



Characteristics of the bacterial microbiota in the upper respiratory tract of children

Wei Cao¹ · Yi Sun¹ · Na Zhao² · Jun Song¹ · Nanfeng Zhang¹ · Long Liu¹ · Qian Liu²

Received: 15 April 2021 / Accepted: 20 July 2021 / Published online: 24 July 2021

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Purpose The respiratory tract microbiota are deemed as the gatekeeper to health. Consequently, microbiota dysbiosis can lead to the development of diseases. To identify the exact origins of the localized pathogenic bacteria, we investigated bacterial composition in the upper airway tract.

Methods Separate mucosal swabs were collected from nostril or oropharynx of each participant. Meanwhile, the lymphoid tissues including adenoids and tonsils were collected during operation. DNAs were extracted from all the samples for the following 16S rRNA analysis.

Results At the phylum level, the basic bacterial structures in the adenoids, tonsils, oropharynx, and nostrils were generally similar: five main phyla *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria* form the majority of the microbiota. However, across these four sites, the microbiota composition differed. More specifically, the bacterial composition in the nostrils was unique. There, *Firmicutes* and *Actinobacteria* were the most abundant phyla, while *Bacteroides* and *Fusobacteria* were the least abundant. At the genus level, *Staphylococcus*, *Dolosigranulum*, *Corynebacterium*, and *Moraxella* were the most plentiful, while *Fusobacteria* was the least ample. Across all sites, *Streptococcus* displayed similar abundances. *Fusobacteria* exhibited higher abundances in the lymphoid tissues and oropharynx. *Haemophilus* and *Neisseria* were more plentiful in the tonsils and oropharynx. Notably, *Klebsiella*, which is normally localized to the gut, was abundant in the adenoids and tonsils.

Conclusion Our data indicate that promising pathogenic bacteria originate from all sites in the upper airway. The upper tract lymphoid tissues, normally considered as immune organs, may also serve as reservoirs for pathogenic bacteria.

Keywords Microbiota · Adenoids · Tonsils · Oropharynx · Nostrils

Introduction

The airway microbiota play a crucial role in local immune function [22]. Microbiota dysbiosis in the upper respiratory tract (URT) has been reported to be involved in disease development. The nasal microbiota are diverse, and the

Wei Cao and Yi Sun have contributed equally to this work.

✉ Long Liu
122044683@qq.com

✉ Qian Liu
qq2005011@163.com

Wei Cao
545749757@qq.com

Yi Sun
zoecharming@126.com

Na Zhao
zhaona_smu@163.com

Jun Song
1178848128@qq.com

Nanfeng Zhang
zhangnf2617@163.com

¹ Department Otorhinolaryngology, Head and Neck Surgery, The Second Hospital of Anhui Medical University, Hefei, China

² Department of Laboratory Medicine, School of Medicine, Renji Hospital, Shanghai Jiao Tong University, Shanghai, China

presence of opportunistic pathogens in the nasal cavity is associated with allergic rhinitis, chronic rhinosinusitis, pneumonia, otitis media, severe obstructive sleep apnea (OSA), and even neurological diseases [2, 6, 9, 43]. The presence of pathogenic bacteria in the adenoids and tonsils contributes to the development of chronic or recurrent middle ear disease, recurrent tonsillitis, and obstructive sleep apnea [13, 37]. Fago-Olsen et al. hypothesized that the adenoids are the main reservoir for otopathogens (e.g., *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*) leading to otitis media [7].

The entire URT is interconnected, with the adenoids and tonsils located at the junction of nose and throat. The bacterial community in the upper airway is affected by many factors, such as age, temperature, and density [3, 16]. For example, before adulthood, the proportion of pathogenic bacteria declines while that of the commensal *Staphylococcus epidermidis* increases [19]. Furthermore, microbiota structure differs across various sites. That is, *Firmicutes* and *Actinobacteria* form the majority of the bacteria in the nostrils, while *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* are more abundant in the oropharynx [18].

Here, we identified the basic microbiota structure in the upper airway in a pediatric population. Samples including the adenoid and palatine tonsils tissues as well as swabs of the nostrils and oropharynx were collected. The microbiota alpha-diversity index in the lymphoid tissues was much higher than that of the swabs. The microbiota compositions differed significantly in the URT. In the nostrils, *Firmicutes* and *Actinobacteria* were the most abundant, while *Bacteroidetes* and *Fusobacteria* were the least. *Proteobacteria* were much less present in the adenoids and tonsils. At the genus level, *Streptococcus* was similarly prolific at all sites. *Fusobacteria*, the primary genus in the adenoids, was more abundant in the lymphoid tissues and the oropharynx. *Haemophilus* and *Neisseria* were more plentiful in the tonsils and oropharynx, while *Staphylococcus* and *Moraxella* were the most abundant genus in the nostrils. Notably, *Klebsiella*, which is usually localized to the gut, was ample in the adenoids.

Materials and methods

Participant enrollment and sample collection

Twenty-five children with hyperplasia of adenoids and tonsils were recruited for the study from Department of Otolaryngology-Head and Neck Surgery, the Second Hospital of Anhui Medical University from January 2020 to July 2020. These children were associated with either OSA (14 cases) or recurrent tonsillitis (11 cases). OSA patients were diagnosed with overnight polysomnography and an

Apnea–Hypopnea Index (AHI) score were calculated [36]. Here, we focused on uncomplicated childhood OSA, associated with adenotonsillar hypertrophy (> 50%) [24]. All enrolled OSA patients were associated with adenotonsillar hypertrophy with OSA AHI score > 5. The exclusion criterion was the use of any antimicrobials within the last two weeks. Infants younger than 1 year of age, with central apnea or hypoventilation syndromes were also excluded. OSA patients associated with other medical disorders including Down syndrome, craniofacial anomalies, neuromuscular disease, chronic lung disease were all excluded in our study. All participants do not have surgeries previously. The participants were unrelated individuals from both sexes. Separate mucosal swabs were collected from nostril or oropharynx of each participant, and the swabs were immediately submerged in 1 ml normal saline. The adenoids and tonsils tissues were collected during adenotonsillectomy operation. All the samples were kept at -80°C until further use. The study was approved by the ethics committee of the Second Hospital of Anhui Medical University (No.20200402), and informed consent was obtained from all participants.

