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Isolation, Identification and Evaluation of Resistance of Bacterial Isolates from Patients with Tonsillitis

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ABSTRACT:

Introduction: Tonsillitis means the pharyngeal tonsils are inflamed which caused by bacteria, viruses and other immunologic factors, like Group A streptococci, *Staphylococcus aureus* was also reported as the commonest and the principal causative agent

Material and Methods: 64 Samples were collected from core tonsils of patients who had tonsillectomy were streaked on different type of CHROM agar™ media. Antibacterial susceptibility test was performed by disk diffusion technique for different type of antibiotics.

Results: Different bacterial isolates have been obtained from patients with Chronic and Recurrent tonsillitis. The genus *Staphylococcus* has the dominance among them n=56 isolates. Antibiotic susceptibility has been introduced for the species *Staphylococcus aureus*, in which most isolates were Methicillin resistant *Staphylococcus aureus* (MRSA), isolates were also 100% resistance to Ampicillin and susceptible to Gentamicin, the response of *S. aureus* isolates were variable to Chloramphenicol.

Conclusion: Core of tonsils alike a store for many bacteria. different genera have recorded in our study. Children have the great chance for infection, may be for many reasons. Drug resistance bacteria especially the type MRSA remain under focus because of the big problems may regard with. We always need to future studies in the war of the resistance to antibiotic used in the human treatment.

KEY WORDS: Tonsillitis, Bacteria, Drug resistance, MRSA

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I. INTRODUCTION

Tonsillitis means the inflammation of the pharyngeal tonsils which caused by bacteria, viruses and other immunologic factors (1). Members of Group A streptococci, such as *Streptococcus pyogenes*, are the most often responsible for human infections, including tonsillitis which are β-hemolysis on blood agar (2). Cleary and Cheng demonstrated that tonsils from children with recurrent tonsillitis were shown to harbor *Streptococcus pyogenes*, in par keratinized epithelial cells (3). Other types of bacteria were recorded by Agrawal *et al.*; *Staphylococcus aureus*, *Pseudomonas* species, *Escherichia coli*, and *Proteus vulgaris* (4).

Staphylococcus aureus was introduced as the commonest and the principal causative agent in the study of Babaiwa *et al.* (5). Tonsillitis also may be caused by the acute respiratory viruses as one of respiratory system disease of human (6). Nada illustrated that the outcome of the infection depends on the virulence and degree of resistance or susceptibility of the pathogen to antimicrobial chemotherapy, and the high prevalence of resistant bacteria may be related to antibiotics uncontrolled usage (7). The study of Romanus *et al.* showed that *Streptococcus* spp. were susceptible to Ciprofloxacin,

Ceftazidime but resistant to Amoxicillin/Clavulanic acid, Ceftazidime and Ampicillin (8). Whereas *Pneumococcus* species in the study of Agrawalet al. were resistant to Cefaclor, Gentamicin and Tobramycin with different rates (4).

Staphylococcus aureus resistance to antibiotics, as reported by Romanuset al. is a worldwide phenomenon especially when it involves MRSA (8).

The aim of recently study has focused on the antibiotic sensitivity pattern of *Staphylococcus aureus* especially MRSA type isolates obtained in high level in patients of tonsillitis at the hospital.

II. MATERIAS AND METHODS

Samples Collection:-

Samples were collected from 64 patients who had tonsillectomy in surgery unite of Basrah teaching hospital from January 2019 to July 2019, all samples were from two genders aged between (3- 20) years. it were transferred under sterile conditions to laboratory for cultured after washing all sample with normal saline then cut by sterile scalpel to take swab from the tonsils' core.

Cultivation and Identification:

Samples were directly immersed in brain heart infusion broth and incubated for 24h at 37⁰c, after that each isolate, was streaked on CHROMagar™ media (CHROMagar™, Paris, France) [CHROMagar™ orientation, CHROMagar *Staphylococcus aureus* and CHROMagar™ *Candida spp.*] using a loop and incubated at 37⁰c for 48h (9-11).

Each swab was cultured onto two specific CHROMagar™ media plate, and according to (9), microbes isolated and identified depended on shape and color of each colony grew on specific agar.

