Journal of Otology 16 (2021) 220–224

Contents lists available at ScienceDirect

Journal of Otology

journal homepage: www.journals.elsevier.com/journal-of-otology/

Nasopharyngeal colonization of otopathogens in South Indian children with acute otitis media – A case control pilot study

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ARTICLE INFO

Article history:
Received 20 January 2021
Received in revised form
22 February 2021
Accepted 25 February 2021

This work was not presented at any scientific meeting.

Keywords:
Acute otitis media
Nasopharyngeal
Otopathogens
Case control study
Streptococcus pneumoniae
Haemophilus influenzae
Moraxella catarrhalis
Staphylococcus aureus

ABSTRACT

Background: Acute otitis media (AOM) is an inflammatory disease of the middle ear causing significant morbidity in early childhood. A pilot study was undertaken to identify the role of various risk factors South Indian children with AOM, especially the role of nasopharyngeal otopathogens.
Methodology: A prospective case control pilot study was conducted in children aged below six years, presenting to a single tertiary care from 2018 to 2019. Fifty cases with AOM and 45 age and gender matched controls were recruited. Two nasopharyngeal swabs were collected, one was processed for bacterial culture. The other swab was processed according to the CDC recommended broth enrichment method to identify carriage of *S. pneumoniae*. Subsequent serotyping was done by Quellung method and conventional sequential multiplex PCR.
Result: Otalgia was the major presentation seen in 92% of the children with AOM. None of the clinical and demographic characteristics were found to be statistically significant between the cases and controls. The most common otopathogen was *S. pneumoniae* (55%) followed by *H. influenza* (29%). The common *S. pneumoniae* serotypes encountered were 11A and 19F.Nasopharyngeal colonization with *S. pneumoniae* [OR 6.57, $p < 0.003$] and *H. influenzae* [OR14.18, $p < 0.003$] were significant risk factors for AOM in children. The risk increased with co-colonization (OR 13.89,$p < 0.003$).
Conclusion: This study strengthens the significant association between nasopharyngeal colonization of otopathogens and AOM as a risk factor that is enhanced by co-colonization.*S. pneumoniae* was the main otopathogen in this population, serotypes 11A and 19F being the most common.

1. Introduction

Acute otitis media (AOM) is a common inflammatory disease of the middle ear causing significant morbidity in early childhood (Ren et al., 2018; Monasta et al., 2012; DeAntonio et al., 2016; Fortanier et al., 2019). It is characterized by rapid onset (<48 h) of otalgia with or without fever along with evidence of middle ear effusion and inflammation (Lieberthal et al., 2013; Hayashi et al., 2020). The most common bacterial otopathogens in AOM are *Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis* (Dunne et al., 2013; Lappan et al., 2018). In addition, other organisms such as *Streptococcus pyogenes* have also been implicated (Hau et al., 2014). A possible role of *Staphylococcus aureus* as an otopathogen and its negative association with pneumococcal carriage has also been reported. (9–11) Since the prevalence and distribution of the otopathogens vary with region, age group, and introduction of vaccination, identifying the role of otopathogens is of vital importance in treating these children. This study was undertaken as a pilot study to identify the role of otopathogens and associated variables in children with acute otitis media. This study specifically focuses on *S. pneumoniae* and its serotypes, *H. influenzae, M. catarrhalis*, and *S. aureus*.

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Peer review under responsibility of PLA General Hospital Department of Otolaryngology Head and Neck Surgery.

https://doi.org/10.1016/j.joto.2021.02.004

Abbreviation

AOM	Acute otitis media
MEF	Middle ear effusion
CDC	Centre for disease control and prevention
PCR	Polymerase chain reaction

