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RESEARCH ARTICLE

Comparative evaluation of culture results and composition of microbiome of removed tonsils due to distant focal disease or other reasons. A prospective pilot study

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ABSTRACT

The aim of this prospective pilot study was to compare culture and microbiome results of the removed tonsils of patients with assumed distant focal disease (11 patients) and those who underwent a tonsillectomy, due to other reasons, such as recurrent tonsillitis, tonsil stones or snoring (nine patients). Aerobic culture was carried out for samples taken from the surface of the tonsils by swabs before tonsillectomy for all 20 patients. The squeezed detritus and the tissue samples of removed tonsils, taken separately for the right and left tonsils, were incubated aerobically and anaerobically. The microbiome composition of tissue samples of removed tonsils was also evaluated. Based on the culture results of the deep samples *Staphylococcus aureus* was the dominating pathogen, besides a great variety of anaerobic and facultative anaerobic bacteria present in the oral microbiota in those patients who underwent tonsillectomy due to distant focal diseases. Microbiome study of the core tissue samples showed a great diversity on genus and species level among patients of the two groups however, *S. aureus* and *Prevotella nigrescens* were present in higher proportion in those, whose tonsils were removed due to distant focal diseases. Our results may support previous findings about the possible triggering role of *S. aureus* and *P. nigrescens* leading to distant focal diseases. Samples taken by squeezing the tonsils could give more information about the possible pathogenic/triggering bacteria than the surface samples cultured only aerobically.

KEYWORDS

focal disease, tonsillar microbiome, *Staphylococcus aureus*, *Prevotella nigrescens*

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INTRODUCTION

The results of the revolution of microbiology in the 19th century indicated many false theories to explain diseases with previously unknown etiology. Such was the theory of "focal infection" [1]. A localized infection, often asymptomatic, may cause disease elsewhere in the host, but such distant focal diseases are fairly uncommon. There are theories attributed many

systemic diseases to the decay products of the microorganisms that colonize special parts of our body. In the 21st century, the evidence supporting distant focal diseases did not increase, but new knowledge about them created additional possible mechanisms such as metastasis of infection, metastatic toxic or immunological damage. All of these can occur simultaneously and may even interact [2, 3]. In some cases, the hair loss, inflammatory skin diseases, arthritis, glomerulonephritis, peri- and myocarditis that cannot be proven for other reasons, the "focal theory" still holds true today. Inflammatory diseases of the palatine tonsils, teeth, and the prostate or ovaries are considered to be the reason of focal diseases of distant organs [4–6]. However, establishing a real cause-and-effect relationship is very difficult.

Nowadays several microbiome studies, conducted with 16S rDNA amplicon sequencing or by metagenomics, also demonstrated possible correlation between the composition of the intestinal or oral microbiota and some physiological disorders of other body sites such as autoimmune disorders or Alzheimer disease [7, 8].

In the field of otolaryngology, among the presumed organs (adenoids, palatine tonsils, lingual tonsils) the palatine tonsil may play an important role in distant focal diseases, due to its histopathological and immunological structure [9]. In the case of distant focal disease, the difficulty is caused by the fact that we only see the symptoms of the distant target organ (e.g. joint, skin, kidney), while the triggering organ, such as the tonsils, is silent and asymptomatic. The first step in tissue-injury processes is the damage of the basal membrane, which triggers the local mucosal inflammation of the palatine tonsils and the distant organ syndrome therefore referred to a tonsil-induced autoimmune/inflammatory process [9, 10]. In many of the chronic diseases, such as chronic rhinosinusitis with nasal polyposis, the local microbial stimulus can initiate the inflammatory immune process [11, 12]. Determining the microbes that play an important role as a trigger, could make it possible to develop a screening test for the daily clinical routine. By removing the local organ, (e.g. by tonsillectomy) the immunological damage of the distant target organ (such as e.g. skin, joint or kidney), can be prevented or reversed [13].

