

Full Length Research Paper

Bacterial identification and antibiotic susceptibility patterns of *Staphylococcus aureus* isolates from patients undergoing tonsillectomy in Malaysian University Hospital

Saad Alasil¹, Rahmat Omar^{1*}, Salmah Ismail², Mohd Yasim Yusof³ and Mahmood Ameen²

¹Department of Otorhinolaryngology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

²Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

³Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

Accepted 21 October, 2019

Diagnosis and treatment of chronic tonsillitis (CT) and recurrent tonsillitis (RT) are common problems seen in any otolaryngology clinic. Antibiotic therapy usually fails to prevent the recurrences of these infections leaving the patient with no choice but surgery (tonsillectomy). This study aims to identify the bacteriology of both tonsillar swabs and biopsies specimens among children and adult patients diagnosed with CT and RT (infected tonsils). In addition to obstructive sleep apnea (OSA) and tonsillar hypertrophy (TH) (non-infected tonsils) all undergoing elective tonsillectomy and to characterize the antibiotic susceptibility patterns of the most common isolate. A total number of 138 surgically removed palatine tonsils were collected from 72 patients. Six types of antibiotics were selected to test the susceptibility of *Staphylococcus aureus* isolates which are methicillin, gentamicin, erythromycin, co-trimoxazole, clindamycin and fusidic acid. The most common isolate was *S. aureus* (190 isolates) followed by *Haemophilus influenza* (83), *Streptococcus* Group B (61), *Haemophilus parainfluenza* (33), *Klebsiella pneumoniae* (32), *Streptococcus* Group G (29), *Streptococcus* Group F (14), *Streptococcus* Group C (12), *Pseudomonas aeruginosa* (10) and *Streptococcus* Group A (9) and *Streptococcus* Group A (9). The number of *S. aureus* isolates in infected tonsils (RT and CT) was 145 (76.3%) while in non-infected tonsils (OSA and TH) was 45 (23.6%). Three susceptibility patterns were found among *S. aureus* isolates in all cases, (89.4%) susceptible to all the selected antibiotics, (10.6%) resistant to fusidic acid only, whereas 0.5% resistant to both methicillin and fusidic acid. There was a 30.5% difference in the type of bacterial isolates between tonsillar swab and biopsies. The high prevalence of *S. aureus* in patients with both infected and non-infected tonsils suggests that this bacterium might not be the aetiological agent of chronic and recurrent tonsillitis. In addition, the high susceptibility rate of methicillin among *S. aureus* isolates could be due to the fact that *S. aureus* represents part of the oropharynx normal flora. The increased rate of resistance 10.6% to fusidic acid among methicillin-susceptible *S. aureus* (MSSA) isolates makes this antibiotic a less potential drug of choice for patients with chronic and recurrent tonsillitis.

Key words: Antibiotic susceptibility, *Staphylococcus aureus*, tonsillectomy, swab and biopsy.

INTRODUCTION

The diagnosis and treatment of chronic and recurrent tonsillitis has been a common problem to otolaryngologist

(Logantham et al., 2006). In the past, chronic tonsillitis was largely a clinical concept but today bacteriological and patho-anatomical considerations are getting more attention (Upal and Bais, 1989). Despite the fact that tonsillitis is frequent, a general agreement to identify the main causative microorganisms and their differences

*Corresponding author. E-mail: o_rahmat@hotmail.com.

between children and adults is still lacking (Logantham et al., 2006). It has been reported that bacteria causing tonsillitis are present in both tonsillar surface and deep tissue.

However, the isolates taken from surface may not always reflect the real pathogens (Inci et al., 2002). *Staphylococcus aureus* is among the leading Gram positive bacteria that can be found in the normal flora of oropharynx and nose (Shanmugam et al., 2008). In recurrent tonsillitis (RT) the tonsillar core contains a large number of bacteria including *S. aureus*, *Haemophilus influenza* and *Streptococcus pyogenes* in which there is a strong correlation between the type of isolate and the tonsillar infection (Lindroos, 2000).