Gram staining with microscopic examination was included.

Disk Diffusion Test

Antibacterial susceptibility test was performed on Muller Hinton Agar by the Kirby Bauer method (12). Antibiotics disc with concentrations used; Chloramphenicol 10µg, Methicillin 10µg, Ampicillin 25µg and Gentamicin 10µg. The isolates of *S. aureus* were picked up using sterile loop and transported in to sterile distilled water, the turbidity of each suspension was done to be 10⁶ CFU/ml then spread on the surface of seeded agar, the antibiotics disc were distributed on the agar plats and incubated overnight at 37⁰c. Then, inhibition zones were determined according to the standards outlined by the Clinical and Laboratory Standards Institute (CLSI) (13).

III. RESULTS

Present research found that the infected patients were n=33 approximately 52% from total 64 tonsillitis patients distributed according ages and genders as seen in table (1). Both gender male and female could be infected, but there is a little different in the rate of infection, shown that in female it may be higher than in male. In addition; Children may revealed more response from adults to the disease.

Table 1: Numbers of infected patients with their ages and gender.

Age (y)	Gender	
	M	F
1-9	8	11
10-19	4	6
20-29	1	2
30-39	1	0

(y): year; M: male; F: female.

This study got 148 microbial isolates from 64 samples, bacteria were n=141 (95.37%) as: *Staphylococcus* (*S. aureus* and *S. epidermidis*), *Citrobacter* spp., *Klebsiella* spp., *Streptococcus* spp., *Enterobacter* spp. and *Pseudomonas* spp. In addition to another microbial isolate which was *Candida* spp. The results showed the prevalence of genus *Staphylococcus* followed by *Citrobacter*. As shown in table (2).

Table 2: Microbial isolates obtained with numbers and percent.

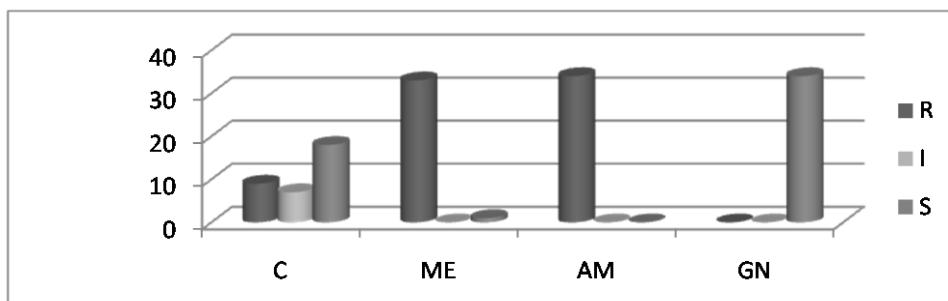
Isolate name	Isolate number	Percentage (%)
<i>Staphylococcus</i> spp.	56	39.7
<i>S. aureus</i>	34	60.71
<i>S. epidermidis</i>	22	39.28
<i>Citrobacter</i> spp.	47	33.33
<i>Klebsiella</i> spp.	12	8.5
<i>Streptococcus</i> spp.	10	7
<i>Enterobacter</i> spp.	9	6.38
<i>Pseudomonas</i> spp.	7	4.96
<i>Candida</i> spp.	7	4.72*

* percentage from the whole microbial isolates

Our findings have shown different responses to the antibiotics used in the examination; resulted in a high rate of Methicillin resistant *S. aureus* MRSA, where most of them were resistance to Methicillin except one isolate. The recordings revealed that *S. aureus* isolates were 100% resistant to Ampicillin, while all of these isolates were susceptible to Gentamicin.

The response of *S. aureus* isolates were variable to Chloramphenicol, where 26.47% were resistance compared with approximately 53% were sensitive and 20.58% intermediate as shown in figure (1).

Figure 1 : Antibiotic sensitivity of *S. aureus* as a result of different inhibition zone.