Our main goal in this pilot study was to evaluate what is the value of the aerobic culture result of the sample taken by a swab from the surface of the tonsils (usually done in many countries before tonsillectomy) or of the more detailed evaluation of the culture results (aerobic and anaerobic) of the detritus and the removed tonsillar tissues. The microbiome composition of the tissue samples of the removed tonsils were also evaluated. We looked for differences by comparing two well-defined groups (patients with and without distant focal disease) based on culture results or composition of the microbiome.

MATERIALS AND METHODS

Patients

Twenty patients were involved in this study in 2020 (Ethical approval No: 178/2017). The tonsillectomy of 11 patients (Group I) was decided due to skin disorders (such as acne, eczema, or loss of the hair) or immunological disorders (arthritis, nephritis) assuming it is related with the tonsils. In the present study only those patients were involved whose focal symptoms significantly improved or disappeared within 4 months after tonsillectomy. The other nine patients (Group II) underwent a tonsillectomy, as they had several serious tonsillitis events earlier or due to the presence of tonsillolith (tonsillar stone) or snoring (due to tonsillar hypertrophy) (Table 1). The tonsillectomy was carried out during the symptom-free period. None of the patients received antibiotics two weeks before the tonsillectomy. Swab sample from tonsillar surface for aerobic culture was taken prior surgery by otolaryngologists, when the patient was already under general anesthesia, but before disinfection. Beside the surface sample of the tonsils, detritus from the right and left tonsils was taken by separate swabs and transferred in anaerobic transport medium in the laboratory. During the whole process of sampling, attention was taken that the swab should not touch oral mucosa, pharyngeal cavity mucosa, or saliva. The tissue samples from the removed right and left tonsils were submitted for culture in sterile Petri dishes within 2 h.

Culture of the samples and identification of the isolated bacteria

Processing of the samples and evaluation of the culture results were carried out according to accepted routine laboratory methods [14, 15] in our bacteriological laboratory. Semi quantitative inoculation of the surface samples of the

Table 1. The main reasons of tonsillectomy in the 20 patients

Group I. (number of the patient)	Sex	Age of the patient (year)	Reason of the tonsillectomy
1.	M	12	acne vulgaris
2.	F	24	eczema
3.	M	17	acne vulgaris
4.	F	38	eczema
5.	F	40	alopecia areata
6.	F	15	alopecia areata
7.	M	18	acne vulgaris
8.	M	24	arthritis
9.	M	27	arthritis
10.	M	28	alopecia areata
11.	M	54	nephritis
Group II.			
12.	M	22	>5 acute tonsillitis/year
13.	F	23	>5 acute tonsillitis/year
14.	F	36	tonsil stone
15.	F	16	>5 acute tonsillitis/year
16.	F	29	tonsil stone
17.	M	49	snoring/tonsillar hypertrophy
18.	F	25	2–3 acute tonsillitis/year
19.	M	39	snoring/tonsillar hypertrophy
20.	M	40	snoring/tonsillar hypertrophy

tonsils was performed within 3 h on aerobic culture media (Columbia agar +5 % sheep blood; chocolate agar Polyvitex [BioMerieux, France]; eosin-methylene blue agar [Condalab, Spain]; Sabouraud agar [Bio-Rad, USA]) and incubated for 48 h in 5% CO_2-containing environment at 37 °C. The detritus taken before the surgery with a swab was homogenized in 1 mL PBS and the tissue samples of the removed tonsils were mechanically homogenized in sterile abrasive mortar and part of it was vortexed in 1 mL PBS for culture. Standardized part of the homogenized tissue samples of the right and left tonsils were immediately transferred in freezer at −80 °C for molecular analysis later. All the right and left detritus and tissue samples were cultured separately on the same way as described above to isolate aerobic and facultative anaerobic bacteria and additionally, for isolating strict anaerobes, Schaedler agar +5 % sheep blood [BioMerieux, France] and Brucella agar with laked sheep blood + kanamycin + vancomycin was used [14]. The anaerobic plates were kept in an atmosphere of 10% H_2, 10% CO_2 and 80% N_2, in an anaerobic chamber (Concept 400, Ruskinn Technology Ltd., Bridgend, UK) for 48–72 h. The identification of the isolated bacteria was carried out by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method (Biotyper, Bruker Daltonics GmbH, Bremen, Germany) using the actual database (MBT Compass Library DB-8468) following the guideline of Bruker Daltonics current User Manual [16–18].

