ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Study Of Chronic Otitis Media With Pleural Effusion In Pediatric Age Group At Tertiary Care Hospital.

Anil Solunke[1], Gajanan Kashid[2], Manoj Waghmare[3], and Anshul Vikram Shrivastava[4]*.

[1]Professor & Head, Department of E.N.T.,DVVPF's Medical College, Ahmednagar, Maharashtra, India.
[2]Professor, Department of E.N.T., DVVPF's Medical College, Ahmednagar, Maharashtra, India.
[3]Associate Professor, Department of Respiratory Medicine, DVVPF's Medical College, Ahmednagar, Maharashtra, India.
[4]Junior Resident, Department of Paediatrics, DVVPF's Medical College, Ahmednagar, Maharashtra, India.

ABSTRACT

Chronic otitis media with effusion, a common pediatric ailment, is characterized by a buildup of fluid behind the eardrum (COME). Recent characterization of the middle ear fluid (MEF) microbiome in pediatric children has led to an improved understanding of the microorganisms present in the middle ear during COME. Samples of the middle ear fluid were obtained from 50 children with chronic otitis media who had myringotomy with tympanostomy tube implantation at DVVPF Medical College, Ahmednagar. Although the taxonomic profiles of children varied greatly, the microbiome's function was remarkably constant in all individuals. Additionally, we found significant differences in the diversity and relative abundance of Haemophilus, Moraxella, Staphylococcus, Alloiococcus, and Turicella in the middle ear microbiome that were associated with a diagnosis of lower airway disease. Further research into the connection between these important pediatric illnesses and the microbiome of respiratory infections is encouraged by these findings. Further research into the connection between these important pediatric illnesses and the microbiome of respiratory infections is encouraged by these findings.

Keywords: Chronic otitis media, Pleural effusion, pediatric age, microbial infection

https://doi.org/10.33887/rjpbcs/2022.13.5.36

Corresponding author

ISSN: 0975-8585

INTRODUCTION

An accumulation of fluid behind the eardrum that lasts for three months or longer is the hallmark of chronic otitis media with effusion (COME), which often shows no symptoms of inflammation (Minovi and Dazert, 2014). According to Monasta et al. (2012), COME is a major contributor to hearing loss in children, particularly in impoverished nations, and it can cause learning impairments and other issues in the classroom (Williams and Jacobs, 2009). By the age of 10, 80 percent of children in the world will have gone through a COME episode (Minovi and Dazert, 2014). AOM, which is typically brought on by bacterial infections with Streptococcus pneumoniae, Moraxella catarrhalis, and Haemophilus influenzae, precedes more than half of COME cases (Minovi and Dazert, 2014; Qureishi et al., 2014). It is less clear how these infections or other elements of the middle ear microbiome contribute to COME. Alloiococcus otitis, Turicella otitidis, and Staphylococcus sp. have been found in middle ear fluid (MEF), in addition to these three infections (Harimaya et al., 2006; Guvenc et al., 2010; Jervis-Bardy et al., 2015; Chan et al., 2016; Lappan et al., 2018).It's interesting to note that several of these same species, particularly M. catarrhalis and H. influenzae, have been connected to the emergence of asthma and the asthma microbiome (Bisgaard et al., 2007; Castro-Nallar et al., 2015; Pérez-Losada et al., 2015). But it is unknown whether there is a connection between asthma and the middle ear microbiota, particularly in the setting of COME. This work adds to our understanding of the MEF microbiota in COME and the MEF microbiome modifications related to bronchiolitis or asthma.

MATERIALS AND METHODS

For this investigation, sample gathering and DNA sequencing procedures were previously described (Krueger et al., 2017). In a nutshell, 50 kids with chronic otitis media who underwent myringotomy with tympanostomy tube implantation at DVVPF Medical College ,Ahmednagar, had samples of their middle ear fluid taken.

The cohort included 34 boys and 16 girls, ranging in age from 3 to 176 months. Nearly three-fourths (36/51) of the 50 kids had a substantial hearing loss.

Two weeks prior to sampling, no antibiotics were administered to any of these kids. The usage of any additional drugs (not antibiotics) was not noted for this study.