2. Methodology

2.1. Selection of participants

We conducted a prospective case-control pilot study in children aged below six years with acute otitis media presenting to single tertiary care from 2018 to 2019. Children, less than six years from Vellore district (Tamil Nadu) presenting to the ENT outpatient with acute otitis media were included in the study. The three criteria for AOM included - a child having rapid onset (<48 h) of otalgia or fever along with signs of inflammation characterized by either purulent ear discharge or bulging/congested tympanic membrane and features of middle ear effusion (MEF) confirmed with tympanometry (Fortanier et al., 2019; Lieberthal et al., 2013; Hayashi et al., 2020). Each child underwent microscopic (Zeiss OPMI Pico microscope) examination of the ear. Tympanometry was performed in all non-discharging ears in the audiology suite (GSI Tympstar Pro) to diagnose middle ear effusion. In children with ear discharge, an ear swab was taken for bacterial culture. Controls included children less than 6 years of age without any ear and nose complaints. They were children who had come to the ENT outpatient for complaints such as sinus or swelling of the head or neck and hearing screening or to the adjacent immunization clinic for immunization. Exclusion criteria included immunocompromised children, children with ciliary dyskinesia, craniofacial anomalies, or syndromic associations. Children who had used antibiotics for any indication in the last one-month were also excluded.

A detailed questionnaire was administered which included demographic characteristics, vaccination status, clinical presentation, socioeconomic status and education of parents, number and age of siblings, day-care attendance, history of breastfeeding, pacifier use, and passive smoking. Socioeconomic status was measured using the Kuppuswamy index (Wani, 2019).

2.2. Microbiological analysis of nasopharyngeal swab

Two nasopharyngeal swabs, one from each nostril were obtained using sterile flocked flexible nylon swab following the CDC guidelines (Satzke et al., 2013). One swab was processed for bacterial culture as per standard protocol (World Health Organization, 2011). Bacterial growth was graded by a semiquantitative method depending on the number of colonies as scanty (<20 colonies), moderate (20–100 colonies) or heavy (>100 colonies). Antimicrobial susceptibility testing (AST) was performed by disk diffusion test for all the antibiotics except penicillin. For penicillin, MIC against *S. pneumonia* was performed by E-test (Biomerieux. Ltd) and interpreted according to Clinical Laboratory and Standards Institute (CLSI) guidelines (CLSI, 2008). The second swab collected in STGG (skim milk tryptone glucose glycerol) transport medium was processed according to the CDC recommended broth enrichment method to identify carriage of *S. pneumonia* (World Health Organization, 2011; Trzciński et al., 2013). Serotyping of *S. pneumoniae* was done by Quellung method using pneumococcal antisera from Staten's Serum Institute, Denmark and the conventional sequential multiplex PCR (Veeraraghavan et al., 2016). The differentiation between typeable and non-typeable*H. influenzae* were based on the presence and absence of the *bexB* gene, respectively using conventional PCR (Falla et al., 1994; Davis et al., 2011).

2.3. Statistics

Data was summarised using Mean (SD)/Median (IQR) for continuous variables depending on normality. Categorical variables were expressed as numbers and percentages. All the variables were compared between the cases and controls and odds ratio with 95% CI is presented. For logistic regression, the inclusion of variables in the multivariate model was fixed as $p \leq 0.3$ and a backward stepwise regression was performed. STATA IC/15.0 software was used for data analysis.

3. Results

A total of 1021 children aged below six years belonging to Vellore district (Tamil Nadu) presented to our Paediatric ENT outpatient from December 2018 to December 2019. After screening, 55 children fulfilled the criteria for acute otitis media. Five caregivers were unwilling for a nasopharyngeal swab and hence these children were excluded from the study. So, 50 children were recruited as cases, and 45 age and gender-matched children without any ear or nose complaints were designated as controls. Comparison of basic demographic characteristics and vaccination details of the study subjects is shown in Table 1. The mean age of children was 3.2 years (range −3 months to 6 years).

