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TITLE PAGE

Tonsillar surface swab bacterial culture results differ from those of the tonsillar core in recurrent tonsillitis.

Running title: Bacterial culture in recurrent tonsillitis

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TITLE PAGE continued**Financial Disclosure statement**

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Conflict of interest statement

The authors disclose no conflicts of interest

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Abstract

Objectives: We aimed to determine whether there was a difference between core and surface bacteriology of Finnish adults with recurrent or chronic tonsillitis, to understand whether a surface swab is worthwhile, and to understand which bacteria are involved.

Methods: Uninflamed tonsillar surface swabs and core biopsies were taken prior to, and during surgery, respectively, in 103 patients aged 16-66 undergoing tonsillectomy for recurrent or chronic tonsillitis. The McNemar test was used to determine differences between the surface and core in the most prevalent bacterial species.

Results: Twenty-seven bacterial species were isolated in addition to normal flora and were more commonly found in the core (1.11 surface, and 4.75 core bacteria isolated per patient). Viridans group streptococci were the most commonly detected bacteria, found in 88% of the patients, mainly in the core. The bacteria in general were mainly isolated from the core. Of the ten most prevalent bacteria, only Group C beta-haemolytic streptococci showed no difference between detection from core and surface swabs. Other bacteria found mainly in the core include *Prevotella melaninogenica*, *Staphylococcus aureus* and *Fusobacteria*.

Conclusions: There is discord between the surface and core bacteria. A different population of bacteria exists in the core, especially anaerobic bacteria, suggesting that a core sample may be useful in evaluating recurrent and chronic tonsillitis.

Level of Evidence

Iib, individual cohort study.

Keywords

Microbiology

Bacterial growth

Biofilms

Staphylococcus Aureus

Viridans group Streptococci

Introduction

Recurrent tonsillitis is a condition with a great impact on the sufferers, resulting in school and work absences. It can be treated with tonsillectomy in those patients in whom it occurs several times a year. The recurrence rate in patients who have had group A streptococcal tonsillitis treated with penicillin is around 20% in younger children, with lower rates in adolescents (1). The exact prevalence of recurrent and chronic tonsillitis in adults is not completely known.

A few previous studies, with samples from 50, 116 and 23 patients, respectively, have found that the surface bacteria do not always correlate with the pathogens causing recurrent tonsillitis (2-4). On the other hand, a more recent study has shown that bacteria on the surface and within the core of the tonsils do not significantly differ with regards to anaerobes, and that the same antibiotics are efficient against both the surface and core bacteria (5).

Given the discord between the previous study results, the aim of this study was to investigate which bacteria are isolated in adult patients with chronic or recurrent tonsillitis, whether there is a correlation between the core and surface bacteria in these patients, to understand the underlying tonsillar microbiotas in these patients, and to identify any differences that may contribute to ongoing symptoms or play a role in the recurrences.

Materials and methods

Ethical Considerations

All procedures performed in this study on human subjects were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed oral consent was obtained from all individual participants included in the study.

Patient samples

Paired bacteriology samples of tonsils (surface swab and core tissue sample) were taken from 103 patients with recurrent or chronic tonsillitis who visited the Laakso Hospital, Helsinki, Finland during the years 1988-1989. The swabs were taken from tonsils that were not acutely infected - either on the day of the clinic visit, when the decision of the operation was done, or on the operation day, prior to the surgery. The core tissue samples were taken perioperatively. Bacterial culture was performed at the Aurora Hospital microbiology laboratory, with samples inoculated into different media, to maximize detection of anaerobic bacteria (6).

Statistical analysis was performed using SPSS (v.23, IBM, New York, USA), with the McNemar test. The Bonferroni multiple test correction was used, therefore a p-value of <0.005 was considered significant.

Results

Seventy percent (n=72) of the patients were female and 30% (n=31) were male. They were aged between 16-66 years, with a median age of 24 at the time of data collection. From the sample cultures, there was growth of normal flora in 92 samples. An additional 27 species of bacteria were singled out, with an average of 4.9 species per patient. One patient's cultured sample yielded only normal flora. Please see **Table 1** for details of the 10 most abundant bacterial species and from where they were detected: either the surface, the core, or both, along with the p-value for the core vs surface comparison. Viridans group streptococci were the most commonly found bacteria, observed more within the core samples ($p=1.034E-25$). Group C beta-hemolytic streptococcus was found on the surface and in the core in the majority of the samples and there was no difference between the core and surface ($p=0.180$). The other most abundant bacteria were found mainly in the cores of the samples.

Other, less abundant bacteria are listed in Supplementary Table 1, including but not limited to other groups of beta-hemolytic streptococci (A, B, F, G), with group A being present in 4/118 cases, and group G being present in 3/118 cases.