One in thirteen of the kids had a diagnosis of a condition affecting the lower airways, such as bronchiolitis or asthma.The kids had to fulfil one of the following descriptions to qualify as being positive for these: (1) a history of asthma with a pulmonary doctor's diagnosis; (2) documented chronic wheezing being treated with a daily inhaler; or (3) a positive PCR for the diagnosis of rhinovirus bronchiolitis. Although bronchiolitis and asthma are regarded as distinct respiratory disorders, there is sometimes a spectrum of disease throughout time, making it difficult to accurately distinguish between them in young children. In this study, we evaluated them as a single entity as lower airway illness.

Using the QiaAmp mini kit (Qiagen), DNA was purified from MEF and extracted using the MiSeq SOP methodology outlined in Kozich et al (2013).Libraries were sequenced using the Illumina MiSeq and the V4 region of the 16S rRNA gene was amplified.

Negative controls did not amplify, proving that there was no bacterial DNA in the reagents and preventing them from being sequenced.

RESULTS

Taxonomic Composition of the Chronic Infection-Related Ear Microbiome As opposed to 84 percent in Krueger et al., an average of 98 percent of sequences were assigned at the genus level (Figure 1).

Achromobacter and Pseudoflavitalea were two of the previously undiscovered taxa that were present. For several ASVs, species level resolution was also attained. These included some ASVs with low abundance (1%), such as Turicella otitidis (6.9 2.2%), Alloiococcus otitis (6.0 1.7%), and

ISSN: 0975-8585

Stenotrophomonas maltophilia (4.6 1.4%).

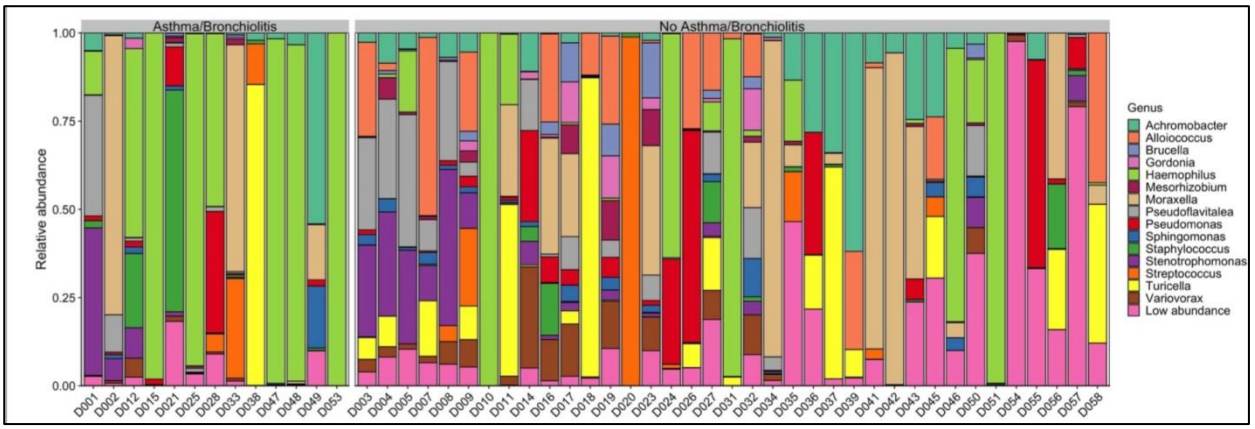

Figure 1) Assignment of sequences at the genus level

Cellular processes (4.7 0.2 percent), environmental information processing (20.4 0.3 percent), genetic information processing (24.8 0.5 percent), and metabolism (33.8 0.3 percent) were the four main categories used to categorise the functional roles of the middle ear microbiome. 16 percent of the samples were not categorised. Membrane transport (19.4 0.3%), translation (13.4 0.4%), amino acid metabolism (7.6 0.2%), and carbohydrate metabolism (6.3 0.1%) were the sub-pathways with the highest relative abundance. All patients' functional characteristics were similar (Figure 2). Neither MaAsLin nor principal component analysis found any conclusive relationships between the functional profiles and clinical factors.

