Similar recovery rates of Fusobacterium necrophorum from recurrently infected and non-infected tonsils

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ABSTRACT

INTRODUCTION: Recent studies implicate the presence of *Fusobacterium necrophorum* (FN) in recurrent tonsillitis (RT), acute tonsillitis and peritonsillar abscess. The objective of the present study was to determine whether FN plays a role in RT by comparing bacteriologic results from patients suffering from RT, tonsillar hypertrophy and halitosis or persistent sore throat syndrome (PSTS). We analyzed both tonsils to determine the degree of concordance.

MATERIAL AND METHODS: A prospective study was conducted in 80 patients aged 8-30 years who were undergoing elective tonsillectomy. The patients were divided into four groups according to indication for surgery. Aerobic and anaerobic cultures from the tonsillar surface and core were analyzed.

RESULTS: FN was detected less frequently in the tonsillar cores of RT patients (22%) than in those of patients without RT (30%) (p = 0.44). FN detection frequencies ranged between 20% and 35% across the four groups. Beta-haemolytic streptococci groups A/C/G (BHS) were detected significantly (p = 0.007) more often in the RT group than in the halitosis/PSTS group.

CONCLUSION: A possible role of FN in RT was not substantiated. Our results indicate that FN is likely to be part of the normal flora. The tonsillar surface and core flora carry considerable interpersonal diversity, but is very similar bilaterally in each individual. Other factors seem to play a major role in the development of the represented tonsillar diseases.

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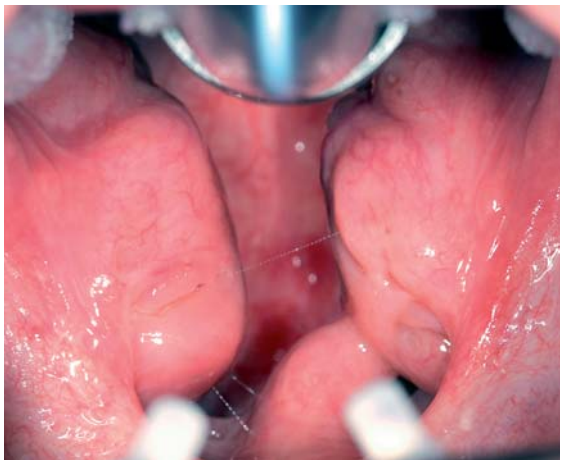

Oropharynx with hyperthrophic tonsils in a 30 year-old male.

Recurrent acute tonsillitis (RT) remains a frequently occurring clinical problem. Penicillin is used to treat acute episodes; however, it often fails to prevent recurrence and many patients ultimately undergo tonsillectomy.

Beta-haemolytic streptococci group A (GAS) is recognised as the primary bacterial pathogen in RT, acute tonsillitis (AT) and peritonsillar abscess (PTA) [1, 2]. Large colony-forming beta-haemolytic streptococci group C and G, *Arcanobacterium haemolyticum* and *Corynebacterium diphtheriae* are also documented causes of bacterial AT [3]. However, within the past five years, a pathogenic role of FN has been suggested in RT, AT and PTA [4-7]. The prevalence of *Fusobacterium necrophorum* (FN) was even higher than that of GAS in 847 PTA patients treated at our Department from 2001 to 2006 [7]. FN differs from the other tonsillar pathogens by being a gram-negative, obligate anaerobic rod. It is the cause of Lemierre's disease, a life-threatening illness with metastatic abscesses secondary to septic thrombophlebitis of the internal jugular vein, where the patient initially presents with a sore throat. However, most FN infections remain localised [8].

Prior studies on the bacteriology of RT and TH have generally focused on the paediatric age group. However, the young adult population is of major interest as the incidence of RT, AT and PTA peaks in this age group [9, 10]. The capture rates of FN and GAS have been shown to be highly age-dependant [4, 11, 12]. Brook et al found significant differences in the bacterial flora of children and adults with RT and suggested that the disease might differ between children and adults [11]. The prevalence of FN peaks in young adults, not only in AT and PTA patients, but also in healthy subjects [7, 12].

