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Tonsillar microbial diversity, abundance, and interrelations in atopic and non-atopic individuals

To the Editor,

While the prevalence of allergic diseases has increased worldwide, their underlying mechanisms are still not fully understood.¹ Tonsils, consisting of lymphoid tissue, play an important role in local and systemic immune response to bacterial and viral pathogens and allergens.² Tonsil microbiota is influenced by age and health status.^{3,4}

Allergic diseases, as many other noncommunicable diseases, have been associated with imbalance of the gut, respiratory tract, and skin microbiotas.¹ However, to our knowledge, microbial profiling of tonsils comparing atopic and non-atopic subjects has not been performed as of yet. Therefore, as tonsillar microbiota can closely modulate immune responses and serve as an effective *in vivo* model in investigating their interactions, we wanted to explore whether there are differences in bacterial diversity, abundance, and interrelations in tonsils between atopic and non-atopic elective tonsillectomy patients.

Human tonsil samples were obtained from two consecutive cohorts between 2008 and 2015 from Satakunta Central Hospital, Pori, Turku University Hospital, Turku, and Salo Regional Hospital, Salo, all in Finland.⁵ Study patients completed a standard questionnaire to collect information on health, medication, and respiratory symptoms within 30 days before the operation. Tonsillectomy was performed by routine clinical practice, and the tonsil samples were stored in RNAlater. Blood samples were drawn for allergy tests, and nasopharyngeal aspirate was obtained for respiratory virology analysis. Atopy was defined as positive immunoglobulin E antibody (>0.35 kU/L, Phadiatop Combi[®], Phadia, Uppsala, Sweden) against any of the common food or aeroallergens. More details in Online Supplementary.

Intratonsillar microbial profiling was performed using RNA sequencing data as previously described.⁶ In total, 53 subjects were included consisting of 24 atopics and 29 non-atopics. After filtering out reads mapped to human, the rest were considered microbial reads (~1 M reads on average, 6% of total). These candidate microbial reads were utilized with Kraken2⁷ for investigating microbial composition with default parameters. Phyloseq⁸ was used to measure diversity of microbial communities and EdgeR to assess differential abundance in atopic and non-atopic subjects. Differences were considered significant based on the corrected *P* value of less than .05. Details in Supplementary methods.

The median age of the study subjects was 11 years (range 2-38) of which 45% were atopic. All the operations were performed during a "cold phase" of chronic tonsil condition. On the operation day, 36 (68%) had no respiratory tract symptoms, 8 (15%) reported mild respiratory symptoms and 9 (17%) had no data. Main indications for

tonsillectomy were recurrent tonsillitis (47%) and tonsillar hypertrophy (45%), see details in Table S1.

Alpha diversity (measures of richness and species distribution in a sample¹) was computed to evaluate the difference in microbial diversity between atopic and non-atopic subjects (Figure 1A). We observed lower alpha diversity in samples from atopic patients compared with non-atopics (*P* = .02, Figure 1A). After adjusting the analysis to age and self-reported allergy, the difference remained significant (*P* = .02). Similar finding was observed when those with clinical allergic disease were compared with non-allergic ones (see Online Supplementary and Figure S2). To investigate the difference in microbial diversity at a group level, we used beta diversity (measure of diversity between samples). However, β -diversity analysis of the tonsillar did not show a significant difference between atopic and non-atopic groups (Supplementary results Figure S3).

The most abundant phylum found in microbial data was Proteobacteria (Figure 1B). No considerable difference (*P* = .3) in the overall bacterial abundance between atopic and non-atopic individuals was observed. However, there was a difference in the expression of single pathogens between the study groups. The differential abundance of two specific taxa, *Clostridium Botulinum* and *Moraxella Osloensis*, was lower (unadjusted *P* < .05, adjusted *P* < .05) in atopic compared with non-atopic study subjects (Figure 1C). The heat maps illustrate correlations between the abundances of pathogenic bacteria in the two study groups (Figure 2A,B; network figures in Figure S4). Positive correlations between pathogenic bacteria were stronger in atopic compared with non-atopic individuals (Figure 2C).