Variation in the Function and Composition of the Ear Microbiome During Chronic Infection Patients with asthma or bronchiolitis had significantly lower levels of the -diversity of the middle ear microbiome as measured by the ASV richness and Shannon diversity indices (Figure 3; p 0.05) than patients without these conditions. The mean ASV richness and Shannon diversity in patients with asthma and bronchiolitis were 16.4 and 0.96, respectively, compared to 31.1 and 1.72 in patients without, respectively. Other clinical factors such as age, gender, substantial hearing loss, mucoid/serous effusion, and the presence of Muc5B and/or Muc5AC did not significantly affect the -diversity.

After taking into consideration the difference in other clinical and demographic factors, differential abundance testing with DESeq2 discovered several ASVs that varied considerably in the relative mean proportions with respect to the presence of asthma or bronchiolitis. Children with asthma or bronchiolitis had significantly greater levels of Haemophilus, Staphylococcus, and Moraxella, while Turicella and Alloiococcus had significantly lower levels.

DISCUSSION

By age 10, up to 80% of children have chronic otitis media with effusion (COME), the most common cause of hearing loss in children (Minovi and Dazert, 2014). Here, we evaluate linkages to identified lower airway disease and offer fresh findings from the 16S data from Krueger et al. (2017) using amplicon sequence variants (ASVs) rather than OTUs. When determining taxonomic assignment, precise sequence variations have higher precision and reproducibility than OTUs (Callahan et al., 2017, 2019; Edgar, 2018; Knight et al., 2018; Xue et al., 2018). Prior OTU-based methods typically worked by grouping sequences according to a similarity threshold of 97 percent, then designating these groups as OTUs based on reference trees. These methods were losing out on important data because they did not factor read statistics or information about sequence quality into taxonomy assignments (Callahan et al., 2017). Exact sequence variants enhance estimates of diversity and taxonomy predictions, particularly for populations that have not received much research attention (Callahan et al., 2017; Caruso et al., 2019).We were able to classify several ASVs at the species level using the methodology used in dada2 (Callahan et al., 2016), three of which were present at a mean relative abundance >1 percent. These include Turicella otiditis and Alloiococcus otitis, which have been recognised as characteristic elements of the OM microbiome in the past (Tano et al., 2008;

von Graevenitz and Funke, 2014; Lappan et al., 2018). In contrast, although being known to be the cause of some human diseases, such as respiratory infections, Stenotrophomonas maltophilia has not been extensively characterised in middle ear microbiomes (Brooke, 2012). S. maltophilia was found in tympanosclerotic plaques isolated from a patient undergoing surgery because to COME, according to recent research by Kalcioglu et al. (2018).As a result, S. maltophilia may be present in some COME patients, especially in those who have tympanosclerosis. S. maltophilia was interestingly not discovered in cholesteatomas in the same investigation, suggesting it may have a particular function (Kalcioglu et al., 2018).

There is no typical middle ear microbiome linked to COME, according to the high inter-patient variation and lack of a core microbiome (Figure1). The middle ear microbiome in this study was highly variable, typically comprising at least two high-abundance genera, in contrast to previous studies that found the middle ear microbiome to be dominated by a single genus, typically from Alloicoccus, Moraxella, or Haemophilus (Guvenc et al., 2010; Jervis-Bardy et al., 2015). The ASV pipeline used in Dada2 has a sharper resolution, which may account for the discrepancy, as well as the larger amount of data kept by not rarefying the counts. Furthermore, fewer than half of the samples from our COME patients included any of the "typical" AOM-associated pathogens, such as Haemophilus, Moraxella, and Streptococcus.These findings show the intricacy of the middle ear microbiota linked to COME, which is probably not surprising considering the large number of risk factors and probable etiologies for COME that have been discovered.

To our knowledge, this is the first description of middle ear microbiome function. Although patient-to-patient taxonomic composition varied significantly, microbiome function was very consistent (Figure 2). This demonstrates that despite varying taxonomic makeup, microbial community functions frequently converge (Human Microbiome Project, 2012; Louca et al., 2018). These will comprise fundamental activities necessary for microbial survival, such as translation (Human Microbiome Project, 2012), which we found to be relatively abundant. It's interesting that "Membrane Transport," which comprises a variety of genes like transporters, the phosphotransferase system, and the bacterial secretion system, was one of the top activities of the middle ear microbial population.In our instance, the majority of the genes fell under the broad category of "Transporters." Membrane transport has been linked to gut microbiome dysbiosis in conditions like irritable bowel syndrome and obesity, as well as being a plentiful component of the healthy laryngeal microbiome, in earlier research on the human microbiome (Jette et al., 2016). (Greenblum et al., 2012). Further investigation is required to determine whether these roles are particular to the microbial community during COME or whether they are shared by the healthy MEF microbiome and AOM.