The majority of studies focusing on tonsillar core bacteriology in young adults have used techniques detecting only aerobic bacteria [13]. Jensen et al have studied the FN incidence in non-streptococcal RT. However, they analyzed swabs of the tonsillar surface [5].

ORIGINAL ARTICLE

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Furthermore, to our knowledge, no studies have been conducted to study both tonsils in patients undergoing elective tonsillectomy. Thus, it had not been explored if the two tonsils harbor similar bacterial flora. Brook et al have shown the necessity of swabbing both tonsils in patients with bilateral AT in order to detect all cases caused by GAS [14]. This indicates some difference between the two tonsils at the time of acute infection.

TABLE 1

Gender and age distribution across the four tonsillar disease groups.

	Group 1	Group 2	Group 3	Group 4	Total
Patients, n	30	20	20	10	80
Males, n (%)	4 (13)	11 (55)	8 (40)	4 (40)	27 (33)
Median age, years	20.0	19.5	17.5	19.5	19.0
Mean age, years	20.4	19.1	18.5	19.3	19.4

Group 1 = recurrent acute tonsillitis.
Group 2 = tonsillar hypertrophy.
Group 3 = recurrent acute tonsillitis and tonsillar hypertrophy.
Group 4 = halitosis or persistent sore throat syndrome.

TABLE 2

Number and distribution of isolates in tonsillar surface swabs and core tissues in 80 elective tonsillectomised patients.

	Isolates, n		Distribution of isolates, n		
Organism	surface swabs	core tissues	surface swabs only	core tissues only	surface swabs & core tissues
Aerobic					
Beta-haemolytic streptococci					
Group A	5	6	1	2	4
Group C	8	10	0	2	8
Group G	6	9	1	4	5
Not grouped	2	2	0	0	2
Total A-C-G	21	27	2	8	19
Streptococcus group B	2	2	0	0	2
Viridans streptococci	145	138	8	1	137
Streptococcus pneumoniae	1	0	1	0	0
Staphylococcus aureus	40	46	0	6	40
Coagulase-negative staphylococci	6	6	3	3	3
Haemophilus influenzae	5	6	2	3	3
Haemophilus parainfluenzae	1	1	0	0	1
Eikenella corrodens	5	14	2	11	3
Neisseria species	65	66	4	5	61
Moraxella catarrhalis	0	1	0	1	0
Corynebacterium species	32	27	7	2	25
Enterobacteria	3	1	2	0	1
Total aerobic isolates	326	335	31	40	295
Anaerobic					
Fusobacterium necrophorum	11	20	3	12	8
Fusobacterium species	55	57	6	8	49
Prevotella species	84	109	4	29	80
Other anaerobes	11	9	3	1	8
Total anaerobic isolates	161	195	16	50	145
Yeast	7	5	3	1	4

Thus, prior studies of RT may have overlooked certain pathogens.

The aim of the present study was to determine whether FN plays a role in RT by comparing the tonsillar surface and core bacteriology of patients suffering from RT with that of patients undergoing elective tonsillectomy for other reasons. Furthermore, our study considers both tonsils to determine the degree of concordance.

MATERIAL AND METHODS

Patients

Patients were enrolled in the study between November 2005 and February 2009 at three ear, nose and throat (ENT) departments in Denmark. The study comprised four distinct diagnostic patient groups undergoing elective tonsillectomy: patients suffering from (1) RT (more than five episodes of AT within two years), (2) TH with a history of airway obstruction, (3) both RT and TH and (4) halitosis or persistent sore throat syndrome (PSTS) without signs or symptoms of infection other than sore throat. Only patients between the ages of eight and 30 years having received no antibiotic treatment during the month preceding surgery and who had no history of PTA were included in the study. None of the patients had signs or symptoms of AT at the time of surgery.

Specimen collection

After the induction of anaesthesia, coal-coated cotton swabs were rubbed thoroughly on the surfaces of each of the tonsils and placed in transport media (Stuart's medium, SSI Diagnostic, Hilleroed, Denmark). The tonsils were removed by blunt dissection and placed separately in sterile containers. None of the patients received antibiotics before the collection of specimens had been completed. Tonsillar tissue and surface swabs in Stuart's media were placed in a –80 ˚C freezer within minutes of collection.

