Original Article

Comparison of core and surface cultures in recurrent tonsillitis

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This prospective study was done to find out the most common organism affecting the tonsils in recurrent tonsillitis and to compare the microorganisms found in the core and surface of such tonsils. A total of 50 patients attending ENT Out patient Department of Tribhuvan University Teaching Hospital (TUTH) from February 2000 to August 2001 were included in the study. All these patients had undergone tonsillectomy under general anaesthesia by dissection method. Among these 39 patients had undergone tonsillectomy for recurrent tonsillitis and 11 had undergone tonsillectomy for reasons other than recurrent tonsillitis. The excised tonsils were kept in agar plates and transported to the microbiology laboratory within half an hour of surgery. The culture was taken from the surface and the core after cutting the tonsils into half by a sterile blade and with another sterile blade only core was extracted in a wedge shaped manner and was cultured in aerobic (5% sheep blood agar, Chocolate agar and McConkey's agar and anaerobic (gas pack jar system) media for 24 - 48 hours. The most common microflora seen in recurrent tonsillitis was *Streptococcus viridans* from the surface, as well as the core. Other common micro organisms prevalent on the surface in recurrent tonsillitis were *Staphylococcus aureus,* and *Brahmnella catarrhalis*. Likewise other common micro organisms in the core in recurrent tronsillitis were *Staphylococcus aureus, Klebsiella pneumonie,* and *Haemophillus influenzae*. There was no statistical difference between the micro organisms found on the surface and the core except for *Brahmnella catarrhalis* which was significantly more on the surface. Anaerobes were isolated only from the core and there was no statistical difference between the micro organisms isolated from the non-infected tonsils and from recurrent tonsillitis

Introduction

Acute tonsillitis is defined as infection of the tonsils that is characterized by sore throat, fever, odynophagia, leucocytosis, congested tonsils with or without enlargement and tender jugulodigastric lymph nodes[1]. It is one of the most common diseases that an Ear Nose and Throat (E.N.T.) surgeon, a pediatrician and a general practitioner encounters in his/her daily practice. This is because the effective treatment depends on the knowledge of the infecting organisms that paints a very confusing scenario. Tonsillar infection may stem from the bacteria within the tonsillar crypts or parynchyma, rather than the bacteria identified on the surface. Pathogens isolated from the surface culture may be colonizing the tonsil but not infecting it.

An alternative possible explanation for not acquiring an accurate culture of pathogenic microorganisms in tonsillitis is that the sole aerobic techniques are not satisfactory and anaerobic technique would increase the isolation of Group A *Beta-hemolytic streptococci.* The failure of penicillin to eradicate the infection due to *Beta-hemolytic streptococci* has also been attributed in part to B-lactamase producing anaerobic bacteria. Regarding the microorganisms affecting the tonsils in recurrent acute tonsillitis, Group A *Beta-hemolytic streptococci* is considered to be the most common. Besides this, *Alpha-hemolytic streptococci* (*Streptococcus viridans*), *Streptococcus pyogenes*, *Hemophilus influenzae* and *Staphylococcus aureus* are the next common. *Brahmnella catarrhalis, Klebseilla pneumonie* and other anaerobic organisms like *peptostreptococci, bacteroids, fusibacterium, Velionelle* are also recovered from many tonsils[2]. Another school of thoughts suggests that a viral infection is the sole agent for acute tonsillitis viz. Adenovirus, E.B. virus or Herpes simplex virus[3].

This study is undertaken to ascertain the common microorganisms affecting the tonsil in recurrent acute tonsillitis, so that the most sensitive antibiotic can be dispensed and the cause of recurrent nature can be identified. Besides the process of tonsillectomy along with its huge expenditure, excruciating post-operative pain and complications leading to morbidity and even mortality can be reduced.

Material and Methods

This was a prospective study consisting of a total of 50 patients, conducted in the E.N.T. Department of T.U. Teaching Hospital, Kathmandu, Nepal. The study period was $1^{1}/_{2}$ years i.e. from March 2000 to August 2001. The patients were divided into two groups. Group A, study group consisted of a total number of 39 cases of recurrent tonsillitis that underwent tonsillectomy. Group B, control group consisted of 11 cases that underwent tonsillectomy for reasons other than recurrent tonsillitis, like sleep apnoea syndrome, keratosis of tonsils, cysts of tonsils, as an approach to elongated styloid process and tonsillar hypertrophy.

