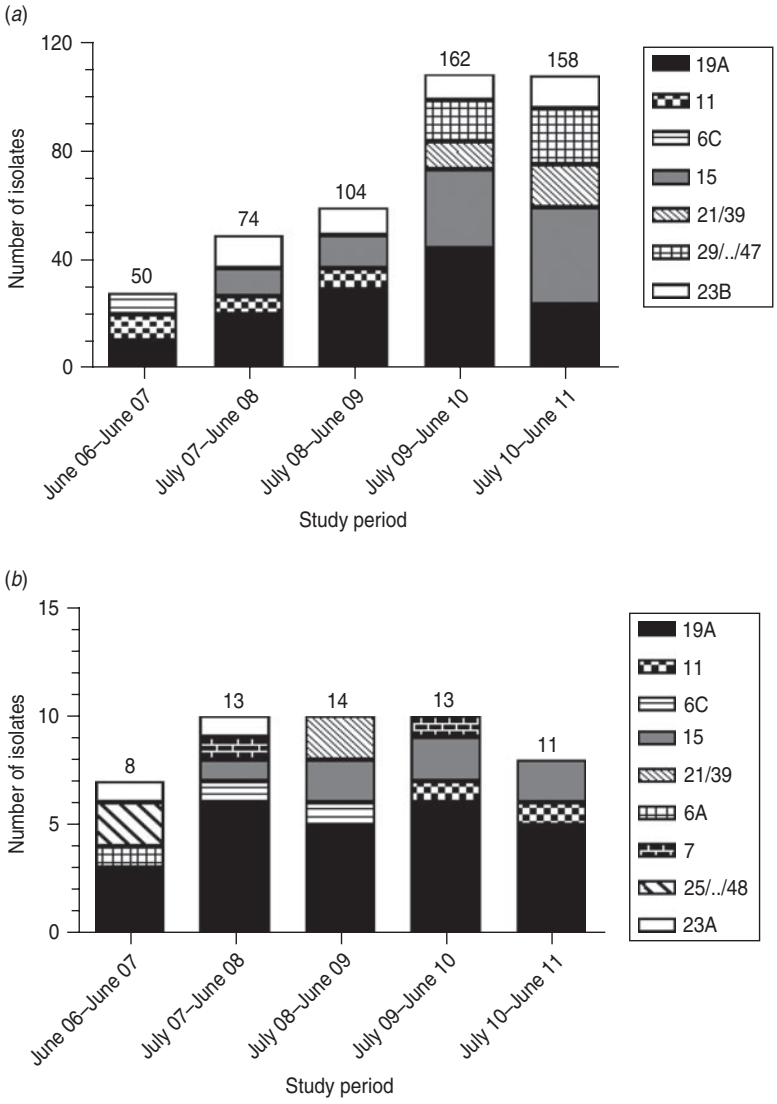

Fig. 1. Number of *S. pneumoniae* isolates by study period and circulating serotypes/serogroups. (*a*) Nasopharyngeal samples (total $n = 548$), (*b*) middle ear fluid samples (total $n = 59$). Not all circulating serotypes/serogroups are represented in this figure, as some were only present in <3 samples.

During the 5-year study, there were 360 visits due to AOM. A Pearson's correlation analysis of bacteria present in both the NP and MEF during these visits indicated that NT*Hi* had the strongest positive correlation (0·44), *S. pneumoniae* had a weak positive correlation (0·25), and *M. catarrhalis* had no correlation (0·19) between presence of the bacteria in the NP and MEF during episodes of AOM.

https://doi.org/10.1017/S0950268813003178 Published online by Cambridge University Press

Table 4. *Predicted outcome of model A (AOM in young children) and model B (*S. pneumoniae *NP colonization in young children)*