Microbiological analysis

Microbiological analysis was carried out at the Department of Clinical Microbiology, Aarhus University Hospital. Samples were stored at –80 °C until the bacteriologic investigations were performed. Specimens were processed in a class-2 laminar airflow safety cabinet using an aseptic technique. Tissue samples and swabs were cultured onto 5% blood agar plates, chocolate agar plates and anaerobic plates (all from SSI Diagnostic, Hilleroed, Denmark). The plates were incubated at 35 °C in either a carbon dioxide (CO_2)-enriched atmosphere for three days or anaerobically for five days using the Concept 400 anaerobic workstation (Fisher Scientific, Denmark). Speciation for microorganisms was performed by standard methods [15] or by using the VITEK 2 system. Special care was taken to differentiate (large

colony) beta-haemolytic group C and G streptococci (Voges-Proskauer test-negative) from *Streptococcus anginosus* (Voges-Proskauer test-positive). The diagnosis of *Fusobacterium* was accepted for an anaerobic, non-spore-forming Gram-negative pleomorphic rod which was penicillin-, kanamycin- and metronidazole-susceptible, vancomycin-resistant, catalase-negative, smell of butyric acid and fluorescence of a chartreuse colour in ultraviolet light.

FN was differentiated from other *Fusobacterium* species by bata-haemolysis on horse blood agar. Antibiotic sensitivities were determined by a standard disc diffusion method using the protocol from the Swedish Reference Group for Antibiotics (SRGA) on isosensitivity plates (Oxoid, Denmark). Organisms of the same species were deemed indistinguishable if they had the same colony morphology, the same basic biochemical features and an identical antibiogram.

Statistical analysis

Fisher's exact test was used for group comparison of bacteriologic findings, and the Kruskal-Wallis test was used for comparison of semi-quantitative growth distribution. Statistical significance was defined as $p < 0.05$.

Trial registration: The study was approved by The Research Ethics Committee of Aarhus County (no. 20050034).

RESULTS

Eighty patients were included in the study. Their mean and median ages were 19.0 and 19.4 years, respectively, and nearly identical across the four groups (**Table 1**).

Mixed aerobic and anaerobic flora was present in 95% of surface swabs and in 99% of core tissues. An average of 5.6 isolates (3.9 aerobes and 1.7 anaerobes) was detected in surface swabs and an average of 6.3 isolates (4.0 aerobes and 2.3 anaerobes) in core tissues. The four groups did not differ significantly with regard to the average number of aerobic and anaerobic isolates.

The aerobic bacteria most frequently isolated from both tonsillar surfaces and cores were viridans *Streptococci, Neisseria* species, and *Staphylococcus aureus* (**Table 2**). The predominant anaerobic bacteria at both sites were *Prevotella* species, *Fusobacterium* species and FN. No consistent pattern of co-isolates was noted at either site.

The various bacterial species were isolated at similar frequencies from the four groups with the exception of beta-haemolytic streptococci group A/C/G (BHS), which were not detected in the halitosis/PSTS group (**Table 3**). A significant difference between the frequency of BHS in the RT group and the halitosis/PSTS group was found ($p = 0.007$, Fisher's exact test). However, the difference between isolation rates of BHS from patients with RT (group 1+3) and patients without RT (group 2+4) was not statistically significant ($p = 0.15$).

FN was isolated from the tonsillar cores of 25% of the patients with detection frequencies ranging from 20% to 35% between the four groups (Table 3). In RT patients with or without TH (group 1+3), FN was detected less often (22%) than in patients not suffering from RT (group 2+4) (30%). This difference was statistically insignificant ($p = 0.44$).

Semi-quantification revealed no significant differences between the four groups regarding heaviness of growth of each bacterial strain. Twenty-three of 26 BHS were isolated as heavy growth and three as moderate growth. Similarly, 19 of 20 FN were isolated as heavy growth and one as moderate growth.

A comparison between the surface and core specimens revealed that tonsillar surface swab detection was reliable for most organisms (Table 2). However, surface swabs failed to detect 30% of BHS, 60% of FN and 79% of *Eikenella corrodens* isolated in core tissues.

Concordance rates between left and right tonsillar

TABLE 3

Isolates from core tissues obtained from 80 patients admitted for elective tonsillectomy. The values are n (%).