Pre-operative clinical examination included detailed general examination, ear, nose and throat examination, neck examination and other systemic examinations. Investigations like hemoglobin, total and differential counts of WBC, bleeding time, clotting time, platelet counts were done followed by blood grouping. Then those who were fit for surgery and general anesthesia were posted for tonsillectomy.

Patients with recurrent acute tonsillitis of any age, sex or race and those who had not taken any form of antibiotics within at least 10 days prior to surgery were included in the study. Patients of any age, sex or race who were subjected to tonsillectomy for reasons other than recurrent tonsillitis were included in the control group. Patients who had taken any form of antibiotics within 10 days before surgery and who had clinical suspicion of malignancy of tonsils were excluded from the study.

Pre-operatively patients were admitted at least 1 day prior to surgery. No pre-operative antibiotics or medication were given. Tonsillectomy was done under general anesthesia by dissection method. Once the tonsils were out, they were placed on the culture plates provided in the Operation Theater. It was transported to the laboratory within half an hour. In the microbiology laboratory, the sample was placed in a sterile petri-dish. The surface sample was taken from the surface of tonsil with the help of a red-flamed loop and streaked in the Chocolate agar, 5% sheep blood agar and McConkey's agar then it was placed in the incubator for 24-48 hours. The remaining sample was cut into two halves by a flamed sterile scalpel. Again with another flamed sterile scalpel a wedge section was cut from the cut surface of the tonsil. [The whole process was done in a clear bench "sterile cabinet"]. Now from the center of this wedge-shaped core area, core sample was taken with the help of a red-flamed loop and streaked in the Chocolate agar, two plates of 5% sheep blood agar and McConkey's agar, with due aseptic precautions it was placed in incubator for 24-48 hours. One of the blood agar plates with core sample was kept in the BBL(Baltimore ,Boston Laboratory) anaerobic gas jar by BBL gas pack method after placing an "Anaerogen". [made by oxoid ltd. Wade Road, Basingstoke, Hampshire, England] for 24-48 hours. The remaining core tissue was inoculated in the thioglycolate and cooked meat media for sub-cultures. After 24 hours the media were taken out and findings were recorded.

Study of Gram's reaction and morphoTypes of bacteria was done. The colony characteristics were studied viz. size, shape, color, odour, lusture , haemolytic nature and colony character was recorded and confirmatory test were done simultaneously. Thioglycolate or cooked meat broth was sub-cultured if turbidity was seen and results were tabulated. In cases where *Beta-haemolytic streptococci* were seen, it was sub-cultured with antibiotic impregnated discs of penicillin, erythromycin, ciprofloxacin and clindamycin and sensitivity was read after 24 hours of incubation.

Results

The age distribution ranged from $2^{1}/_{2}$ years to 51 years, with mean age of 26 years.

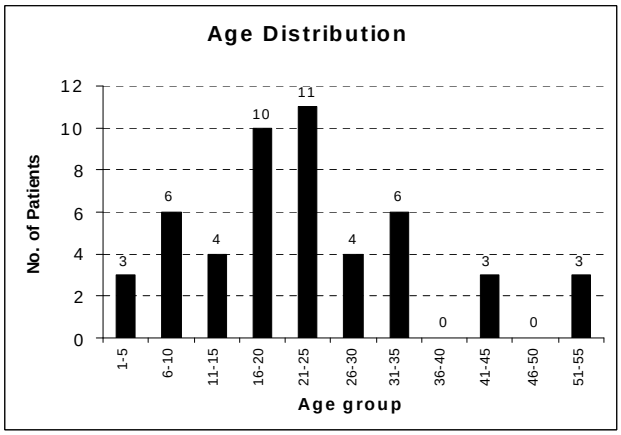

Fig 1 Age distribution

In the age group of 2-10 years *Streptococcus viridans* was the most common pathogen infecting the tonsils. This was followed by *Streptococcus pneumoniae, Brahmnella Catarrhalis* and *Staphylococcus aureus.* In the age group 11-20, *Staphylococcus aureus* was the most common pathogen isolated which was followed by *Streptococcus viridans* and *Streptococcus pneumoniae.* In the age group more than 20 years, the most common pathogen was *Streptococcus viridans* followed by *Staphylococcus aureus*. Beside these, *Klebsiella pneumoniae* and *Haemophilus influenza were* more significantly seen in >20 age group than others *(Fig:2).*