DNA extraction

All tissue samples of the right and left tonsils stored at −80 °C were homogenized in 1 mL PBS and DNA extractions were carried out from 200 µL homogenates by the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) with an elution volume of 100 µL. Afterwards DNA concentrations were determined by the Qubit dsDNA BR kit and fluorimeter (Thermo Scientific).

Next-generation sequencing

The amplification and sequencing of the prokaryotic hypervariable V3–V4 region of 16S rRNA gene were performed as described in "Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System" standard protocol provided by the supplier (Illumina, San Diego, CA, USA). Detailed description of the applied method can be found in our previous article [19, 20].

Amplicon sequence analysis

Amplicon sequencing data were handled in house-developed bioinformatics pipeline, containing three modules. 1) Sequencing preparation, trimming: raw sequences were trimmed by Fastp (v default settings and minlen: 150) and checked with FastQC (v.0.11.8) [21]. 2) Taxonomic annotation: amplicon sequences were annotated at species level with the combination of Kraken2 (v.2.0.8: -confidence 0.95) and Bracken on Greengenes database [22, 23]. 3) Filtration and normalization: Copy number normalization was done through the rrnDB (v.5.6) database [24]. MetagenomeSeq (v.1.16.0) was used to create normalized and scaled output of microbial abundances (–rel 0.1, –scale 1,000) [25].

Statistics and visualization

For statistical calculation and visualization R program microeco package was employed [26]. We use Bray-Curtis dissimilarity test to calculate differences in beta-diversity (significant differences between groups was detected by Wilcox rank sum test $P \leq 0.05$). To assess the significance of differences between microbiomes of the two groups (i.e.: Group I and II), we used LEfSeR (the R package for linear discriminant analysis effect size calculator; version 4.3 inside microeco, with a significance threshold of $P \leq 0.05$ (trans_diff: alpha = 0.05, p_adjust_method = fdr, boots = 30, taxa_level = species) [27].

RESULTS

Microbial analysis of tonsil samples: aerobic and anaerobic culture results

During the aerobic culture of the surface swabs of the 20 patients' tonsils (Tables 2 and 3) revealed only one sample, which was positive for *Staphylococcus aureus* beside the normal flora of the pharynx. When the culture results of the detritus and the tissue samples were evaluated, they were merged according to the right or left side of the patients, as a very similar distribution of aerobic and anaerobic species were present in both deep samples on the same side. High colony counts (>10^5 CFU/mL) of *S. aureus* were found on both sites in seven patients, belonging to Group I, whose tonsils were removed due to different distant focal diseases (Table 2). Out of this seven patients only one had *S. aureus* detectable in the surface sample of the tonsils. In case of two patients in this group *Haemophilus influenzae,* whereas in two other patients *Streptococcus pyogenes* was found in the deep samples of both removed tonsils with the presence of low colony counts of α-haemolytic streptococci and some coagulase-negative staphylococci cultured aerobically (Table 2). In Group II there were two patients whose right or left deep samples were also positive for *S. aureus* with low colony counts (10^2 CFU/mL in one or two samples) (Table 3). There was only another patient with high colony counts of *H. influenzae* in the deep samples in both sites in this group (Table 3). During aerobic culture only very low numbers of other aerobic bacteria, belonging to the normal oral flora, were isolated from the deep samples of the tonsils of all 20 patients.