DNA extraction and 16S rRNA gene amplification

All mucosal swabs were centrifuged at 10,000 g for 10 min after completely vortex. The samples were dissolved in buffer ALT with lysozyme (1.25 mg/ml) for 30 min at 37°C . For the tissue samples, the tissue was excised and homogenized in buffer ALT. After centrifugation at 6000 g for 10 min, the supernatant was incubated with lysozyme (1.25 mg/ml) for 30 min at 37°C . All the samples were used for the following DNA extraction according to the QIAamp DNA Mini Kit (Qiagen) protocol. Bacterial DNA was amplified using primers 341F (CCTACGGGNBGCASCAG) and 805R (GACTACHVGGGTATCTAATCC). The amplicon-specific V3 and V4 hypervariable regions of the bacterial 16S rRNA gene were amplified and sequenced using the Illumina HiSeq 2500 platform according to the standard protocols. The raw sequencing data were submitted to the GenBank database under accession number PRJNA689096.

Processing of bacterial 16S rRNA sequenced data

After de-multiplexing and quality trimming, the forward and reverse sequences were combined using Fast Length Adjustment of SHort reads (FLASH). Low-quality reads were filtered by fastq_quality_filter ($-p\ 90 -q\ 25 -Q33$) in FASTX Toolkit 0.0.14. Usearch 64 bit (v8.0) was applied for quality control and chimera filtering. The cleaned FASTA sequences were combined and clustered into OTUs using Uclust. After normalizing based on the smallest size of samples by random subtraction, the numbers of reads for each sample were selected to construct the OTU abundance

table. The taxonomic classification from the phylum to the genus level for the same number of reads was performed based on the Silva 16S rRNA v128 database using the RDP Classifier. The estimated sampling saturation and rarefaction curves were generated. Finally, the alpha-diversity and beta-diversity indices were calculated in the quantitative insights into microbial ecology (QIIME) and calculated based on weighted and unweighted Unifrac distance matrices. We used the Metastats method to identify species that show statistically significant differential abundances among groups.

Results

Demographic data of all the subjects

To identify the microbiota composition of different parts from one population, we enrolled 25 children who need operation due to the hyperplasia of adenoids and tonsils.

Table 1 Demographics characteristics of all the participants

Parameter	OSA	Recurrent tonsillitis
Total cases	14	11
Male/female	9/5	8/3
Mean age(y)	7.43	7.82
Previous surgeries	0/14	0/11
AHI score		
1–4.9	0/14	7/11
5–8.9	3/14	4/11
>9	11/14	0/11

OSA obstructive sleep apnea, AHI Apnea–Hypopnea index

The tonsillar tissue and adenoids of 25 children undergoing adenotonsillectomy due to OSA (14 cases) or recurrent tonsillitis (11 cases) were collected during operation. The average ages for all the enrolled participants are 7.43 and 7.82 y, respectively. AHI scores were calculated for all the participants (Table 1).

16S ribosomal ribonucleic acid (rRNA) sequencing

The microbiota from different sites of the same individuals were determined. URT samples (adenoids, palatine tonsils, oropharynx swabs, and nasal swabs) were collected from 25 children and underwent 16S rRNA sequencing. After quality control and chimera filtration, we finally got the following sample sizes 21 adenoids, 23 palatine tonsils, 25 oropharynx swabs, and 25 nasal swabs. The rarefaction curve for the number of sequences was generated (Supplementary Fig. 1), which confirmed that our data were large enough for subsequent analysis.

Bacterial diversity

We first analyzed microbiota diversity. In terms of alpha diversity, the operational taxonomic units (OTUs) and Chao index revealed that community richness in the adenoids and palatine tonsils was higher than in the oropharynx and nostrils (Fig. 1a, b). The Shannon diversity index, taking into account both community richness and evenness, was calculated to determine the nature and range of the temporal variability in microbial diversity across individuals. The Shannon index indicated a lower microbial diversity in the nostrils compared to the other sites (Fig. 1c).

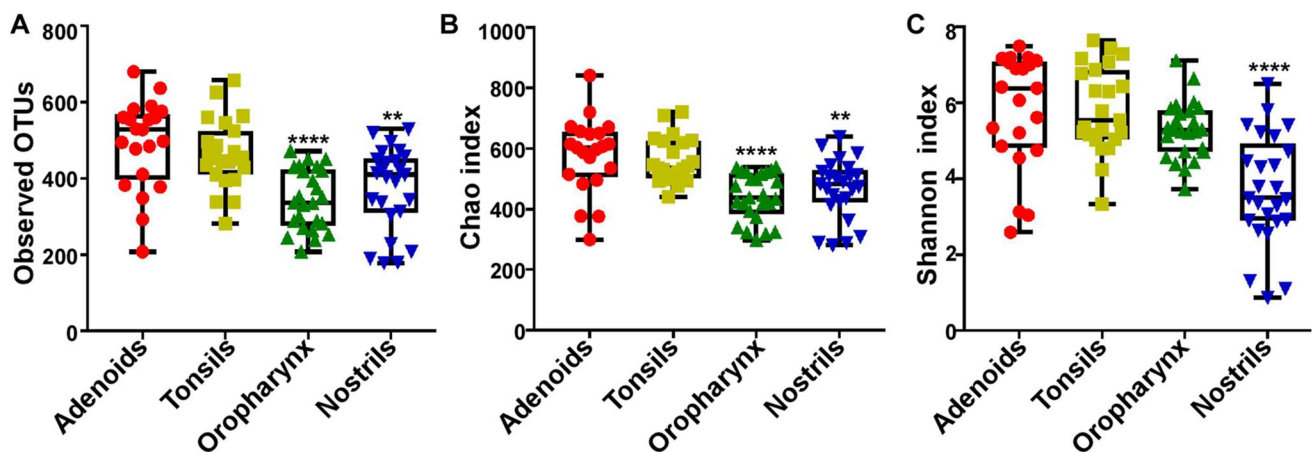


Fig. 1 Alpha-diversity indices of microbiotas across different sites. The operational taxonomic units (A) and Chao index (B) revealed a higher community richness in the adenoids and tonsils compared to

the other two sites. C The Shannon index indicated that the microbial diversity was lower in the nostrils compared to the other three sites. ** $p < 0.01$, **** $p < 0.0001$

Multivariate analysis of the microbial community across different sites

Principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity distance was used to analyze differences in inter-sample diversity. Samples from the nostrils had bacterial community structures that significantly differed from those of the adenoids. The bacterial compositions between the tonsils and oropharynx overlapped. Altogether, microbiota significantly differed in the URT of one pediatric population (analysis of similarities, $R=0.455$, $p=0.001$, Fig. 2).