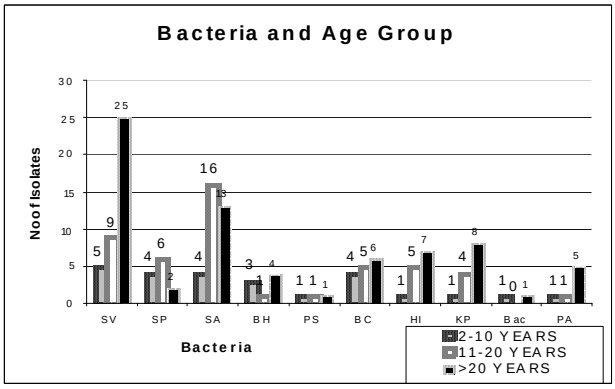

Fig. 2 Bacteria and Age Group.

Where SV is *Streptococcus viridans,* SP is *Streptococcus pneumoniae*, SA is *Staphylococcus aureus,* BH is *Beta-haemolytic streptococcus*, PS is *Peptostreptococci*, BC is *Brahmnella catarrhalis,* HI is *Hemophilus influenzae*, KP is *Klebsiella pneumoniae,* Bac is Bacteroids, and PA is *Pseudomonus aeruginosa*.

Gender Ratio:

In group A (study group) i.e. patients who had undergone tonsillectomy for recurrent acute tonsillitis, there were 23 males and 16 females. Whereas in group B or the control group, there were 5 males and 6 females

Results of culture in Group A: Study group

	SA	SV	BC	SP	KP	PA	BH	HI	PS	Bac
Surface	14	16	11	5	3	4	3	4	0	0
Core	13	15	6	3	7	4	3	5	2	2
P value	0.762	0.927	0.033	0.411	0.376	1.00	0.743	0.556	0.117	0.241

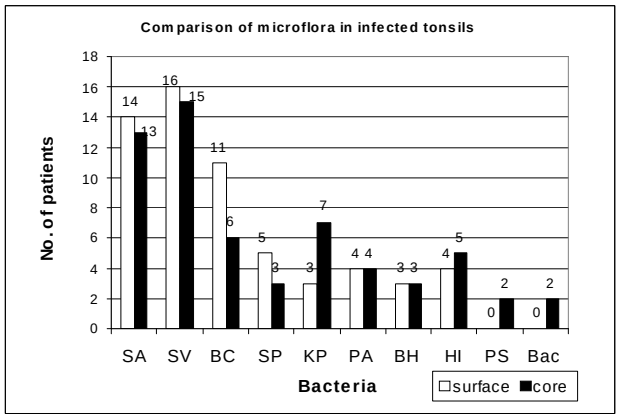

Fig 3 Comparison of microflora in infected tonsils.

Results of culture in Group B: Control Group

SA	SV	BC	SP	KP	PA	BH	HI	PS	Bac

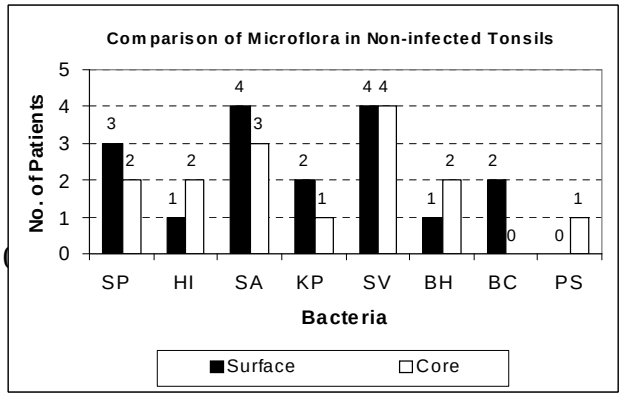

Fig 4 Comparison of microflora in non-infected Tonsils

Surface	4	4	2	3	2	0	1	1	0	0
Core	3	4	0	2	1	0	2	2	1	0

Group A *Beta-hamolytic Streptococci* (BH*)* was identified in total of 9 isolates (6 in Group A and 3 in Group B) and all of them were sensitive to Penicillin, Erythromycin, Ciprofloxacin and Clindamycin. Only Beta-haemolytic Streptococci were taken for culture and sensitivity because they were thought to be the most common microorganisms affecting the tonsils in recurrent tonsilitis in the past.

