

A STUDY TO FIND OUT THE BACTERIOLOGY OF TONSILLAR SURFACE AND CORE, AMONG PATIENTS UNDERGOING TONSILLECTOMY AT A TERTIARY CARE HOSPITAL IN SOUTH INDIA

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ABSTRACT

BACKGROUND

The study was aimed at finding out the bacteriology of the tonsillar surface and core, in those patients undergoing tonsillectomy at a tertiary care hospital. The study was also planned to look for the antibiotic resistance pattern among organisms identified and to see whether a tonsillar surface swab can be used as a surrogate for finding out the aetiopathogenesis of tonsillar infections.

METHODS

This is a 2-year cross-sectional study of 100 patients undergoing who consented for the study, after taking a smear from the surface of the tonsil. Tonsillectomy was done by dissection and snare method. The core of the tonsil is then biopsied and microbiological culture and sensitivity is done. The bacteria were identified by morphology and staining characteristics and cultured using standard laboratory procedures. Antimicrobial susceptibility was done by Kirby-Bauer disc diffusion method. The data was digitalised using a data entry platform using Epidata and analysed using SPSS 16. Relativity between organism identified as the surface and core was tested using Chi-square tests and Kappa statistics.

RESULTS

There were significant differences between the organisms identified from the tonsillar surface and the core; out of the 200 specimens, only 48 showed a similar bacteriology. The viability between the surface and the core specimens were measured using Kappa statistics and was found to be 0.04, which shows a very poor agreement between them.

Assessment of antibiotic sensitivity shows that Staph aureus was resistant to most of the commonly used antibiotics. H. influenza isolated showed 70% susceptibility to erythromycin and cotrimoxazole. While group A β haemolytic streptococci showed 95% susceptibility to ampicillin and 100% to erythromycin.

CONCLUSION

Staph. aureus, H. influenza, Streptococci pneumonia were the major pathogenesis identified from the tonsil of the patients undergoing tonsillectomy, and bacteriology was different between the tonsillar surface and the core. Also the staphylococcal showed high level of resistance towards first line antibiotics.

KEYWORDS

Tonsillar Surface and Core, Tonsillectomy, Bacteria, Antibiotic Resistance.

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INTRODUCTION: Tonsillectomy is one of the most commonly performed surgeries in the world, and studies suggest that the rates of surgery has increased since the 1970s.⁽¹⁾ Data from United States of America show that the rate of tonsillectomy increased from 126 per 100,000 person years in 1970 to 153 per 100,000 person years in 2005. The rate of adenotonsillectomy showed an even dramatic increase from 243 per 100,000 person years in 1970 to 485

per 100,000 person years in 2005.⁽¹⁾ The indications for tonsillectomy has also undergone a change in recent years.

Among the paediatric age group, upper airway obstruction has supplanted infection as the most common primary indication for surgical intervention. But in older age groups, recurrent tonsillar infection is still the prime reason for patients to undergo a tonsillectomy.⁽²⁾ But some other studies have shown a progressive decrease in the number of tonsillectomies and adenotonsillectomies since the early eighties. This fall in numbers is more in case of adenoidectomies and adenotonsillectomies when compared to tonsillectomies alone.⁽³⁾ This may be partially due to mounting evidence which shows only a marginal benefit for those undergoing these procedures. A Cochrane review showed that the average incidence of sore throat in those who underwent these surgical procedures were 3 in the six

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months following surgery compared to 3.6 in the control group. Also, the children who underwent surgery has 18 days of sore throat in one year after the intervention compared to 23 days in the control group.

