Tissue microbiota in nasopharyngeal adenoid and its association with pneumococcal carriage

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\textbf{ABSTRACT}

The microbial colonization in the nasopharynx is a prerequisite for the onset of infectious diseases. For successful infection, pathogens should overcome host defenses as well as compete effectively with the resident microbiota. Hence, elucidating the richness and diversity of the microbiome at the site of pathogen colonization is pivotal. Here, we investigated the adenoidal tissue microbiota collected through adenoidectomy to evaluate the impact of \textit{Streptococcus pneumoniae}. Prospectively, children with sleep-disordered breathing (SDB) and otitis media with effusion (OME) were enrolled. During adenoidectomy, the nasopharyngeal swab and adenoid tissues were collected to determine the pneumococcal carriage and tissue microbiota, using multiplex PCR and 16S ribosomal RNA (16S rRNA) pyrosequencing. A total of 66 pediatric patients comprising 38 children with SDB and 28 children with OME were enrolled. There was no difference between the bacterial cultures from the surface of the nasopharyngeal adenoid in the SDB and OME groups. Thirty-four samples (17 SDB and 17 OME) underwent 16S rRNA pyrosequencing and fulfilled the criteria for further analysis. The Shannon diversity index for the samples from the SDB patients was found to be higher than that observed for the samples from OME patients, although the difference was not significant ($p = 0.095$). The Shannon diversity index for the samples negative for the pneumococcal carriage was significantly higher than that for the samples positive for pneumococcal carriage ($p = 0.038$). \textit{Dialister} was significantly less present in the adenoid tissue positive for the pneumococcal carriage. \textit{Streptococcus pneumoniae}, one of the most common pathogens of the airway, significantly influences the composition and diversity of the microbiota in the nasopharyngeal adenoid. Thus, bacterial community analysis based on 16S rRNA pyrosequencing allows for better understanding of the relationship between the adenoidal microbial communities.

\section{Introduction}

Sleep-disordered breathing (SDB) is a breathing disorder wherein the upper airways are compromised particularly during sleep as the pharyngeal muscle relaxes [1,2]. SDB ranges from simple snoring to obstructive sleep apnea syndrome (OSAS) in extreme conditions.

\textit{Abbreviations:} IL, interleukin; OME, otitis media with effusion; OSAS, obstructive sleep apnea syndrome; OTU, operational taxonomic unit; rRNA, ribosomal RNA; SDB, sleep-disordered breathing.

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Adenoid hypertrophy, along with enlarged palatine tonsils, is a major factor for airway obstruction in children with SDB [3]. To date, adenoidectomy and tonsillectomy are the standard surgical procedures in clinical practice for OSAS in children with hypertrophy of adenoid and tonsils [4].

Otitis media with effusion (OME) is another common disease in pediatric otolaryngology characterized by the accumulation of fluid in the middle ear [5]. OME affects the hearing in children along with further distorted language and behavioral development [6–9]. Although the pathogenesis of OME is not fully understood, the dysfunction of the Eustachian tube (ET) and adenoid hypertrophy are presumed to be the most important contributing factors [5]. A shorter and more straightened ET is susceptible to the microbial transfer from the nasopharynx into the middle ear cavity in children. The hypertrophic nasopharyngeal adenoid obstructing the orifice of the ET and prolonged infections in the adenoid leading to tubal edema may predispose children to OME [10, 12].

Most OME cases resolve spontaneously; however, some persist and require surgical drainage with tympanostomy tube placement [13]. Several pieces of evidence have shown that concurrent adenoidectomy effectively reduces the need for repeated tympanostomy tube placement in OME [14, 15]. Therefore, adenoidectomy is assumed to reduce the burden of resident bacteria in the adenoid, thereby restoring the function of the ET and diminishing the chance of retrograde seeding of bacteria from the ET in the middle ear [10, 12].

The microbial environment of the nasopharynx is the usual niche of various important airway pathogens such as Neisseria meningitidis, Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus, Streptococcus pneumoniae, and various streptococci [16, 17]. Although this microbial colonization is mostly asymptomatic, often this colonization in the nasopharynx acts as a source of infectious diseases, including otitis media and sinusitis in the upper airway as well as invasive or systemic diseases of pneumonia, bacteremia, and meningitis [18, 19].