	Model A (AOM)		Model B (*S. pneumoniae*)	
Parameters	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (months)				
<9	Ref.		Ref.	
9–18	0·99 (0·67–1·46)	0·95	1·01 (0·77–1·34)	0·92
>18	1·08 (0·68–1·70)	0·75	1·13 (0·82–1·57)	0·46
Daycare				
No	Ref.		Ref.	
Yes	1·29 (0·86–1·92)	0·08	**2·27 (1·66–3·10)**	**<0·01**
Family history of OM				
No	Ref.		—	—
Yes	**1·59 (1·06–2·37)**	**0·02**	—	—
NT*Hi* in the NP				
No	Ref.		Ref.	
Yes	**3·37 (2·33–4·88)**	**<0·01**	1·10 (0·82–1·48)	0·51
AHS in the NP				
No	Ref.		Ref.	
Yes	**0·24 (0·15–0·39)**	**<0·01**	1·17 (0·80–1·72)	0·40
S. pneumoniae in the NP				
No	Ref.		n.a.	n.a.
Yes	**2·39 (1·70–3·36)**	**<0·01**	n.a.	n.a.
M. catarrhalis in the NP				
No	Ref.		Ref.	
Yes	**1·87 (1·31–2·67)**	**<0·01**	**1·82 (1·43–2·33)**	**<0·01**
Breastfeeding				
<6 months	Ref.		—	—
⩾6 months	1·44 (0·96–2·15)	0·08	—	—
Siblings in the home				
None	—	—	Ref.	
<5 years old	—	—	**1·60 (1·14–2·21)**	**0·01**
⩾5 years old	—	—	0·90 (0·51–1·56)	0·70
Both	—	—	**1·65 (1·01–2·68)**	**0·04**
Tobacco exposure				
No	—	—	Ref.	
Yes	—	—	0·90 (0·57–1·41)	0·64
History of AOM episodes				
No	—	—	Ref.	
Yes	—	—	**1·99 (1·46–2·72)**	**<0·01**
Random effects† constant	0·71 (0·46–1·10)	0·16	0·83 (0·63–1·09)	0·12

AOM, Acute otitis media; OR, odds ratio; CI, confidence interval; NT*Hi*, non-typable *Haemophilus influenzae*; NP, nasopharynx; AHS, alpha-haemolytic streptococci; Ref., reference group; n.a., not applicable.
*Bold odds ratios indicate *P* value ⩽0·05.
† For random effects: estimate and 95% CI are given as well as the standard error.

Multilevel modelling

Repeated-measures logistic regression models predicting the risk of AOM by any otopathogen and the risk of *S. pneumoniae* colonization in the NP are shown in Table 4. In the model for risk of AOM, the following factors increased the risk of AOM: family history of OM ($P = 0{\cdot}02$), NT*Hi* in the NP ($P < 0{\cdot}01$), *S. pneumoniae* in the NP ($P < 0{\cdot}01$) and *M. catarrhalis* in the NP ($P < 0{\cdot}01$). The presence of AHS in the NP decreased

https://doi.org/10.1017/S0950268813003178 Published online by Cambridge University Press

the risk of AOM ($P<0{\cdot}01$). In this model, breastfeeding for $\geqslant 6$ months increased the odds of AOM unexpectedly, but the result was not statistically significant.

In the second model (model B), the following factors increased the risk of *S. pneumoniae* colonization in the NP: children who attended daycare ($P< 0{\cdot}01$) and who had *M. catarrhalis* present in the NP ($P= 0{\cdot}01$). Children with siblings aged <5 years had an increased risk of *S. pneumoniae* NP colonization compared to children with no siblings ($P=0{\cdot}01$). In this model, children with a history of $\geqslant 1$ AOM episodes had an increased risk of *S. pneumoniae* colonization in the NP ($P<0{\cdot}01$). The correlation between *S. pneumoniae* colonization in the NP and history of AOM episodes was weak at 0·17.

We tested for linear age dependence by splitting the children into three age groups: <9 months, 9–18 months, and >18 months. Using the youngest group as the reference group in modelling we found age was not a significant factor in the models.

