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Association of *Streptococcus pneumoniae* nasopharyngeal colonization and other risk factors with acute otitis media in an unvaccinated Indian birth cohort

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SUMMARY

In order to study the epidemiology of acute otitis media (AOM) and *Streptococcus pneumoniae* nasopharyngeal colonization in the first 2 years of life, we followed up an unvaccinated birth cohort monthly and at visits when sick, with otoscopy to detect AOM and performed nasopharyngeal swabbing to detect *S. pneumoniae*. Serotyping of positive cultures was also performed. Of 210 babies who were enrolled at birth, 61 (29·05%) experienced 128 episodes of AOM [relative risk 2·63, 95% confidence interval (CI) 1·21–5·75] with maximum incidence in the second half of the first year of life. Episodes ranged from 1 to 7 (mean 2·1 episodes). Most (86·9%) babies with AOM had a positive culture swab giving an odds ratio (OR) of 1·93 (95% CI 1·03–3·62, $P = 0·041$) for this association. Other risk factors identified for AOM were winter season (OR 3·46, 95% CI 1·56–7·30, $P = 0·001$), upper respiratory infection (OR 2·43, 95% CI 1·43–4·51, $P = 0·005$); residents of small households were less likely to develop AOM (OR 0·32, 95% CI 0·17–0·57, $P < 0·01$). Common *S. pneumoniae* serotypes isolated during episodes were 19, 6, 15, 35, 7, 23, 9 and 10 which indicated a theoretical coverage for pneumococcal vaccines PCV10 and PCV13 constituent serotypes of 62·8%. We conclude that AOM in Indian infants is often associated with *S. pneumoniae* colonization of the nasopharynx as well as other risk factors.

Key words: Ear infections, epidemiology, immunization (vaccination), pneumococcal infection.

INTRODUCTION

Otitis media is one of the most common diseases of childhood in developed and developing countries [1, 2] and occurs in an acute or chronic form. Previous studies have shown that the prevalence of chronic suppurative otitis media and otitis media with effusion in children is high in India [3–6], and African countries [7, 8]. However, the incidence of acute otitis media (AOM) has generally been less well studied in developing countries. Studies from Western centres have shown that for children who have not received pneumococcal vaccination, the disease commences in infancy with a maximum incidence in the second half of the first year of life [9]. Comparative data regarding the epidemiology of AOM in Indian babies is lacking.

One of the prerequisites for both AOM and sinusitis is colonization of the nasopharynx by *Streptococcus pneumoniae* which is a natural occurrence whose incidence varies with age [10–13], season [12, 13] and family size [11]. This factor assumes clinical importance as it is a recognized precursor to invasive infections

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such as meningitis and pneumonia which are associated with considerable morbidity and mortality. *S. pneumoniae,* together with the other nasopharyngeal colonists *Haemophilus influenzae* and *Moraxella catarrhalis*, are responsible for most episodes of AOM in infants.

In an unvaccinated population it is important to ascertain the pattern and frequency of constituent vaccine serotypes of *S. pneumoniae* before the introduction of the vaccine in order to assess vaccine-induced changes in serotype patterns. In general, immunity is serotype specific, but cross-protection between related serotypes can occur. In India, although the pneumococcal conjugate vaccines (PCV) PCV10 and PCV13 are currently available, they did not form part of the mandatory immunization schedule prescribed by governmental agencies during the period of the study [14], with the result that large numbers of children were unprotected by pneumococcal vaccination. Moreover, few studies have analysed the pattern of nasopharyngeal colonization of normal Indian babies and compared this with described patterns across the world [13, 15].

The present study aimed to examine the association between *S. pneumoniae* colonization and AOM in an unvaccinated birth cohort which to the best of our knowledge has not previously been investigated in Indian infants. In addition, we sought to identify other risk factors for AOM in the same birth cohort.

MATERIAL AND METHODS

The study was conducted at the Rural Unit for Health and Social Affairs which is part of the Community Health Department, Christian Medical College, Vellore. The geographical area covered was K.V Kuppam rural development block located in the Vellore district of Tamil Nadu state in south India. The block comprises 90 villages and is divided into 18 peripheral service units, each of which has an average population of 5000–7000. The geographical area is completely rural and agriculture is the main occupation of the population.

