Antibiotic susceptibility of *Streptococcus pyogenes* and *Staphylococcus aureus* isolated from Pharyngitis and Tonsillitis patients in Nasiriyah City, Iraq

Baidaa Rasoul Dakhil* and Saad Salman Hamim**

* Biology Department-College of Education for Pure Sciences-University of Thi-Qar, Iraq  
** Pathological analysis Department- College of Sciences- University of Thi-Qar, Iraq

**ABSTRACT**

The present study aimed to isolation and identification and antibiotic susceptibility test for *Streptococcus pyogenes* and *Staphylococcus aureus* bacteria isolated from Pharyngitis and Tonsillitis patients. 276 swabs were collected from the patients aged from 10 to 70 years in Nasiriyah City during the period from November 2014 to May 2015. 96 isolates showed positive culture for both Strep. pyogenes and Staph. aureus, divided to 34(35.42%) and 62(64.58%) for Strep. pyogenes and Staph. aureus, respectively. All Strep. pyogenes isolates were sensitive to both Vancomycin and Ceftriaxon. While, completely resistance to Ampicillin and Amikacin. On the other hand, sensitivity of Staph. aureus isolates were 100% to both Clindamycin and Amikacin. While, resistance to Ampicillin and Augmentin (Amoxicillin/Clavulanic acid) with a percentages (100%) for both.

**Keywords:** Pharyngitis, Group A *Streptococcus*, *Staph. aureus*, Tonsillitis, Antibiotic susceptibility

**INTRODUCTION**

Acute Pharyngitis/tonsillitis, characterized by inflammation of posterior pharynx and tonsils, is a common condition observed in outpatients seeking healthcare provision. Its main symptoms are sore throat and fever. When inflamed, tonsils become swollen and red with a grayish or yellowish coating on its surface. Several viruses and bacteria can cause acute Pharyngitis and Tonsillitis in children and adults. Viruses cause approximately 75% of Pharyngitis cases, *Staph. aureus* and *Strep. pyogenes* are the most common bacteria that cause Tonsillitis (Kocaturk et al., 2003; Logathan et al., 2006; Wessels, 2011; Low, 2012). *Streptococcus pyogenes* Lancefield group A β-hemolytic Streptococci, is capable of causing a wide variety of diseases, such as Scarlet fever, Pharyngitis, Impetigo, Acute rheumatic fever (ARF), Rheumatic heart disease (RHD), Glomerulonephritis, Necrotizing fasciitis and Toxic shock syndrome (Carapetis et al., 2005). GABHS Pharyngitis is most common in individuals aged 5-15 years, although adults may also acquire the disease (Tart et al., 2007; Alter et al., 2011). GAS produces a wide variety of virulence factors, which allowed the evasion of the host immune response. The cell wall associated M protein is a major virulence determinant of GAS because it provides resistance to phagocytosis (Hondorp and McIver, 2007). *Staphylococcus aureus* (including Methicillin Resistant *Staphylococcus aureus* MRSA) can able to cause a large diversity of both benign and lethal infections in humans and animals because of a wide range of virulence factors that include various toxins and enzymes. It also has a major impact as a causative agent of a variety of serious infections including those of the skin and soft tissue (Bal and Gould, 2005; Brook and Foote, 2006; Miller and Kaplan, 2009). Because most acute Pharyngitis cases are caused by viruses, antibiotic treatment is completely useless. Antibiotics help to reduce the severity and duration of symptoms and limit disease spread (Wessels, 2011; Nakhoul and Hickner, 2013). Narrow-spectrum penicillins are the first choice for treatment because of the rarity of documented resistance to penicillin by group A *Streptococcus* during Pharyngitis treatment and because of their low cost (Chiappini et al., 2011; Roggen et al., 2013). A recent literature review shows that the administration of new generation antibiotics such as Azithromycin for 3-6 days has comparable efficacy as the standard 10-day course of oral penicillin (Wessels, 2011; Altamimi et al., 2013). Some experts recommend combination therapy...
with a penicillinase-resistant Penicillin or Cephalosporin (in case the organism is Methicillin-sensitive Staph. aureus [MSSA]) and Clindamycin or a quinolone. As data accumulate, Clindamycin may become the preferred outpatient antibiotic therapy (compared with TMP-SMX) in regions with a relatively low incidence of Clindamycin resistance (Elliott et al., 2009; Lee et al., 2011; Williams et al., 2011).