The clinical spectrum of AOM varied from an early stage when there are clear signs of TM inflammation and MEF accumulation, to more severe AOM when purulent MEF under pressure causes bulging of the TM, to spontaneous rupture of TM with otorrhoea. Otalgia was the major presentation seen in 46 (92%)of the children with AOM. It was associated with fever in 35 (70%) children. Ear discharge was present in 21 (42%) children. Ear swab culture revealed no growth in 18, two grew *H. influenzae* and one *Pseudomonas aeruginosa*. Nasal symptoms of rhinorrhoea were present in 38/50 (76%) of the cases. Table 2 compares the different social and demographic variables between the cases and controls which could have contributed to the pathogenesis of AOM.

The analysis of the culture-positive specimens in the nasopharyngeal culture showed that 13 (10 cases and 3 controls) specimens were monomicrobial and an equal number (n = 13; 12 cases and 1 control)were polymicrobial. Bacterial culture identified 52

Table 1
Baseline characteristics of cases and controls.

	Cases (n = 50) No.(%)	Control (n = 45) No.(%)
Age		
0-≤6 months	3 (6)	4 (8.9)
>6 months − ≤2 years	7 (14)	6 (13.3)
>2 years-≤6 years	40 (80)	35 (77.8)
Gender		
Female	26 (52)	22 (48.9)
Male	24 (48)	23 (51.1)
Season		
Apr–July	20 (40)	16 (35.5)
Aug–Nov	25 (50)	8 (17.8)
Dec–March	5 (10)	21 (46.7)
Vaccination status		
Pneumococcal vaccine	27 (54)	24 (53.3)
Influenza vaccine	24 (48)	15 (33.3)

Season was categorized by months.
Of the 51 children who received pneumococcal vaccine, PCV 13 was the most common (41/51). In 10 children the type of pneumococcal vaccine was not clear from the vaccination records.

Table 2
Comparison of social, family, economic and medical history between cases and controls.

	Variables	Cases (50) N (%)	Controls (45) N (%)	Odds ratio (95% CI)	P value
Socioeconomic status	Upper middle	18 (36)	23 (51.1)		
	Lower middle	30 (60)	22 (48.9)	1.72 (0.76–3.89)	0.19
	Lower	2 (4)	0 (0)	6.35 (0.29–140.5)	0.24
Sibling (<5 years of age)	Present	32 (64)	28 (62.2)	1.07 (0.47–2.46)	0.86
GERD	Present	5 (10)	4 (8.89)	1.11 (0.29–4.15)	0.87
Daycare attendance	Present	21 (42)	30 (66.7)	0.37 (0.16–0.85)	<0.01
Feeding history	Exclusive breast feeding	29 (58)	29 (64.4)		
	Breastfeeding along with formula feeding	12 (24)	7 (15.6)	1.66 (0.60–2.44)	0.32
	Only formula feeding	9 (18)	9 (20)	0.51 (0.19–1.44)	0.21
Passive smoking	Present	2 (4)	4 (8.9)	0.47 (0.09–2.36)	0.36

otopathogens and 95% of the isolates were moderate or heavy growth. An additional ten cases had *S. pneumoniae* identified by the broth enrichment method, for which the routine nasopharyngeal culture result showed the growth of normal flora. Altogether of the 62 [53 in cases and nine in controls] bacterial isolates; *S. pneumoniae* (n = 34) was the most common otopathogen isolated followed by *H. influenzae* (n = 18), *S. aureus* (n = 5), and *Moraxella catarrhalis* (n = 5). Table 3 analyses the growth of bacterial otopathogens in the nasopharyngeal culture. The results of logistic regression analysis showed that odds of having AOM was 6.57 (95% CI, 2.43–17.78) times if *S. pneumoniae* and 14.18 (95%, CI 2.51–79.99) times if *non-typeable H. influenzae* was isolated from the nasopharynx. Growth of *M. catarrhalis* and *S. aureus* was seen in five cases and none of the controls. Twelve cases showed co-colonization with two organisms while among the controls only one showed co-colonization with two organisms. *S. pneumoniae* with nontypeable *H. influenzae* followed by *S. pneumoniae* and *S. aureus* were the most common otopathogens in cocolonization.