Comparison of core and surface cultures

In Group A (study group) the most common organism from the core and surface was *Streptococcus viridans* (SV) followed by *Staphylococcus aureus.* In Group B (control group), the most common organism from the surface were *Streptococcus viridans* and *Staphylococcus aureus* (SA) and from the core were *Streptococcus viridans.* . Species like P*eptostreptococci* and B*acteroides* were seen only in the core of both infected tonsils and non-infected tonsils. *Pseudomonus aeruginosa* (PA) was isolated from the surface and core of infected tonsils only. Same organisms were found in the surface and core in case of recurrent acute tonsillitis in 54.5% of isolates and different organisms in 27% of isolates. In 18.4% of isolates partially same organisms from core and surface were isolated.

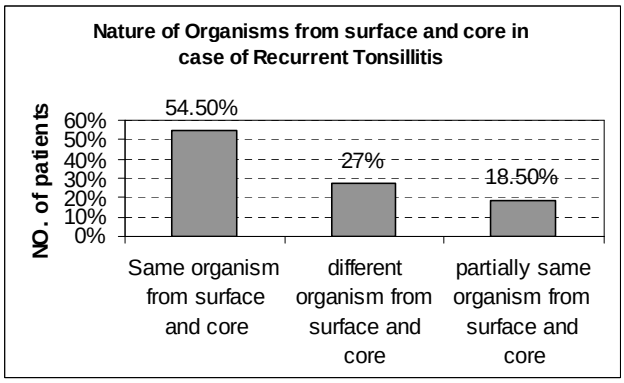

Fig 5: Nature of organisms from Core and Surface in Case of Recurrent Tonsillitis.

Likewise same organisms were found in surface and core of non-infected tonsil in 56% of cases and in 36% of cases different organisms were form the surface and core. Partially same organisms were found from the surface and core in 8% of non-infected tonsils.

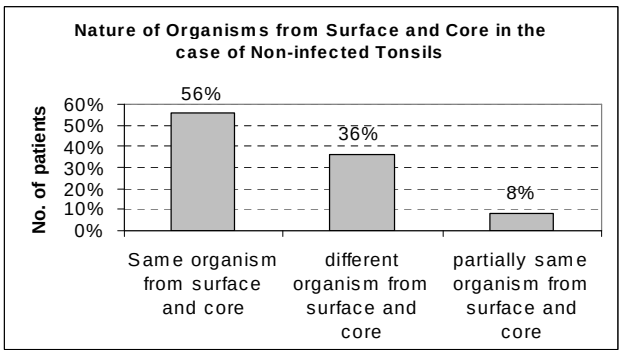

Fig 6: Nature of organisms from core and surface of Non-infected tonsils

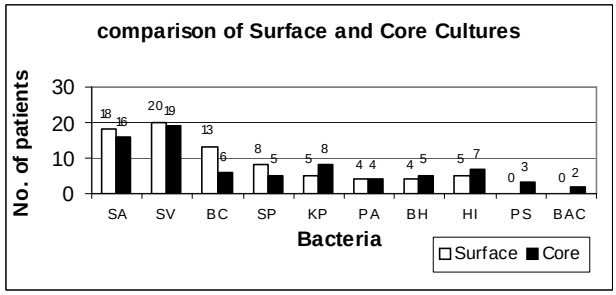

Fig 7: Comparison of Surface and Core Culture

In this study, the comparison between the common organisms in the surface and the core showed not much of difference in which *Streptococcus viridans* (SV) was found in the surface in 20 isolates out of 77, i.e. 30% and *Staphylococcus aureus* (SA) in 18 isolates among 77 i.e. 23%. *Brahmnella catarrhalis* (BC) was significantly more in the surface than the core i.e, 12 out of 77 isolates i.e. 17%. In the core, *Streptococcus viridans* was seen to be in 19 isolates out of 75 i.e. 30% and *Staphylococcus aureus* in 16 out of 75 i.e. 21%. Likewise *Klebsiella pneumoniae* (KP) and *Haemophilus influenzae* (HI) were significantly more in the core culture. *Klebsiella pneumoniae* in 8 out of 75 i.e. 10% and *H. influenzae* in 7 out of 75 i.e. 9.3%. In 4 isolates from the core, and 3 isolates from surface *Beta-haemolytic streptococci* were isolated, all sensitive to penicillin, ciprofloxacin, erythromycin and clindamycin. *Peptostreptococci* and *Bacteroids* (Bac) were found only in the core. All Group A B-haemolytic species were sensitive to the tested antibiotics.