**Materials and Methods**

**Samples Collection:** Two hundred and seventy six swabs were collected from patients infected with Pharyngitis and Tonsillitis whom admitted to ENT department in Al–Habboby Teaching Hospital in Nasiriyah City, Thi-Qar province, Iraq during the period from November 2014 to May 2015. Swabs were collected from patients by disposable transport media and directly transported to laboratory for diagnosis.

**Identification of Strep. pyogenes and Staph. aureus:** Strep. pyogenes and Staph. aureus were identified by culture on Blood agar and Mannitol salt agar media, then incubated at 37 °C for 24-48 hr. Biochemical tests were done according to (Maclaffin, 2000). Identification of Strep. pyogenes was performed by colony morphology, β-haemolysis on blood agar, Bacitracin susceptibility. Identification was confirmed by using API 20 Strept. and API 20 Staph. tests.

**Mast Strep. Kit:** This test was done for differentiation between of Strep. pyogenes (group A Streptococci) and others groups to Streptococci. It’s a rapid slide latex agglutination test that was performed according to the instruction of the manufacturing company (Mast/United Kingdom).

**Antibiotics Susceptibility Test:** This test was done by using Amikacin (30mg), Vancomycin (30mg), Ampicillin (10mg), Augmentin (Amoxicillin/Clavuacnic acid) (30mg), Clindamycin (2mg), Tetracyclin (30mg), Methicillin (10mg), Cloramphenicol (30mg), Pencilin (10U.), Rifaxmin (5mg), Ceftriaxon (30mg), Azthromycin (15mg) and Gentamycin (10mg). The test was done by agar disc diffusion method as described by (Kirby and Bauer, 1966).

**Results and Discussion**

Two hundred and seventy six swabs (included 65 Pharyngitis and 211 Tonsillitis) were collected from 142 males and 134 females infected with Pharyngitis and Tonsillitis. The results of the present study showed that no significant difference according to gender distribution (P>0.05) (Table 1). The basic function of tonsils and adenoids is to help the body to build up immunity against infectious organisms entering into the body through the mouth or nose. They protect the throat and lungs from infection. The tonsils work as a filter which fights and protects the entire human system against the foreign organisms (Loganathan et al., 2006). The results of the present study were agreed with Al-Tamimi, (2013) in Diyala province, Iraq founded that most of the patients had Tonsillitis followed by Pharyngitis. Babaiwa et al., (2013) whom founded that males were represented 53%. While, results of Alasil et al., (2011) and Elsherif et al., (2011) recorded that adults females patients were the most dominant in recurrent/chronic tonsillitis.

Table 1: Distribution of Pharyngitis and Tonsillitis according to gender.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
<th>Total (%)</th>
<th>χ² (Cal.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharyngitis</td>
<td>Male</td>
<td>35 (53.85%)</td>
<td>30 (46.15%)</td>
<td>65 (100%)</td>
<td>0.385</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>30 (46.15%)</td>
<td>30 (46.15%)</td>
<td>60 (100%)</td>
<td></td>
</tr>
<tr>
<td>Tonsillitis</td>
<td>Male</td>
<td>107 (50.71%)</td>
<td>104 (49.29%)</td>
<td>211 (100%)</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>104 (49.29%)</td>
<td>104 (49.29%)</td>
<td>208 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Male</td>
<td>142 (100%)</td>
<td>134 (100%)</td>
<td>276 (100%)</td>
<td>0.232</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>134 (100%)</td>
<td>134 (100%)</td>
<td>268 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

df: 1, α: 0.05, Tab. χ²: 3.84
Also, the results showed that age group of <10 years recorded the highest infection rate compared with other age groups with 70 (25%) cases. Also, Urban areas recorded the highest infection rate in comparison to Rural areas. There was a statistically differences according to age and residence (P≤ 0.05) (Figures 1 and 2). The high infection rates in Urban can be explained by the overpopulation in Urban areas. In addition to, lack of interest in general hygiene or frequent waste places nearby residential areas compared with Rural areas. These results of the present study were agreed with Al-Aawaadi, (2014) whom reported that age group of (1-10 years) recorded the highest infection rates with 68 patients (28.94%) and Elsherif et al., (2011) mentioned that the most infections rates was in the age group of (2-9 years). Whereas, the results were disagreed with Agrawal et al., (2014) who recorded that age group of (11-20 years) was the most affected with Tonsillitis and recorded 57 (40.72%) cases.