A birth cohort of 210 babies born in K.V Kuppam block between February 2009 and August 2009 was recruited. The same birth cohort that was recruited in two previously published studies [13, 16] was also used in this study. Children whose homes were located outside the study area were excluded. In every alternate week babies were examined in a peripheral clinic within 5 km of the mother's home. At sick visits the baby was brought to the peripheral clinic or main hospital for evaluation and treatment. All babies received routine immunization in the first year of life with diphtheria, pertussis, tetanus, BCG, polio, mumps and measles vaccines, but not pneumococcal vaccine as this was unavailable in Vellore district during the study period.

Baseline sociodemographic data regarding gender, birth weight, type of house (thatched or tiled/terraced), parental education and occupations, exposure to tobacco smoke, use of firewood at home, and number of family members were recorded in previously prepared proformas. Informed consent was obtained from all parents. Patient information forms were distributed to parents who were interviewed at each visit regarding recurrent colds, earache or other medical problem in the child. Parents were asked if the child rubbed the ear and cried in order to make a determination of earache. Babies with a congenital birth defect, immunodeficiency, known syndrome or neonatal illness such as sepsis or asphyxia were excluded from the study.

At birth and at monthly scheduled visits, nasopharyngeal swabbing was performed by trained study personnel with a sterile swab with a flexible aluminum wire shaft and a calcium alginate tip (Hardwood Products LLC, USA) using a standard technique described previously [16]. Both ears were examined by otoscopy either by a trained doctor or nurse.

Bacteriological processing

Standard techniques described previously were used [16]. Swabs in 1 ml transport medium STGG (skimmed milk, tryptone, glycerol, glucose) broth (as recommended by the WHO) were transported to the laboratory on the same day before 17:00 hours and were frozen immediately at –70 °C. The next day, the samples were thawed, vortexed, and a loopful of sample (25 µl) was streaked on trypticase soy base with 5% sheep blood agar (TSBA) plates, one of which contained 5 mg/ml gentamicin to optimize recovery of *S. pneumoniae* in mixed flora from middle ear swabs as recommended by Peled & Yagupsky [17]. The plates were incubated at 37 °C in 5% CO_2 overnight and examined for bacterial growth. Presumptive *S. pneumoniae* were characterized by small α-haemolytic colonies. Isolates were further screened for optochin sensitivity and those with borderline susceptibility were confirmed as *S. pneumoniae* by the bile solubility test and serogrouped by the

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coagglutination technique with antisera obtained from Statens Serum Institut (Copenhagen, Denmark).

Statistical analysis

Descriptive statistics and frequency and percentages were reported for categorical variables, respectively. Relative risk was calculated for AOM episodes. A curve was plotted to show the cumulative incidence of AOM during the first 2 years of life. Generalized estimating equations (GEE) with binomial distribution with logit link were performed to analyse repeated measures for the same child with AOM using population-averaged effects of covariates. Independent correlation structure was used.

Quasi-likelihood under independent model criterion (QIC) was computed using initially two models for risk factor analysis. In the first model, using the risk factors which were significant on univariate analysis with adjustment for age, the QIC obtained was 489·256. In the second model, the same risk factors with adjustment for age were used but excluded breastfeeding, and gave a QIC of 488·953. The latter model was found to have the best fit and was utilized for the final risk factor analysis. Data were analysed using SPSS 16.0 for Windows (SPSS Inc., USA).

Definitions

Acquisition was defined as a positive nasopharyngeal swab for the first time during the course of the study. Upper respiratory infection was diagnosed by the mother's history of the child having a runny nose with/without fever and not feeding well or loose stools, accompanied by the presence of mucoid/mucopurulent secretion in the nasal cavity. AOM was diagnosed by the presence of both otoscopic findings and systemic symptoms. Otoscopic findings included a bulging or cloudy/opaque, congested tympanic membrane or congested, perforated tympanic membrane with discharge, and systemic symptoms and signs included acute onset of fever and one or more of the following: incessant crying, ear rubbing, not feeding well or loose stools. A positive nasopharyngeal swab associated with AOM referred to the swab that was taken at the time of diagnosis of AOM.

The study design and protocol was approved by the Institutional Review Board and Ethics Committee of Christian Medical College, Vellore, India.

RESULTS

Baseline characteristics of this birth cohort have been reported previously [13, 16]. A total of 210 infants, 121 (57·6%) boys and 89 (42·4%) girls, were recruited at birth and followed up for 26 months. Babies attended the peripheral clinic monthly and half of them had at least 16 visits, with 70% attending at least once every quarter. There was a drop-out rate of 19% after birth, primarily because of relocation of parents to a new place of residence. There were a total of 17 extra visits when babies were brought with complaints consistent with an upper respiratory infection as defined earlier. Otoscopy and nasopharyngeal swabbing were also performed at these visits.

