Antimicrobial susceptibility of clinical *Staphylococcus aureus* isolated from patients with an ear infection in Misan

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ABSTRACT

The present study was targeted to examine the prevalence of multi-drug resistant *Staphylococcus aureus*, which has been carried out in Misan, Iraq at a local hospital from February 2016 to January 2017. A hundred and eighty ear swabs have been obtained from patients with ear infections with or without discharges. Cultivating and identifying the causative agents, as well as the antibiotic sensitivity profile, have been done on the specimens. Swabs were collected under sterile conditions and instantly transferred to the laboratory sealed in brain heart broth tubes. The initial isolation was done on selective media to *S. aureus* (mannitol salt agar) at a temperature of 37°C for 24 - 48 hours and then the biochemical tests and identification were done in accordance with the standard monotonous techniques. Antibiotic susceptibility tests were done by the disk diffusion method. A hundred and forty-four isolates diagnosed with *Staphylococcus aureus* and eighteen isolates as other bacteria. *S. aureus* isolates tested for antibiotic susceptibility showed high resistance to ampicillin, carbenicillin and amoxicillin, mild resistance to co-trimoxazole and were susceptible to norfloxacin, rifampicin, and ciprofloxacin. Additionally, *S. aureus* isolates showed multiple antibiotic resistance (MAR). The MAR index of the isolates found to range between 0.35 and 0.7. In conclusion, an ear infection is mostly caused by *Staphylococcus aureus* and most of these isolates showed a high level of antibiotics resistance, which eventually may lead to too many health-related consequences in Misan, Southern Iraq and expose the needs for further studies to lessen the resistance to antibiotics.

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INTRODUCTION

Ear infection can be defined as an ear inflammation which is associated with ear discharge as one of the most common signs of ear infection (De et al., 2002). Around the world, as reported by the World Health Organization (WHO), approximately 300 million people develop an ear infection and around two-thirds of them had a significant impairment to the hearing ability. Other complications of ear infection have also been reported such as facial nerve paralysis, meningitis, brain abscess and even death which is estimated to reach 28000 deaths per year (Acuin and Organization, 2004). The health and economic consequences of ear infection are severe in Africa and other developing nations where the prevalence of the disease is reported to be approaching 11% (Akinpelu et al., 2008). Regardless of age, an ear infection is frequently diagnosed with different percentages in various countries (Bluestone and Klein, 2007). According to the survey conducted by WHO, countries can be categorized as those with a low prevalence of ear infection (1-2%) and countries with high prevalence...
rate (3-6%) (Acuin and Organization, 2004). Considering the etiological microorganisms, an ear infection is mainly caused by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Haemophilus influenza*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Escherichia coli* with different prevalence in different countries (Mittal et al., 2015). One of the most frequently isolated bacteria among patients with an ear infection is *S. aureus*, and it has been shown to be the responsible for more than 50% of nosocomial ear infections (Iseh and Adegbite, 2004, Wolter et al., 2008). Nasal carriage of *S. aureus* has been identified as a risk factor for community-acquired as well as nosocomial infections (Cole et al., 2001).

One of the severe problems associated with staphylococcal ear infections is treatment as there is an increase in the incidence of resistance to several antibiotics among these bacteria (Alghaithy et al., 2000). Throughout the world, MAR *S. aureus* strains have been isolated including strains that were resistant to methicillin, aminoglycosides, macrolides, quinolones, and even to a combination of those antibiotics (Von Eiff et al., 2001). Accordingly, our scope in this study was to determine the local prevalence of s. aureus as a causative agent for an ear infection and to study the antibiotic sensitivity trends among this bacteria.

**MATERIALS AND METHODS**

**Study Design**

A retrospective study was performed at Al- sadder teaching hospital over the period from February 2016 till January 2017 in Misan city in the south of Iraq.

**Culture and Identification**

A hundred and eighty patients were included in this study. The ear, nose and throat specialist supervised the diagnosis of ear infection. The microbiological study includes culture and identification of causative bacteria and antibiotic susceptibility tests. The collected samples were taken under the sterile condition and then transferred to the lab by brain heart broth tubes. *Staphylococcus aureus* was primarily isolated on specific media (mannitol salt agar) at 37°C for 24 - 48 hours and subsequently the identification and the biochemical tests were performed in accordance with the standard routine techniques (Cole et al., 2001). Well, isolated colonies were taken up and stored at 4°C in nutrient agar slant. Single colony were picked from the stored isolation cultures to perform the pure culture.

**Media and Culture Conditions**

Nutrient agar, blood agar, nutrient broth, mannitol salt agar, Muller Hinton agar (Hi-media, India). Brain-Heart infusion broth, and Urease based agar. Sterilized by autoclave 121 under 15 lbs pressure for 15 min. Bacterial culture was done at 37°C.