The nasopharyngeal adenoid is a part of the Waldeyer’s ring, consisting of a mass of lymphoid tissue in the critical area that serves as the first line of immune defense in the upper airway [20]. Recent evidence suggests a central role of the CD4+ T cells that produce cytokines of the interleukin (IL)-17 family, the helper T 17 cells involved in the host defense against mucosal microbial colonization, especially S. pneumoniae, and the alteration of the microbiome in the upper airway [21–23]. The insufficient response of IL-17-mediated mucosal clearance of S. pneumoniae, one of the most common respiratory pathogens, may lead to prolonged or chronic carriage and vulnerability of the middle ear to OME development in children [24–26].

Carriage studies in children have demonstrated that microbiobioare competition and symbiosis occur in the upper airway [27]. For initial colonization, a pathogen should not only overcome host defenses but also compete effectively with the resident microbiota [28]. Thus, studies on the richness and diversity of the microbiome at the site of pathogen colonization are needed. Besides, bacteria have been found intracellularly in the adenoids [29] and associated with the pathogenesis of the adenoid hyperplasia [30]. The prevailing knowledge regarding the microbiology of the upper airways has been derived from culture-based studies, which reflect only a very small fraction of the bacteria present on the mucosal surface. Culture-independent molecular surveys based on 16S ribosomal RNA (16S rRNA) pyrosequencing are now being employed to determine the microbiota both on the surface and within the tissue of adenoids [31].

A previous study has demonstrated the powerful influence of IL-17 signaling on the composition of the nasal microbiome before and after pneumococcal colonization using a murine model [23]. Our previous study also reported the difference in adenoid response in the IL-17-mediated mucosal clearance of S. pneumoniae between children with OME and those with SDB [26]. In this study, we hypothesized that there would be a difference in the composition and diversity of nasopharyngeal tissue microbiota between children with OME and those with SDB, and that S. pneumoniae may drive the changes in the nasopharyngeal microbiome composition and diversity. Therefore, in the present study, our aim was to investigate the adenoidal tissue microbiota collected from children with OME and SDB and evaluate the impact of S. pneumoniae on the adenoidal tissue microbiota of these children.

2. Material and methods

2.1. Patients

Children with SDB and OME were prospectively enrolled from April 2015 to October 2018. Children who met these inclusion criteria were recruited: (1) age 3–12 years; (2) presenting significant symptoms of OSAS or persistent OME; and (3) scheduled for adenoidectomy and myringotomy with ventilation tube insertion or adenotonsillectomy. Children who met these exclusion criteria were excluded: (1) usage of antibiotics in the recent 4 weeks; (2) congenital anomalies; or (3) persistent major medical disorders or chronic illnesses, such as autoimmune disorders, diabetes, immunodeficiency, nephrotic disease, and malignancy. The Institutional Review Board approved this study (approval numbers: 103–4773B and 201601090A3C501). All participants and/or their legal guardians provided written informed consent. All protocols followed the relevant guidelines and regulations of the institutional review board.

2.2. Bacteriologic study of nasopharyngeal adenoid

During transoral endoscopic adenoidectomy, cultures for aerobic and anaerobic bacteria on the surface of the nasopharyngeal adenoid were obtained with sterile swabs before the removal of adenoid tissue. Swabs were then stored in Amies Transport Medium (Copan Italia, Brescia, Italy) and were transported to the Department of Laboratory Medicine (Chang Gung Memorial Hospital) and placed on blood agar plates (tryptic soy agar, 5% sheep’s blood, 10 μg/mL neomycin; BD Biosciences, Bedford, Massachusetts, USA), Columbia colistin-nalidixic acid agar biplates, and eosin methylene blue plates. The plates were then cultured at 37 °C with 5% CO2 atmosphere, and normal atmosphere for 48 h. Bacterial identification was determined using the Bruker LT microflex MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) with Bruker BioTyper 3.0 system software (Bruker Daltonics) [22, 23].

Another swab obtained just before adenoidectomy was sent to the laboratory for S. pneumoniae serotyping, determined by multiplex PCR, as previously described [25, 34]. In brief, the nucleic acids were extracted by a QIAamp genomic DNA kit (Qiagen, Valencia, CA, USA), and kept frozen at −70 °C until further use. Primer pairs of 35 serotypes were designed as previously described [25]. A primer pair targeting cpsA (capsular polysaccharide A), which was found in all known serotypes, was used as a positive control. PCR conditions were set as follows: an initial incubation at 94 °C for 4 min, followed by 30 cycles of 94 °C for 45 s, 54 °C for 45 s, and 65 °C for 2 min 30 s. PCR products were measured with 1.4% agarose gel electrophoresis at 120 V for 45 min. The gel was then visualized by ultraviolet transillumination after staining with ethidium bromide. The oligonucleotide primer sequences were published and are available online on the Centers for Disease Control and Prevention (Atlanta, GA, USA). (https://www.cdc.gov/streptab/pneumococcus/index.html).

