Isolation, identification and Antibiogram study of pathogenic bacteria mediated Children Chronic Tonsillitis at Qena University Hospital, Egypt.

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Abstract:

Background: Inflammation of tonsils is a common disease of the throat that occurs predominantly in the children. Sothis prospective study was carried out to determine the pattern of bacterial strains and their antibiotic sensitivity amongst children with tonsillitis admitted to the E.N.T Department, Faculty of Medicine,Qena University Hospital for elective tonsillectomy.

Patients and Method(s): Thirty-five children (2 to 17 years old) suffering from chronic tonsillitis were sampled. The bacterial isolates were identified phenotypically based on morphological, hemolysis pattern and biochemical characters using different media or identified genotypically through 16S rRNA gene sequencing.

Results: The bacterial strains were identified as Staphylococcus aureus (40%) followed by Streptococcus pyogenes (35%), Streptococcus australis (15%), and Streptococcus pneumoniae (10%). The susceptibility of the isolates was tested towards 8 different types of antibiotics (Bioanalyse®). The results showed that most bacterial isolates were multidrug-resistant bacteria that showed resistant to three or more tested antibiotics.

Conclusion: The present study revealed that the ß-haemolytic Streptococci (BHS) is a significant causal agent of tonsillitis in our environment which causes a potential danger of rheumatic fever and rheumatic heart disease.

Keywords: Tonsillitis, ß-haemolytic Streptococci, antibiotics, multidrug-resistant bacteria.

Introduction:

Tonsillitis is inflammation of the tonsils, typically of rapid onset (**Ferri, 2015**). It is most common in school-aged children and typically occurs in the fall and winter months (**Windfuhr*et al.,* 2016**). Tonsillitis is generally the result of an infection, which may be viral or bacterial (**Anderson and Paterek, 2019**).Bacterial infections are typically due to group A beta-hemolytic *Streptococcus* (GABHS), but *Staph aureus*, *S. pneumoniae*, and *Haemophilus influenza* have also been cultured (**Wang *et al.,* 2017**). A"carrier state" is defined by a positive pharyngeal culture of group A beta-hemolytic *Streptococcus pyogenes* (BHS), without evidence of an antistreptococcal immunologic response (**Alasmari *et al.,* 2017**). While tonsillitis is typically symptomatically managed with good clinical outcome, complications do occur. Abscesses, rheumatic fever, scarlet fever, and acute glomerulonephritis are known complications. The problem of antibiotic resistance is a global problem which is implicated in elevated morbidity and mortality rates as well as in the increased treatment costs (**Chandra *et al.,* 2017).** Several of various recent studies revealed that almost 400 different bacteria have demonstrated about 20,000 possible resistant genes (**Davies and Davies, 2010**). *Staphylococcus aureus* isolated from clinical samples are now showing resistance to more than three drugs and are considered as multiple-drug-resistant bacteria (**Styers *et al.,* 2006**). In the case of *Streptococcus pyogenes* isolates, the resistance rates for penicillin are 50% and for erythromycin is 80% globally (**Chandra *et al.,* 2017**). The current study was carried out to isolate, identify bacterial strains involved in

tonsillitis after tonsillectomy using biochemical tests or 16S rRNA. Also, screening for antibiotics resistance of the isolated strains among children was assessed.

Materials and Methods

Collection of samples: Patients suffering from chronic tonsillitis wereguided to otolaryngology Department of Qena University Hospital for elective tonsillectomy. The study was carried out during the period from December 2016 to February 2017. Tonsils samples were taken from 35 children with age ranged from 2 to 17 years. Samples from tonsils specimens were taken during surgery in a disposable container. Any patient who had taken antibiotics during the previous 10 days was excluded from the study. The samples were labeled with the required information about each patient such as name, age, sex, date of sample collection.

Isolation of bacteria (**Fujimori *et al.,* 1996**): Immediately after the tonsillectomy independent sterile swabs sticks were rubbed on the interior of the right and left tonsils (as shown in Fig. 1) to obtain the tonsillar deep culture. The swabs immediately streaked onto two types of media; Blood agar with 5% sheep blood (Sigma–Aldrich, 70133) (**Facklam, 1980**) and Selective Strep agar (selective media for GAS, Acuemedia, 7405) (**Roantree *et al.,* 1958**) with 5% sheep blood. The cultures were incubated at 37°C overnight under 5% CO_2 effect (STIK Co2 Incubator IL 161 CT, China) in case of selective media for group A streptococci (GAS).

Fig. 1: Tonsils sample after tonsillectomy.

