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Original Article

Study of common bacterial agents and antibiotic susceptibility in patients with chronic and repeated tonsillitis

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ABSTRACT

Background and Aims: The aim of this study was to evaluate the bacterial sensitivity of the tonsillar core and to evaluate the antibiotic susceptibility to determine the currently selected antibiotic and to treat chronic tonsillitis and to reduce the need for surgery and to correct the resistance caused by inappropriate use of antibiotics. Methods: This prospective study included the analysis of 96 patients with chronic and recurrent tonsillitis who underwent tonsillectomy in Imam Khomeini Hospital of Ahvaz. In this study, 96 patients were enrolled. The average age was about ten years. 41 patients were female (42%) and 45 (58%) were male. The youngest age was 5 years and the maximum age was 25 years. The cause of tonsillectomy (35%) was obstructive and 61 cases (64%) were infectious. Results: In this study, about 11% have negative culture and, fortunately, about 89% have positive culture reported. The positive Gram-positive strains cultured were Staphylococcus aureus and Staphylococcus coagulase. The negative Germ-free cultivars Includes Pseudomonas and klebsiella pneumonia and klebsiella oxytoca. No sample of staphylococcus epidermidis was reported. The most isolated were Staphylococcus aureus with about 50%. Other isolates included Pseudomonas and Klebsiella and Staphylococcus coli. The most isolated isolate was Staphylococcus aureus. Conclusion: Our study suggests that the type of isolates and antibiotic resistance are changing and requires a new look at the appropriate antibiotic selection and requires more studies. In most isolates, antibiotic resistance was high, which is probably due to misuse of antibiotic regimens in recent years. On the other hand, there was resistance to very new antibiotics, and if this trend continues falsely, it will lead to the fate of the resistance of antibiotics.

Keywords: Antibiotic Susceptibility, tonsillar core, antibiotic resistance

Introduction

Tonsils are vital structures of the immune system and their infection is one of the frequent diseases in humans. [1-3] Tonsillitis is inflammation of the tonsils which is a common clinical state caused by bacterial or viral infections. [4, 5] There are three types of tonsillitis: acute, sub-acute, chronic, and recurrent. [4] Chronic tonsillitis is referred to enlargement of the tonsils along with recurrent infective attacks. [6, 7] It is the most common throat disease which is mainly observed in younger individuals. [1, 8] Recurrent tonsillitis is among the most prevalent diseases in children. Despite that antimicrobial treatments are prescribed for these children, they are generally insufficient and they need to undergo surgery. [9] Infection stability is due to insufficient or inappropriate antibiotic treatment and tonsillectomy is mostly the sole remained solution for this problem.[1, 3, 6, 7, 10] It is not clear why antibiotic therapy does not work for patients with chronic and recurrent tonsillitis. The presumable reason can be the lower concentration of antibiotics in central tissue of the tonsil which is due to scars resulted from the recurrent infections that decreases the distribution of antibiotics. [11] There is no

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convincing evidence suggesting that exclusive antibiotic therapy in recurrent attacks of tonsillitis is ineffective. Medical treatment of recurrent tonsillitis has remained a far-fetched objective and tonsillectomy is still the selective therapy in managing recurrent and chronic tonsillitis. [4] Quite apart from complications of the respective surgery, tonsillectomy affects patients and their families mentally and financially. [1-3] Thus, medical treatment seems a more appropriate option to eradicate infections and suitable medical therapies can prevent many tonsillectomies. [12, 13] Today medical treatment is the first step in managing recurrent tonsillitis and surgery is reserved for cases where the medical treatments fail. Medical management of tonsillitis demands a perfect knowledge of infective organisms. [1, 12, 14] Numerous studies suggest a remarkable difference between the external and central pathogen flora of tonsils and tonsils' diseases are probably caused by bacteria in center of the tonsils rather than by identified surface bacteria. [1, 8, 10, 13-16] As a result, it is not appropriate to choose antibiotics based on surface swabs. Patients have common prevalent pathogens in their tonsils' tissue. [9] The most dominant organisms in center of tonsils that cause infection in recurrent tonsillitis are probably different through various areas; however, it has been reported that when the type and number of the isolated organisms are considered, right and left tonsils do not differ at all. [9, 17] In recurrent tonsillitis the center of tonsils contains a lot of bacteria including staphylococcus aureus, Haemophilus influenzae, and staphylococcus pyogenes. [8] There is a meaningful relationship between the isolated species and tonsillitis. [9] The main organisms are Haemophilus influenzae and S. aureus. Microorganisms other than group a beta-hemolytic streptococcus are presumably the reason for chronic tonsillitis. [18] The recent decade has observed an increased resistance among widespread pathogens as well as increased unusual suspects. Especially recently, beta lactamase-producing bacteria such as S. aureus and Haemophilus influenzae are dominant in micro flora causing resistance to penicillin. [9, 14] Some researchers claim that antibiotic therapy failure is possibly due to underestimating the resistant microorganisms. [9] Probably, this scenario demands a wiser application of antibiotics and restoration of surgical procedure. [18] Currently penicillin therapy as the first-line treatment for tonsillopharyngitis does not conform to minimal standards of U.S FDA which necessitates eradication of 85% or more by the end of treatment. [19] The recent outcomes of amoxicillin therapy indicate that its efficiency is about to end. Cephalosporin alone or cephalosporin coupled with metronidazole, when anaerobes incorporate, enjoys the most bacteriologic and clinical efficiency. There is strong anatomic evidence of bacterial biofilms existence in the tonsils of patients with chronic diseases. [8, 16]