The overall microbiota structure in the upper airway

The microbiota distribution down to the genus level was analyzed across the different sites. In total, 18 phyla, 37 classes, 56 orders, 89 families, and 214 genera were detected from all the samples.

The analysis of 10 major microbial phyla revealed that the URT microbiota were composed of five predominant phyla: *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria*. *Firmicutes* (around 50%) were the most abundant phylum across all sites. Generally, the microbiota composition in the nostrils significantly differed from that of the other three sites (Fig. 3a). In the nostrils, *Firmicutes* and *Actinobacteria* were the most plentiful, while *Bacteroidetes* and *Fusobacteria* were the least (Fig. 3b). Notably, *Proteobacteria* was much less prolific in lymphoid tissues compared to the nostrils or oropharynx (Fig. 3b).

Bacteria at the genus level were also analyzed across the different sites. The distributions of the top 16 bacterial

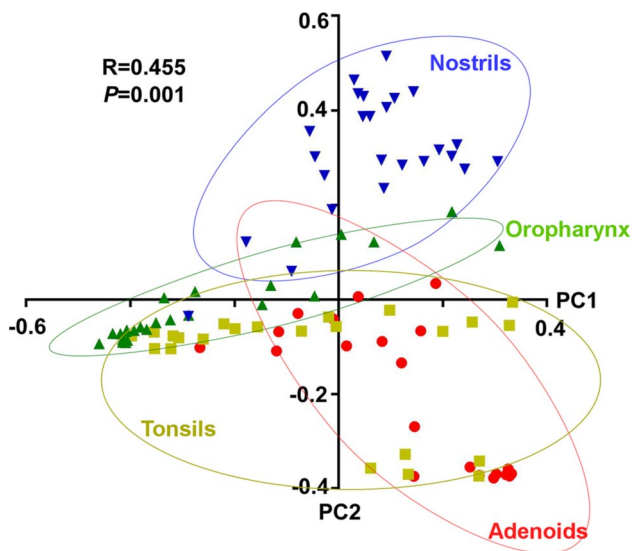


Fig. 2 Principal coordinates analysis based on Bray–Curtis dissimilarity among the four groups. Statistical analysis is by Analysis of Similarities (ANOSIM) test

genera for all areas are shown in Fig. 4a. Similar to the phyla analysis, the top 16 genera in the nostrils were completely different from the tonsils, adenoids, and oropharynx. More specifically, the top three genera *Staphylococcus* (phylum *Firmicutes*), *Moraxella* (phylum *Proteobacteria*), and *Corynebacteria* (phylum *Actinobacteria*) in the nostrils were absent from the top 10 genera of the other three sites (Fig. 4b). Although *Firmicutes* was much more prolific in the nostrils than in the other areas, *Streptococcus* (phylum *Firmicutes*) was similarly abundant across all sites.

Moraxella, *Klebsiella*, *Haemophilus*, and *Neisseria* are all pathogenic genera belonging to the *Proteobacteria* phylum. Although the abundance of *Proteobacteria* was very low in the adenoids, *Klebsiella* was the third-most ample genus at that site. *Haemophilus* and *Neisseria* were the most prevalent genera in the tonsils and oropharynx, respectively, and *Moraxella* was the second-most abundant genus in the nostrils (Fig. 4b), indicating that the entire upper tract serves as a possible reservoir for pathogenic bacteria.

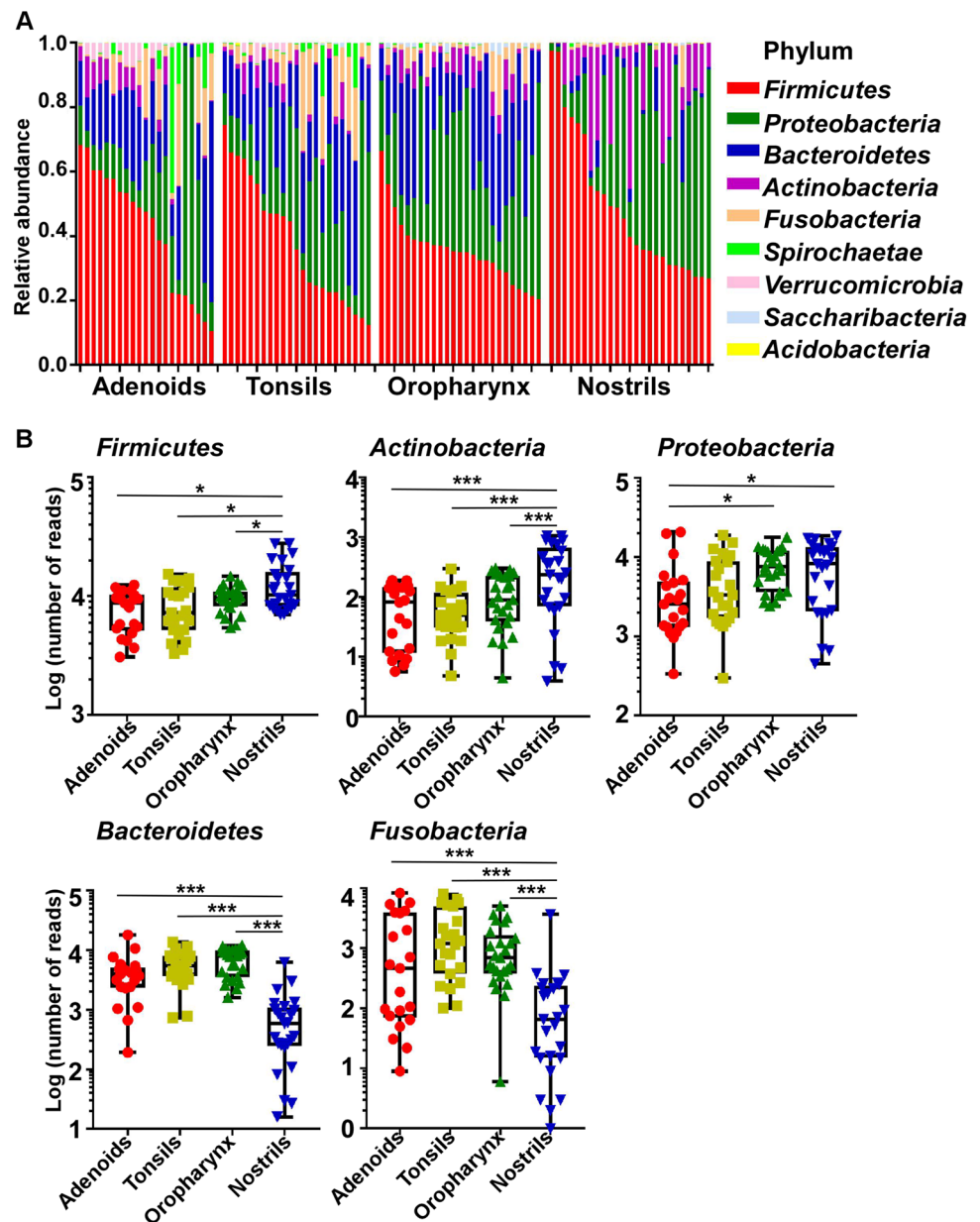
The composition of the *Bacteroidetes* phylum is diverse: the *Bacteroides*, *Prevotella* 7, and *Porphyromonas* were all in the top 10 genera present in the adenoids, tonsils, and oropharynx; however, their amounts were very low in the nostrils (Fig. 4). In addition, *Fusobacterium*, which belongs to the *Fusobacteria* phylum, was more abundant in the adenoids and tonsils (Fig. 4b).