Identification of the bacterial isolates: The bacterial isolates isolated from the tonsils samples after tonsillectomy were identified phenotypically based on morphological, hemolysis pattern and biochemical characters [Catalase (**Koneman *et al.,* 1988**), Oxidase (**MacFaddin, 1976**), Urea hydrolysisand Oxidative fermentative tests (**Hugh and Leifson's, 1953**)] using different media and identified genotypically through 16S rRNA gene sequencing.

Antimicrobial Susceptibility tests: The antibiogram of all the recovered isolates was determined as described earlier by **NCCLS (2002**) using the disc diffusion method (**Bauer *et al.,* 1966**). The susceptibility of the isolates was tested towards 8 different types of antibiotics {Cefadroxil, Cephradine, Amoxicillin/Clavulanic acid, Ampicillin/Sulbactam, Cephalexin, Erythromycin, Ofloxacin, Ciprofloxacin} (Bioanalyse®). Pure colonies of bacterial isolates were transferred into 5 ml tryptic soy broth. The culture was incubated at 37°C until it matches the turbidity of 0.5 McFarland standards (**McFarland, 1907**). Optimally, within 15 min. after adjusting the turbidity, the dried surface of a Muller-Hinton agar plate (Muller-Hinton agar containing 5% sheep blood for testing Streptococci) was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. Using sterile forceps, the selected antibacterial discs were placed on the plates. The plates were incubated at 37°C for 24 h. After incubation, each plate was examined and the inhibition zones were measured. Interpretation of the results was performed according to the Clinical and laboratory standard institute (**CLSI, 2011**) to determine if the strain is resistant or susceptible to the tested antibiotics.

Results

A total of 35 tonsils samples were collected from patients attending Qena University Hospital in Egypt after tonsillectomy. The commonest age group of tonsillitis in this study is between 2 to 17 years classified into two groups; group 1 (2-10 years) represent 65% and group 2 (11-17 years) 35% from the total samples. Out of 35 tonsils samples, 30 samples (85.7%) were showed bacterial infection with one or two isolates.

Forty bacterial strains isolated from the tonsils samples after tonsillectomy were identified through their morphological, hemolysis

pattern, cultural and biochemical characters for Gram-positive bacteria *Streptococcus pyogenes* and *Streptococcus pneumoniae* using Selective Strep Agar media as shown in figure (2) and the biochemical reactions confirm thepresence of *Streptococcus pyogenes* (streptococci) and *Streptococcus pneumoniae* (diplococci) (negative for oxidase, urease and catalase) and through 16S rRNA based on molecular technique for bacterial strains isolated by using Blood Agar media.

(a)*S. pneumoniae* α-hemolysis (b)*S. pyogenes* β-hemolysis

Fig. 2: (a) α-hemolysis and (b) β-hemolysis of isolated bacteria on Selective Strep Agar media

To identify and confirm *Staph. aureus* and *S. australis* at the molecular level, 16S rRNA gene region was amplified and sequenced. The generated phylogenetic trees categorized all isolates into five well-supported clades (Fig.3), showing that the isolated bacterial strains from tonsils samples after tonsillectomy were *Staph aureus* and *S. australis*.

The most common isolated organism as Gram-positive bacteria including *Staphylococcus aureus*16 (40%), *Streptococcus pyogenes*14 (35%), *Streptococcus pneumoniae* 4 (10%), *Streptococcus australis* 6 (15%).

The antibiotic resistance of isolated pathogenic bacteria against 8 antibiotics with different disc potency was determined by the disc diffusion method (Table 1). Allisolates of *S. australis* were susceptible to all antibiotics used in this study (Fig. 4).

Discussion

Tonsillitisis an inflammation of the tonsils, typically of rapid onset (**Ferri, 2015**). Many organisms can induce inflammation of the tonsils. Because the oropharynx is colonized by many organisms, most infections are polymicrobial (**Wessels, 2016**).