Sociodemographic data

Most parents were labourers, residing in tiled/pucca houses (66·7%), and had at least high school education. Smoke exposure was minimal as most parents did not smoke cigarettes or beedis (92·9%) and did not use firewood for cooking (59·5%). Most households had >3 members.

Prevalence and cumulative incidence of AOM

Sixty-one of the 210 babies experienced at least one episode of AOM during the follow-up period of 26 months giving an overall prevalence of 29·1%. Of a total of 128 episodes of AOM, 93 occurred in the first year and 35 in the second year. The relative risk of developing AOM in the first year of life was 2·63 [95% confidence interval (CI) 1·21–5·75] compared to the second year. Bilateral AOM was seen in the majority (68·8%). The total number of AOM episodes experienced by each affected child ranged from 1 to 7, with a mean of 2·1 episodes. All AOM episodes occurred in the presence of upper respiratory infection and all were diagnosed by study staff alone, either during routine or sick visits.

The graph of the cumulative incidence of AOM during the first 2 years of life showed that the first episode of infection occurred at age 4 months and progressively increased, especially in the second half of the first year (Fig. 1*a*) but there was no subsequent increase in the incidence of AOM. When analysed by the child's age, most AOM episodes occurred after the age of 6 months (Fig. 1*b*). However, a comparison of those babies who had $\geqslant 3$ episodes of AOM ($n = 6$) with those who had $\leqslant 3$ episodes of AOM ($n = 55$)

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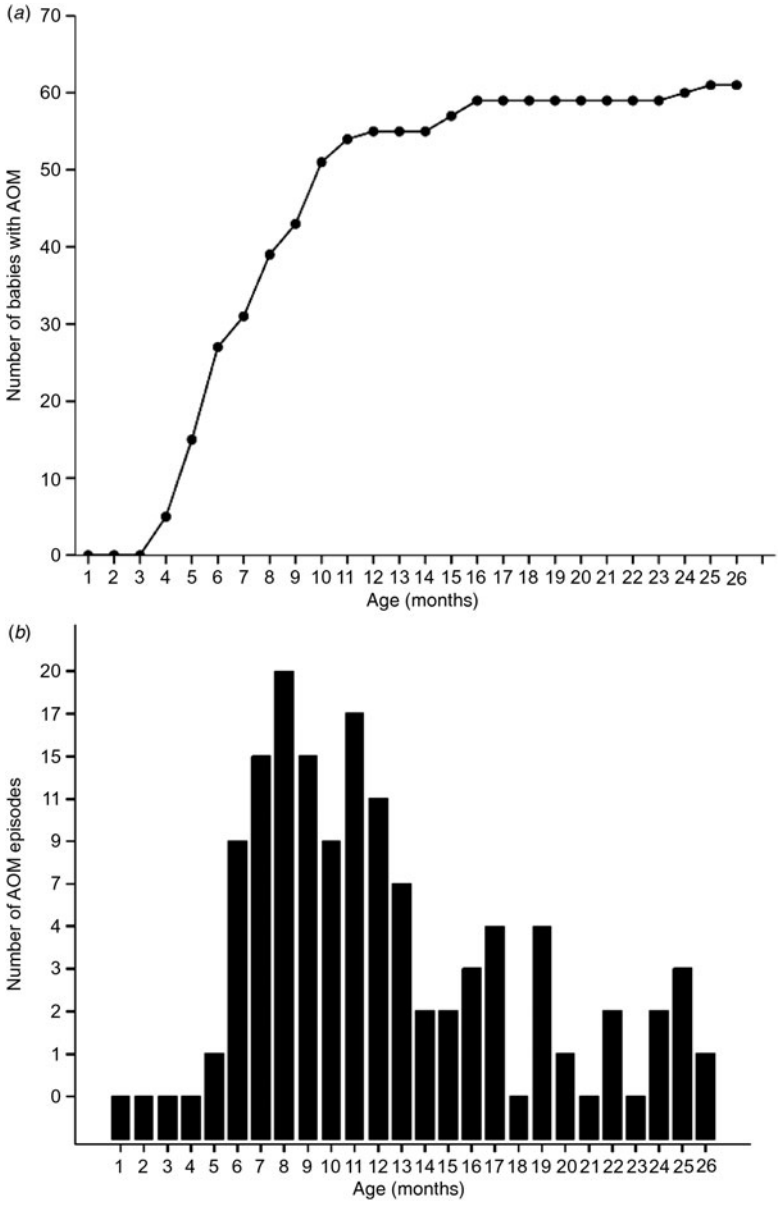