Antibiotic therapy usually fails to prevent the recurrence of chronic tonsillitis (CT) and recurrent tonsillitis (RT) because inappropriate usage against the pathogen in deep tonsillar tissue leads to the continuation of infection and re-inoculation causing recurrence leaving the patient with no choice but surgery (Mostafa et al., 2009).

Even though clinical cases with infected tonsils like RT and CT are different with non-infected tonsils like obstructive sleep apnea (OSA) and tonsillar hypertrophy (TH) in terms of the course of disease and symptoms, they share a common aspect of surgery (tonsillectomy) as a treatment of choice.

The bacteriology of tonsils among RT, CT and TH patients has been previously reported by Andreas et al. (2010); Kurien et al. (2000) and Lindroos (2000), respectively. In our study we added the clinical case of OSA in order to identify the bacteriology of tonsillar swabs and biopsies and to characterize the antibiotic susceptibility patterns of the most common isolate among patients undergoing elective tonsillectomy at University of Malaya Medical Center (UMMC). Determining the bacteriology of tonsillar biopsy (core) is important for several reasons because failure to identify and eradicate pathogens in the core will allow persistence of core infection or re-inoculation of initially sterilized surface.

MATERIALS AND METHODS

Patient selection

This is a cross sectional study performed on 72 patients diagnosed with four types of clinical cases undergoing elective tonsillectomy. Infected tonsils were collected from RT and CT cases while non-infected tonsils from OSA and TH for a period of 9 months (October 2009 to June 2010) at the University of Malaya Medical Center (UMMC) situated in Kuala Lumpur, Malaysia. Upon approval from UMMC Medical Ethics Committee (PPUM/UPP/300/02/02Ref. No. 744.11), a written consent was taken from all the selected patients. The age of patients to be considered as adults was more than 12 years. Inclusion criteria included three or more severe recurrent attacks of tonsillitis in two consecutive years (Loganathan et al., 2006). Patients are considered to have severe illness if at least three associated symptoms, that is high fever, snoring during acute attacks, unable to take normal diet, absence from school and work, and admission to hospital, are present. However, unilateral enlargement, pregnancy and immune compromised patients were

considered as exclusion criteria (Loganathan et al., 2006).

Specimen collection

The selected patients undergoing general anesthesia were followed by retraction of the uvula and soft palate (Figure 1). Swabs were taken from the surface of palatine tonsils with a sterile cotton-tipped applicator then placed in a transport medium. Tonsillectomy was performed and biopsies were collected and rinsed with phosphate buffered saline. Biopsy specimens were cut into appropriate size using surgical scalpel before being kept in a sterile labeled container in order to be processed. All specimens were sent immediately to the microbiology unit of Clinical Diagnostic Laboratory (CDL) at UMMC.

Bacterial identification

Biopsies were placed in a sterile Petri dish and minced until complete homogenization with nutrient broth before being cultured onto blood agar (BA), chocolate agar (CA), MacConkey's agar (Mac), fastidious anaerobic agar (FAA) and thioglycollate broth. All the plates were incubated for 24 to 48 h according to the type of media used. For *S. aureus*, isolates were Gram stained and further characterized using coagulase and DNase tests. Non *S. aureus* isolates including *Streptococcus* species were identified via Optochin test, bile solubility test and bacitracin tests; Gram negative bacilli via kligler's iron agar, indole, citrate, malonate utilization, urease, oxidase, methyl red (MR) and voges-proskauer (VP) tests; and *Haemophilus* species via XV factor test.