Staph aureus was the leading Gram-positive bacteria that can be found in the normal microbiota of oropharynx and nose (**Shanmugam *et al.*, 2008**). In the current study, the highest percentage of tonsillitis was caused by *Staph aureus*16 (40%) of all samples which agree with **Bakir and Ali**

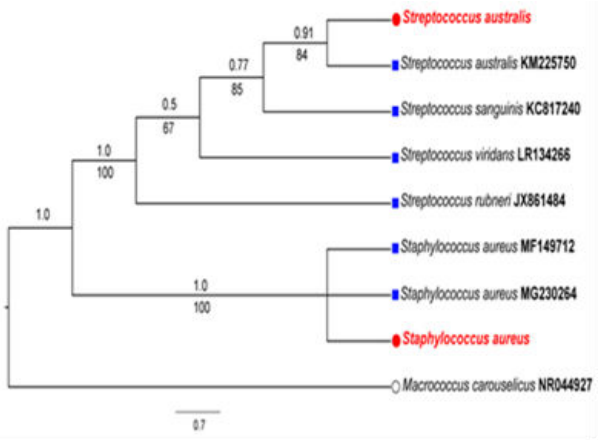
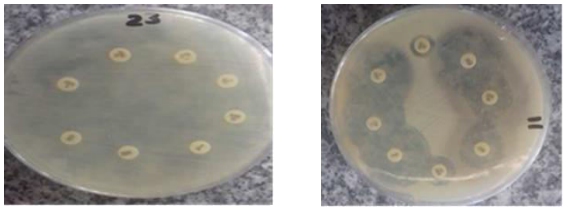


Figure (3): Strict consensus cladogram the Most Parsimonious (MP) treesfound using the 16S ribosomal RNA sequence data showing the phylogenetic relationships among the studied strains of *Staph. aureus* and *S. australis*. The newly generated sequences are preceded by red circle. The GenBank sequences are preceded by blue squares. The GenBank accession number appears after the species name. Values above the branches indicate Bayesian posterior probabilities, and the maximum parsimony bootstrap support values are given below the branches. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The outgroup used for tree constructionpreceded by an empty circle.Tree length= 724; Consistency index (CI) = 0.9986; Homoplasy index (HI) = 0.0014; Retention index (RI) = 0.9994; Rescaled consistency index (RC) = 0.9980.



Staph aureus **resistant strain** *S. australis***sensitive strain**

Fig. (4): Antibiotics resistance and sensitivity of the isolated bacterial strains.

(2016) from Iraq and **Babaiwa and JO(2013)** from Nigeria showed *Staph aureus* was the most common bacteria isolated from Tonsillitis in the rate of (30.5%) and (70%) from all patients in their study. ***S. pyogenes*** came second after *Staph aureus* as a tonsillitis causal agent; it accounts for more than 37% of all diagnosed sore throat cases in children and up to 5–10% in adult's reports millions of cases per year worldwide **(Wijesundara and Rupasinghe 2019)**. It has been established that tonsillitis caused by *S. pyogenes* occur in children than in adults **(Shaikh *et al*., 2010**). These results in agreement with **Bakir and Ali (2016)** and, **Babaiwa and JO (2013)** reported that *S. pyogenes* represented 9 and 14% respectively from bacterial tonsillar microbiota in recurrent tonsillitis. Indifference to our study results, **Agrawal *et al.*** (**2014**) showed that *S. pyogenes* isolated from patients with chronic tonsillitis represent 2.14%.

In the present work, *S. pneumoniae* was represented 10% of the total isolates. **Bakir and Ali (2016)** investigated that the percentage of *S. pneumoniae* isolated from positive culture from throat infection as 4.9%. **Agrawal *et al.*** (**2014**) showed that *S. pneumoniae* was isolated from 5% of the total isolates.

In the case of *S. australis* isolated from tonsillar swabs samples after tonsillectomy was at the rate of 15%. *S. australis* an oral streptococcal species was first described in 2001 from strains isolated during a microbiological survey of saliva samples from healthy children attending the United Hospital in Sydney, Australia (**Willcox *et al.,* 2001**). *S. australis* belongs to the *S. mitis* group, one of the five groups of viridans *streptococci* described by **Facklam** (**2002**). **Héry-Arnaud *et al.*** (**2011**) from France reported the first documented case of invasive *S. australis* infection resulting in meningitis.

Antibiotic drugs have reduced the burden of common infectious diseases and become essential for many medical interventions (**Laxminarayan *et al.,* 2013**). However, antibiotic-resistant pathogens have emerged and spread among human and animal populations worldwide (**WHO,2015**). Centers for Disease Control and Prevention (CDC) stated that antibiotic resistance is responsible for around 2 million infections, more than twenty thousand deaths and, cost $55 billion each year in the United States **(Reta *et al.,* 2019)**. In this study, *Staphaureus* exhibited resistance to all antibiotics with different percentages ranging from 43.75 to 87% of all isolates. These findings harmony with other studies in different parts of the world. In this respect, **Bakir and Ali (2016)** from Iraq and **Agrawal *et al.* (2014)** from India have established the resistances of *Staph aureus* to β-lactams antibiotics are 92.5% and 75% respectively. Also, they reported that the resistances against the second generation of quinolones were 1.8% and 62.5%.