Discussion

In this study it has been seen that the age of the patients undergoing tonsillectomy has shifted from the 1st decade of their life to the 2nd and 3rd decade of their life. This is more likely because T.U.T.H. entraps more of adult population and children usually visit children's hospital located near by, thus eluding our attention. Other probability is that the use of antibiotics whenever there is an attack of acute tonsillitis, may have postponed the procedure of tonsillectomy. Similar age group distribution is seen in the study by Attallah in which age range was 1-47 years with the mean age of 23 years[4].

When the study group was divided into three parts-2-10 years, 11-20 years, and 21 years and above, it was found that the most common organism affecting these younger groups were *Streptococcus viridians*, *Streptococcus pneumoniae* and *Staphylococcus aureus.* Whereas in 11-20 years groups, the most common organisms were *Staphylococcus aureus*, followed by *Streptococcus viridians and* then *Streptococcus pneumoniae*. In the age group above 21 years of age, *Streptococcus viridans* was

the most common followed by St*aphylococcus aureus* and then *Klebsiella pneumoniae.*

Gaffney *et al* have done a similar study consisting of 262 patients and divided into 3 groups 2-7 years, 8-14 years, and more than 15 years of age. Here mixed microflora was seen in all the groups like this study and *H. influenzae* and *Staphylococcus aureus* were the most common in the 2-7 years and 8-14 years group respectively. In this study also *Staphylococcus aureus* was the commonest in 10-20 age groups. *Beta-hemolytic Streptococci* was found to be more common in younger age group whereas anaerobic species were more in the older age groups. In this study, *Bacteroids* and *Peptostreptococci* were found only above 20 years age group. The findings were almost similar as this study [5].

In the infecting organisms cultured from the core and surface of the non-infected and the infected tonsils taken from the core and the surface, there was no significant difference seen. In this study, *Streptococcus viridans* (28%) and *Staphylococcus aureus* (25%) were isolated from the surface of infected as well as non-infected tonsils and *Streptococcus viridans* (25%) and *Staphylococcus aureus* (25%) were isolated from the core of infected as well as in non-infected tonsils. There was no significant difference seen in the infecting organisms cultured from the core and the surface of non-infected and infected tonsils. *Bacteria* and *Peptostreptococci* like anaerobic species were isolated from the core of both Types of tonsils. Similar study by Stjernquist-Desatnik, *et. al* showed that among his 34 patients who had undergone tonsillectomy for recurrent tonsillitis (n=17) sleep apnoea syndrome (n=17), the growth of aerobic, anaerobic and facultative organisms, in both groups did not differ significantly in the mean number of isolates per patients, either of aerobic species (3.8 vs. 4.3) or anaerobic species (5.2 vs. 4.7). Nor did the two subgroups differ significantly in the proportion of patients whose specimens manifested Beta-lactamase producers (71% vs. 59%) [6].

Regarding the comparison of the microflora in the surface and core of infected and non-infected tonsils, this study revealed that the most common microorganism infecting the surface of the tonsil was alpha-haemolytic streptococci (*Streptococcus viridans* in both groups was followed by *Staphylococcus aureus* i.e. 23% of the total isolates. The third common was *Brahmnella catarrhalis* 17% of the isolates from the total isolates. In the core specimen the most common microorganism was again *Streptococcus viridans* i.e. 29% of the core isolates, followed by *Staphylococcus aureus* in 21% of the total isolates. This was followed by *Klebsiella pneumoniae* in10% of the total isolates, which was followed by *Haemophilus influenzae* in 8% of the total isolates. *Streptococcus viridans* did not show significant difference between the surface and core cultures [with p=0.9279]. *Staphylococcus aureus* showed p value of 0.7642. *Streptococcus pneumonie* showed p value of 0.4119. Brahmnella catarrhalis was significantly more (p=0.0338) on the surface than core of total isolates. *Klebsiella pneumonie*, *beta-haemolytic streptococci* and *Haemophilus influenzae* were found more in the core isolates than the surface but was not statistically significant (with a p value of 0.03576, 0.7438, 0.5562 respectively). Likewise the anaerobic organisms like *Peptostreptococci* and *Bacteroid* species were only isolated from the core cultures. In a study by Mitchelmore *et. al* swabs from surface and core of 50 patients who had undergone tonsillectomy, were taken. In this study he found out that the total number of isolates from surface and core samples were similar (9.2 and 8.8) like in this study (with 8 and 10). The range of species isolated was also similar for both surface and core samples, as was the proportion of organisms producing Beta-lactamase from each site (10.7% and 29.5%) respectively[7].