Fig. 1. (*a*) Cumulative incidence of acute otitis media (AOM) during the first 2 years of life. (*b*) Distribution of all AOM episodes in affected children by age.

showed no significant difference in age of onset of the first episode in both groups ($P = 0{\cdot}39$).

Risk factor analysis

Table 1 shows the associations of 14 risk factors with the occurrence of AOM in the patient cohort. As we did not study an entire year cohort, age was adjusted for by including it as a variable to reduce its confounding effect on other studied variables. On univariate analysis, the risk factors that were significantly associated with AOM were >3 members in the household, winter season, breastfeeding for <6 months, a positive swab for *S. pneumoniae* and presence of upper respiratory infection. While age showed some association with AOM on univariate analysis, it was not found to be statistically significant [odds ratio (OR) 1·02, 95% CI 0·99–1·05, $P = 0{\cdot}086$]. After adjusting for age, breastfeeding was no longer significant on multivariate logistic regression analysis but the other factors remained significant.

A positive nasopharyngeal swab for *S. pneumoniae* was seen at least once in 165 (78·6%) babies. Most (86·9%) with AOM had a positive swab and the

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Table 1. *Multivariate logistic regression analysis of risk factors for acute otitis media*

	Unadjusted analysis		Adjusted analysis	
Risk factors	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (months)	1·02 (0·99–1·05)	0·086	0·97 (0·92–1·02)	0·29
Sex				
Male	0·99 (0·62–1·59)	0·972	—	—
Female (reference)	1			
Parental occupation				
Nil/labourer/small business/marginal farmer/big business	1·55 (0·81–2·99)	0·190	—	—
Salaried/professional/others (reference)	1			
Father's education				
Illiterate/primary school	1·05 (0·52–2·12)	0·893	—	—
High school and above (reference)	1			
Mother's education				
Illiterate/primary school	1·26 (0·55–2·85)	0·585	—	—
High school and above (reference)	1			
Type of house				
Thatched	0·83 (0·48–1·41)	0·485	—	—
Tiled/terraced/grouped house (reference)	1			
Birth weight				
⩽2·5 kg	1·05 (0·63–1·73)	0·852	—	—
>2·5 kg (reference)	1			
Members				
⩽3	0·43 (0·26–0·70)	0·001*	0·32 (0·17–0·57)	<0·01*
>3 (reference)	1		1	
Passive smoking				
Yes	1·53 (0·54–4·37)	0·426	—	—
No (reference)				
Firewood use at home				
Yes	0·93 (0·56–1·54)	0·784	—	—
No (reference)	1			
Water source				
Bore well	0·78 (0·48–1·25)	0·293	—	—
River/open well (reference)	1			
Season				
November–February (winter)	3·68 (2·10–6·44)	<0·01*	3·46 (1·56–7·30)	0·001*
June–October	0·45 (0·20–1·03)	0·058	0·57 (0·19–1·74)	0·326
March–May (reference)	1		1	
Breastfeeding for <6 months				
Yes	2·01 (1·04–3·87)	0·038*	—	—
No (reference)	1			
Swab for *S. pneumoniae*				
Positive	3·57 (2·26–5·65)	<0·01*	1·93 (1·03–3·62)	0·041
Negative (reference)	1		1	
Upper respiratory tract infection				
Present	5·11 (2·25–7·48)	<0·01*	2·43 (1·31–4·51)	0·005*
Absent (reference)	1		1	

OR, Odds ratio; CI, confidence interval.

odds of a child with AOM having a positive swab was 1·93 (95% CI 1·03–3·62, $P = 0·056$). A diagnosis of AOM was made at five of the 17 (29·4%) sick visits but only two swabs were positive during those visits. Carriage rates of *S. pneumoniae* were found to be increased both in the presence of upper respiratory infection and AOM and all babies had an upper respiratory infection at diagnosis making this a significant risk factor for AOM (OR 2·43, 95% CI 1·31–4·51, $P = 0·005$). Regarding other risk factors, AOM