Comparison of potential pathogenic bacteria abundances in the upper airway

We observed a significant difference in microbiota composition in the nostrils compared to the other three sites; accordingly, the top four genera were analyzed. Consistent with the increased presence of the *Firmicutes* and *Actinobacteria* phyla, *Staphylococcus* (phylum *Firmicutes*) was approximately 29.6, 17.4, and 14.0 times more plentiful in the nostrils, respectively, than in the adenoids, tonsils, and oropharynx. Similarly, *Dolosigranulum* (phylum *Firmicutes*) was approximately 252, 191, and 161 times more abundant than in the adenoids, tonsils, and oropharynx, respectively. Finally, *Corynebacterium 1* (phylum *Actinobacteria*) was present in amounts approximately 305, 489, and 820 times higher, respectively, than in the adenoids, tonsils, and oropharynx. Altogether, these three genera displayed the highest abundance in the nostrils compared to the other three sites (Fig. 5). Although the abundance of *Proteobacteria* was the lowest in the nostrils, the abundance of *Moraxella* (phylum *Proteobacteria*) was the highest (approximately 16, 178, and 30 times higher than the adenoids, tonsils, and oropharynx, respectively).

The pathogens reportedly involved in URT diseases were further analyzed. Traditional culture-based studies

Fig. 3 The overall microbiota structure at the phylum level of the upper airway of children. **A** Relative abundance of the ten major phyla in subjects divided by age. **B** Abundance of Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, and Fusobacteria across different sites in single individuals. * $p < 0.05$, *** $p < 0.001$