Organism	Group 1 (n = 30)	Group 2 (n = 20)	Group 3 (n = 20)	Group 4 (n = 10)
Aerobic				
Beta-haemolytic streptococci				
Group A	2 (7)	2 (10)	2 (10)	0
Group C	6 (20)	3 (15)	1 (5)	0
Group G	4 (13)	2 (10)	3 (15)	0
Not grouped	2 (7)	0	0	0
Total A-C-G	14 (47)[a]	7 (35)	6 (30)	0[a]
Streptococcus group B	1 (3)	1 (5)	0	0
Viridans streptococci	47 (90)	37 (100)	35 (95)	19 (100)
Staphylococcus aureus	14 (47)	11 (55)	14 (70)	7 (70)
Coagulase-negative staphylococci	3 (10)	0	1 (5)	2 (20)
Haemophilus influenza	4 (17)	0	2 (10)	0
Haemophilus parainfluenzae	1 (3)	0	0	0
Eikenella corrodens	3 (10)	4 (20)	4 (20)	3 (30)
Neisseria species	24 (80)	18 (90)	15 (75)	9 (90)
Moraxella catarrhalis	0	1 (5)	0	0
Corynebacterium species	10 (33)	10 (50)	5 (25)	2 (20)
Enterobacteria	0	1 (5)	0	0
Anaerobic				
Fusobacterium necrophorum	6 (20)	7 (35)	5 (25)	2 (20)
Fusobacterium species	22 (73)	14 (70)	12 (60)	9 (90)
Prevotella species	41 (90)	26 (100)	27 (100)	15 (100)
Other anaerobes	5 (17)	3 (15)	0	1 (10)
Yeast	1 (3)	1 (5)	3 (15)	0

Group 1 = recurrent acute tonsillitis; Group 2 = tonsillar hypertrophy; Group 3 = recurrent acute tonsillitis and tonsillar hypertrophy; Group 4 = halitosis or persistent sore throat syndrome.
a) Significantly different (Fisher's exact test; $p = 0.007$).

core isolates for aerobic and anaerobic bacteria were 86% and 90%, respectively (**Table 4**). Concordance rates for surface isolates were 88% for aerobes and 79% for anaerobes.

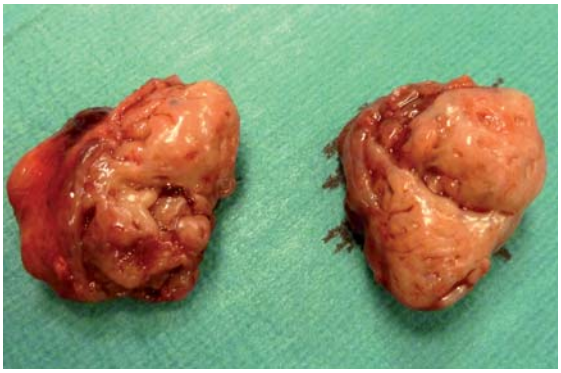
DISCUSSION

This study demonstrates the presence of polymicrobial aerobic and anaerobic flora on the tonsillar surface and in the tonsillar cores of young adults suffering from RT, TH, both RT & TH, and halitosis/PSTS.

In contrast to our expectations, FN was actually detected more frequently in the groups of patients without RT than in those with RT, although the difference was not statistically significant.

BHS were detected significantly more often in the RT group than in the halitosis/PSTS group, which was expected as BHS are accepted as major pathogens in RT, while the relation of BHS to halitosis/PSTS is less recognized. Apart from this finding, the comparison of core tissues and surface swabs across the four tonsillar disease groups revealed no significant differences in isolation frequencies or quantities of either aerobic or anaerobic organisms.

Some researchers have failed to isolate FN in healthy subjects [6], which has left some investigators doubtful as to whether FN is part of normal tonsillar flora or not [16, 17]. However, using polymerase chain reaction (PCR), Jensen et al found FN in 21% of tonsillar surface swabs from healthy young adults [5]. In another PCR-study, Ludlam et al detected FN in 10.5% of tonsillar surface swabs from healthy university students [12]. Our culture results, which were obtained from clinically non-infected tonsils, further support the belief that FN is part of the normal tonsillar flora in young adults with a carriage rate of 10-25%. This is important to take into account both for researchers and for clinicians when interpreting tonsillar culture results.