Disk diffusion test

Susceptibility of *S. aureus* isolates was determined by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Bacterial colonies from each isolate were transferred into a suspension medium adjusted to 0.5 McFarland turbidity standards (1.5×10^8 CFU/ml). Inoculum was swabbed on the entire surface of agar plates followed by the application of six selected commercially available antibiotic disks of methicillin (30 μ m), gentamicin (10 μ m), erythromycin (15 μ m), co-trimoxazole (1.25 + 23.75 μ m), clindamycin (30 μ m) and fusidic acid (10 μ m) using sterile forceps (5 disks per plate). Plates were inverted and incubated for 18 to 24 h at 37°C. The antibiotic vancomycin was not included in the susceptibility testing of methicillin-resistant *S. aureus* (MRSA) isolates. Zones of inhibition were determined according to the standards outlined by the Clinical and Laboratory Standards Institute (CLSI) (Cockerill et al., 2010).

Statistical analysis

Statistical significance differences of *S. aureus* susceptibility between age and gender groups were assessed using one-way ANOVA for which all values were reported as standard error mean (S.E.M) \pm . A probability value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Based on our results, 10 types of bacterial isolates were identified from 138 surgically removed tonsils of 72 patients. The age of patients ranged from 4 to 38 years with a mean of 14.8 years. Adult female patients

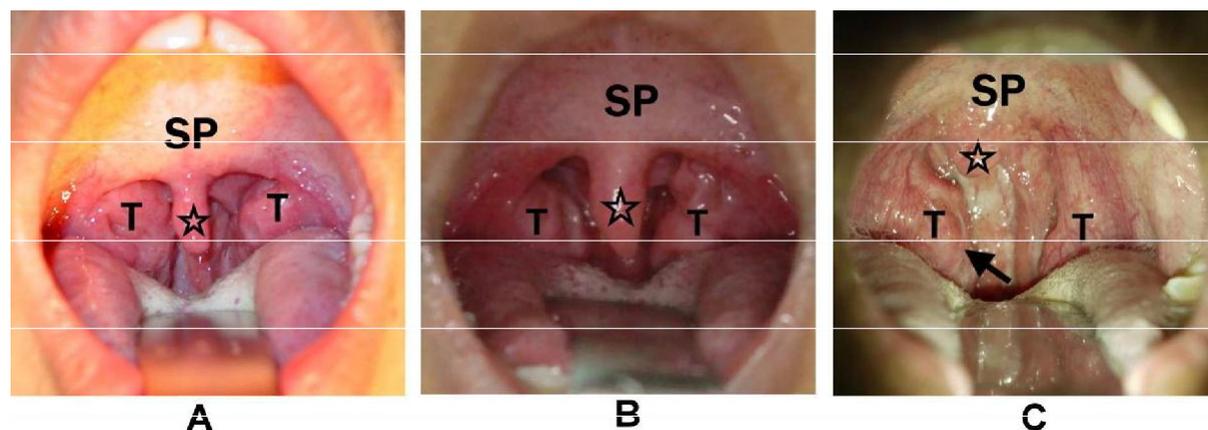


Figure 1. (A) Tonsillar enlargement due to recurrent tonsillitis. (B) Tonsillar enlargement in Obstructive Sleep Apnoea. (C) Unilateral/asymmetric tonsillar hypertrophy. (T= tonsil, SP- soft palate, star- uvula).

Table 1. Prevalence of clinical cases among patients.

Clinical diagnosis	Children		Adult		Total		All count (%)
	Male count (%)	Female count (%)	Male count (%)	Female count (%)	Male count (%)	Female count (%)	
Recurrent Tonsillitis (RT)	9 (12.5)	12 (16.6)	8 (11.1)	20 (27.7)	17 (23.6)	32 (44.3)	49 (68)
Chronic Tonsillitis (CT)	3 (5.5)	0 (0.0)	1 (1.3)	3 (4.05)	4 (6.8)	3 (4.05)	7 (9.8)
Obstructive Sleep Apnea (OSA)	4 (5.5)	2 (2.7)	0 (0.0)	4 (5.5)	4 (5.5)	6 (8.3)	10 (13.8)
Tonsillar Hypertrophy (TH)	2 (2.7)	2 (2.7)	1 (1.3)	1 (1.3)	3 (4.05)	3 (4.05)	6 (8.4)

were the most dominant in all clinical cases 28 (38.8%) followed by 19 children male patients (26.3%), 16 children female (22.2%) and 10 adult male (13.8%). Prevalence of clinical cases according to gender and age groups is shown in Table 1. *S. aureus* was the most common bacteria (190 isolates) in both infected and non-infected tonsils followed *H. influenza* (83), *Streptococcus* Group B (61), *H. parainfluenza* (33), *Klebsiella pneumoniae* (32), *Streptococcus* Group G (29),