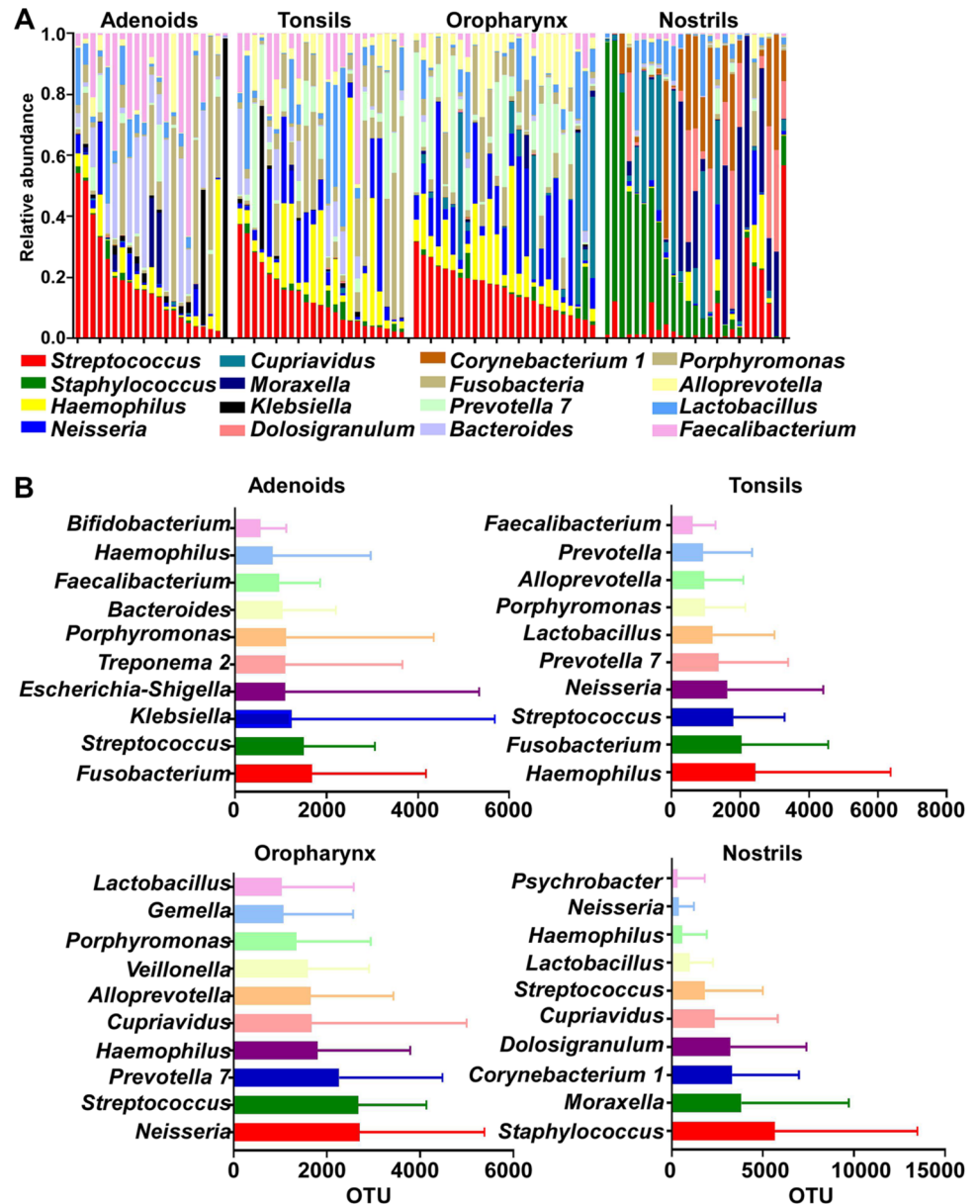


have shown that *S. pneumoniae*, *H. influenzae*, and *Neisseria* spp. are predominately isolated in patients with persistent otitis media with effusion [8]. Here, we observed that *Streptococcus* was similarly abundant across all sites, while *Haemophilus* and *Neisseria* were much more plentiful in the tonsils and oropharynx (Fig. 5). Notably, we observed that *Klebsiella* and Fusobacteria, normally localized to the alimentary canal, were much more ample in the adenoids as well as in the tonsils and oropharynx (Fig. 5), indicating that their presence in the throat, which connects the oral cavity and gastrointestinal tract, affects the localization of bacteria in lymphoid tissues.

Discussion

The URT microbiota influence human health. Commensal bacteria, no doubt, promote the maturation of the local immune system and the microbiota dysbiosis causes many diseases [32]. The adenoids are even coined the “pathogen reservoir” in children [13]. However, most of the current studies were based on the comparison of the microbiota between lymphoid tissues and middle ear specimens in patients. The exact distributions of the promising pathogens in the whole URT in children are still unknown. In this study, we analyzed the microbiota of the URT in a

Fig. 4 The overall microbiota structure at the genus level of the upper airway of children. (A) Relative abundance of the 16 major genera in the subjects divided by age. (B) Abundance of the top 10 genera across the different sites in single individuals

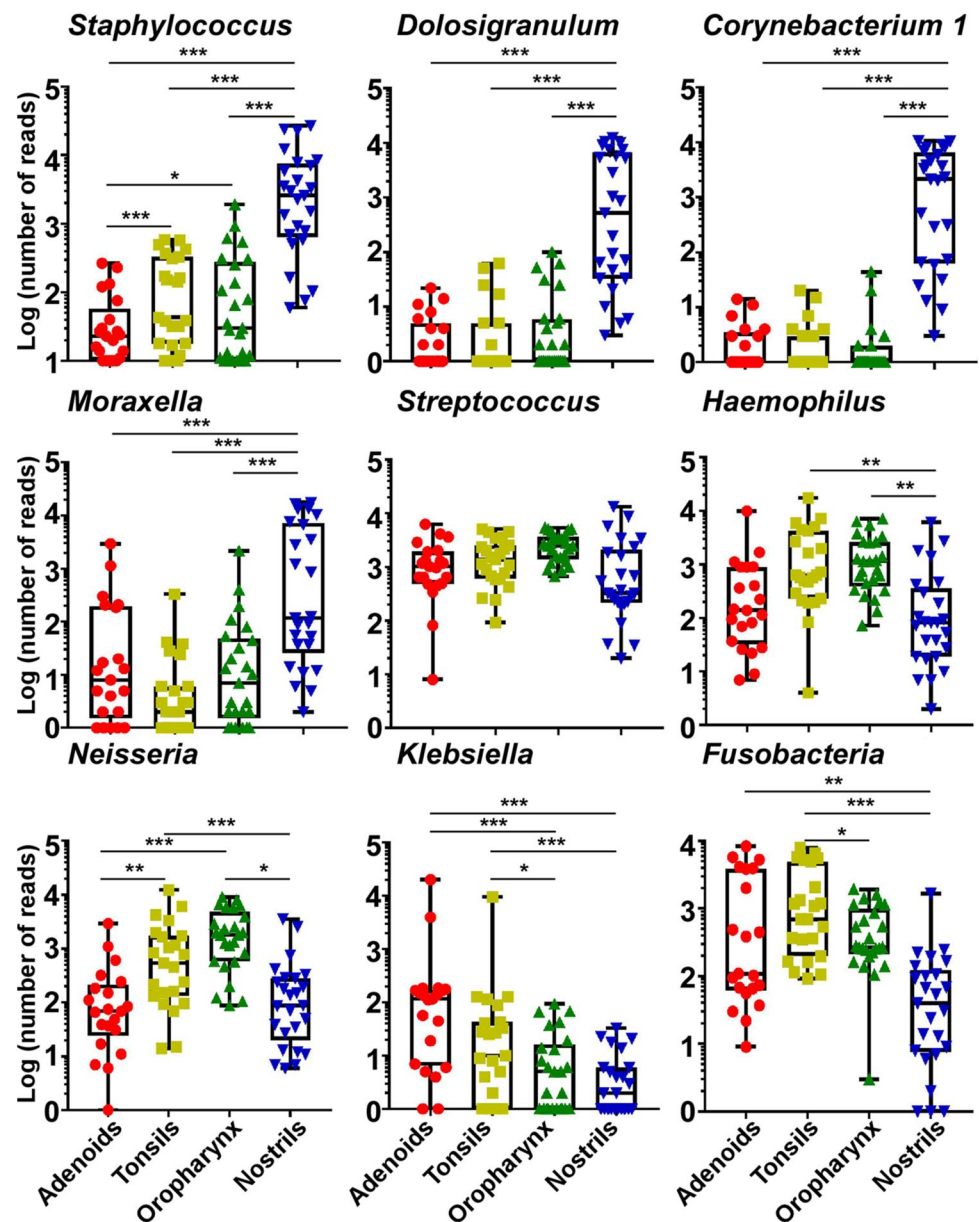


group of children without infections. The hyperplasia of adenoids and tonsils in these children were associated with OSA or recurrent tonsillitis. We found that *Staphylococcus*, *Corynebacterium*, *Dolosigranulum* and *Moraxella* were mainly present in the nostrils, while *Haemophilus* and *Neisseria* were mostly found in the tonsils and oropharynx. Notably, *Fusobacteria* and *Klebsiella*, which are normally present in the alimentary canal, exhibited high abundance in the adenoids. We concluded that promising pathogenic bacteria localize in the entire URT, and that specific bacteria are prone to localize in specific sites.