In this study we attempt to bacteriologically compare the center of the tonsils in chronic and recurrent tonsillitis. Additionally, we assess Antibiogram in patients with chronic and recurrent tonsillitis. The present survey will aim to bacteriologically evaluate the center of the tonsil and assess the antibiotic sensitivity in order to define the preferred antibiotic, treat chronic infection of the tonsils (chronic tonsillitis), decrease the need for surgery, and modify the resistance trend due to inappropriate prescription of antibiotics.

Methodology

Study Design & Studied Population

This prospective study included 96 patients with chronic and recurrent tonsillitis who had undergone tonsillectomy in Therapeutic and Educational Imam Khomeini Hospital of Ahwaz.

Medical histories of all participants were gained and they were clinically examined. Moreover, informed consents were obtained. The respective tests including assessing hemoglobin, random blood sugar, blood urea, serum creatinine, and blood type were carried out.

Tonsil tissues were removed during tonsillectomy and after being washed with sterile saline were cut into two pieces, next they were immersed in distilled water for 30-45 seconds and were used to be microbiologically evaluated. After that, biopsy specimen of the center of the tonsils were taken and the tissue was stored in a sterile sample container and immediately sent to a microbiology laboratory to be cultivated and examined for allergic tests.

In microbiology lab first a direct slide was taken from the sample and after fixing it was stained with Gram's Method to be morphologically examined. The prepared tissue sample was initially homogenized with sterile distilled water in a sterile plate and a section of it was incubated to three culture media: EMB, Choclate Agar, and Blood Agar. Plates of Blood Agar and Choclate Agar were placed in co2 jar and incubated at 37°C for 24-48 hours along with EMB culture medium. A direct slide was prepared from grown colonies after incubation and stained by Gram's Method. Isolates were next identified by standard biochemical tests. Sensitivity and resistance of isolates were assessed through disk diffusion techniques and based on CLSI instruction in Mueller Hinton Agar.

Data Collection

For this purpose first the microbial suspension was prepared according to McFarland standards and next it was spread onto Mueller Hinton medium by a cotton sterile swab laced with the microbial suspension. After that, suitable antibiotic disks (MAST, England) were placed on the medium and plates were incubated for 24 hours at 37°C. In this examination antibiotic discs of azithromycin (15 µ gr), ceftriaxone (30 µ gr), ciprofloxacin (5 µ gr), tetracycline (30/1.25 µ gr), ceftazidime (30 µ gr), nalidixic acid (30µ gr), gentamycin) 10 µ gr) , co-terimoxazole (23.75 µ gr) were used.

Data analysis

Besides descriptive statistics such as average and standard deviation as well as frequency distribution tables, Chi-Squared

and independent t-tests were also applied all using SPSS version 22.

Ethical issue

It is certified that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Informed written consent was obtained from all participants and the Ethical Committee of Ahvaz Jundishapur University of Medical Sciences approved this study at 2015. The study protocol conforms to the ethical guidelines of the 2008 Declaration of Helsinki.