Streptococcus Group F (14), *Streptococcus* Group C (12), *Pseudomonas aeruginosa* (10) and *Streptococcus* Group A (9). Among all 72 patients, only 14 did not show *S. aureus* isolates from their tonsillar specimens. The number of *S. aureus* isolates in infected tonsils (RT and CT) was 145 (76.3%) while in non-infected tonsils (OSA and TH) was 45 (23.6%).

Three susceptibility patterns were found among *S. aureus* isolates in all cases, (89.4%)

susceptible to all the selected antibiotics, (10.6%) resistant to fusidic acid only whereas 0.5% resistant to both methicillin and fusidic acid. Susceptibility of *S. aureus* isolates and their distribution are shown in Table 2. There was no significance in the difference of susceptibility between swab and biopsy specimens among adults 2.00 ± 0.23 , children 2.18 ± 0.98 , male 1.92 ± 0.916 and female 2.37 ± 0.74 in all the four clinical cases.

Table 2. Susceptibility patterns of *S. aureus* isolates and their distribution among age groups.

Susceptibility pattern	Children		Adult		Total		All count (%)
	Swab count (%)	Biopsy count (%)	Swab count (%)	Biopsy count (%)	Swab count (%)	Biopsy count (%)	
Susceptible	37 (19.4)	39 (20.5)	45 (23.6)	48 (25.2)	82 (43.1)	87 (45.7)	169 (89)
Resistant	4 (2.06)	8 (4.1)	5 (2.6)	4 (2.06)	9 (4.7)	12 (6.18)	21 (11)

There was a 30.5% difference in the type of bacterial isolates between tonsillar swab and biopsies.

DISCUSSION

The reason we have selected UMMC as a site for our study is because it represent a tertiary care hospital in addition to the fact that antibiotic resistance among bacteria varies between different geographic areas even within the same country (Lim, 2003). Donnelly et al. (1994) found that recurrent tonsillitis was the commonest indication for tonsillectomy. Same observation was found with our study. In a previous study, the most frequent isolate from tonsillar surface was found to be *Streptococcus pneumoniae* (66.6%) followed by group A β -hemolytic streptococci (62.5%), *S. aureus* (38.4%) and *H. influenzae* (27.2%) (Mustafa et al., 2007). In contrast, Gaffney et al. (1991) reported that *H. influenzae* was the most common isolate from tonsillar core followed by *S. aureus*. Kumar et al. (2005), reported *S. aureus* as the common isolate from both tonsillar surface and core followed by β -haemolytic streptococci. However, in our study we have showed that *S. aureus* was the most frequent isolate in both tonsillar swabs and biopsies among all the four clinical cases followed by *H. influenzae* then *Streptococcus* Group B with no significance between age and gender which

gives an indication that the type of tonsillar microorganisms may vary due to the antibiotic regiments that are being prescribed in that clinical setting. This highlights the importance of monitoring tonsillar bacteriology using reliable diagnostic techniques. Determining the bacteriology of tonsillar biopsy (core) is important because failure to identify and eradicate pathogens in the core, whether it is from inappropriate antibiotic choice or from insufficient penetration into the core, will allow persistence of core infection or re-inoculation of initially sterilized surface (Kurein et al., 2000).

A study by Brook and Foote in "Microbiology of normal tonsils" concluded that there is polymicrobial flora in normal and infected tonsils but their number and encapsulation is increased in inflammatory process (Brook and Foote 1990). Our study showed that *S. aureus* was found in 33.2% of children with CT and RT (infected tonsils) compared to 13.6% children with OSA and TH (non-infected tonsils). In contrast, *H. influenzae* was found in 32% of children with recurrent tonsillitis compared to 48% of children without tonsillar disease (Bista et al., 2006).