2.3. Adenoid sample collection

Adenoidectomy was performed under general anesthetic without the usage of intravenous antibiotics at induction. Under the transoral endoscopy approach, samples of adenoid tissue were obtained using a sterile blade, put into sterile cryotubes, and stored at −70 °C until further use.
2.4. DNA extraction

Forty-two adenoidal tissue samples (21 SDB and 21 OME) were thawed on ice and put into a sterile Lysing Matrix E tube (MP Biomedicals, Australia). DNA extraction was performed using the Qiagen Blood Mini Kit (Qiagen) according to the manufacturer’s instructions. The resultant DNA quality and quantity were measured using 1% agarose gel electrophoresis and a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The quality and quantity of genomic DNA were measured on a NanoDrop 3300 fluorospectrometer (Thermo Scientific, Barrington, IL, USA).

2.5. Bacterial 16S rRNA gene sequencing

Total DNA extracted from the 42 adenoidal tissue samples were subjected to PCR amplification using the V3 forward primer 5'-CCTACGGGGNGGCWGCAG-3' and V4 reverse primer 5'-GACTACHVGGGTATCTAATCC-3', yielding a 300 bp amplicon covering the highly variable V3–V4 region on the 16S rRNA gene. By using the Illumina platform, paired-end sequence data in FASTQ format were obtained. A FASTX Toolkit was used to assess sequence quality. Raw reads were demultiplexed by barcodes, and adaptor sequences were removed. A minimum Phred quality score (Q score) of 20 was applied to trim low-quality bases.

2.6. Bacterial community analysis

QIIME2 was applied for alpha diversity, beta diversity, and principal coordinate analyses (PCoA) using the Bray–Curtis distance. Deseq2 in QIIME2 was used to identify operational taxonomic units (OTUs) that differed between two patient groups (OME vs. SDB; positive vs. negative for pneumococcal carriage). The prevalence of a specific taxon was calculated by the number of samples containing the taxon divided by the total number of samples. The annotated OTUs were used for showing the taxonomy composition in adenoidal tissues from both the SDB and OME groups.

2.7. Statistical analyses

Data were presented as mean ± standard deviation. Categorical data were compared using the Chi-square test or Fisher’s exact test, as appropriate, using the GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA). Diversity indices (including Shannon—Wiener index and Inverse-Simpson index) and rarefaction curves were calculated for all samples from OTU tables using QIIME2. The differences with p value < 0.05 were considered statistically significant.

3. Results

3.1. Clinical characteristics of the study population

In total, 66 consecutive pediatric patients comprising 38 children with SDB who underwent adenotonsillectomy and 28 children with OME who underwent adenoidectomy and myringotomy with ventilation tube insertion were enrolled during the study period. The mean age was 6.4 ± 2.1 years (6.7 ± 2.1 and 6.1 ± 2.2 years in SDB and OME, respectively). The ratio of males to females was 43:23, 27:11, and 16:12 for total cases, SDB, and OME groups, respectively. No statistical differences in age, gender, allergy, BMI, Adenoid/Nasopharynx (A/N) ratio, and pneumococcal conjugate vaccination were observed between the two groups.

3.2. Bacteriological study of nasopharyngeal adenoid

Bacterial cultures from the surface of the nasopharyngeal adenoid are presented in Table 1. H. influenzae (43.9%), S. aureus (39.4%), and