Discussion

Synopsis of main findings and comparison with other studies

The purpose of this study was to clarify the bacteriology of adult patients with recurrent tonsillitis, specifically whether there is a difference between the bacterial composition on the surface and in the core of tonsils in Finnish patients. Microbiological analysis of tonsils is challenging due to the great diversity and differences in the normal flora between patients. This diversity is reflected in our data. Additionally, it has been suggested that even normal flora can cause tonsillitis (7), although this could be interpreted as a change in the local microbiome. Our results also showed a range of aerobes and anaerobes, with anaerobes typically found within the core and less on the surface. The importance of anaerobes within tonsillitis is being more recognized than previously, with anaerobes being isolated from recurrent tonsillitis (2, 5) and predominating in tonsillar abscesses, often without aerobes (8). In our study *Fusobacteria* species were found in 40% of patients (**Table 1**), typically in the core, as oppose to the surface ($p=2.8376E-10$). *Fusobacteria* are increasingly recognized as pathogens in recurrent tonsillitis, especially *Fusobacterium necrophorum*, which is an agent of Lemierre's syndrome and also implicated in up to 60% of peritonsillar abscesses (9).

In this study, nearly all the bacterial species found can be considered to be part of the normal pharyngeal flora (10). However, some of the bacteria are potential pathogens, for example group A streptococci, *Haemophilus influenzae* and pneumococci. The ten most common bacteria concurred with the common bacteria identified in other studies (11-13). Interestingly, the most common bacterium found on the surface was group C streptococcus, and there was no difference between the surface and core ($p=0.180$). It is unclear whether the group C streptococci actually play a role in recurrent tonsillitis or whether they only are local commensals. Nevertheless Group C streptococci were also found in 12% of Finnish patients

with a sore throat (14). Group A streptococci are well recognised in their preponderance in acute throat infections (8), however the prevalence of group A streptococci in our cohort of non-inflamed tonsils of 3.9% is in keeping with previous research in the Finnish population, where a prevalence of 2% was found using a routine throat swab test, or 6% using a novel point-of-care test (15).

In this study, the tonsillar surface and core tissue bacteria did not correspond well with each other. In all of the most abundant bacteria except for *Group C beta-hemolytic streptococci* there was a discrepancy between the core and surface, with these bacteria usually being found in the core (see **Table 1** for p-values). This result differs from other studies, where *group C beta-hemolytic streptococci* were found to be of low prevalence, and only rarely in both the surface and core of the same patients, and *S. aureus* was found most frequently in both the surface and the core (2). Possible reasons for the differences between surface and core samples could be (i) core conditions favoring anaerobes and microaerophiles, (ii) other growth conditions such as food and drink supporting the growth of surface bacteria, (iii) surface bacteria being washed away with saliva. The finding could also be a consequence of toothpaste use, as this can alter the oral microflora (16). It is also possible that group C streptococci were not classified similarly in the different studies.

Potential mechanisms for chronicity and recurrence of tonsillitis

Tonsillitis could become chronic for various reasons. Fibrotic tissue or other anatomic changes in the tonsils from multiple infections could lead to inadequate penetration of antibiotics into the tonsillar core (13). Bacteria could be protected from the microbicidal or growth restricting effects of antibiotics by being intracellular or deeply inserted in the tissues, as has been shown for *S. pyogenes* (group A streptococci) in recurrent tonsillitis. This may

occur not only in epithelial cells, but also within macrophage-like cells in the tonsillar crypts (17), possibly acting as a reservoir for re-infection. Penicillin may be inadequate in treating some of the bacteria causing recurrent tonsillitis, particularly as many of them are resistant to penicillin. Beta-lactamase-secreting strains of four of the species found in our cases (*Bacteroides fragilis*, *Fusobacterium* sp., *Haemophilus influenzae* and *Staphylococcus aureus*) have been found in 73-80% of children with recurrent tonsillitis (8).

Our samples clearly show a difference in the composition of bacteria between the core and surface areas of tonsils. Some of the bacteria have increasing rates of penicillin resistance in Finland such as *Staphylococcus aureus* (18) and *fusobacteria* (19), but this is not the case for all: *S. viridans* group has a resistance rate of only 2.3% (20). Although we do not have the antibiotic sensitivity results for bacteria found in this study, it is also possible that from repeated antibiotic courses, strains become resistant to the typical antibiotic treatments. Our samples also show prevalence of biofilm-producing bacteria such as *Staphylococcus aureus*, which require longer courses of antibiotics. It may be simply that the choice of antibiotic is failing to cover and adequately treat the underlying pathogens found in the core.