The correlation between chronic and recurrent tonsillitis and the presence of *S. aureus* in both children and adults was very strong yet the high prevalence of *S. aureus* in patients with both infected and non-infected tonsils suggests that this bacterium might not be the aetiological agent of chronic and recurrent tonsillitis.

The high susceptibility rate of methicillin among

S. aureus isolates could be due to that fact that *S. aureus* is part of the oropharynx normal flora. On the other hand it is emphasized that among any large population of *S. aureus* isolates, resistance to fusidic acid will occur (Mette et al., 1990) and development of fusidic acid resistance during therapy has been described especially when it was used systemically as a single agent (Pattison et al., 1973). Fusidic acid is recommended to be used as combination therapy with clindamycin, rifampicin or trimethoprim (Rohani et al., 2000). Its usage as combination therapy did not lead to the development of resistance (Rosdahl, 1988).

In Malaysian hospitals, the resistance rates for fusidic acid and rifampicin individually were reported to be between 3 to 5% in the years 1992 to 1996 as these antibiotics represent an alternative therapy to vancomycin in Malaysia (Norazah et al., 2002). Similarly in our study, an increased rate of resistance to fusidic acid was found to be 10.6% among methicillin-susceptible *S. aureus* (MSSA) isolates which suggests making it a less potential drug of choice for patients with chronic and recurrent tonsillitis. A similar finding was reported among MSSA isolates in Bristol with a resistance to fusidic acid increasing from 6% in 1998 to 11.5% in 2001 due to the frequent use of this antibiotic in topical applications (Brown and Thomas 2002). Educating the medical staff on the appropriate prescription of fusidic acid and rifampicin represents an important step to prevent further emergence of these resistant strains.

The reason why patients with chronic and recurrent tonsillitis do not respond to antibiotic therapy remains unclear. Few explanations could be due to the low concentrations of antibiotics in the tonsillar core due to the presence of scar tissue after each infection causing less diffusion of the antibiotic into the core (Usamah et al., 2005). In addition to the presence of normal flora that produces protective enzymes and the formation of biofilms (Andreas et al., 2010). Further studies are needed to assess the etiological agent of the infected and non-infected tonsils among larger sample size to eliminate the discrepancies between surface and biopsy (core) isolates.

ACKNOWLEDGEMENT

Authors would like to express their gratitude to the staff of Microbiology Unit at the Clinical Diagnostic Laboratory in University of Malaya Medical Centre (UMMC) for their role in conducting the required tests according to the standards of the University of Malaya and for their financial support.