S.pyogenes (GAS strains) exhibited high resistance to β-lactams, macrolides, and quinolone antibiotics. This resistance is ranging from 92.85% to 50%. These findings are in agreement with different studies such as **Bakir and Ali (2016)** and **Al-Saidiand Hameed** (**2010**) showed that 100% and 94% resistance to β-lactams antibiotics. In contrast to our study, all isolates of *S. pyogenes* isolated from tonsillitis/pharyngitis patients were sensitive to β-lactams antibiotics (100%) showing no resistance completely as reported by numerous authors **(Wu *et al.* 2014 and Khosravi *et al.* 2016).** Worldwide resistance rates of Group A β hemolytic *streptococci* isolates were resistant to macrolides Erythromycin was reported to range from 10-95% **(Elsherif *et al.* 2011 and Choi *et al.* 2015).** In contrast, **Agrawal *et al.* (2014)** from India reported that beta-hemolytic *streptococci* were found 100% sensitive to Erythromycin. In the case of quinolones, a variable rate of resistance was detected as 57.14% Ofloxacin

and 50% to Ciprofloxacin. These results in the range of different studies worldwide that found that the resistance of *S. pyogenes* to Ciprofloxacin in the range of 16-63% (**Bakir and Ali 2016; Karaky *et al.*, 2014).**

S. pneumoniae showed high resistance to all antibiotics used ranging from 100% to 50%. These finding in agreement with **Bakir and Ali (2016) and Agrawal *et al.* (2014),** they noted that *S. pneumoniae* was exhibited high resistant to Amoxicillin/Clavulanic acid, Ampicillin, and Erythromycin 100% and 57.14%. On the otherwise, **Bakir and Ali (2016)** reported 100% sensitivity to Ciprofloxacin. Whereas, **Agrawal *et al.* (2014)** reported that Ciprofloxacin resistance was 57.14%.

One reason in antibiotics treatment failure is β-lactamase producing bacteria in polymicrobial infections. They have a direct pathogenic impact in causing the infection as well as an indirect effect through their abilities to produce the enzyme β-lactamase and protect other penicillin-susceptible bacteria from penicillin by releasing the free enzyme into their environment (**Brook, 2009**). The problem of bacterial resistance to commonly used antibiotics was apparent among Gram-positive isolates of the current study. The problem of bacterial resistance to commonly used antibiotics is worldwide. Antimicrobial resistance is projected to reach epidemic proportions on a global scale by 2050, accounting for 10 million deaths **(Sugden*et al.*, 2016)**.

Table (1), the antibiotic resistance of isolated pathogenic microorganisms against the used 8 antibiotics with different disc potency, was determined by the disc diffusion method.

No. of bacterial isolate	Ampicillin\Sulbactam SAM	Amoxicillin\Clavulanic acid AMC	Cefadroxil CFR	Cephalexin CL	Cephradine CE	Erythromycin E	Ofloxacin OFX	Ciprofloxacin CIP
Staph. aureus (16)*	(14)* 87%	(13)* 81.25%	(10)* 62.5%	(10)* 62.5%	(8)* 50%	(8)* 43.75%	(8)* 50%	(8)* 50%
S. pyogenes (14)*	(13)* 92.85%	(12)* 85.71%	(10)* 71.42%	(10)* 71.42%	(12)* 85.71%	(12)* 85.71%	(8)* 57.14%	(10)* 71.42%
S. pneumoniae (4)*	(4)* 100%	(4)* 100%	(3)* 75%	(3)* 75%	(2)* 50%	(4)* 100%	(2)* 50%	(4)* 100%
S. australis (6)*	0%	0%	0%	0%	0%	0%	0%	0%

-*refer to the total number of isolates.

Conclusions

The current study indicated that there is a considerable rate of bacterial tonsillitis and prevalence of multidrug-resistant bacteria among children in Egypt. The problem of bacterial resistance to commonly used antibiotics was apparent among Gram-positive isolates of the current study. *Staphaureus* was the commonest bacterial isolate (40%) followed by *S. pyogenes* (35%), *S. australis* (15%), and *S. pneumoniae* (10%). ß hemolytic Streptococci (BHS) is a significant cause of pharyngitis and tonsillitis in our environment and therefore poses a potential danger of rheumatic fever and rheumatic heart disease, non-suppurative sequelae of BHS. Therefore, investigations are needed for antibacterial agents that offerbroad-spectrum activity against resistant strains of microorganisms.

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