Clinical applicability of the study

From this study, it is clear that there is a difference between the local core and surface microbiome. It may be that an aspiration sample could be considered in patients with recurrent tonsillitis to get a better understanding of the etiology at an earlier stage, to prevent further illness and complications. The complications of tonsillitis, although uncommon, can be severe, with peritonsillar- and deep neck space abscesses, Lemierre's syndrome, rheumatic fever among the more severe. However, aspiration may be too invasive for the first episode of tonsillitis, as not all episodes recur. Another option would be to commence a broader

spectrum, and better tissue penetrating antibiotic in cases of recurrent tonsillitis. The problem, however, would be the potential emergence or selection of antibiotic resistant bacteria and induction of disturbances in the normal intestinal microbiome that could lead to antibiotic-associated diarrhea or colitis.

Strengths and limitations of the study.

The strengths of the study are the sample size and homogeneity in terms of it being a single-center series with sequential patients. The weaknesses of the study are the lack of background information on the patients, such as past medical history, smoking and lifestyle information, and previous antibiotic use. In order to determine whether potential pathogens could contribute to the disease or are just part of the normal flora, it would be useful to have a control group, such as patients undergoing tonsillectomy for snoring or sleep apnea.

Conclusions

Determining the significance of tonsillar bacteriology is challenging because of the wide spectrum of bacteria. However, the results from this study confirm that anaerobes are harbored in the core of the tonsil in adults with recurrent or chronic tonsillitis. In addition, the results show that there is a clear difference between the bacteria on the surface and in the core of the tonsils in these patients. Therefore, possibly a core aspirate would be useful to determine the types of bacteria harbored to tailor antibiotic choices in case of reinfection or in chronic cases. However, further research is needed, ideally incorporating a control group to really assess the differences in commensal bacteria, as well as testing the bacterial profiles during acute episodes of tonsillitis. A more recent sample series would help us to improve understanding of the current bacterial landscape including antibiotic sensitivities, to aid in the treatment decisions.

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Table 1 Surface and core swab growths of the 10 most abundant tonsillar bacteria, in order of prevalence. Percentages are expressed in terms of patients out of the 103 total patients.

The McNemar test was performed for each bacterial species (p-values shown). The remaining bacteria identified can be found in Supplementary Table 1.

Bacteria	Surface only		Core only		Both		Total		p-value
	%	n	%	n	%	n	%	n	
Viridans group streptococci	0	0	81.6	84	6.8	7	88.4	91	1.034E-25
Beta-haemolytic streptococci (group C)	1.9	2	6.8	7	44.7	46	53.4	55	0.180
<i>Prevotella melaninogenica</i>	0	0	42.7	44	5.8	6	48.5	50	1.1369E-13
<i>Staphylococcus aureus</i>	0	0	32.0	33	12.6	13	44.7	46	2.3283E-10
Fusobacteria	1.0	1	35.9	37	2.9	3	39.8	41	2.8376E-10
<i>Neisseria</i> sp. (apathogenic)	0	0	34.0	35	1.0	1	35.0	36	5.8208E-11
<i>Peptostreptococcus</i> sp.	0	0	32.0	33	2.9	3	35.0	36	2.3283E-10
<i>Haemophilus influenzae</i>	4.9	5	22.3	23	3.9	4	31.1	32	0.000912

<i>Bacteroides</i> (not including <i>B. fragilis</i>)	1.9	2	25.2	26	1.0	1	28.2	29	0.000003
<i>Haemophilus parainfluenzae</i>	0	0	21.2	22	1.9	2	23.3	24	4.7684E-7

Supplementary Table 1.

Bacteria name	Surface (%)	Surface (n)	Core (%)	Core (n)	Both (%)	Both (n)	Total (%)	Total (n)
Coagulase-negative staphylococci	1	1	8.7	9	0	0	9.7	10
Propionibacteria	0.0	0	4.9	5	2.9	3	7.8	8
Diphtheroids	1.0	1	6.8	7	0	0	7.8	8
<i>Moraxella catarrhalis</i>	1.9	2	1.9	2	1.0	1	4.9	5
β -haemolytic streptococcus (group A)	1.9	2	0	0	1.9	2	3.9	4
β -haemolytic streptococcus (group B)	1.0	1	0	0	2.9	3	3.9	4
<i>Veillonella</i>	0	0	3.9	4	0	0	3.9	4
<i>Bacteroides fragilis</i>	0	0	3.9	4	0	0	3.9	4
β -haemolytic streptococcus (group F)	0	0	3.9	4	0	0	3.9	4
<i>Micrococcus</i>	0	0	3.9	4	0	0	3.9	4
β -haemolytic streptococcus (group G)	0	0	0	0	2.9	3	2.9	3
<i>Enterobacter cloacae</i>	0	0	1.9	2	0	0	1.9	2
<i>Staphylococcus epidermidis</i>	0	0	1.9	2	0	0	1.9	2
Pneumococci	0	0	1.0	1	0	0	1.0	1
<i>Escherichia coli</i>	0	0	1.0	1	0	0	1.0	1
<i>Klebsiella pneumoniae</i>	0	0	1.0	1	0	0	1.0	1
<i>Haemophilus haemolyticus</i>	0	0	1.0	1	0	0	1.0	1