| Table 1: Bacteriology of nasopharyngeal adenoid surface in patients with SDB and OME. |
|----------------------------------|----------|----------------|-----|-----|
| Total (n = 66) | SDB (n = 38) | OME (n = 28) | P value |
| Aerobic | | | |
| Haemophilus influenzae | 29 (43.9) | 17 (44.7) | 12 (42.9) | 0.879 |
| Staphylococcus aureus | 26 (39.4) | 18 (47.4) | 8 (28.6) | 0.122 |
| Streptococcus mitis | 20 (30.3) | 9 (23.7) | 11 (39.3) | 0.173 |
| Moraxella catarrhalis | 10 (15.2) | 7 (18.4) | 3 (10.7) | 0.388 |
| Neisseria species | 10 (15.2) | 6 (15.8) | 4 (14.3) | 0.866 |
| Viridans streptococcus | 10 (15.2) | 8 (21.1) | 2 (7.1) | 0.119 |
| Streptococcus pneumoniae | 7 (10.6) | 4 (10.5) | 3 (10.7) | 1 |
| Anaerobic | | | |
| Prevotella melaninogenica | 50 (75.8) | 28 (73.7) | 22 (78.6) | 0.647 |
| Prevotella species | 11 (16.7) | 5 (13.2) | 6 (21.4) | 0.373 |
| Veillonella species | 9 (13.6) | 3 (7.9) | 6 (21.4) | 0.153 |
| Actinomyces odontolyticus | 9 (13.6) | 5 (13.2) | 4 (14.3) | 1 |
| Prevotella nanceiensis | 6 (9.1) | 1 (2.6) | 5 (17.9) | 0.076 |
| Actinomyces oris | 5 (7.6) | 3 (7.9) | 2 (7.1) | 1 |
| Actinomyces species | 5 (7.6) | 3 (7.9) | 0 (0) | 0.067 |
| Veillonella dispar | 5 (7.6) | 2 (5.3) | 3 (10.7) | 0.256 |
| Fusobacterium species | 3 (4.5) | 3 (7.9) | 0 (0) | 0.072 |
| Prevotella histicola | 3 (4.5) | 0 (0) | 3 (10.7) | 1 |
| Veillonella parvula | 3 (4.5) | 2 (5.3) | 1 (3.6) | 1 |
| Others | 9 (13.6) | 7 (18.4) | 2 (7.1) | 1 |

Data were presented with the number (%). †Variables were compared using the Chi-square test or Fisher’s exact test, as appropriate, between SDB and OME groups. SDB, sleep disordered breathing; OME, otitis media with effusion.

Streptococcus mitis (30.3%) was the most common aerobic bacteria. Prevotella melaninogenica (75.8%), Prevotella sp. (16.7%), Actinomyces odontolyticus (13.6%), and Veillonella sp. (13.6%) were the most common anaerobic bacteria. There was no difference in the bacteriology measured by traditional culture between the SDB and OME groups. S. pneumoniae was detected in swabs collected from 7 children (10.6%) (4 SDB and 3 OME) by traditional culture, and in 20 children (30.3%) (11 SDB and 9 OME) by multiplex PCR. It was not feasible to compare the difference in serotypes between the SDB and OME groups due to the small sample size and large number of serotypes.

3.3. Tissue microbiota of the nasopharyngeal adenoids

A total of 42 adenoid samples (21 SDB and 21 OME) were analyzed. Two of them had no detectable V3–V4 amplicon during PCR amplification. The remaining 40 samples (20 SDB and 20 OME) were completely sequenced. After demultiplexing and quality control assessments, 1 489 033 sequence reads were obtained from the samples, with a median of 32 002 reads and a median read length of 415 bp per sample. Filtered criteria included: 1) each feature was present in at least 2 samples; 2) each feature at least contained 0.01% (# reads) of total reads; 3) each sample contained at least 1500 reads. Four samples were excluded due to inadequate total reads. A total of 173 features and 34 samples (17 SDB and 17 OME) met the criteria and were subjected to further analysis. The taxonomic compositions at the phylum level in adenoid tissues from both the SDB and OME groups are shown in Fig. 1. The PCoA was performed using the Bray—Curtis distance matrix to determine the relationships between various bacterial communities in the two groups (Fig. 2). Adenoidal samples in the OME group were more...
closely clustered than in the SDB group. The difference in Shannon diversity index between the samples collected from SDB and OME patients are presented in Fig. 3, in which the Shannon diversity index observed for the samples from SDB patients was higher than that observed for the samples from the OME patients, although the difference was not significant. Moreover, the differences in Shannon diversity (alpha diversity) between adenoidal samples positive for and negative for pneumococcal carriage were evaluated. The Shannon diversity index observed for the samples negative for pneumococcal carriage was higher than that observed for the samples positive for pneumococcal carriage (Fig. 4).

The differential abundance microbiome analysis evaluated the dominant OTUs in samples positive or negative for the pneumococcal carriage. 