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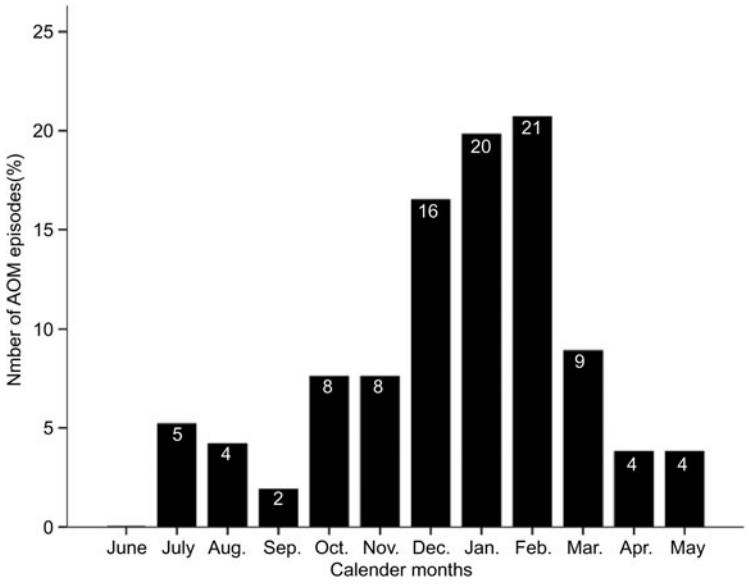

Fig. 2. Seasonal distribution of acute otitis media (AOM) episodes during the first 2 years of life.

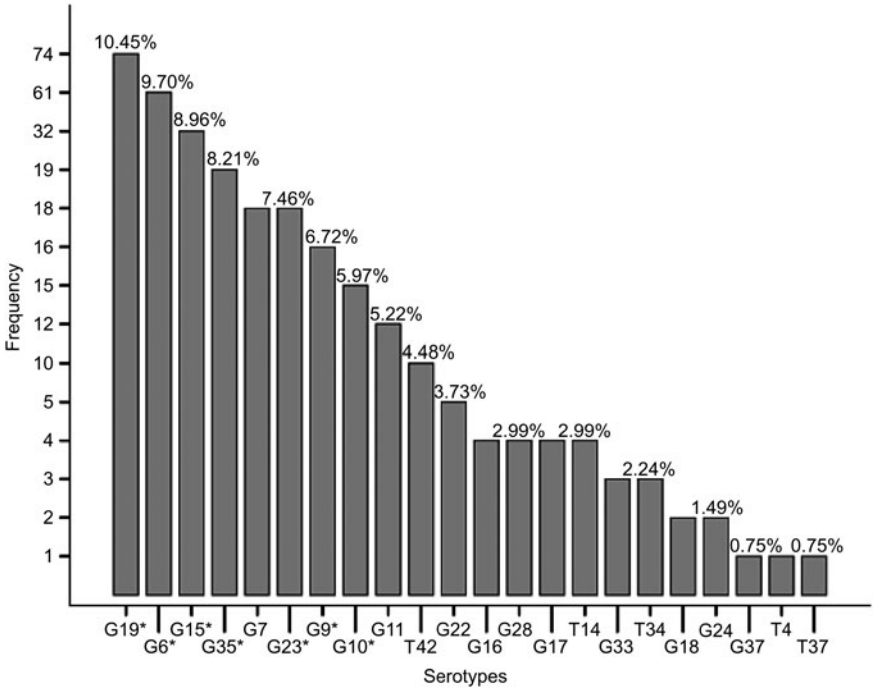

Fig. 3. Distribution of serotypes of *S. pneumoniae* isolated during acute otitis media episodes.

episodes were most frequently seen during November–February (winter months) with a significant difference in incidence (OR 3·46, 95% CI 1·56–7·30, $P = 0·001$) compared to the rest of the year (Fig. 2). Babies from families with ⩽3 members were at a decreased risk for developing AOM than those with families having >3 members (OR 0·32, 95% CI 0·17–0·57, $P < 0·01$). On univariate analysis, babies who had been breastfed for <6 months were more prone to develop AOM than those breastfed for longer periods ($P = 0·038$), but this difference was not seen in multivariate analysis.