The adenoids and tonsils are lymphoid tissues that play important roles in immune defense [31]. The adenoids surface is colonized by commensal microbiota, which serve as a gateway to the respiratory tract [42]. However,

the potential pathogenic bacteria localized to the adenoids and tonsils are involved in the development of various diseases in the pediatric population [23]. In fact, the adenoids are coined the “pathogen reservoir” in children due to its specific role in middle ear infections [13]. This “pathogen reservoir hypothesis” is supported by Ren et al. By surveying 16S rRNA, they found that the common pathogens including *Haemophilus*, *Streptococcus*, *Moraxella*, and *Staphylococcus* are prevalent and abundant in the adenoids [34]. However, others have cast doubt on the adenoid “pathogen reservoir hypothesis” because they failed to find a correlation between adenoid and middle ear microenvironments [1, 4, 12, 29]. Here, we observed that several pathogenic bacteria, such as *Streptococcus* and *Haemophilus*, were indeed localized to the adenoids at

Fig. 5 Mean abundance of the promising pathogenic genera in the human upper respiratory tract microbiota across the four sites. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$



high amounts, supporting the pathogen reservoir hypothesis. However, *Staphylococcus aureus* and *M. catarrhalis*, bacteria frequently cultured from children with otitis media [25, 40], were the most abundant in the nostrils (Fig. 4 and 5), indicating that the potential pathogenic bacteria may also originate from the nostrils.

In our study, subjects including OSA or recurrent tonsillitis with the hyperplasia of adenoids and tonsils were recruited. It is reported that tonsils of patients with OSA present a defective regulatory B cell (Breg) compartment. Importantly, bacterial species isolated from the tonsils harbor the ability to breach the epithelial barrier [35]. We cannot exclude the possibility that the disease status affects the microbiota composition. The limit for our study is the lack of samples collected from healthy pediatric population. This

constrains the conclusions of our study to the children with the hyperplasia of lymphoid tissues.

Notably, the top three genera in the adenoids included *Fusobacterium* and *Klebsiella* (Fig. 4b). *Fusobacterium* commonly inhabit the oral cavity and are involved in the development of colorectal cancer [14]. *Klebsiella* is commonly in the gut and is one of the most common pathogens in nosocomial infections [20]. The prevalence of gastroesophageal reflux disease is associated with otitis media in children [26, 40]. We hypothesize that these bacteria, normally localized to the alimentary canal, are also present in the adenoids due to the gastroesophageal reflux and contribute to the progression of infectious diseases in children.

Jensen et al. reported that the tonsillar crypts microbiota of children (2–4 years) are as diverse as that of adults.

Pathogenic bacteria including *H. influenzae*, *Haemophilus haemolyticus*, *Neisseria* spp., and *S. pneumoniae* were detected in the tonsillar crypts of children [11]. Here, we observed a similar microbiota pattern in the tonsils and oropharynx, where *Haemophilus* and *Neisseria* were the most abundant pathogenic genera (Fig. 4 and 5). We hypothesize that pathogens localized to the tonsillar crypts partially originate from the oropharynx.

Patients with rhinosinusitis display reduced *Corynebacterium* levels compared to healthy individuals [21, 38]. The existence of bacterial interference between *Corynebacterium* and the pathogenic *S. aureus* and *Streptococcus* has been reported. *Corynebacterium* implantation can help eradicate *S. aureus* from the nasal cavities [33, 39]. Indeed, various *Corynebacterium* spp. can inhibit the growth or the virulence of *S. aureus* and *S. pneumoniae* [16], suggesting that the higher amounts of *Corynebacterium* in the nostrils may maintain nostril microbiota balance. Here, we observed that *Corynebacterium* and *Staphylococcus* were most plentiful in the nostrils, while *Streptococcus*, which is reported to be a prevalent pathogen in children [28], displayed similarly high abundance across all sites (Fig. 4b). *Corynebacterium* may help maintain microbiota balance in the nostrils by eradicating the pathogenic bacteria there. Due to limitations in sequencing, we were unable to identify the exact *Staphylococci* spp. In children, the carriage rate of pathogenic *S. aureus* is very high [19]. The pathogen *S. aureus*, commonly detected in patients with chronic sinusitis, nasal polyposis, or otitis media [5, 41], may mainly originate from the nasal carriage.

Dolosigranulum and *Moraxella* were the most abundant in the nostrils. *Dolosigranulum pigrum*, an unusual Gram-positive bacterium, is involved in nosocomial pneumonia and septicemia [10, 17]. *M. catarrhalis* is a common cause of otitis media and chronic obstructive pulmonary disease [27]. Coagulase-negative staphylococci (CoNS) can defend against pathogenic bacteria, such as *Dolosigranulum* and *Moraxella*, by producing antimicrobial peptides [15, 30]. Notably, the nostril microbiota are constantly changing because the nostrils are open to the air [3]. We hypothesize that the main *Staphylococcus* species, CoNS, is crucial in maintaining microbiota balance in the nostrils. However, why certain bacteria are prone to localizing at specific sites remain unknown. Further studies should focus on how microbiota balance maintenance affects human health.

In conclusion, our data shed light on the characteristics of the bacterial microbiota in the URT by analyzing the samples collected from the different sites of the same population. Our data confirm that upper tract lymphoid tissues, normally considered immune organs, may also serve as reservoirs for pathogenic bacteria.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00405-021-07013-y>.