Results

The present study included 96 patients. The average age of patients was nearly 10. Forty one participants were females (42%) and 45 were males (58%). The youngest patient was 5 and the oldest one was 25. Thirty five of cases were caused by obstructive difficulties and 61 cases caused by a bacterial infection. Nearly 11% of cultivations were negative and fortunately 89% were reported positive. The cultivated gram-positive species included staphylococcus aureus and coagulase-negative staphylococcus. Gram-negative bacteria included pseudomonas, Klebsiella pneumonia, and Klebsiella oxytoca. In nearly 11% of cases there was no bacterial growth observed on culture media. There was no reported case of Staphylococcus epidermidis. Table 1 illustrates the frequency of isolates extracted from patients. Staphylococcus aureus had the highest frequency with 50%. Other isolates included pseudomonas, klebsiella, and coagulase-negative staphylococcus.

Table 2 shows the antibiotic resistance pattern of staphylococcus aureus isolates extracted from patients. The cultivated species were 100% resistant to ampicillin, G-penicillin, and ampibactam. There was 14% resistance to imipenem, 8% to linezolid, 22% to co-trimoxazole, 30% to ofloxacin, 28% to ciprofloxacin, and 4% to rifampin.

Table 3 shows the pattern of antibiotic resistance of pseudomonas isolates of patients. This species revealed 94% resistance to ceftriaxone, 100% to cefpirome and cefotaxime, 33% to imipenem, 22% to doripenem, 5% to piperacilin tazobactam, 77% to co-trimoxazole, 44% to ceftazidime, and 11% to ofloxacin. However, 100% of isolates were sensitive to ciprofloxacin.

Table 4 demonstrates the pattern of antibiotic resistance of klebsiella isolates extracted from patients. This isolate was 6% resistant to cefotaxime, 43% to cefpirome, 18% to impenem, 14% to doripenem, 25% to piperacilin tazobactam, 43% to co-trimoxazole, 25% to ceftazidime, 12% to ofloxacin, and 12% to ciprofloxacin.

Coagulase-negative staphylococcus was resistant to ampicillin, ampibactam, and penicillin; however, it was sensitive to imipenem, doripenem, linezolid, co-trimoxazole, rifampin, ofloxacin, and ciprofloxacin. Klebsiella oxytoca species was resistant to imipenem and doripenem and sensitive to ceftriaxone, cefotaxime, cefpirome, tazobactam and co-trimoxazole.

Discussion

The most common extracted isolate was staphylococcus aureus. The strength of the present study is that it is prospective and unlike majority of studies anti-BIOGRAM tests were also carried out. On the other hand instead of using tonsil smear and throat swab culture, the freshly-cut tissue of tonsil after tonsillectomy was used for cultivation. No Haemophilus influenzae isolate was extracted. Since no staphylococcus epidermis was extracted and all extracted isolates were pathogen, it can be claimed that sampling has been accurately carried out without any error and contamination. Unlike nasal mucus where staphylococcus isolates can exist as the normal flora, it does not apply to tonsil's tissue and mucus, therefore the presence of staphylococcus isolate is an indication of pathogen existence. Regarding the prevalence of staphylococcus aureus, the results of the current study are in accordance of outcomes of similar studies. However, pseudomonas percentage and klebsiella prevalence were higher and antibiotic resistance was much higher. [20, 21]

Our study indicates that the type of isolates and antibiotic resistance are changing which demands a new perspective to select the right antibiotic and carry out more extensive studies. Most isolates had a higher antibiotic resistance which is likely due to inappropriate prescription and consequently inaccurate consumption of antibiotics in recent years. On the other hand, resistance was also observed in very new antibiotics and if this trend continues they will have the same fate as older ones. The next problem was the high resistance to penicillin, the most frequent medicine prescribed for tonsillitis treatment, in addition that this is an alarm for clinical specialists, it notifies the need to change the selected medicine for curing recurrent and chronic tonsillitis.

The cultivated bacteria were not meaningfully different regarding age and indication of surgery. Additionally, since isolates reveal a high resistance to penicillin if we tend to decrease the size of tonsils without surgery, for any reason, the antibiotic selection matters. Therefore, based on present findings penicillin is not a suitable choice.