Serotype analysis

Analysis of the serotypes of *S. pneumoniae* recovered from babies with AOM showed a predominance of serotypes 19, 6, 15, 35, 7, 23, 9 and 10 (Fig. 3). Five

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of these serotypes are also represented in PCV10 and PCV13 and assuming cross-protection between serotypes, this would give an overall coverage of 62·8% for the cohort. There was no significant difference in the serotype distribution between those with AOM and those without.

DISCUSSION

Previously, for the same birth cohort, we reported an increase in nasopharyngeal colonization with *S. pneumoniae* in the second half of the first year of life [13]. In the present study we have extended this observation to show a clearcut association between nasopharyngeal colonization with this organism and AOM, with a coincident peak incidence in the same age group. By age 1 year most Indian babies appear to have been exposed to a variety of *S. pneumoniae* serotypes which with the development of immunity leads to a fall in both colonization and AOM thereafter [13]. Similar results have been reported by other authors [12, 15, 18]. Revai *et al.* [19] found that a child colonized with two of three organisms (*S. pneumoniae*, non-typable *H. influenzae* or *M. catarrhalis*) was 2·6 times more likely to develop AOM compared to one whose nasopharyngeal swab cultures were negative; this is in accord with our finding of a likelihood value of 2·2 for such subjects.

Early colonization of the nasopharynx by bacteria has largely been reported from developing countries [13] and some ethnic communities [20]. Some authors have found a strong association between those babies colonized with nasopharyngeal flora before age 3 months and the development of AOM [18]. It is noteworthy that despite the fact that many of the birth cohort babies had previously been shown to be colonized within the first year [13], no such association was evident for the development of AOM. Moreover, as has been noted by some authors [9, 18], no association between recurrent episodes of AOM and early age of onset of the condition was found in the present study. However, as only six in the cohort had recurrent AOM and only three had onset of the first episode before age 6 months, it is not possible to confirm or refute such an association from this survey.

Multivariate logistic regression analysis of risk factors for AOM indicated that besides nasopharyngeal colonization with *S. pneumoniae*, upper respiratory infection, winter season and crowding at home were statistically significant. We had previously noted a strong association between upper respiratory infection and nasopharyngeal colonization by *S. pneumonia* [13, 16]. The anatomical continuity of the eustachian tube, nasal cavity and nasopharynx facilitates the development of AOM in the presence of an upper respiratory infection. Furthermore, given the observed seasonal increase in both *S. pneumoniae* colonization and upper respiratory infection [13, 16], accompanied by greater viral and bacterial load in affected children, an increased predisposition to AOM is not surprising, and has been noted in other studies [12, 19].

Crowding is a recognized risk factor for the development and spread of pneumococci in households [11]. The latter study showed a statistically significant difference in *S. pneumoniae* carriage in households comprising $\leqslant 3$ persons (range 29–46%) compared to those with >3 persons (range 38–50%), and this association was borne out here for babies living in larger households who were more prone to develop AOM. Although the duration of breastfeeding was not confirmed to be a significant risk factor by multivariate analysis, breast milk is known to possess antimicrobial, anti-inflammatory and immunomodulatory properties. The evidence in the literature regarding the benefit of breastfeeding in preventing AOM is, however, variable, with one meta-analysis of risk factors for AOM suggesting that even 3 months of breastfeeding reduced the risk of the infection (relative risk 0·87, 95% CI 0·79–0·95, $P = 0·003$) [21], but this is not supported by a recent comprehensive meta-analysis which found that neither breastfeeding for <6 months, nor breastfeeding at all, were important risk factors for otitis media [22].

The overall prevalence of AOM (29·1%) in the present birth cohort is considerably less than that reported from Western countries where rates of 65% in Finnish babies aged <2 years [12] have been recorded, and the more global prevalence estimate for AOM in children aged 1–4 years of 61% [23]. Our low prevalence begs the question as to whether AOM is truly less common in developing countries. The difference may be more apparent than real. It is likely that in the West there is better reporting of cases of AOM by paediatricians, family practice doctors and ENT specialists resulting in increased awareness of the infection by parents of sick children. A previous study in the same south Indian community, as described here, found that 26·4% of parents of children attending daycare, aged around 3 years, gave no treatment if the child had earache; 67·2% relied on home remedies such as plant juice and oil, while 6·4% visited an allopathic practitioner, although a medical doctor's opinion was sought for 50% of

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cases with a discharging ear [24]. As a consequence, despite clear instructions to parents of our study cohort to bring the ill child to the base hospital, it is possible that some parents did not follow the advice given. If many of these children actually had AOM, such practices may explain the relatively low incidence of AOM, particularly during sick visits, in the study subjects. It is also possible that a number of cases of AOM are asymptomatic and therefore go undiagnosed in children from developing countries as described for Aboriginal communities [20].