Acknowledgements This study was supported by the Cooperative research promotion plan of basic medicine and clinical medicine of Anhui Medical University (2020XKJT036), the National Natural Science Foundation of China (grant 81772139, 82072235), Shanghai Rising-Star Program (20QA1405900), the Shanghai Committee of Science and Technology, China (grant 20ZR1432800).

Declarations

Conflicts of interest The authors declare no conflict of interest.

References

- Ari O, Karabudak S, Kalcioğlu MT, Gunduz AY, Durmaz R (2019) The bacteriome of otitis media with effusion: does it originate from the adenoid? Int J Pediatr Otorhinolaryngol. <https://doi.org/10.1016/j.ijporl.2019.109624>
- Bell JS, Spencer JJ, Yates RL, Yee SA, Jacobs BM, DeLuca GC (2019) Invited review: from nose to gut—the role of the microbiome in neurological disease. Neuropathol Appl Neurobiol 45:195–215
- Bomar L, Brugger SD, Lemon KP (2018) Bacterial microbiota of the nasal passages across the span of human life. Curr Opin Microbiol 41:8–14
- Chan CL, Wabnitz D, Bardy JJ, Bassiouni A, Wormald PJ, Vreugde S, Psaltis AJ (2016) The microbiome of otitis media with effusion. Laryngoscope 126:2844–2851
- Coleman A, Wood A, Bialasiewicz S, Ware RS, Marsh RL, Cervin A (2018) The unsolved problem of otitis media in indigenous populations: a systematic review of upper respiratory and middle ear microbiology in indigenous children with otitis media. Microbiome 6:199
- Dimitri-Pinheiro S, Soares R, Barata P (2020) The microbiome of the nose—friend or foe? Allergy Rhinol (Providence) 11:2152656720911605
- Fago-Olsen H, Dines LM, Sorensen CH, Jensen A (2019) The adenoids but not the palatine tonsils serve as a reservoir for bacteria associated with secretory otitis media in small children. mSystems. <https://doi.org/10.1128/mSystems.00169-18>
- Giebink GS, Juhn SK, Weber ML, Le CT (1982) The bacteriology and cytology of chronic otitis media with effusion. Pediatr Infect Dis 1:98–103
- Hilty M, Qi W, Brugger SD, Frei L, Agyeman P, Frey PM, Aebi S, Muhlemann K (2012) Nasopharyngeal microbiota in infants with acute otitis media. J Infect Dis 205:1048–1055
- Hoedemaekers A, Schulin T, Tonk B, Melchers WJ, Sturm PD (2006) Ventilator-associated pneumonia caused by *dolosigranulum pigrum*. J Clin Microbiol 44:3461–3462
- Jensen A, Fago-Olsen H, Sorensen CH, Kilian M (2013) Molecular mapping to species level of the tonsillar crypt microbiota associated with health and recurrent tonsillitis. PLoS ONE. <https://doi.org/10.1371/journal.pone.0056418>
- Johnston J, Hoggard M, Biswas K, Astudillo-Garcia C, Radcliff FJ, Mahadevan M, Douglas RG (2019) Pathogen reservoir hypothesis investigated by analyses of the adenotonsillar and middle ear microbiota. Int J Pediatr Otorhinolaryngol 118:103–109
- Johnston JJ, Douglas R (2018) Adenotonsillar microbiome: an update. Postgrad Med J 94:398–403
- Kelly D, Yang L, Pei Z (2018) Gut microbiota, *fusobacteria*, and colorectal cancer. Diseases 6:109