REFERENCES

- Andreas Z, Merit K, Gerhard S, Silva H, Hagen F, Claudia M, Bernd K, Hans P, Andreas P (2010). Intracellular Persisting *Staphylococcus aureus* is the Major Pathogen in Recurrent Tonsillitis. PLoS ONE., 5(3): 1-16.
- Bista M, Amatya RCM, Basnet P (2006) Tonsillar microbial flora: A comparison of infected and non-infected tonsils. Kathmandu Univ. Med. J., 4(1): 18-21.
- Brook I, Foote PA Jr (1990) Microbiology of "normal" tonsils. Ann Otol Rhino Laryngol., 99: 980-983.
- Brown EM, Thomas P (2002). Fusidic acid resistance in *Staphylococcus aureus* isolates. Lancet., 359(9308): 803.
- Cockerill F, Wikler M, Bush K, Dudley M, Eliopoulos G, Hardy D, Hecht D, Hindler J, Patel J, Powell M, Thomson R, Turnidge J, Weinstein M, Zimmer B, Ferraro M and Swenson J (2010). Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Suppl., 30: 1-153.
- Donnelly MJ, Quraishi MS, Shane MC (1994). Indications for paediatric tonsillectomy GP versus Consultant perspective. J. Laryngol. Otol., 108: 131-134.
- Gaffney RJ, Freeman DJ, Walsh MA, Cafferkey MT (1991). Differences in tonsil core bacteriology in adults and children: A prospective study of 262 patients. Respir. Med., 85: 383-388.
- Gul M, Okur E, Ciragil P, Yildirim I, Aral M, Akif Kilic M (2007). The comparison of tonsillar surface and core cultures in recurrent tonsillitis. A. J. Otolaryngol. Head Neck Med. Surg., 28: 173-176.
- Inci E, Karakullukcu B, Aygun G (2002). Surface and core microflora of the tonsils in children undergoing tonsillectomy for recurrent tonsillitis. Turkish. Arc. Otolaryngol., 40: 247- 251.
- Kumar A, Gupta V, Chandra K, Gupta P, Varshney S (2005) Clinco Bacteriological Evaluation of Surface and Core Microflora in Chronic Tonsillitis. Ind. J. Otolaryngol. Head Neck Surg., 57(2): 118-120.
- Kurien M, Stanis A, Job A, Brahmadathan, Thomas K (2000). Throat Swab in the Chronic Tonsillitis: How Reliable and Valid is it? Singapore Med. J., 41(7): 324-326.
- Lim V (2003). Antibiotic Resistance in the Community. Med. J. Malaysia., 58(2): 156-158.
- Lindroos R (2000). Bacteriology of the Tonsil Core in Recurrent Tonsillitis and Tonsillar Hyperplasia— A Short Review. Acta. Otolaryngol., 543: 206-208.
- Loganathan A, Arumainathan D, Raman R. (2006) Comparative study of bacteriology in recurrent tonsillitis among children and adults. Singapore Med. J., 47(4): 271-275.
- Mette F, Rosdahl VT (1990). Susceptibility of fusidic acid among Danish *S. aureus* strains and fusidic acid consumption. J. Antimicrob. Chemother., 25: 7-14.
- Mostafa H, Zeinab A, Seham A, Mosaad A and Manal F (2009). Chronic Tonsillitis Bacteriology in Egyptian Children Including Antimicrobial Susceptibility. Aust. J. Basic Appl. Sci., 3(3): 1948-1953.
- Norazah A, Lim V, Koh Y, Rohani M, ZUridah H, Spencer K, NG P and Kamel A (2002). Molecular fingerprinting of fusidic acid and rifampicin-resistant strains of methicillin-resistant *Staphylococcus aureus* (MRSA) from Malaysian hospitals. J. Med. Microbiol., 51: 1113-1116.
- Pattison JR, Mansell PE (1973). Fucidin-resistant staphylococci in current hospital practice. J. Med. Microbiol., 6: 235-44.
- Rohani M, Raudzah A, Lau M, Zaidatul A, Salbiah M, Keah K, Noraini A, Zainuldin T (2000). Susceptibility pattern of *Staphylococcus aureus* isolated in Malaysian hospitals. Int. J. Antimicrob. Agents 13: 209-213.
- Rosdahl VT (1988). Epidemiology of resistance among gram positive bacteria: a special reference to staphylococcal infections. J. Hosp. Infect., 11: 235-244.
- Shanmugam J, Gopan R, Senthilkumar S (2008). The prevalence, antibiogram and characterization of *Staphylococcus aureus* including MRSA among the healthy staff, medical students and patients from Sri Manakula Vinayagar Medical College and Hospital (SMVMCH) Pudu Cherry. DSTE project report.
- Upal K, Bais AS (1989). Tonsillar microflora—superficial surface vs deep. J. Laryngol. Otol., 103: 175-177.
- Usamah H, Michel E, Marwan U, Nabil F, Ghassan M (2005). Characteristics of Pathogens Recovered from the Tonsils and Adenoids in a Group of Lebanese Children Undergoing Tonsillectomy and Adenoidectomy. J. Appl. Res., 5(3): 473-480.