The low prevalence of positive cultures for *S. pneumoniae* during the sick visits was unexpected. Of five such AOM episodes diagnosed at sick visits, only two yielded positive cultures despite the fact that the same study staff made the diagnosis of AOM during both routine or sick visits using the same swabbing technique. This might be attributed to the slightly increased transportation time for specimens to the laboratory if the sick visit occurred over a weekend or alternatively, organisms other than *S. pneumoniae*, which were not tested for, were responsible for these infections. Revai *et al*. [19] showed a higher isolation rate of *H. influenzae* (OR 2·2, 95% CI 1·6–3·1, $P < 0·001$), *M. catarrhalis* (OR 1·9 (1·4–2·8, $P < 0·001$), and *S. pneumoniae* (OR 1·8, 95% CI 1·3–2·6, $P < 0·001$) in children with AOM complicating upper respiratory infection and also suggested a possible major role for viruses, particularly in culture-negative children.

The efficacy of PCV against AOM has been widely investigated in countries where the vaccine has been available for over a decade. The systematic study of Taylor *et al*. [25] of 18 relevant publications reported an efficacy of PCV7 against all-cause AOM episodes of 0–9% in randomized trials and 17–23% in non-randomized trials. A more recent determination of efficacy of PCV7 and PCV10 in young Australian Indigenous children showed that children vaccinated with the latter vaccine had less suppurative otitis media than children vaccinated with PCV7 [26]. In another trial in young Latin American children, PCV10 efficacy against all clinically confirmed AOM was 16·1% and this rose markedly to 67·1% for infections due to vaccine serotypes [27]. There are few large-scale data for PCV13 but a significant reduction in AOM visit rates was noted in children aged <2 years in the USA in 2010–2011 following the introduction of this vaccine in 2010 [28]. We found no significant difference between the serotype distribution in those with and without AOM but constituent vaccine serotypes in PCV10 and PCV13 accounted for only 62·8% of those serotypes recovered from babies with AOM. Thus, the efficacy of PCV10 or PCV13 to significantly reduce the burden of AOM due to *S. pneumoniae* in the South Indian population remains doubtful. In Aboriginal children the effect of PCV7 or PCV10 in reducing the prevalence of otitis media was limited, although the reduction by PCV10 was more pronounced [26] suggesting that apart from serotypes not included in PCV10, other organisms such as *H. influenzae* may be responsible for some cases.

It is clear from the above that further studies are required in developing countries and ethnic populations to chart the natural history of AOM in otitis-prone children and determine the value of vaccination and antibiotics in preventing the progression of AOM to a chronic suppurative state which is one of the known sequelae of AOM. A perforation that fails to heal, or persistent otitis media with effusion, may lead to chronic mucosal or squamosal forms, respectively. Both acute and recurrent otitis media were shown to increase the risk of chronic otitis media by a factor of 11·13 (95% CI 1·06–116·44, $P = 0·04$) in one meta-analysis [22].

A limitation of the study is our reliance on simple otoscopy rather than pneumatic otoscopy, tympanometry or otomicroscopy for the diagnosis of AOM as the sensitivity, specificity and predictive value of simple otoscopy is lower than the other techniques. However, we were constrained to make use of a technique most suitable for the field situation. Nevertheless, the presence of clouding/opacification of the tympanic membrane as visualized by simple otoscopy has been shown to have a sensitivity of 81% and specificity of 95% for the diagnosis of AOM [29].

In conclusion, this is the first study from India on the epidemiology of AOM and its association with *S. pneumoniae* nasopharyngeal colonization in unvaccinated Indian babies in the first 2 years of life. Our results, extrapolated from results of Western studies on infants who have been vaccinated with PCVs, suggest a possible role for vaccination to prevent AOM and protect against its sequelae, although the degree of reduction in AOM episodes needs to be carefully ascertained. As the prevalence of chronic suppurative otitis media in preschool Indian children is high it is possible that there may be another peak for AOM between ages 2 and 5 years or that organisms other than *S. pneumoniae* may play a role in causing middle ear disease.

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DECLARATION OF INTEREST

None.

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