**Antimicrobial Susceptibility Testing**

Susceptibility tests to antibiotics were performed on Mueller-Hinton agar using disk diffusion method as explained by Kirby Bauer (Von Eiff et al., 2001) and the used antibiotics discs shown in the table (1).

**RESULTS**

A total of 180 swabs obtained from patients with an ear infection. Bacteria found to be the cause of infection in 162 cases (90%). The bacterial isolates were then grown on mannitol agar plates and the hemolytic test was done on blood agar. Gram staining and catalase test also were performed to spot the gram-positive, β-hemolytic and catalase positive strains. Based on the cultural characteristics and colony morphology on blood agar, the colonies were circular, and it was found microscopically that the microorganisms were coordinated in pairs or short chains and clusters, exhibited golden yellow pigment on nutrient agar, and the isolates were coagulase, β-Galactosidase and DNase positive. These characteristics were found in 144 (88.88%) isolates and thus were identified as *Staphylococcus aureus* as shown in Table. 2.

![Figure 1: Multiple antibiotic resistance among *S. aureus* isolates](image)

Regarding the antibiotic susceptibility patterns which were done by the antibiotic disc diffusion method, all of the 144 *S. aureus* isolates were tested in vitro. All of the staphylococcal isolates (100%) were resistant to ampicillin, amoxicillin, and carbenicillin. On the other hand, none of the isolates demonstrated resistance to norfloxacin, ciprofloxacin and rifampicin. These antibiotics were highly effective against *S. aureus* collected during the study period, while ceftriaxone showed resistance (72.2%), cephalaxin (63.8%), tobramycin (63.8%), tetracycline (56.9%), clindamycin (55.5%), gentamicin (34.7%), oxacillin (56.9%), imipenem (43%), erythromycin (65.2%), teicoplanin (41.6%), vancomycin (34.7%), neomycin (34.7%),
Table 1: Antibiotics discs

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Concentration</th>
<th>Antibiotics</th>
<th>Concentration</th>
<th>Antibiotics</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>10 μg</td>
<td>Imipenem</td>
<td>10 μg</td>
<td>Clindamycin</td>
<td>2 μg</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>25 μg</td>
<td>Erythromycin</td>
<td>15 μg</td>
<td>Oxacillin</td>
<td>1 μg</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>100 μg</td>
<td>Tetracycline</td>
<td>30 μg</td>
<td>Kanamycin</td>
<td>30 μg</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10 μg</td>
<td>Ciprofloxacin</td>
<td>5 μg</td>
<td>Vancomycin</td>
<td>30 μg</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30 μg</td>
<td>Neomycin</td>
<td>30 μg</td>
<td>Teicoplanin</td>
<td>30 μg</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>30 μg</td>
<td>Gentamicin</td>
<td>10 μg</td>
<td>Co.trimoxazol</td>
<td>30 μg</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>10 μg</td>
<td>Rifampicin</td>
<td>5 μg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Frequency of S. aureus and other bacteria isolated from an ear infection

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>No. of Staphylococcal isolates</th>
<th>Others (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>162 (90)</td>
<td>144 (88.88)</td>
</tr>
</tbody>
</table>

Table 3: Susceptibility of clinical isolates of S. aureus to 20 different antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (AM)</td>
<td>144</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin (AX)</td>
<td>144</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Carbenicillin (PY)</td>
<td>144</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Norfloxacin (NOR)</td>
<td>0</td>
<td>72</td>
<td>50</td>
</tr>
<tr>
<td>Ceftriaxone (CRO)</td>
<td>104</td>
<td>36</td>
<td>25</td>
</tr>
<tr>
<td>Cephalaxin (CL)</td>
<td>92</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Tobramycin (TOB)</td>
<td>92</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>Imipenem (IPM)</td>
<td>62</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin (E)</td>
<td>94</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>Tetracycline (TE)</td>
<td>82</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>0</td>
<td>72</td>
<td>50</td>
</tr>
<tr>
<td>Neomycin (N)</td>
<td>50</td>
<td>64</td>
<td>44</td>
</tr>
<tr>
<td>Gentamicin (CN)</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rifampicin (RA)</td>
<td>0</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Clindamycin (DA)</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oxacillin (OX)</td>
<td>82</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>Kanamycin (K)</td>
<td>80</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Vancomycin (VA)</td>
<td>50</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Teicoplanin (TEC)</td>
<td>60</td>
<td>72</td>
<td>50</td>
</tr>
<tr>
<td>Co.trimoxazol (COT)</td>
<td>30</td>
<td>93</td>
<td>64</td>
</tr>
</tbody>
</table>

kanamycin (55.5%), and co.trimoxazol (20.8%) as shown in Table 3.

MAR was found in all S. aureus isolates. Such that, thirty-two isolates were resistant to seven antibiotics, thirty-two isolates resisted eight antibiotics, twenty isolates resisted nine antibiotics, ten isolates resisted ten antibiotics, twenty isolates resisted thirteen antibiotics, and thirty isolates resisted fourteen antibiotics as in Table 4 and Fig.1. MAR index found to be within the range 0.35-0.7 as presented in Table 5.