All isolates of *H. influenzae* were non-typeable and suspectible to amoxicillin/clavulanic acid, as were the isolates of *M. catarrhalis*. Only 17% of *Streptococcus pneumoniae* were susceptible to penicillin by oxacillin disk diffusion test (Surrogate marker as per CLSI) as opposed to 96% of the isolates being susceptible to levofloxacin. All the isolates were susceptible to penicillin by MIC. Three of five *S. aureus* isolates were MRSA. Among the subjects who are vaccinated (n = 51) and non-vaccinated (n = 44) with PCV, *S. pneumoniae* was grown in 43% and 27% respectively. Serotype 11A and15 B/C were the major non-vaccine serotypes associated with both the vaccinated and non-vaccinated.Fig. 1 shows the serotypes of *S. pneumoniae* that were isolated. Carriage of non-typeable*H. influenzae* was similar (n = 9) in those vaccinated with PCV (n = 51) and those non-vaccinated (n = 44).

4. Discussion

This pilot case-control study was done to identify the role of nasopharyngeal colonization with otopathogens in the etiopathogenesis of AOM. Screening of all children from the study district aged below six years visiting our clinic revealed that 55 out of 1021 (5.3%) children aged below six years had AOM. This percentage reflects the incidence of AOM in this age group presenting to a tertiary care center and is not a reflection of the incidence of AOM in the community. There is wide variation noted in the prevalence of AOM worldwide and particularly from India. While the prevalence worldwide has been reported to be around 10.8%, in India it has been reported to be between 4 and 43% based on a questionnaire and otoscopic assessment (Ren et al., 2018; Monasta et al., 2012; DeAntonio et al., 2016; D'silva et al., 2013; Gaur et al., 2009; Sophia et al., 2010). This variation could be because of different ways of reporting AOM (Sophia et al., 2010; Srikanth et al., 2009). The present study uses AOM criteria based on acute symptoms, presence of MEF, and signs of TM inflammation which are in concordance with the 2004 AAP guidelines but only included children presenting to the tertiary care center (Fortanier et al., 2019; Lieberthal et al., 2013). While otalgia was the most common presenting symptom, in almost half of them it was associated with fever. More than three quarters (76%) of our cases had coexistent rhinitis. Rhinitis is a consistent significant factor associated with AOM with studies showing high odds of developing AOM during URTI (Sophia et al., 2010; Kalu et al., 2011). Since our controls were specifically chosen as those without nasal symptoms, the significance could not be calculated.

On univariate analysis day care attendance was significantly more in controls. This is in contrast to previous studies such as one by Kong and Coates where daycare attendance has been referred to as a mini epidemic for AOM (Kong and Coates, 2009). However, multivariate analysis to understand its exact significance could not be done as only one factor was significant No substantial differences in other demographic characteristics were noted between cases and controls contrary to that noted in the literature (Bogaert et al., 2004a; Sophia et al., 2010; Rupa et al., 2016). Most of the cases belonged to lower and lower-middle socioeconomic strata in the current study. Quintero et al. have opined that a combination of nasopharyngeal colonization with otopathogens in a low socioeconomic setting puts them at risk for more invasive disease (Bogaert et al., 2004a).

Table 3
Distribution of otopathogens grown in the nasopharyngeal culture of cases and controls.

Otopathogen	Cases (n = 50)	Controls (n = 45)	Odds ratio (95% CI)	*P* value
	N (%)	N (%)		
Streptoccocus pneumoniae	27 (54)	9 (20)	6.57 (2.43–17.78)	<0.003
Haemophilus influenzae	16 (32)	1 (2.2)	14.18 (2.51–79.99)	<0.003
Moraxella catarrhalis	5 (10)	0 (0)	NA	NA
Staphylococcus aureus	5 (10)	0 (0)	NA	NA
Double organism growth	12 (24)	1 (2.3)	13.89 (1.73–111.89)	<0.003
S. pneumoniae + H. influenzae	9 (18)	1 (2.2)		
S. pneumoniae + S. aureus	2 (4)	0 (0)		
S. pneumoniae + M. catarrhalis	1 (2)	0 (0)		