Alloprevotella, Staphylococcus, Moraxella, and Neisseriacea were significantly dominant in the samples positive for pneumococcal carriage. Dialister was significantly less present in the adenoid tissue samples positive for the pneumococcal carriage. (Fig. 5).

4. Discussion

To the best of our knowledge, this is the first study to investigate the tissue microbiota of the nasopharyngeal adenoid in children with SDB and OME and its association with S. pneumoniae. Our results demonstrated that the adenoid samples from OME patients depict a lower level of Shannon diversity index than those from SDB patients, although the difference was not significant (p = 0.095). Furthermore, by using Bray—Curtis distance matrix to determine the relationships between various bacterial communities in the two groups, the adenoidal samples in the OME group were found to be more closely clustered than in the SDB. Our previous study had revealed children with OME exhibited insufficient response of IL-17-mediated mucosal clearance to the common airway pathogen S. pneumoniae, leading to a prolonged or chronic carriage, and thus may be vulnerable to OME development [25,26]. However, the adenoidal samples positive for pneumococcal carriage demonstrated a significantly lower Shannon diversity index than those negative for the pneumococcal carriage. Alloprevotella, Staphylococcus, Moraxella, and Neisseriacea were significantly dominant in the samples positive for pneumococcal carriage. Dialister was significantly less present in the adenoid tissue positive for pneumococcal carriage. Taken altogether, these results indicated that in children with OME, there is an insufficient response of nasopharyngeal mucosal clearance to pathogens. This may result in the dominance of specific microbes and reduction of microbiota diversity. As one of the most common pathogens of the airway, S. pneumoniae presents a powerful influence on the composition and diversity of the microbiota in the nasopharyngeal adenoid, as well as the interspecific symbiosis between several bacterial species.

Previous studies have shown that IL-17 plays an important role in host defense against extracellular bacterial pathogens, especially S. pneumoniae [35–40]. Furthermore, one study has reported specific gene polymorphisms of IL-17, such as G-152A, which were associated with an increased colonization rate of S. pneumoniae in young children [41]. Similarly, our previous study has shown that children with OME exhibited insufficient responses of IL-17 to S. pneumoniae [26]. Besides, a previous study also demonstrated that changes in nasal microbiome composition driven by IL-17 could be an important factor in successful resistance to pneumococcal colonization, which could be potentially manipulated to enhance host defense against airway pathogens [25]. When colonization begins, a pathogen needs to overcome host defenses but also needs to compete or synergize effectively with the environmental microbiota [28]. Thus, it is important to elucidate the microbe-microbe competition and symbiosis occurring in the upper airway especially in children with an insufficient mucosal clearance of airway pathogens [27]. A complete study of the nasopharyngeal microbiota may aid in identifying those children susceptible to OME development and improve the therapeutic strategy for those refractory to current treatment.

M. catarrhalis has been reported to play important roles in the co-colonization and persistence of the other major otopathogens, such as S. pneumoniae and non-typeable H. influenzae [42,43]. However, S. pneumoniae and S. aureus reportedly interfere with one another during nasopharyngeal colonization [44]. The S. pneumoniae carriage was negatively associated with S. aureus carriage in children [45]. Besides, the interaction between S. pneumoniae and Alloprevotella, Neisseriacea, and Dialister was not clear in the present study. The implications of these findings require further investigation.

A previous culture-based study showed that the bacteriology of nasopharyngeal swabs and inner part of the adenoid tissue were similar.
A culture-independent bacterial 16S rRNA gene sequencing study also suggested that there is no significant difference in the relative abundance or diversity of the microbial community between an adenoid surface mucosal swab and a tissue sample. Accordingly, it may be sufficient to use an adenoid surface swab or tissue sample to assess the adenoid microbiome as a whole.

Similar studies using adenoid surface swabs have been conducted and reported in the literature. Fago-Olsen et al. analyzed adenoid surface microbiota from children undergoing surgery for adenoid/tonsillar hyperplasia and subjects with secretory otitis media, and demonstrated that *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* were significantly more abundant in the adenoids and almost absent from palatine tonsils, indicating that adenoids but not palatine tonsils may be the main reservoir of pathogens for otitis media. A study by Walker et al. investigated the nasal microbiome in children with OME and revealed a higher abundance of pathogens and a lower abundance of commensals in children with OME than those in the control group. A study by Kim et al. also showed that the diversity index, mean OTUs, and Shannon index were lower in the OME group than in children undergoing tonsillectomy and adenoidectomy due to obstructive symptoms such as snoring and mouth breathing.