The anaerobic culture of the detritus and the tissue samples of the right and left tonsils resulted a very mixed population of gram-negative and gram-positive anaerobic bacteria (Tables 2 and 3). *Fusobacterium nucleatum* was present almost in all samples of the patients belonging to the Group I and II. *Fusobacterium necrophorum* was isolated with high colony counts (≥10^5 CFU/mL) from all deep

Table 2. Culture results of tonsils of 11 patients (Group I) with tonsillectomy due to distant focal diseases

Isolated bacteria	Culture results		
	Surface swab of the tonsils	Detritus and tissue of the right tonsils	Detritus and tissue of the left tonsils
Aerobic bacteria			
Staphylococcus aureus	1	7	7
Streptococcus pyogenes	0	2	2
Haemophilus influenzae	0	2	2
Other aerobic bacteria*	10	8	6
Anaerobic bacteria			
Fusobacterium nucleatum	nd	11	11
Fusobacterium necrophorum	nd	0	0
Fusobacterium periodonticum	nd	2	1
other *Fusobacterium* spp**	nd	2	2
Prevotella buccalis	nd	5	4
Prevotella nigrescens	nd	7	6
Prevotella intermedia	nd	1	2
Prevotella melaninogenica	nd	2	4
other *Prevotella* spp***	nd	8	11
Veillonella atypica	nd	2	2
other *Veillonella* spp****	nd	1	1
Gram-positive anaerob cocci (GPAC)	nd	2	2
Actinomyces odontolyticus	nd	4	2
other *Actinomyces* spp*****	nd	1	3
Other anaerobic bacteria	nd	1	2

nd – not done.
* mixed population of α-haemolytic streptococci, coagulase-negative staphylococci (considered normal flora).
***F. naviforme* (1), *Fusobacterium* sp (1).
****P. salivae* (3), *P. denticola* (1), *P. pallens* (1), *P. veroralis* (1), *P. maculosa* (1), *P. histicola* (1), *P. oulorum* (1), *P. oris* (1), *P. jejuni* (1), *P. dentalis* (1), *P. baroniae* (1), *Prevotella* spp (4).
*****V. dispar* (1), *V. parvula* (1).
****** *A. gerencseriae* (1) *A. neuii* (1), *A. meyeri* (1).

Table 3. Culture results of tonsils of nine patients (Group II) with tonsillectomy due to different other reasons than distant focal disease

Isolated bacteria	Culture results		
	Surface swab of the tonsils	Detritus and tissue of the right tonsil	Detritus and tissue of the left tonsils
Aerobic bacteria			
Staphylococcus aureus	0	2	1
Streptococcus pyogenes	0	0	0
Haemophilus influenzae	0	1	0
Other aerobic bacteria*	9	5	6
Anaerobic bacteria			
Fusobacterium nucleatum	nd	7	6
Fusobacterium necrophorum	nd	3	3
Fusobacterium periodonticum	nd	3	2
other *Fusobacterium* spp**	nd	3	3
Prevotella buccalis	nd	4	5
Prevotella nigrescens	nd	2	1
Prevotella intermedia	nd	4	6
Prevotella melaninogenica	nd	2	2
other *Prevotella* spp***	nd	11	13
Veillonella atypica	nd	7	9
other *Veillonella* spp****	nd	1	1
Gram-positive anaerob cocci (GPAC)	nd	4	6
Actinomyces odontolyticus	nd	4	6
other *Actinomyces* spp*****	nd	1	0
Other anaerobic bacteria	nd	1	1

nd – not done.
* mixed population of α-haemolytic streptococci, coagulase-negative staphylococci (considered normal flora).
***F. naviforme* (1), *Fusobacterium* sp (2).
****P. salivae* (4), *P. denticola* (2), *P. pallens* (2), *P. histicola* (2), *P. jejuni* (2), *P. maculosa* (1), *P. oulorum* (1), *P. loescheii* (1).
*****V. dispar* (1), *V. parvula* (1).
****** *A. graevenitzii* (1).

samples of three young adult patients (with age 16, 22 and 23 years) belonging to the Group II. All three patients had several tonsillitis events/year before the tonsillectomy has been decided (Table 1). Other *Fusobacterium* spp were also identified on species level or only on genus level in one or more samples of the patients. From all deep samples of patients belonging to Group I and II a great number of different *Prevotella* spp were obtained. The most frequently isolated species were *Prevotella buccae, Prevotella nigrescens*, *Prevotella intermedia* and *Prevotella melaninogenica*, but several further *Prevotella* spp were also identified in the deep samples of the tonsils in low numbers (Tables 2 and 3). *Veillonella atypica* was much more frequently found in patients belonging to Group II, than in those belonging to Group I, beside some other *Veillonella* spp. *Actinomyces odontolyticus* was the most frequently found *Actinomyces* spp beside some other species of this genus (Tables 2 and 3). We could not find significant differences in the anaerobe population of the deep tissue of the tonsils of the patients belonging into these two groups, based on the semi-quantitative culture procedure.