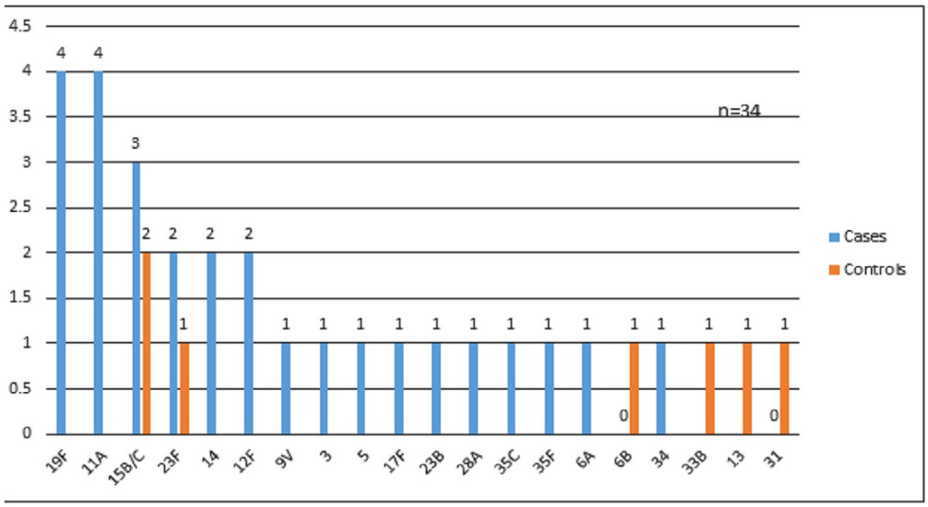

Fig. 1. Distribution of streptococcal serotypes among cases and controls.

Microbiological analysis revealed that 70% of the cases in our study had otopathogens isolated from their nasopharynx. This is similar to the study by Barkai et al. where 64% of the episodes of AOM had at least one otopathogen (Barkai et al., 2009). The major otopathogens identified in cases were *S. pneumoniae* and non-typeable*H. influenzae* which is similar to previous studies (Dupont et al., 2010; Thornton et al., 2011; Leibovitz et al., 2010; Chonmaitree et al., 2017; Faden et al., 1991). The recent increase in non-typeable*H. influenzae* has been attributed to the introduction of pneumococcal vaccine (28). Amongst healthy controls, only 19% had otopathogens isolated from their nasopharynx which was mostly *S. pneumoniae*. The prevalence of pneumococcal carriage has been reported to be between 7 and 70% in healthy children depending on age, ethnicity, and region of study (Bogaert et al., 2004a, 2004b). When we looked individually at each organism, nasopharyngeal colonization with *S. pneumoniae* (OR: 6.57: CI: 2.43–17.78; $P < 0.003$) and *H. influenzae* (OR 14.18; CI: 2.51–79.99; $p < 0.003$) were significant risk factors for AOM in children. A similar odds ratio could not be calculated for *M. catarrhalis and S. aureus as* these otopathogens were absent in our control group. However, the fact that these otopathogens were present only in cases and absent in controls hint at a contributing role in the pathogenesis of AOM. A negative association of *S. aureus* with pneumococcal colonization has been reported by some authors but this was not demonstrated in our results (Lappan et al., 2018; Quintero et al., 2011; Wani, 2019).