DISCUSSION

The present study was directed to explore the distribution and antibiotic susceptibility of S. aureus isolated from patients with an ear infection, one of the most commonly encountered infection in health practices (Roland, 2002, PV Hirapure, 2014). In this study, Staphylococcus aureus isolated in about 88 % of the samples collected from patients with an ear infection. This finding is in agreement with other studies that showed Staphylococcus aureus as the most common cause of ear infection followed by Pseudomonas aeruginosa (Khalil et al., 2013, Ahmad, 2013, Kumar et al., 2014, Shetty and Shetty, 2014). However, it contradicts other studies that reported Pseudomonas aeruginosa as the most frequent causative bacteria followed by Staphylococcus aureus ( Sharma et al., 2004, Madana et al., 2011, Ahn et al., 2012). The geographical differences might explain this contradiction in the results as these studies were performed in different countries (Mittal et al., 2015).

Additionally, the susceptibility of S. aureus isolated from this study patients was tested against twenty different antibiotics. Resistance rates were highly variable among the antibiotic tested. All the tested isolates demonstrated resistance to ampicillin, amoxicillin and carbenicillin, while none of the isolates resisted ciprofloxacin, norfloxacain and rifampicin. Similarly, other studies have reported such
high resistance to ampicillin (Khalil et al., 2013). Additionally, studies have demonstrated high levels of susceptibility demonstrated by S. aureus to rifampicin, co-trimoxazole (Campos et al., 1995), ciprofloxacin and norfloxacin (PV Hirapure, 2014), but quinolones resistance differs in another study (Udden et al., 2018). Resistance rates for amoxicillin, tetracycline and erythromycin also found to be dangerously high as other studies have shown (PV Hirapure, 2014). Vancomycin and ceftriaxone resistance rates found to be comparable to another study performed in Ethiopia (Argaw-Denboba et al., 2016). Moreover, oxacillin and clindamycin resistances were comparable to Tadesse et al. study (Tadesse et al., 2018). Overall, the isolated S. aureus in this study showed very high resistance rates to most of the antibiotic tested indicating that further researches have to do in order to genetically determine the genes responsible for such resistance and also further multi-centre studies have to be performed to update the antibiogram and minimize the spread of the resistant strains and the associated health and cost related consequences.

Regarding MAR, all the investigated S. aureus in this study showed resistance to more than six antibiotics. A finding that is in coordination with Deyno et al. study (Deyno et al., 2017). Resistance rates to all β-lactam antibiotics tested in this study were very high and all of the isolates resisted with variable rates these antibiotics, this may be explained by the ability of S. aureus to produce β-lactamases, an enzyme that deactivates the β-lactam antibiotics (Barber and Rozwadowska-Dowzenko, 1948). Additionally, resistance to oxacillin reported in this study exposed the presence of methicillin-resistant S. aureus (MRSA), and the mechanism behind this resistance is due to the acquisition of modified penicillin-binding protein with altered affinity for β-lactams, a protein that is produced by meca gene (Hartman and Tomasz, 1981). The meca gene contains sites that accept plasmids mediating resistance to multiple non-β-lactam antibiotics (Chambers, 2001).

Moreover, vancomycin, a glycopeptide antibiotic, also found with reduced sensitivity in this study where the results showed that fifty isolates were also resistant to vancomycin reflecting the development of vancomycin-resistant strains (VRSA). VRSA developed resistant to vancomycin through modification of the cell wall and trapping of the drug within the cell wall by gaining vanA gene (Weigel et al., 2003). The development of VRSA is a health-related severe problem as it massively limits its treatment options since vancomycin is one of the few highly effective anti-staphylococcal drug with activity against MRSA (Akanbi and Mbe, 2013). Several reasons could explain the development of multi-drug resistance strains such as the ability of these strains to rapidly spread in hospitals through health-care professionals or infected persons or contaminated environments (Tadesse et al., 2018). Additionally, misuse of antibiotics has also been considered as a factor in increasing the chance of developing resistance among bacteria (Cameron et al., 2011).

**CONCLUSION**

This study exposed that ear infection is mostly caused by S. aureus. Most of the isolates demonstrated high rates of resistance to the commonly used antibiotics and there is a high prevalence of MAR strains, which eventually lead to treatment-related problems and may increase the length of hospital stay. All of isolated S. aureus found to be resistant to ampicillin, amoxicillin and carbenicillin which might be due to the irrational use of these antibiotics and none of them should be used for treating a staphylococcal ear infection. Based on the results of this study, further restriction to the use of antibiotic has to be done and all health-care professionals must strictly follow the results of culture and sensitivity in an attempt to minimize the high prevalence of antibiotic resistance. Additionally, more studies are warranted to explore the nationwide spread of antibiotic resistance among different bacterial isolates in order to update the treatment guidelines.
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REFERENCES