Amplicon sequence analysis of tonsillar microbiota: taxonomic resolution and comparative results

The composition of the microbiota in the tissue samples of the right and left tonsils were evaluated separately. The metagenomic (16S rDNA amplicon sequencing) results are presented in a sequence that follows the higher taxonomic resolution of the microbial community of the tissue samples of the removed tonsils, followed by the more detailed results. The two main describing dimensions of principal coordinate analysis calculation represent 41.5% of the microbiome variation between samples of patients belonging in the Group I and Group II (Fig. 1A). The individual microbiomes of the patients were scattered, influenced probably by numerous additional external factors, (e.g. diet, age, gender, systemic health conditions, medications, etc.). According to Bray-Curtis dissimilarity test, the two sample group's beta-diversity are significantly different ($P < 0.001$) (Fig. 1B).

Genera abundances revealed distinct differences between the two groups (Group I and Group II), even though the microbiomes of the right and left tonsillar tissue from the same patient exhibited considerable similarity (Fig. 2A). The most abundant Operational Taxonomy Unit (OTU) was the genus *Prevotella*, which is generally observed in human oral cavity. It is noteworthy, that the majority of predominant taxa belonged to oral pathogens/normal flora members [20]. Some of them apparently did not indicate abundance difference between patients belonging to Group I and II, such as the genera *Rothia, Porphyromonas, Streptococcus* and *Fusobacterium*. Although, there were pronounced patient dependent abundances difference observed in the case of the genera *Porphyromonas* and *Treponema* (Fig. 2A).

The top 12 identified OTUs at species level and their distribution between the patient's samples is shown in Figure 2B and 2C. It is clearly seen, that the patterns of the 12 most abundant bacterial species harbored in the left and right tonsils were much more related (Fig. 2B), than the similarity among individual subjects or between patients belonging to Group I and II (Fig. 2C). Significantly different biomarker species were detected, which may have important diagnostic relevance in removed tonsils microbial analysis (Fig. 2D). In the patients belonging to Group I, whose tonsils were removed due to distant focal diseases *S. aureus*, and *P. nigrescens* were the dominating species, whereas in the "control group" beside *P. intermedia* and *V. atypica* five further species were present in higher proportion in patient's removed tonsils, beside the substantial heterogeneity of the individual tonsillar microbiomes.

DISCUSSION

The indication of the tonsillectomy has changed during the past decades, but even today carried out very often in symptom free period, due to recurrent serious/acute tonsillitis in children, but also in young adults. However, there are many other reasons initiating tonsillectomy such as awareness of obstructive problems in children, or in adults due to the obstructive sleep apnea syndrome with snoring [28, 29]. Many studies support the idea that immunological consequences of chronic tonsillar infection or just symptomless presence of some microorganisms in the deep tonsillar tissue may cause distant focal diseases [4, 6, 9, 10, 30, 31]. Many of these symptoms may dramatically improve after