In a previous case-control study from the same region, an association between nasopharyngeal colonization of *S. pneumoniae* with AOM in an unvaccinated birth cohort has been demonstrated (Rupa et al., 2016). Our study further reinforces this association. Besides, it was noted that the presence of multiple organisms i.e. co-colonization with 2 or more organisms increases the chances of acute otitis media (OR 13.89, CI (1.73–111.89), $p < 0.003$). Multiple growth or co-colonization was found in 28 percent and the majority of these were with *S. pneumoniae* and *H. influenzae*. These results signify that co-colonization with multiple otopathogens is a significant risk factor in the development of AOM. The co-colonization of otopathogens especially in the presence of viral infection is postulated to modify the innate and adaptive immune response of children predisposing to AOM (Casey et al., 2010). The possibility of biofilms in the nasopharynx facilitates co-colonization is another explanation and is under study in many centers including ours (Barkai et al., 2009; Ralte et al., 2019). All otopathogens can be treated with amoxicillin. However, in cases of co-colonization with inherent beta lactamase producing organisms such as *M. catarrhalis* and *H. influenzae*,beta-lactam inhibitor combinations like amoxicillin/clavulanic acid are preferred than amoxicillin alone (Veeraraghavan et al., 2020).

There was no concordance between the cultures from spontaneous otorrhoea and nasopharynx with most cultures from the ear being negative. This could be because the ear culture was obtained from spontaneous otorrhoea and not by tympanocentesis. No significant differences in culture positivity by vaccination status were noted in this study. Sierra et al. also noted an equal number of vaccinated and unvaccinated children to be culture positive for otopathogens (Sierra et al., 2011). The number of PCV13 vaccine serotypes were similar within the vaccinated and non-vaccinated, (40%,9/22) and (33%,4/12) respectively. Since the number of doses of pneumococcal vaccine could not be elucidated from the records in all cases, no further inference regarding the role of vaccination and nasopharyngeal colonization of vaccine serotypes could be made out. Besides, the community was in the stage of partial immunization hence the role of herd immunity could also not be ruled out. Cohort studies with more cases will help predict trends and throw light on the reported beneficial role of vaccination in AOM (DeAntonio et al., 2016; Quintero et al., 2011; Rupa et al., 2016; Faden et al., 1991; Norhayati et al., 2017).

The most common pneumococcal serotypes identified in our series were 11A, 19F and 15B. Globally, serotypes 3, 6A, 6B, 9V, 14, 19A, 19F, and 23F have been reported from different regions to cause AOM in young children, of which serotype 19A pneumococcal AOM has increased predominantly after the introduction of PCV7 (Dupont et al., 2010; Casey et al., 2010). The variation in the serotypes could be because of region wise variation in immunization and colonization of nasopharynx. No correlation could however be established with respect to their vaccination status with pneumococcal vaccine serotype and with the presence or absence of AOM. However it is to be noted that the expected serotype coverage for by PCV13, PCV10 (Serum Institute of India), and PCV15 is around 38% with respect to the serotypes isolated in this study. It is expected to be higher (70%) with the new proposed PCV 20 vaccine.

The major limitation of this study is the small sample size. This was because we designed this as a pilot study and wanted to get quick results about role of nasopharyngeal colonization. In addition, it was a hospital based study, therefore regional characteristics were not reflected. However, doing a hospital-based study had the

advantage of confirmed diagnosis of AOM as per the guidelines. The small sample size and the fact the community was is the stage of partial immunization makes it difficult to predict trends with vaccination. In addition, regarding the vaccinated, we were not able to capture the data on number of doses and interval s between the doses of PCV13, so the benefits of vaccination cannot be commented upon. Further prospective studies are being planned to understand the benefit of immunization in this population.

5. Conclusion

This case control study further strengthens the association of nasopharyngeal colonization with otopathogens as a significant risk factor for AOM. The main otopathogens in this population continued to be *S. pneumoniae* followed by *H. Influenzae*. Of the pneumococcal serotypes isolated 11A and 19F were the most common. Co-colonization with two or more otopathogens increased the risk for AOM.

Ethical considerations

The study was approved by our institutional review board (IRB No10947/17) and all the subjects provided informed consent.

Financial disclosure

None of the authors have any financial connections, direct or indirect in connection with the contents of this paper.

Declaration of competing interest

None.

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