A significant body of research points to a connection between OM, and specifically COME, and atopic illness. Allergy rhinitis (Alles et al., 2001), eczema (MacIntyre et al., 2010), and asthma (Gamble et al., 1992; Eldeirawi et al., 2010; MacIntyre et al., 2010; Bjur et al., 2012) are among these associated atopic diseases. However, the same associations are not always reproduced between studies (Zernotti et al., 2017). According to Gamble et al. (1992), COME in asthmatics is not a separate condition but rather a symptom of an atopic disease that affects the mucociliary system throughout the respiratory tract.Similar to bronchiolitis, inflammation from bronchiolitis may compromise mucociliary clearance and increase the susceptibility of the middle ear to infections. The intriguing correlation between a decline in two ear-trophic OM pathogens (Alloiococcus and Turicella) and an increase in pathogens linked to asthma and bronchiolitis (Haemophilus, Moraxella, and Staphylococcus) in asthma and bronchiolitis patients lends credence to the idea that COME linked to respiratory diseases may have a different aetiology from COME in children without respiratory illnesses (Gamble et al., 1992; Zernotti et al., 2017).Our results demonstrate that COME in children with lower airway disease is characterised by a distinct microbiome, even though the directionality of this relationship is unknown. This finding may also help to explain the higher frequency of COME complications like mucoid effusion and COME recurrence seen in asthma patients (Gamble et al., 1992). Future research should look into how these diseases relate to the microbiomes of their respective hosts.

CONCLUSION

We undertook a thorough analysis of the middle ear microbiota in children with COME in

this study to determine its makeup and purpose. In comparison to OTUs, we used an ASV pipeline to attain higher resolution at the species and genus levels. Children's taxonomic profiles differed considerably, but the function of the microbiome was impressively consistent across all patients. Additionally, we discovered important variations in the middle ear microbiome linked to a diagnosis of lower airway disease, including - and -diversity and relative abundance of Haemophilus, Moraxella, Staphylococcus, Alloiococcus, and Turicella. These findings encourage further investigation into the relationship between these significant paediatric disorders and the microbiome of respiratory infections.

REFERENCES

[1] Alles, R., Parikh, A., Hawk, L., Darby, Y., Romer, J. N., and Scadding, G. (2001). The prevalence of atopic disorders in children with chronic otitis media with effusion. Pediatr. Allergy Immunol. 12, 102–106

[2] Andrade, M. A., Hoberman, A., Glustein, J., Paradise, J. L., and Wald, E. R. (1998). Acute otitis media in children with bronchiolitis. Pedatrics. 101, 617–619.

[3] Barbera, P., Kozlov, A., Czech, L., Morel, B., Darriba, D., Flouri, T., et al. (2019). EPA-ng: massively parallel evolutionary placement of genetic sequences. Syst. Biol. 68, 365–369.

[4] Bisgaard, H., Northman Hermansen, M., Buchvald, F., Loland, L., Brydensholt Halkjaer, L., Bonnelykke, K., et al. (2007). Childhood asthma after bacterial colonization of the airway in neonates. N. Engl. J. Med. 357, 1487–1495.

[5] Callahan, B. J. (2018). Silva Taxonomic Training Data Formatted for DADA2. Silva version 132.

[6] Callahan, B. J., McMurdie, P. J., and Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11, 2639–2643. doi: 10.1038/ismej.2017.119

[7] Hasegawa, K., Linnemann, R. W., Mansbach, J. M., Ajami, N. J., Espinola, J. A., Petrosino, J. F., et al. (2017). Nasal airway microbiota profile and severe bronchiolitis in infants: a case-control study. Pediatr. Infect. Dis. J. 36, 1044–1051.

[8] Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15:550.

[9] Lozupone, C. A., Hamady, M., Kelley, S. T., and Knight, R. (2007). Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. Appl. Environ. Microbiol. 73, 1576–1585.