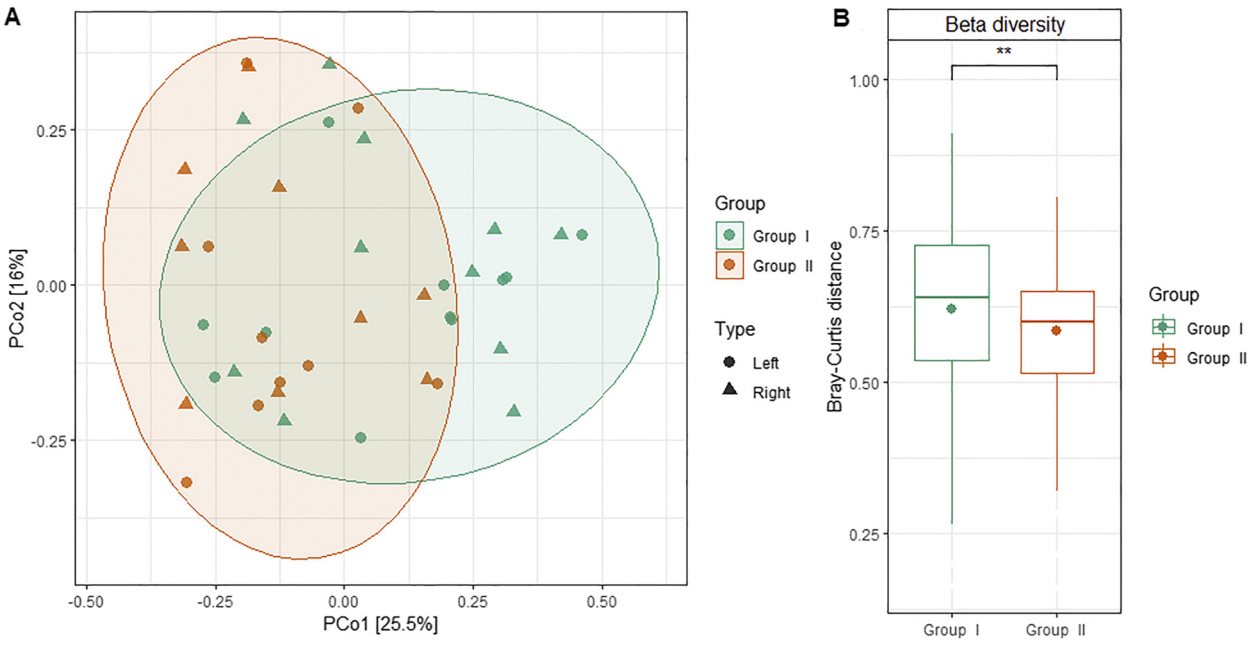

Fig. 1. Principal coordinate analysis (PCoA) and microbiome diversity. **A.** Principal coordinate analysis of microbiome data from the left and right tissue samples of the tonsils removed from patients belonging to Group I and II. **B.** Shows the beta-diversity of the microbiomes of the patients belonging in the two groups. The difference is statistically significant: $P < 0.001$ (**)