DISCUSSION

Here we report for the first time the results of the first 5 years of our ongoing prospective study on pneumococcal NP colonization and middle ear infections in children to the end of the PCV7 era in the USA. Results from this analysis show that *S. pneumoniae* was the most prevalent bacteria isolated from the NP during episodes of AOM and *M. catarrhalis* was the most prevalent bacteria isolated from the NP in healthy samples. The most prevalent serotype of *S. pneumoniae* isolates was 19A, in agreement with our earlier smaller study [26] and others [27, 28]. A new finding is the emergence of serogroups 15 and 11 as increasingly common NP colonizers and causes of AOM.

In our model, children with a family history of OM had an increased risk of AOM (OR 1·59, $P= 0{\cdot}02$). A similar result was observed in families in Greenland [29] and researchers have suggested a genetic link for OM risk [30]. In our study population we found that breastfeeding for $\geqslant 6$ months did not diminish the risk for AOM more than breastfeeding for a shorter time. We have no explanation for the non-significant trend of more frequent AOM in breastfed infants. In our study population we have previously reported a protective effect of breastfeeding [31].

Our results from the model for AOM confirm previous reports regarding the increased risk of AOM in children with *S. pneumoniae, M. catarrhalis*, and NT*Hi* colonization in the NP [32, 33]. The model also showed that AHS colonization in the NP decreased the risk of AOM. This result is in agreement with our previous study [34] and others [35] that upper respiratory commensals like AHS interfere with the colonization of potential otopathogens.

Host factors that increased the risk of *S. pneumoniae* colonization in the NP included daycare attendance and having siblings aged <5 years. Children who attend daycare and have siblings aged <5 years most likely attend daycare with their siblings; therefore, both children are exposed to outside pathogens. Daycare attendance is a known risk factor for AOM [20, 36].

The risk of *S. pneumoniae* NP colonization increased during concurrent *M. catarrhalis* NP colonization. Previous studies have observed a similar positive association between *S. pneumoniae* and *M. catarrhalis* [37]. Our group has found a synergistic (positive) association between *M. catarrhalis* and *S. pneumoniae* NP colonization in healthy children [38]. We have also observed a negative association between NT*Hi* and *S. pneumoniae* and *M. catarrhalis* and NT*Hi* NP colonization during episodes of AOM [38]. Our models indicated that a history of AOM episodes increases the risk of *S. pneumoniae* NP colonization in children. There may be genetic or epigenetic factors that influence the innate and/or adaptive immune response in the NP environment caused by intercurrent viral upper respiratory bacteria that facilitates the occurrence of AOM. Our group is now analysing the changing NP environment before and after AOM in otitis-prone children.

In late 2010, PCV13 became available in the USA ending the PCV7 era. The study period of June 2010–June 2011 showed a small reduction in serotype 19A prevalence in NP samples following the introduction of PCV13. With the anticipated reduction in serotype 19A, we predict other serogroups not included in PCV13 will fill this niche, similar to the serotype replacement observed after the introduction of PCV7 [39].

This study is not without limitations. The study occurred predominantly in one, suburban paediatric practice site, although it is the only site in the USA conducting such prospective analyses of NP colonization and AOM events. Due to the voluntary basis of this study, not all patients were observed at all-time points (visits). The majority of children enrolled in the study who did not complete all seven visits were

https://doi.org/10.1017/S0950268813003178 Published online by Cambridge University Press

older than 6 months when enrolled and some children moved out of the Rochester area, therefore sample collection for these children was not complete.

CONCLUSION

This prospective, longitudinal study of a large cohort of children over the 5-year time-span 2006–2011 provides insight into the epidemiological risk and protective factors for NP colonization and AOM by pneumococci in children during the second half of the PCV7 era.

The study is unique due to the use of tympanocentesis to definitively prove every AOM infection. There were no other identical or similar studies of this nature ongoing in the USA or in the world to our knowledge so these are the only data collected for the time-frame of this investigation. The results reported here are highly relevant to those countries still exclusively or predominantly using PCV7. As we continue this study during the next 5 years in the same community, recruiting from the same population, using the same methods we will be able to compare the results reported here as we move into the PCV13 era.