The review concludes that the potential benefit from the surgery should be rigorously analysed against the risk of morbidity due to the surgical intervention.⁽⁴⁾

The bacterial aetiology of recurrent tonsillar infection is well documented. Studies have demonstrated that a wide array of organisms are involved in aetiopathogenesis of recurrent tonsillitis, with *Haemophilus influenzae*, *Staphylococcus aureus* and *Streptococcus pyogenes* dominating the list.⁽⁵⁾ Other studies have demonstrated that swabs taken from tonsillar surface of those suffering from recurrent tonsillitis, mainly yielded normal bacterial flora while the core showed considerable presence of pathologic organisms. Again, *Haemophilus influenzae* and *Staphylococcus aureus* were the predominant aetiological agents found in the core tissue of tonsils.⁽⁶⁾ Another study, reported from South Korea tried to differentiate between the bacterial aetiology of recurrent tonsillitis and tonsillar hypertrophy. It was found that *Staphylococcus aureus* was the predominant organism in recurrent tonsillitis and *Haemophilus influenzae* was the main culprit in cases of tonsillar hypertrophy. Also, it was found that *Streptococcus pneumoniae* was relatively common in children, while *Klebsiella pneumoniae* was commoner in adults. It was reported that penicillin resistance is common among the identified organisms while susceptibility to third generation cephalosporins were almost universal.⁽⁷⁾

The present study was aimed at finding out the bacterial aetiology of tonsillar problems in those patients undergoing a tonsillectomy procedure at a tertiary care hospital in south India. The study was also planned to look for the antibiotic resistance pattern among the organisms identified during the study, and to see whether a tonsillar surface swab can be used as a surrogate for finding out the aetiopathogenesis of tonsillar infections.

MATERIALS AND METHODS: A cross-sectional study was carried out in the Department of Otorhinolaryngology, Pushpagiri Medical College, Thiruvalla during the period of September 2010 to August 2012. A total of 100 patients undergoing tonsillectomy or adenotonsillectomy for any indication were included in the study, through systematic random sampling. Those patients with history of antibiotic intake in the preceding week before surgery is excluded from the study. Written informed consent was taken from all participants or their immediate caregivers, whichever was applicable.

The recruitment was done one day before the surgery and all participants were administered a standard and structured questionnaire, which collected socio-demographic and clinical data of the participants. All patients underwent tonsillectomy by dissection and snare method. Once the patient was anaesthetised, mouth is opened with Boyle-Davis mouth gag and fixed with Draffin's bipods. Using a sterile cotton swab, a smear from the surface of the tonsil was obtained by the principal investigator. Following this, the

tonsillectomy procedure was carried out by the respective surgeon. The dissected tonsil tissue was dipped in povidone iodine solution for 30 to 45 seconds, after which it was rinsed with sterile saline solution and sectioned into two pieces. The core of the tonsil was then biopsied, and this tissue was collected in a sterile glass container and sent immediately to the Microbiology laboratory for culture and sensitivity testing. The transportation process was completed under one hour in all cases to ensure that microbiological yield is good and contamination does not occur.

In Microbiology lab of the institution, a smear was made on a glass slide and Gram staining was done immediately. Aerobic cultures of the specimens were done after plating them on Blood agar, Nutrient agar and MacConkey agar. All plates were incubated aerobically at 37°C and evaluated at 24 hours, 48 hours and 72 hours and the plates were discarded if there was no growth. The specific identification of bacterial pathogens was done based on microscopic morphology, staining characteristics, cultural and biochemical properties using standard laboratory procedures. When multiple organisms were obtained, the predominant organism among them was noted. Antimicrobial susceptibility of the bacterial isolates to commonly used antibiotics was done by Kirby-Bauer disc diffusion method.

The data was digitalised using a data entry platform created using Epidata, and was analysed using SPSS 16.0. The baseline socio-demographic and clinical correlates were tabulated and expressed as numbers and percentages. The bacteriology of the tonsillar surface and core was also found out to be expressed as frequencies. Relationship between the organism identified on the surface and that from the core, was tested using Chi-square tests and Kappa statistics. The antibiotic sensitivity of the organism to commonly used drugs were assessed.