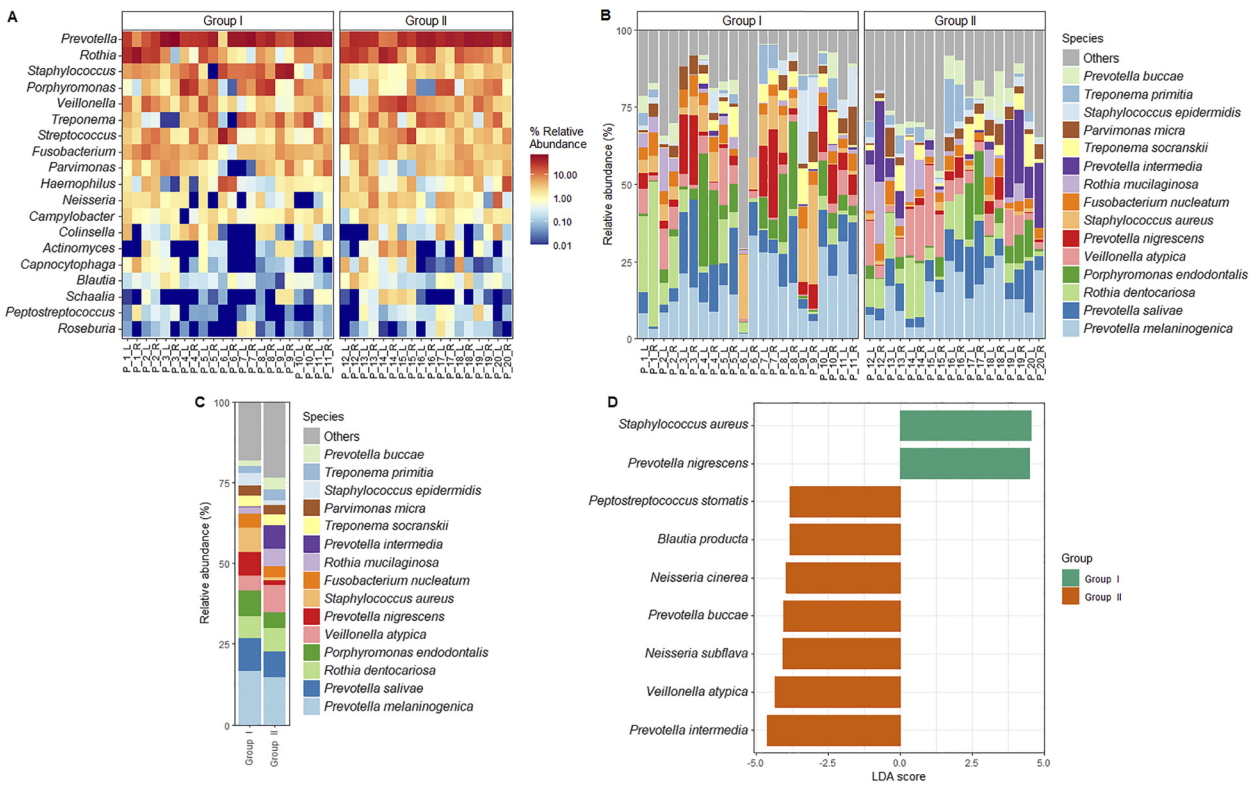

Fig. 2. Analysis of the microbiome composition of the two groups. The patients are coded within groups (P_1-P_20). L and R distinguishes left and right tonsillar tissue samples. **A.** The heat map shows the microbiome composition at genus level of the individual samples of the two groups (top 19 genera). **B.** Microbiome composition of individual samples from the two groups at species level. The figure shows the percentage distribution of the abundance of the top 15 species in each sample. **C.** Average species-level composition of the microbiome of the two groups. **D.** The distribution of species is significantly different between the two groups (LEfSe: $P < 0.05$)

tonsillectomy [6, 10, 32]. It has been shown that tonsillectomy revealed an improvement in 76.7% of 77 patients suffering from different skin disease and the most effective results were found in palmoplantar pustulosis [31]. The indication for tonsillectomy is often based on the distant focal disease hypothesis even in the case of otherwise completely asymptomatic tonsils. In other words, we perform tonsillectomy without the "source of the disease" being really verifiable. Symptomatic changes after surgery can be the proof.

The classical culture-based evaluation of the bacteriological background of different inflammatory processes of the tonsils or their triggering role in distant focal diseases has been discussed by many earlier or recent publications [4, 6, 9, 10, 33]. They try to find correlation between bacteria isolated from the surface of the tonsils (throat swab) usually taken in operating theatre before surgery or from the deep tissue taken by needle aspiration or with squeezing out the detritus [33–36]. Many of these studies prove the disadvantages of routine surface sampling of tonsils specially if only aerobic culture is carried out. Samples from the tonsillar core and crypts, obtained before surgery are more favorable for culturing in aerobic and anaerobic environment [33, 36]. However, culture results are highly dependent on what kind of laboratory methods are used and how detailed species determination is carried out.