ACKNOWLEDGEMENTS

This study was supported by NIH NIDCD RO1 08671. We thank the nurses and staff of Legacy Pediatrics and the collaborating paediatricians from Sunrise Pediatrics, Westfall Pediatrics, Lewis Pediatrics and Long Pond Pediatrics, the parents who consented and the children who participated in this study. We also thank Katerina Czup for her assistance.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Centers for Disease Control and Prevention (CDC).** Pneumococcal disease. In: *Epidemiology and Prevention of Vaccine-Preventable Diseases: 'The Pink Book'*, 12th edn, 2012, pp. 233–248.
2. **Casselbrant M, Mandel E.** Epidemiology. In: Rosenfeld R, Bluestone C, eds. *Evidence-Based Otitis Media*. Hamilton, Ontario: BC Decker, 2003, pp. 147–162.
3. **Teele DW, Klein JO, Rosner B.** Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study. *Journal of Infectious Disease* 1989; **160**: 83–94.
4. **Vergison A.** Microbiology of otitis media: a moving target. *Vaccine* 2008; **26** (Suppl. 7): G5–10.
5. **Chonmaitree T, *et al.*** Viral upper respiratory tract infection and otitis media complication in young children. *Clinical Infectious Disease* 2008; **46**: 815–823.
6. **Arola M, Ziegler T, Ruuskanen O.** Respiratory virus infection as a cause of prolonged symptoms in acute otitis media. *Journal of Pediatrics* 1990; **116**: 697–701.
7. **Winther B, Doyle WJ, Alper CM.** A high prevalence of new onset otitis media during parent diagnosed common colds. *International Journal of Pediatric Otorhinolaryngology* 2006; **70**: 1725–1730.
8. **Heikkinen T, Ruuskanen O.** Temporal development of acute otitis media during upper respiratory tract infection. *Pediatric Infectious Disease Journal* 1994; **13**: 659–661.
9. **Massa HM, Cripps AW, Lehmann D.** Otitis media: viruses, bacteria, biofilms and vaccines. *Medical Journal of Australia* 2009; **191** (9 Suppl.): S44–49.
10. **Heikkinen T, Chonmaitree T.** Importance of respiratory viruses in acute otitis media. *Clinical Microbiology Review* 2003; **16**: 230–41.
11. **Acuin J.** Chronic suppurative otitis media. *Clinical Evidence (Online)* 2007; **2007**: 0507.
12. **Monasta L, *et al.*** Burden of disease caused by otitis media: Systematic review and global estimates. *PLoS One* 2012; **7**: e36226.
13. **Centers for Disease Control and Prevention (CDC).** Invasive pneumococcal disease in children 5 years after conjugate vaccine introduction – eight states, 1998–2005. *Morbidity and Mortality Weekly Report* 2008; **57**: 144–148.
14. **Pai R, *et al.*** Active Bacterial Core Surveillance Team. Post vaccine genetic structure of streptococcus pneumoniae serotype 19A from children in the United States. *Journal of Infectious Disease* 2005; **192**: 1988–1995.
15. **Pichichero ME, Casey JR.** Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. *Journal of the American Medical Association* 2007; **298**: 1772–1778.
16. **Pilishvili T, *et al.*** Active Bacterial Core Surveillance/Emerging Infections Program Network. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *Journal of Infectious Disease* 2010; **201**: 32–41.
17. **Beall BW, *et al.*** Shifting genetic structure of invasive serotype 19A pneumococci in the united states. *Journal of Infectious Disease* 2011; **203**: 1360–1368.
18. **Moore MR, *et al.*** Population snapshot of emergent Streptococcus pneumoniae serotype 19A in the united states, 2005. *Journal of Infectious Disease* 2008; **197**: 1016–1027.
19. **Sharma D, *et al.*** Pneumococcal carriage and invasive disease in children before introduction of the 13-valent conjugate vaccine: Comparison with the era before 7-valent conjugate vaccine. *Pediatric Infectious Disease Journal* 2013; **32**: e45–53.