Bacterial isolation: After culture, Microscopic, biochemical and serological tests were performed. 96 isolates were identified, which included 34 (35.42%) isolates of Strep. pyogenes divided to 21 (61.76%) and 13 (38.24%) isolated from Pharyngitis and Tonsillitis, respectively. On the other hand, 62 (64.58%) of Staph. aureus was isolated that showed the highest rate in Tonsillitis with 60 (96.77%) isolates, while in Pharyngitis recorded 2 (3.23%) isolates. There was a statistically difference between diseases according to isolated bacteria (P≤0.05) (Table 2).
Table (2): Distribution of isolated bacteria in both diseases.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Disease</th>
<th>Pharyngitis</th>
<th>Tonsillitis</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strep. pyogenes</td>
<td>Pharyngitis</td>
<td>21</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>(61.76%)</td>
<td></td>
<td>(38.24%)</td>
<td>(35.42%)</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>Tonsillitis</td>
<td>2</td>
<td>60</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>(3.23%)</td>
<td></td>
<td>(96.77%)</td>
<td>(64.58%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>23</td>
<td>73</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>(23.96%)</td>
<td></td>
<td>(76.04%)</td>
<td>(100%)</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td>$\chi^2$ (Cal.)</td>
<td>15.69</td>
<td>30.26</td>
<td>45.95</td>
</tr>
</tbody>
</table>

df: 1, p value: 0.05, $\chi^2$ (Tab.): 3.84

Strep. pyogenes (Lancefield group A β-hemolytic Streptococci) is the only agent that requires an etiologic diagnosis and specific treatment (Wessels, 2011; Low, 2012). Staph. aureus is one of the most common and most virulent causers of Tonsillitis, is can live as a commensal organism on the skin and in the nose and throat. Approximately 30% of healthy people are asymptomatically colonized by Staph. aureus (Chambers, 2001). Babaiwa et al., (2013) demonstrated that the Staph. aureus the principal causative agent of Tonsillitis they were found it at high percentage 70%. While, Abidali, (2014) recorded that most agents of Tonsillitis were Strep. pyogenes followed by Staph. aureus with a percentages of 20.2% and 19.3%, respectively. Agrawal et al., (2014) reported that 11.43% of Staph. aureus isolates from Tonsillitis patients. All of 34 Strep. pyogenes isolates were used for susceptibility test to 12 antibiotic drugs that used in the present study. As showed in (Fig. 3), the present results showed that all isolates were completely sensitive to both Vancomycin and Ceftiraxone with a percentages of (100%).

Vancomycin is a second-line drugs for the treatment of bacterial infections caused by Streptococci, is consists of complex tricyclic glycopeptides, which work the inhibition of bacterial cell wall synthesis by link with the terminal carboxyl group on D-alanine of glycopeptide in the bacterial cell wall, which leads to growth impaired of peptide chain and inhibition growth of bacterial cell (Shahen and Shahen, 2008). These results were similar to the results were recorded by Bahman et al., (2011) and Ksia et al., (2012) whom founded that sensitivity of Strep. pyogenes isolates to Vancomycin was 100%. Kedo and Al-Amori, (2012) reported that 78% of isolates were sensitive to Ceftriaxone. While, showed completely resistance to both Ampicillin and Amikacin. Resistance may be caused by the production of enzymes ANT (4')-1, in addition to, reducing the accumulation inside vital to antibiotic either change the permeability of the outer membrane of the cell or owning effective payment system in gram positive and negative bacteria. Amikacin more resistant to enzymes encoded to plasmids which mediates resistance for each of Streptomycin and Gentamycin (Katzung, 2001). Al-Tamimi, (2013) founded that all of isolates were resistance to Ampicillin. Also Hassan et al., (2010) mentioned that 88% and 60% of isolates were resistance to Ampicillin and Amikacin, respectively. The present study results for antibiotic sensitivity test to 62 Staph. aureus isolates as in (Fig.4), showed that all of isolates were sensitive to Clindamycin and Amikacin. Alasil, (2013) and Al-Awadi, (2014) reported that 100% of isolates were sensitive to Amikacin. The present study results demonstrates that all Staph. aureus isolates were completely resistance to Ampicillin and Agumentin (Amoxicillin/ Clavulanic acid). These results were similar to the results of many previous studies as study done by Babaiwa et al., (2013) whom founded that all of Staph. aureus isolates were resistance to Agumentin. Al-Hamdani and Hamad, (2012) reported that 100% of isolates were resistance to Ampicillin. One of main mechanisms by which microorganisms exhibit resistance to antimicrobials is: alteration of metabolic pathway: for example, some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides, instead, like mammalian cells, they turn to using preformed folic acid. Reduced drug accumulation: by decreasing drug permeability or increasing active efflux (pumping out) of the drugs across the cell surface (Li and Nikaido, 2009).
REFERENCES