C: Chloramphenicol, ME: Methicillin, AM: Ampicillin, GN: Gentamicin, R: resistant, I: Intermediate, S: susceptible

IV. DISCUSSION

Tonsillitis is referred as an acute infection of the palatine tonsils in the study of Yapet *et al.* mentioned that it is more common in children than adults (14), supported our observations, where the children have shown the higher infectious rate, but with little differences between male and female, besides; it may be identical to the results of Dakhil and Hamim (15) in which patients of <10 years had the highest in compared with other disagreement of Agrawal *et al.* who found that age of (11-20 years) was the most affected with Tonsillitis (4). Vijayashree *et al.* suggested some reasons for his acute tonsillitis study on children in schools; such low immunity in the children, overcrowded class rooms and poor ventilation inside (16).

Regarding bacterial identification CHROMagar medium was used. Merlino *et al.* reported that CHROMagar orientation medium facilitates visual detection of mixed isolates in culture, in addition for the differentiation and presumptive identification of genera and species of certain bacteria on the basis of color and morphology alone (9).

Present study found that from 141 bacterial isolates, *Staphylococcus* spp. has the prevalence with high percentage 56(39.7%), hence, that met with the findings of Yousef *et al.* where the genus *Staphylococcus* was the most commonly bacteria in the tonsils core and/or surface (17). Despite the dominance of species *S. aureus* in that study, in our study the genus has been divided in to two groups: *S. aureus* (n=34) and *S. epidermidis* (n=22). *Klebsiella* spp. and *Enterobacter* has been recorded in the both above too.

As in our results; *Pseudomonas* spp. appeared also in Alasil *et al.* despite the different rates. As well as the detection of *Citrobacter* spp. (18), in the present research with high rate of (33.33%); Alasil *et al.* recorded it in small number during his investigations from hypertrophied tonsils, however; it seems to be rare (19). On the other hand, *Streptococcus* spp. has been found in little percent of 7%, which may disagree with other study like Vijayashree *et al.*, in which *Streptococcus* was the dominant bacteria, but from children with acute tonsillitis (16).

Present results revealed other microorganisms like candida spp. Concerning with antibiotic sensitivity of *S. aureus*, our records has revealed that all of isolates selected were sensitive to Gentamicin, besides the distribution for numbers of isolates against Chloramphenicol, ranging from moderate, resistant to the higher number of sensitive bacteria 20.58%, 26.47% and 53% respectively, whereas; near all isolates were resistant to Methicillin and Ampicillin.

About 5 and 6 isolates from coagulase positive *Staphylococci* were sensitive to Gentamicin, Chloramphenicol respectively, in the study of Vijayashree *et al.* that may meet in somewhat with our results, but disagreed in the sensitivity for Ampicillin of 6 isolates recorded in that study (16). Similar to present results has been found in Yousef *et al.* where most of *S. aureus* were resistant to Ampicillin 58 out of 65 (17). In study of Romanus *et al.* there is an identity to recent findings of 100% sensitivity to Gentamicin, while the 60% of Ampicillin is in contrasting with our recorded (8). Results had been demonstrated by Dakhil and Hamim that; all *S. aureus* isolates were resistant to Ampicillin in sensitivity of 0% whereas obtained 23%, 81% and 97% for Methicillin, Chloramphenicol and Gentamicin respectively (15). The analyzing of sensitivity pattern for 16 isolates of *Staphylococcus*, as reported by Agrawal *et al.* was; sensitivity of 68.75% to Gentamicin, 100% resistance to Chloramphenicol with 68.75% to Ampicillin and 37.50% were Methicillin resistant *S. aureus* (MRSA) (4). In many bacteria, the increasing incidence for drug resistance as suggested by Vijayashree *et al.*, could be due to bacterial production of β -lactamase, that cleave the activity for antibiotics and resistance transfer factors which could have taken up by the susceptible isolates during the process of recombination (16). Hasan conducted that MRSA isolates were more in the age (1-9) years, supposing some reasons; bad using of antibiotics, giving antibiotics without following the caregiver's instructions of antibiotic medicine giving for a long time or give it when the child does not need them, which led germs to become resistant (20).

CONCLUSION

Core of tonsils resemble a store for many bacteria. In present study, different genera have appeared. Children have the great chance for infection, may be for low immune system or a little care by health. However; Bacterial drug resistance especially the type MRSA remain under spot light because of the big problems may regard with. We always need to a lot of experimental future studies in the war of the resistance to antibiotic used in the human treatment.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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