20. **Cohen R, *et al.*** Risk factors for serotype 19A carriage after introduction of 7-valent pneumococcal vaccination. *BMC Infectious Disease* 2011; **11**: 95.
21. **Pichichero ME, Casey JR.** Evolving microbiology and molecular epidemiology of acute otitis media in the pneumococcal conjugate vaccine era. *Pediatric Infectious Disease Journal* 2007; **26** (10 Suppl.): S12–16.
22. **Centers for Disease Control and Prevention (CDC).** Licensure of a 13-valent pneumococcal conjugate vaccine (PCV13) and recommendations for use among children – advisory committee on immunization practices (ACIP), 2010. *Morbidity and Mortality Weekly Report* 2010; **59**: 258–261.
23. **Shapiro ED.** Prevention of pneumococcal infection with vaccines: An evolving story. *Journal of the American Medical Association* 2012; **307**: 847–849.
24. **Kaur R, *et al.*** Simultaneous assay for four bacterial species including alloiococcus otitidis using multiplex-PCR in children with culture negative acute otitis media. *Pediatric Infectious Disease Journal* 2010; **29**: 741–745.
25. **Office of the Federal Register NAaRA.** Code of Federal Regulations, Title 21, Part 11.1., 2012.
26. **Casey JR, Adlowitz DG, Pichichero ME.** New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. *Pediatric Infectious Disease Journal* 2010; **29**: 304–309.
27. **Pelton SI, *et al.*** Emergence of 19A as virulent and multidrug resistant pneumococcus in Massachusetts following universal immunization of infants with pneumococcal conjugate vaccine. *Pediatric Infectious Disease Journal* 2007; **26**: 468–472.
28. **Hanage WP, *et al.*** Carried pneumococci in Massachusetts children: The contribution of clonal expansion and serotype switching. *Pediatric Infectious Disease Journal* 2011; **30**: 302–308.
29. **Homoe P.** Otitis media in Greenland. Studies on historical, epidemiological, microbiological, and immunological aspects. *International Journal of Circumpolar Health* 2001; **60** (Suppl. 2): 1–54.
30. **Casselbrant ML, Mandel EM.** The genetics of otitis media. *Current Allergy Asthma Report* 2001; **1**: 353–357.
31. **Sabirov A, *et al.*** Breast-feeding is associated with a reduced frequency of acute otitis media and high serum antibody levels against NTHi and outer membrane protein vaccine antigen candidate P6. *Pediatric Research* 2009; **66**: 565–570.
32. **Ruohola A, *et al.*** Bacterial and viral interactions within the nasopharynx contribute to the risk of acute otitis media. *Journal of Infection* 2013; **66**: 247–254.
33. **Pettigrew MM, *et al.*** Upper respiratory tract microbial communities, acute otitis media pathogens, and antibiotic use in healthy and sick children. *Applied and Environmental Microbiology* 2012; **78**: 6262–6270.
34. **Friedel V, *et al.*** Impact of respiratory viral infections on alpha-hemolytic streptococci and otopathogens in the nasopharynx of young children. *Pediatric Infectious Disease Journal* 2013; **32**: 27–31.
35. **Tano K, *et al.*** Bacterial interference between pathogens in otitis media and alpha-haemolytic streptococci analysed in an in vitro model. *Acta Otolaryngologica* 2002; **122**: 78–85.
36. **Millar EV, *et al.*** Nasopharyngeal carriage of streptococcus pneumoniae in navajo and white mountain apache children before the introduction of pneumococcal conjugate vaccine. *Pediatric Infectious Disease Journal* 2009; **28**: 711–716.
37. **Jacoby P, *et al.*; Kalgoorlie Otitis Media Research Project Team.** Modelling the co-occurrence of streptococcus pneumoniae with other bacterial and viral pathogens in the upper respiratory tract. *Vaccine* 2007; **25**: 2458–2464.
38. **Xu Q, *et al.*** Nasopharyngeal bacterial interactions in children. *Emerging Infectious Diseases* 2012; **18**: 1738–1745.
39. **Wroe PC, *et al.*** Pneumococcal carriage and antibiotic resistance in young children before 13-valent conjugate vaccine. *Pediatric Infectious Disease Journal* 2012; **31**: 249–254.