Most of the current knowledge on the microbiology of the upper airways has been derived from culture-based studies, which reflect only a very small fraction of the bacteria present on the mucosal surface. Moreover, bacteria have been reported to be present intracellularly in the adenoids, which would be difficult to detect by conventional culture. In the current study, we have used culture-independent, 16S rRNA pyrosequencing to elucidate the richness and inter/intra sample diversity of the microbiota in adenoid tissue and identify microbial differences between patient groups. The introduction of culture-independent technique has allowed for investigation of entire bacterial communities, leading to better comprehension of the role of resident flora. However, some limitations should be taken into account when interpreting data derived from these studies. First, the culture-independent technique cannot determine whether identified taxa are active or inactive, and alive or dead; thus, functional information is restricted. Second, taxonomic resolution is usually limited to the family or genus level when a short segment of 16S rRNA gene is amplified and sequenced. Third, there may be great variability in the data depending on technical aspects, such as sampling, storage, region selection, amplicon size, sequencing approach, and bioinformatic analysis.

There are several limitations to this study. First, the study was conducted on a relatively small number of children, and our results need to
be confirmed in larger studies. Second, the taxonomic analysis of the adenoid samples was limited to the genus level and not the species level. However, the main aim of this study was to compare bacterial diversity and composition between children with these two diseases and pneumococcal carriage status. Future studies should use bacterial-specific PCR to confirm the species of specific OTUs. Third, this study lacked a normal healthy control group. There are difficulties in recruiting healthy children because of the ethical considerations involved in performing an invasive biopsy on the adenoid tissue. Instead, our data compared two different diseases and their associations with pneumococcal carriage status.

5. Conclusions

*S. pneumoniae*, one of the most common pathogens of the airway, significantly influences the composition and diversity of the microbiota in the nasopharyngeal adenoid. Thus, bacterial community analysis based on 16S rRNA pyrosequencing allows for better understanding of the relationship between the adenoidal microbial communities.

Declaration of competing interest

The authors declare no competing interests.
Fig. 4. (a) Rarefaction curve (alpha diversity) between samples positive for and negative for pneumococcal carriage. (b) The Shannon diversity index observed for the samples negative for the pneumococcal carriage was higher than that observed for the samples positive for pneumococcal carriage ($p = 0.038$).

**Glossary**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>OME</td>
<td>otitis media with effusion</td>
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<tr>
<td>OSAS</td>
<td>obstructive sleep apnea syndrome</td>
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<tr>
<td>OTU</td>
<td>operational taxonomic units</td>
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<td>rRNA</td>
<td>ribosomal RNA</td>
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<td>SDB</td>
<td>sleep-disordered breathing</td>
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**Availability of data and materials**

The original source data and material will be available upon reasonable request.

**Ethics approval and consent to participate**

The Institutional Review Board of Chang Gung memorial hospital approved of this study (approval numbers: 103–4773B and 201601090A3C501). All participants and/or their legal guardians provided written informed consent. All protocols followed the relevant guidelines and regulations of the institutional review board.

**Author statement**

All authors have read the manuscript, and agree to publish the manuscript.

**Authors’ contributions**

CH Chiu and CC Huang designed the study. TJ Lee, CL Chen, and PW Wu helped in collecting the data. CC Huang, ML Liou, CY Lee, and TH Chang interpreted the data. CC Huang, PW Wu, and TH Chang wrote the manuscript. All authors participated in scientific discussion and approved the final manuscript.
The differential abundance microbiome analysis evaluated the dominant operational taxonomic units (OTUs) in the samples positive or negative for the pneumococcal carriage. *Alloprevotella*, *Staphylococcus*, *Moraxella*, and *Neisseriaceae* were significantly dominant in the samples positive for pneumococcal carriage than those negative for the pneumococcal carriage.

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### References

23. N.D. Ritchie, U.Z. Ijaz, T.J. Evans, IL-17 signalling restructures the nasal microbiome and drives dynamic changes following Streptococcus pneumoniae colonization, BMC Genom. 18 (1) (2017) 807.
29. M. Stopinska, O. Olaszewska-Sosińska, M. Lau-Dworak, et al., Identification of intracellular bacteria in adenoid and tonsil tissue specimens: the efficiency of...