Another study by Hascilik *et. al* (1991) where 52 tonsillectomy cases were studied under aerobic and anaerobic condition. Group A beta-haemolytic Streptococci was the most common and isolated from the core more than the surface. In this study also the beta-haemolytic was isolated more from the core[8]. Similar study by Tekerlan*. et. al*, showed that the predominant pathogen isolated in the patient were: beta-haemolytic streptococci and *S. aureus*. The surface and the core cultures yielded the same pathogenic microorganisms in 36% of the cases in the patient group. Statistically there was no significant difference between the patients and the control group[9].

Likewise study by Brook *et. al*, had taken specimen from both the surface and the core of tonsils from 23 children with recurrent tonsillitis and cultured for aerobic and anaerobic microorganisms. Mixed aerobic and anaerobic flora was obtained from all patients. The predominant anaerobic isolates were *Bacteroides species*, *Fusobacterium nucleatum*, anaerobic Gram positive cocci, and Eubacterium species. The predominant aerobic ioslates were alpha-haemolytic streptococci, Staphylococcus aureus, beta-haemolytic streptococci, *Haemophilus influenzae*. 70% of the aerobic isolates were recovered only in the core, compared to 57% of the anaerobic isolates. Ten percent of aerobes were recovered only in the core, compared to 33% of anaerobes, and 20% of the aerobes in the microflora only, compared with 10% of anaerobes. These data demonstrates the discrepancies between surface and core cultures in the isolation of anaerobic bacteria, and also raises the question whether surface cultures can accurately predict the presence of Beta-lactamase producing organisms in recurrent acute tonsillitis[2].

The above study coincides with this study in the following aspects:

Commonest aerobic organism found was alpha-haemolytic

streptococci followed by *Staphylococcus aureus* and *Group A beta-haemolytic streptococci* and *Haemophilus influenzae*. Among the anaerobic organisms, the commonest was *Bacteroides* and *Peptostreptococci* were the next common. Beta-haemolytic streptococci was isolated more from the core of total isolates and anaerobic organism was isolated from the core only. About the drug sensitivity, only *beta-haemolytic Streptococci* were studied and all were sensitive to Penicillin, Erythromycin, Clindamycin and Ciprofloxacin signifying no Beta-lactamase producers in the cultured strain

Conclusion

The conclusions drawn from this study were that the most common microflora seen on the surface of recurrent tonsillitis was *Streptococcus viridans* (30%) followed by *Staphylococcus aureus* and *Brahmnella catarrhalis*. Common organisms from the core in recurrent tonsillitis were *Staphylococcus aureus* followed by *Klebseilla pneumonae* and *Haemophilus influenzae* .The microorganisms commonly found in the surface and core of non-infected tonsils were *Streptococcus viridans* followed by *Staphylococcus aureus*. There were no statistical difference between the microorganisms found on the surface and the core of infected and non-infected tonsils with no significant P value except for *Brahmnella catarrhalis.* This was significantly more on the surface of infected and non-infected tonsils. There were no statistical difference between the microorganisms found in the infected and the non infected tonsils. Beta-haemolytic organisms were found more in children and were more in the core, though statistically not significant. *Klebsiella pneumonie* and *Haemophylus influenzae* were found more in the core and more in adults, but it was not statistically significant. All the strains of *Group A beta haemolytic streptocci* were sensitive to penicillin, amoxicillin, and climdamycin so resistant strains have not fully developed in our context. *Pseudomonas aeruginosa* was the organism isolated both from the surface and core of infected tonsils but was not isolated from the non-infected tonsils. This study has been done during the remission phase of tonsillitis, so we cannot say for sure which organism is responsible for causing recurrent acute tonsillitis. In this study *Streptococcus viridans* has been seen to be the most common organism found on the surface and core of both the infected and non-infected tonsils. Thus we can presume that when the immune status of the patient becomes low, the same organisms can become virulent and cause the disease. Similar studies in the future are mandatory to compliment this study further.

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