RESULTS: A total of 100 potential participants were approached for the study, and none of them denied consent. Majority (50%) of the participants were from 11 to 20 age category, and only 25% were aged 21 years or above. The sex ratio among the participants were fairly equal, with 46% being males and 54% females. Recurrent tonsillitis was the predominant indication for surgery, followed by obstructive symptoms like dysphagia or excessive snoring. [Table 1]

Characteristic	Frequency	Percentage
Age		
4 to 10 years	25	25%
11 to 20 years	50	50%
21 years and above	25	25%
Sex		
Male	46	46%
Female	54	54%
Indications for surgery		
Recurrent tonsillitis	72	72%
Obstructive symptoms	20	20%
Recurrent tonsillitis and obstruction	8	8%

Table 1: Baseline Clinico-demographic Characteristics

The specimens were plated on agar plates and observed for growth at the end of 24, 48 and 72 hours. Among surface swabs, 28% grew *Staphylococcus aureus*, 12% grew *Haemophilus influenzae*, 10% had various species of Group A beta haemolytic streptococci and 9% had species of *Klebsiella*. Among the specimen from tonsillar core, 34% of the samples grew *Staphylococcus aureus*, 14% grew *Haemophilus influenzae*, 12% grew species of Group A beta haemolytic streptococci and 6% had *Klebsiella*. Interestingly, 23% of surface and core samples did not show any bacteriological growth. [Table 2]

Organism	Surface (Number and Percentage)	Core (Number and Percentage)
<i>S. aureus</i>	28(28%)	34(34%)
<i>H. influenza</i>	12(12%)	14(14%)
<i>Streptococcus</i> spp.	10(10%)	12(12%)
<i>Klebsiella</i> spp.	9(9%)	6(6%)
<i>B. catarrhalis</i>	5(5%)	5(5%)
<i>Pseudomonas</i> spp.	2(2%)	3(3%)
<i>S. epidermidis</i>	8(8%)	3(3%)
<i>Enterococcus</i> spp.	3(3%)	0(0%)
No growth	23(23%)	23(23%)
Table 2: Bacteriology of Tonsillar Surface and Core		

When antibiotic sensitivity of the samples were assessed, it was found that the *Staphylococcus aureus* isolates were resistant to most of the commonly used antibiotics. Only around 10% were susceptible to Ampicillin, around 20% to Erythromycin and almost 30% to Cotrimoxazole. Among the *Haemophilus influenzae* isolates, all were resistant to Ampicillin but more than 70% were susceptible to Erythromycin and Cotrimoxazole. Among the various species of Group A beta haemolytic streptococci, the antibiotic susceptibility levels to common antibiotics were quite high with 95 % of the samples being susceptible to Ampicillin and 100% to Erythromycin. An interesting aspect of the study was that 80% of the isolates of *Pseudomonas* were resistant to a third generation cephalosporin such as Cefotaxime, which shows a high possibility of Extended Spectrum Beta Lactamase (ESBL). [Table 3]

Organism and susceptibility	Ampicillin	Cotrimoxazole	Erythromycin	Tetracycline	Gentamycin	Ofloxacin	Cefotaxime
<i>B. catarrhalis</i> (8)	8(100%)	8(100%)	8(100%)	8(100%)	8(100%)	8(100%)	8(100%)
<i>Streptococcus</i> spp.(22)	21(95.45%)	17(77.27%)	22(100%)	22(100%)	22(100%)	22(100%)	22(100%)
<i>H. influenzae</i> (20)	0(0%)	14(70%)	15(75%)	4(20%)	20(100%)	20(100%)	20(100%)
<i>Enterococci</i> spp.(3)	3(100%)	2(66.66%)	3(100%)	3(100%)	3(100%)	2(66.66%)	3(100%)
<i>Pseudomonas</i> spp.(5)	0(0%)	0(0%)	0(0%)	0(0%)	3(60%)	2(40%)	1(20%)
<i>Klebsiella</i> spp.(13)	1(7.69%)	3(23.07%)	2(15.38%)	2(15.38%)	12(92.30%)	13(100%)	13(100%)
<i>S. epidermidis</i> (10)	4(40%)	2(20%)	7(70%)	1(10%)	10(100%)	10(100%)	10(100%)
<i>S. aureus</i> (42)	4(9.52%)	12(28.57%)	9(21.42%)	9(21.42%)	31(73.80%)	42(100%)	30(71.42%)
Table 3: Antibiotic Sensitivity Pattern of the Identified Organisms							