15. Krismer B, Weidenmaier C, Zipperer A, Peschel A (2017) The commensal lifestyle of *staphylococcus aureus* and its interactions with the nasal microbiota. *Nat Rev Microbiol* 15:675–687
16. Kumpitsch C, Koskinen K, Schopf V, Moissl-Eichinger C (2019) The microbiome of the upper respiratory tract in health and disease. *BMC Biol* 17:87
17. Lecuyer H, Audibert J, Bobigny A, Eckert C, Janniere-Nartey C, Buu-Hoi A, Mainardi JL, Podglajen I (2007) *Dolosigranulum pigrum* causing nosocomial pneumonia and septicemia. *J Clin Microbiol* 45:3474–3475
18. Lemon KP, Klepac-Ceraj V, Schiffer HK, Brodie EL, Lynch SV, Kolter R (2010) Comparative analyses of the bacterial microbiota of the human nostril and oropharynx. *MBio* 1:e00129–e210
19. Liu Q, Liu Q, Meng H, Lv H, Liu Y, Liu J, Wang H, He L, Qin J, Wang Y, Dai Y, Otto M, Li M (2020) *Staphylococcus epidermidis* contributes to healthy maturation of the nasal microbiome by stimulating antimicrobial peptide production. *Cell Host Microbe* 27:68–78
20. Ma YX, Wang CY, Li YY, Li J, Wan QQ, Chen JH, Tay FR, Niu LN (2020) Considerations and caveats in combating escape pathogens against nosocomial infections. *Adv Sci (Weinh)* 7:1901872
21. Mahdavinia M, Engen PA, LoSavio PS, Naqib A, Khan RJ, Tobin MC, Mehta A, Kota R, Preite NZ, Codispoti CD, Tajudeen BA, Schleimer RP, Green SJ, Keshavarzian A, Batra PS (2018) The nasal microbiome in patients with chronic rhinosinusitis: Analyzing the effects of atopy and bacterial functional pathways in 111 patients. *J Allergy Clin Immunol* 142:287–290
22. Man WH, de Steenhuisen Piters WA, Bogaert D (2017) The microbiota of the respiratory tract: Gatekeeper to respiratory health. *Nat Rev Microbiol*. <https://doi.org/10.1038/nrmicro.2017.14>
23. Marchica CL, Dahl JP, Raol N (2019) What's new with tubes, tonsils, and adenoids? *Otolaryngol Clin North Am* 52:779–794
24. Marcus CL, Brooks LJ, Draper KA, Gozal D, Halbower AC, Jones J, Schechter MS, Sheldon SH, Spruyt K, Ward SD, Lehmann C, Shiffman RN (2012) Diagnosis and management of childhood obstructive sleep apnea syndrome. *Pediatrics* 130:576–584
25. Minami SB, Mutai H, Suzuki T, Horii A, Oishi N, Wasano K, Katsura M, Tanaka F, Takiguchi T, Fujii M, Kaga K (2017) Microbiomes of the normal middle ear and ears with chronic otitis media. *Laryngoscope* 127:E371–E377
26. Miura MS, Mascaro M, Rosenfeld RM (2012) Association between otitis media and gastroesophageal reflux: a systematic review. *Otolaryngol Head Neck Surg* 146:345–352
27. Murphy TF, Parameswaran GI (2009) *Moraxella catarrhalis*, a human respiratory tract pathogen. *Clin Infect Dis* 49:124–131
28. Nistico L, Kreft R, Gieseke A, Cotichchia JM, Burrows A, Kham-pang P, Liu Y, Kerschner JE, Post JC, Lonergan S, Sampath R, Hu FZ, Ehrlich GD, Stoodley P, Hall-Stoodley L (2011) Adenoid reservoir for pathogenic biofilm bacteria. *J Clin Microbiol* 49:1411–1420
29. Oshima N, Hamada H, Hirose S, Shimoyama K, Fujimori M, Honda T, Yasukawa K, Ishiwada N, Ohkusu M, Takanashi JI (2021) Pantone-valentine leukocidin-positive novel sequence type 5959 community-acquired methicillin-resistant *staphylococcus aureus* meningitis complicated by cerebral infarction in a 1-month-old infant. *J Infect Chemother* 27:103–106
30. Parlet CP, Brown MM, Horswill AR (2019) Commensal staphylococci influence *staphylococcus aureus* skin colonization and disease. *Trends Microbiol* 27:497–507
31. Perry M, Whyte A (1998) Immunology of the tonsils. *Immunol Today* 19:414–421
32. Pettigrew MM, Laufer AS, Gent JF, Kong Y, Fennie KP, Metlay JP (2012) Upper respiratory tract microbial communities, acute otitis media pathogens, and antibiotic use in healthy and sick children. *Appl Environ Microbiol* 78:6262–6270
33. Ramsey MM, Freire MO, Gabriliska RA, Rumbaugh KP, Lemon KP (2016) *Staphylococcus aureus* shifts toward commensalism in response to corynebacterium species. *Front Microbiol* 7:1230
34. Ren T, Glatt DU, Nguyen TN, Allen EK, Early SV, Sale M, Winther B, Wu M (2013) 16s rRNA survey revealed complex bacterial communities and evidence of bacterial interference on human adenoids. *Environ Microbiol* 15:535–547
35. Sarmiento Varon L, De Rosa J, Rodriguez R, Fernandez PM, Billoordo LA, Baz P, Beccaglia G, Spada N, Mendoza FT, Barberis CM, Vay C, Arabolaza ME, Paoli B, Arana EI (2021) Role of tonsillar chronic inflammation and commensal bacteria in the pathogenesis of pediatric OSA. *Front Immunol*. <https://doi.org/10.3389/fimmu.2021.648064>
36. Schwengel DA, Dalesio NM, Stierer TL (2014) Pediatric obstructive sleep apnea. *Anesthesiol Clin* 32:237–261
37. Subtil J, Rodrigues JC, Reis L, Freitas L, Filipe J, Santos A, Macor C, Duarte A, Jordao L (2017) Adenoid bacterial colonization in a paediatric population. *Eur Arch Otorhinolaryngol* 274:1933–1938
38. Ta LDH, Yap GC, Tay CJX, Lim ASM, Huang CH, Chu CW, De Sessions PF, Shek LP, Goh A, Van Bever HPS, Teoh OH, Soh JY, Thomas B, Ramamurthy MB, Goh DYT, Lay C, Soh SE, Chan YH, Saw SM, Kwek K, Chong YS, Godfrey KM, Hibberd ML, Lee BW (2018) Establishment of the nasal microbiota in the first 18 months of life: Correlation with early-onset rhinitis and wheezing. *J Allergy Clin Immunol* 142:86–95
39. Uehara Y, Nakama H, Agematsu K, Uchida M, Kawakami Y, Abdul Fattah AS, Maruchi N (2000) Bacterial interference among nasal inhabitants: Eradication of *staphylococcus aureus* from nasal cavities by artificial implantation of corynebacterium sp. *J Hosp Infect* 44:127–133
40. Vanneste P, Page C (2019) Otitis media with effusion in children: Pathophysiology, diagnosis, and treatment. A review *J Otol* 14:33–39
41. Vickery TW, Ramakrishnan VR, Suh JD (2019) The role of *staphylococcus aureus* in patients with chronic sinusitis and nasal polyposis. *Curr Allergy Asthma Rep* 19:21
42. Winther B, Gross BC, Hendley JO, Early SV (2009) Location of bacterial biofilm in the mucus overlying the adenoid by light microscopy. *Arch Otolaryngol Head Neck Surg* 135:1239–1245
43. Wu BG, Sulaiman I, Wang J, Shen N, Clemente JC, Li Y, Laumbach RJ, Lu SE, Udasin I, Le-Hoang O, Perez A, Alimokhtari S, Black K, Plietz M, Twumasi A, Sanders H, Malecha P, Kapoor B, Scaglione BD, Wang A, Blazoski C, Weiden MD, Rapoport DM, Harrison D, Chitkara N, Vicente E, Marin JM, Sunderram J, Ayappa I, Segal LN (2019) Severe obstructive sleep apnea is associated with alterations in the nasal microbiome and an increase in inflammation. *Am J Respir Crit Care Med* 199:99–109

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

All Rights reserved. For the use of registered medical practitioners in India only. Although greatest possible care has been taken in compiling, checking and developing the content to ensure that it is accurate and complete, the authors, the publisher, its servants or agents, or Sanofi are not responsible or in any way liable for any injury or damage to any persons in view of any reliance placed on or action taken basis of the information in this publication or any errors, omissions or inaccuracies and/or incompleteness of the information in this publication. The expert comments expressed in this publication are solely the views of the authors. No part of this content may be reproduced, transmitted or stored in any form or by any means either mechanical or electronic, including photocopying, recording or through an information storage and retrieval system, without the explicit written permission of the copyright holder. Sanofi India has taken the requisite permissions from Springer Nature India Pvt. Ltd. for hosting the digital version of this content on Sanofi's educational website.