In our pilot study the tonsillar surface samples were cultured only aerobically as in many routine laboratories. The culture results showed clearly that in 20 patients involved in this study, underwent a tonsillectomy during symptomless period, very few aerobic bacteria considered as pathogens, were detected from the surface swab of the tonsils (1 of 20), whereas from the core samples (detritus and tissue samples) 14 of the 20 patients showed the presence of possible pathogenic aerobic bacteria (Tables 2 and 3). *S. aureus* was the dominating aerobic pathogen found in all deep samples with high colony counts in seven patients belonging to Group I, where the tonsillectomy was carried out due to distant focal disease. In Group II there were two further patients where *S. aureus* was found with low CFU in the deep samples. These data highly support the findings of Sarkar et al. [36] that even aerobic bacteria such as *S. aureus* can be detected more frequently from the core specimen of the tonsils than from the surface swab. They showed that out of the 10 most prevalent bacteria, only group C β-hemolytic streptococci showed no difference between detection from core and surface swabs [36]. In our study even *S. pyogenes* (2 cases) and *H. influenzae* (3 cases) were only detected from the deep samples of the tonsils.

The high prevalence of *S. aureus* in the deep samples of patients belonging to Group I found by the culture method (Table 2) was also confirmed by the microbiome evaluation of the tissue samples of the right and left tonsils. The mean

proportion of *S. aureus* and *P. nigrescens* was higher in patient's tonsillar microbiome whose tonsils were removed due to distant focal diseases (Group I), on the contrary the mean proportion of the *P. intermedia* and *V. atypica* were higher in patient's microbiome ranged to Group II (Fig. 2D). This could also be observed during the culture based evaluation (Tables 2 and 3). According to the present study these 4 species are likely the best choices for the detection of taxonomic differences between the two clinically distinct patients represented in Group I and II. The high prevalence of *S. aureus,* in the present study in deep tonsillar samples of patients, whose tonsillectomy was decided due to distant focal diseases, raises the possibility that the different toxins of *S. aureus* may triggering inflammatory responses and activate inflammatory cells (such as keratocytes, helper T cells, innate lymphoid cells, macrophages, dendritic cells, mast cells, neutrophils eosinophils and basophils) which can express various cytokines and induce an inflammatory response leading to distant focal diseases [12, 37, 38]. Recent studies have shown connection between *Prevotella* strains, specially *P. nigrescens* present in the oral cavity and different distant diseases such as rheumatoid arthritis, systemic lupus erythematosus, cystic fibrosis or the potential inflammatory etiology of Alzheimer's disease [39–41]. The validation of the role of *S. aureus* and *P. nigrescens* as signaling species in patients with distant focal disease is needed on a much larger, carefully selected cohort of subjects with appropriate clinical anamnestic history.

In our study the culture results of the core samples of the tonsils (detritus and the removed tissue samples) on the same side showed a very similar distribution of aerobic and anaerobic bacteria identified on species level. This shows that a sample taking for culture before tonsillectomy is advisable to be carried out by squeezing of the detritus and collected by a swab. Given that tonsils are located between the oral cavity and the laryngopharynx at the gateway of the alimentary and respiratory tracts, tonsillar tissue may be affected by complex microbiota from both the oral cavity (saliva) and the alimentary tract. In our study we found a great variety of the well-known oral/dental anaerobic flora (including *F. nucleatum,* several *Prevotella* spp*, Veillonella* spp, Gram-positive anaerobic cocci (GPAC), *Actinomyces* spp) to be present in the deep samples of the removed tonsils. This was also found during the microbiome evaluation of the tissue samples on genus or species levels (Figure 2A and 2B).

Remarkable finding was the isolation of *F. necrophorum* (with high CFUs) from the deep tonsillar samples of 3 young adults belonging in Group II (Tables 1 and 3). Several culture based [42, 43] and also microbiome based [44] study confirmed the pathogenic role of *F. necrophorum* in acute and chronic tonsillitis primarily in young adult age as well as in peritonsillar abscess [45, 46].

CONCLUSION

Our prospective, pilot, multi-method study clearly showed that the samples taken by squeezing the tonsils give more information about the possible pathogenic/triggering bacteria, why tonsillectomy is carried out, than the surface samples cultured only aerobically. The culture and amplicon sequencing data obtained from deep tissues of tonsils in this study showed higher rate of colonization by *S. aureus* and *P. nigrescens* in patients with distant focal diseases. These bacteria may play a triggering role in the immunological cascade mechanism and could be an indication for tonsillectomy due to distant focal diseases. Further clinical studies are needed to prove their importance in this respect.

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