Our finding of low microbial diversity in tonsils of the atopic patients is in line with previous studies on other tissues. For example, exacerbation of atopic dermatitis has been shown to associate with decreased microbial diversity of the skin when compared to healthy skin¹ and asthma has been linked to imbalance of detrimental and beneficial microbes of the respiratory and gastrointestinal epithelium when compared to healthy controls.¹ Therefore, as tonsil tissue contains rich microbiome, it is logical that atopy status is also associated with lower bacterial diversity in tonsils. Collectively, these findings suggest that systemic dysbiosis, extending to local lymphoid tissue level, is associated with allergic diseases.¹ It should be noted, however, that our study analyzed gene expression by RNA sequencing whereas many earlier studies¹ have analyzed DNA and genetic information.

In our study, the most abundant phylum was Proteobacteria and the most abundant genera were *Salmonella*, *Klebsiella* and *Sodalis*.

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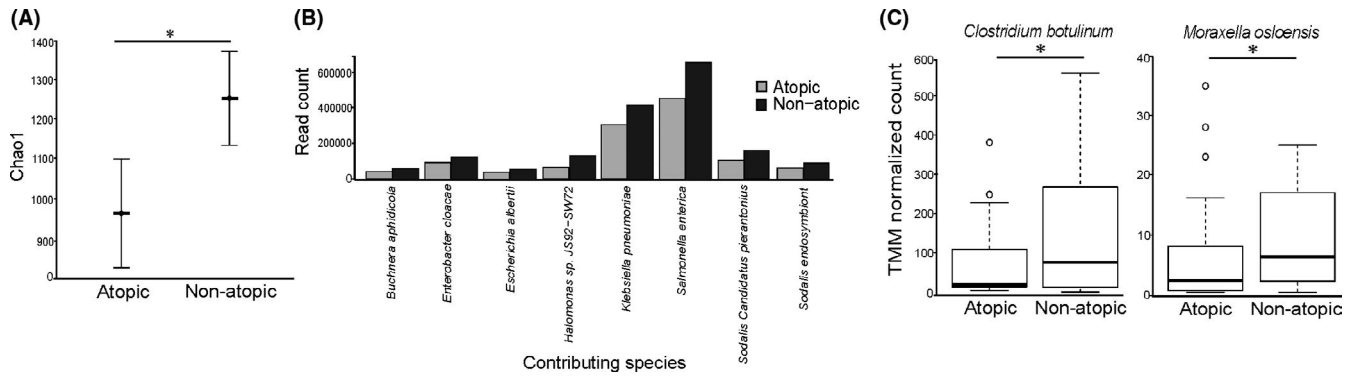


FIGURE 1 Overview of Microbiome data: (A) Atopic subjects ($n = 24$) had lower microbial alpha diversity than non-atopic subjects ($n = 29$) computed with Chao 1 index (estimate of within-group diversity). Data are shown in mean and SEM. B, At phylum level, atopic subjects had a non-significantly lower abundance of Proteobacteria, the largest phylum, than non-atopic subjects ($P = .3$). C, Of all single bacteria, *Clostridium botulinum* and *Moraxella osloensis* showed lower abundance in atopic subjects than in non-atopic subjects. Data are shown in median, quartiles, and range. Please see the statistics in detail in the Online Supplementary. * $P < .05$