Hyperthrophic tonsils.

Jensen et al detected a significantly larger load of FN DNA in patients with recurrent non-streptococcal tonsillitis than in patients with acute non-streptococcal tonsillitis [5]. Ludlam et al found that the magnitude of the copy counts of FN could give an indication of the likelihood of active clinical infection although there was much overlap of copy counts between asymptomatic subjects and AT patients [12]. Our semi-quantified culture results revealed no differences between the four groups and could not support the findings of Jensen et al. A pronounced growth of FN was found in 19 patients who had no signs or symptoms of acute infection. This finding supports the large overlap found by Ludlam et al. As FN and GAS is abundant in a substantial proportion of patients without clinical signs or symptoms of current infection and in different patient groups, other factors seem to play a major role in the development of clinical infection.

The best standard of reference for the bacteriologic tonsillar findings in RT patients would be tonsillar tissue from healthy subjects without current infection and no history of tonsillar disease. For ethical reasons, such specimens were unfortunately unobtainable in the present study and only a very limited number of studies have compared core tissue from "normal tonsils" with equivalent tissue from RT and TH patients [13, 18, 19]. These studies have demonstrated a polymicrobial flora with frequent colonization of potential oropharyngeal pathogens, including BHS, *Staphylococcus aureus,* and *Haemophilus influenzae* in quantities indistinguishable from those found in TH and RT patients in a clinically non-infected phase.

No previous studies have compared left and right tonsillar core bacteriology. We found high inter-tonsillar concordance of both aerobic (86%) and anaerobic (90%) isolates. Thus, the bacterial flora of the right and left

TABLE 4

Selected bacterial concordances between left and right tonsillar core tissues/surface swabs.

	Isolates, core/surface, n			
Bacterium	**left tonsils only**	**right tonsils only**	**both left & right tonsils**	**Concordance, core/surface, %**
Aerobic				
Beta-haemolytic streptococci				
Total group A-C-G	2/3	3/1	22/17	81/81
Viridans streptococci	8/3	4/5	126/137	91/94
Staphylococcus aureus	1/0	3/4	42/36	91/90
Haemophilus influenzae	1/1	0/1	5/3	83/60
Eikenella corrodens	2/3	4/1	8/1	57/20
Neisseria species	4/6	3/1	59/58	89/89
Total aerobic isolates	23/19	22/21	288/285	86/88
Anaerobic				
Fusobacterium necrophorum	0/3	3/1	17/7	85/64
Fusobacterium species	2/2	1/8	54/45	95/82
Prevotella species	4/6	5/8	100/70	92/83
Total anaerobic isolates	8/10	11/22	176/129	90/80

tonsil was very similar and the information missed by previous studies of a single tonsil per patient seems modest.

As FN has been implicated in RT, AT and PTA [4-7], evidence of this bacterium from surface swabs is of great interest in the effort to ensure proper antibiotic treatment. Our study shows that surface swabs are, unfortunately, unreliable in detecting FN, at least in clinically non-infected tonsils, since only 40% of FN isolated in the cores was detected in surface swabs. This is not surprising because FN is anaerobic, which facilitates growth in tonsillar crypts rather than on air-exposed surfaces.

We were able to isolate 88% of core aerobes and 74% of core anaerobes in surface swabs. These percentages are higher than those reported by most other researchers [20] which may reflect that surface swabs were taken under optimal conditions. However, some potential pathogens were missed in surface swabs and our results highlight the advantage of swabbing both tonsils to increase the likelihood of detecting pathogens harbored in the cores.

In conclusion, a possible role of FN in RT was not substantiated as the incidence of FN in tonsillar cores of RT patients was not significantly different from that found in non-RT patients. Our results support previous studies which indicate that FN is part of the normal flora. The tonsillar surface and core flora show much interpersonal diversity, but are bilaterally very similar in each individual. Other factors seem to play a major role in the development of the represented tonsillar diseases. Studies exploring the possible roles of host factors and virus in these diseases are warranted.

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