It is recommended that future studies have more subjects participate in their surveys and examine anaerobic isolates as well. Moreover, we suggest the biofilm microscopy. In addition, we recommend that antibiotics which demonstrated sensitive BIOGRAMS in this very study are tested on patients in real environment-not the laboratory medium- and results are compared with those of control patients and their post-surgery impact on isolates are also examined.

Conclusion

Our study suggests that the type of isolates and antibiotic resistance are changing and requires a new look at the appropriate antibiotic selection and requires more studies. In

most isolates, antibiotic resistance was high, which is probably due to misuse of antibiotic regimens in recent years. On the other hand, there was resistance to very new antibiotics, and if this trend continues falsely, it will lead to the fate of the resistance of antibiotics.

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"Disclosure of interest."

The authors report no conflicts of interest.

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Appendix:

Table 1. Table of isolates extracted from patients.

Species	Number	Percent
Staphylococcus aureus	48	50
Pseudomonas aeruginosa	18	17.75
Klebsiella pneumonia	16	16.6
Negtive culture	11	11.45
Negative-coagulase staphylococcus	2	2
Klebsiella oxytoca	1	1

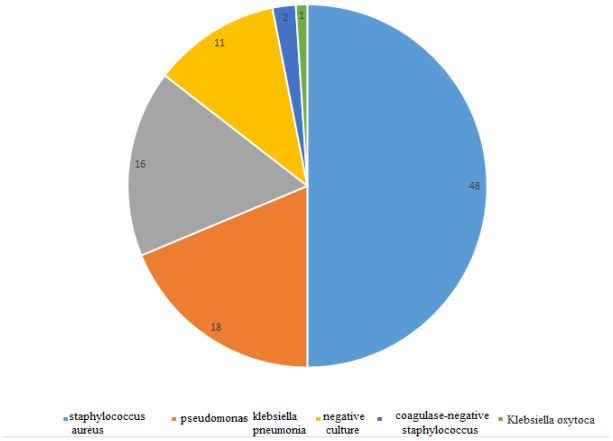

Figure1: Frequency of the extracted isolates

Table 2. Antibiotic resistance pattern of staphylococcus aureus isolates extracted from patients

Antibiotics	Resistant (%)	Sensitive (%)
Ampicillin	49(%100)	0
Ampicillin Sulbactam	49(%100)	0
Penicillin G	49(%100)	0
Imipenem	7(14.28%)	42(85.72%)
Doripenem	6(12.24%)	43(87.76%)
Linezolide	4(8.1%)	45(91.9%)
Cotrimoxazole	11(22.4%)	38(77.6%)
Rifampicin	2(4%)	47(98%)
Ofloxacin	15 (30.61%)	34(69.39%)
Ciprofloxacin	14(28.57%)	35(71.43%)

Table 3. Antibiotic resistance pattern of pseudomonas aeruginosa isolates extracted from patients

Antibiotic	Resistant (%)	Sensitive (%)
Ceftriaxone	17(94.4%)	1(5.6%)
Cefotaxime	18(100%)	0
Cefpirome	18(100%)	0
Imipenem	6(33.3%)	12(66.6%)
Doripenem	4(22.2%)	14(77.8%)
Piperacillin Tazobactam	1(5.6%)	17(94.4%)
Cotrimoxazole	14(77.8%)	4(22.2%)
Ceftazidime	8(44.4%)	10(55.6%)
Ofloxacin	2(11.1%)	16(88.9%)
Ciprofloxacin	0	18(100%)

Table 4. Antibiotic resistance pattern of klebsiella pneumonia isolates extracted from patients

Antibiotic	Resistant (%)	Sensitive (%)
Ceftriaxone	1(6.25%)	15 (93.75%)
Cefotaxime	1(6.25%)	15 (93.75%)
Cefpirome	7 (43.75%)	9 (56.25%)
Imipenem	3 (18.75%)	13(81.25)
Doripenem	7 (43.75%)	9(56.25%)
Piperacillin Tazobactam	4 (25%)	12 (75%)
Cotrimoxazole	7 (43.75%)	9 (56.25%)
Ceftazidime	4 (25%)	12 (75%)
Ofloxacin	2 (12.5%)	14 (87.5%)
Ciprofloxacin	2 (12.5%)	14 (87.5%)