There were significant differences between the organisms identified from the tonsillar surface and the core. Out of the total of 200 specimens, only 48 specimens showed growth of the same organism in surface and core samples. The variability between the surface and core specimens was measured using Kappa statistics and it was found to be 0.04, which shows very poor agreement between them. [Table 4]

Organism		Pathological bacteria cultured from core		
		Present	Absent	Total
Pathological organism cultured from surface	Present	48	52	100
	Absent	51	49	100
	Total	99	101	200
Table 4: Difference in Organisms Identified from Tonsillar Surface and Core				

DISCUSSION: Majority of the study participants were aged between 11 to 20 years, which is different from statistics from other studies which shows a predominance of children.⁽⁸⁾ Tonsillectomies in children are progressively coming down throughout the world, and this may be a reason for lower number of children undergoing surgical intervention for tonsil problems. The major indications for tonsillectomy were recurrent tonsillitis and signs of upper airway obstruction, and is similar to other studies from across the world.⁽²⁾

Staphylococcus aureus, *Haemophilus influenzae* and *Streptococcus pneumoniae* were the predominant species identified from surface and core specimens. A significant number of *Klebsiella pneumoniae* were also cultured from the samples. This bacteriological profile conforms to findings from across the world, which blames these organisms for tonsillar infections.^(6,7) Almost quarter of the specimens from the tonsillar surface and core did not yield any organism, and this may be partially attributed to the fact that anaerobic culture was not done due to lack of facilities at the institution concerned.⁽⁹⁾

Almost 12% of the surface samples and 3% of the core samples yielded commensal organisms, which may not have been a part of the aetiological process.

The *Staphylococcus aureus* samples showed considerable resistance to first line antibiotics, only around 10% were susceptible to Ampicillin, 21 % to Erythromycin and 28% to Cotrimoxazole. In contrast, the Streptococcal isolates were much more susceptible to antimicrobial therapy with 95% being susceptible to Ampicillin, 100% to Erythromycin and 77% to Cotrimoxazole. All the samples of *Haemophilus influenzae* were susceptible to Gentamycin and third generation cephalosporins. Other studies have also found similar results with Staphylococcal specimens showing high levels of anti-microbial resistance, and Streptococcal species being relatively susceptible to first line antibiotics.⁽¹⁰⁾ Another interesting aspect of drug susceptibility testing was that 80% of the *Pseudomonas* samples were resistant to third generation cephalosporins, indicating a possible presence of Extended Spectrum Beta Lactamase (ESBL). Phenotyping and Polymerase Chain Reaction (PCR) would have been the obvious choice of looking for ESBL, but it was not done as institutional facilities were not available. But as seen in other studies, ESBL producing Gram-negative bacteria are being increasingly isolated from respiratory tract of individuals suffering from infective pathology.⁽¹¹⁾ Also, as proved in studies of similar nature, it was found that the bacteriological profile obtained from surface swabs is vastly different from that obtained through culture of tonsillar core specimens.⁽¹²⁾

CONCLUSION: In conclusion, *Staphylococcus aureus*, *Haemophilus influenzae* and *Streptococcus pneumoniae* were the major pathogens identified from the tonsils of those patients undergoing tonsillectomy, and the bacteriological profile was different between tonsillar surface and core. Also, the Staphylococcal isolates showed high level of resistance towards first line antibiotics.

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