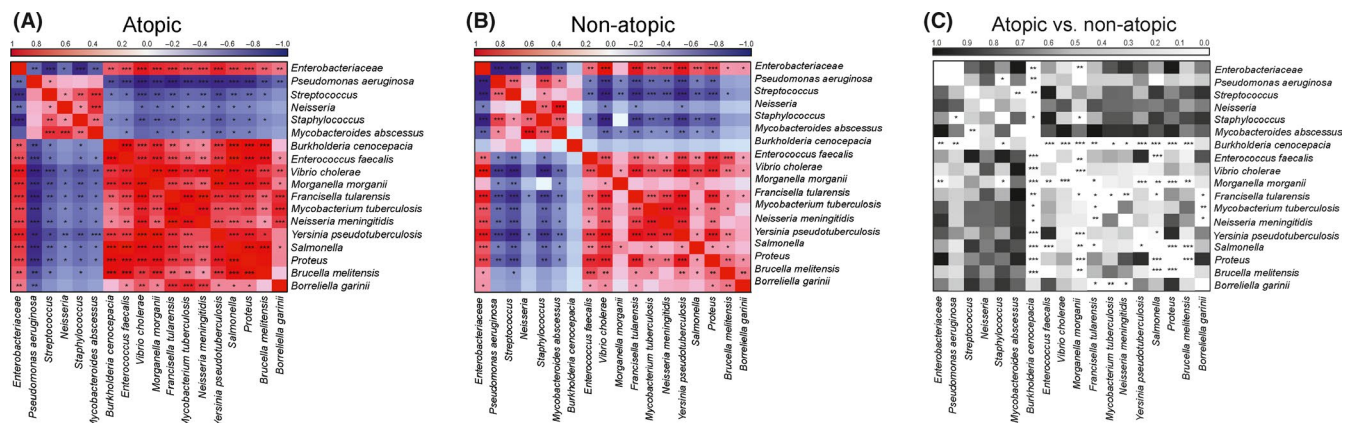


FIGURE 2 Heat maps of intratonsillar pathogenic bacteria-to-bacteria interrelations in atopic subjects (A), non-atopic subjects (B) and comparison of former two (C). Data show stronger positive interrelations between pathogenetic bacteria in atopic than non-atopic subjects. The color scale (blue-red and gray-scale) shows the strength and direction of the correlations. P values $< .001$, $.01$, and $.05$ are indicated with ***, **, and *, respectively. Please see more details in the Online Supplementary

This is in line with previous studies, in which Proteobacteria have been highly associated with tonsillar hyperplasia and recurrent tonsillitis in children.^{3,4} Also in earlier studies, the increase in *Moraxella* at genus level has been linked to asthma.¹ However, in our study, the differential abundance of taxa *Moraxella Osloensis* was lower in atopic patients as well as taxa *Clostridium Botulinum*. Comparing the interrelations of pathogenic bacteria, we found more significant correlations in the atopic group. The existence of atopy slightly increased the complexity of bacteria-to-bacteria networks. Moreover, intra-individual variation of microbiome has been demonstrated comparing classical secretory otitis media pathogens in adenoid and tonsil tissues.⁹

This study shows four important findings in elective tonsillectomy patients. First, tonsil tissue contains rich bacterial species in direct contact with immune system. Second, this is a first study to demonstrate that atopic individuals have lower tonsillar bacterial diversity than non-atopics. Third, our data indicate that lower overall bacterial abundance and fourth, stronger positive interrelations between pathogenetic bacteria are associated with atopy. Our study

provides interesting insights into the microbiome of human tonsils and suggests differences in microbial balance between atopic and non-atopic subjects. Further studies are warranted to investigate interactions between microbes and lymphoid tissue function.

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CONFLICTS OF INTEREST

The authors have no conflict of interest in connection with this paper.


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REFERENCES

- Huang YJ, Marsland BJ, Bunyavanich S, et al. The microbiome in allergic disease: Current understanding and future opportunities—2017 PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology. *J Allergy Clin Immunol*. 2017;139(4):1099-1110.
- Masters KG, Lasrado S. *Anatomy, Head and Neck, Tonsils*. Treasure Island, FL: StatPearls Publishing; 2019. <http://www.ncbi.nlm.nih.gov/pubmed/30969614>. Accessed May 29, 2019.
- Johnston J, Hoggard M, Biswas K, et al. Paired analysis of the microbiota between surface tissue swabs and biopsies from pediatric patients undergoing adenotonsillectomy. *Int J Pediatr Otorhinolaryngol*. 2018;113:51-57.
- Jensen A, Fagö-Olsen H, Sørensen CH, Kilian M. Molecular mapping to species level of the Tonsillar crypt microbiota associated with health and recurrent tonsillitis. Xu P, ed. *PLoS ONE* 2013;8(2):e56418.
- Jartti T, Palomares O, Waris M, et al. Distinct regulation of tonsillar immune response in virus infection. *Allergy* 2014;69(5):658-667.
- Hanif T, Dhaygude K, Kankainen M, et al. Birch pollen allergen immunotherapy reprograms nasal epithelial transcriptome and recovers microbial diversity. *J Allergy Clin Immunol*. 2019;143(6):2293-2296.
- Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biol*. 2014;15(3):R46.
- McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* 2013;8(4):e61217.
- Fagö-Olsen H, Dines LM, Sørensen CH, Jensen A. The adenoids but not the palatine tonsils serve as a reservoir for bacteria associated with secretory otitis media in small children. Cotter PD, ed. *mSystems*. 2019;4(1):00169-218.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.