NASAL CARRIAGE OF METHICILLIN RESISTANT STAPH AUREUS IN FOOD PROVIDER IN RESTURANT AT SAMARA CITY

Prof. Abdulghani Mohamed Alsamarai*, Hayder Mudheher Abbas, Qanat Mahmood Atia

1Department of Medicine, Tikrit University College of Medicine, Tikrit, Iraq.
2Department of Biology, College of Science, Tikrit University, Tikrit, Iraq.

ABSTRACT

Methicillin resistant *S. aureus* (MRSA) emerged in a few years following clinical use of methicillin. MRSA is considered as a major cause of nosocomial infections and associated with high morbidity and mortality. This study conducted to clarify the problem of MRSA in food handlers in Samara city to prevent the spread of such microorganism. The carrier rate of *S. aureus* was 28% in food handlers in Samara restaurants. Unfortunately, all the isolates were resistant to methicillin. Thus this study indicated a high rate (100%) of MRSA in food handlers, which represent a health problem with impact on health care. In conclusion, MRSA represent a health care problem in food handlers in Samara City.

KEY WORDS: *S. aureus*, MRSA, Resistance.

INTRODUCTION

Methicillin resistant *S. aureus* (MRSA) emerged in a few years following clinical use of methicillin.[1] MRSA is considered as a major cause of nosocomial infections and associated with high morbidity and mortality.[2-4] MecA gene that encodes for penicillin – binding protein mediated methicillin resistance in *Saph aureus* and subsequently lead to reduction in affinity for the betalactam antibiotics including the penicillinase-resistant penicillin, therefore methicillin resistant Staph aureus form one of the important cause of hospital infections,[5,6] with a major health impact.
Humans are the main source of the Staph aureus with prevalence of 30% to 50% of the population and the main reservoir sites are skin and nares.[7] *Staphylococci* present as normal flora in the throat, nasal area and also under the fingernails.[8] *Staphylococcus aureus* responsible for a variety of cutaneous and systemic infections and food poisoning.[9] Contaminated hands from nasal carriers are a major source of cross-contamination in the food service kitchen.[10,11] Food handlers are usually regarded as the source of staphylococcal food poisoning. MRSA emerged following the widespread use of methicillin and other semisynthetic penicillins and continue to persist in both the healthcare and community environments.[12]

MRSA who are multiple drug resistant may cause an infections that are difficult to treat and characterised with high morbidity and mortality.[13] MRSA strains have the ability to induce biofilms and this resulted in increase in their virulence.[14,15] This study conducted to clarify the problem of MRSA in food handlers in Samara city to prevent the spread of such microorganism. The study was approved by ethical committee of College of Science and informed consent taken from individuals included in the study.

**MATERIALS AND METHODS**

**Culture media**

Manitol Salt agar (Oxoid, England), Blood Base agar (Oxoid, England), Muller hinton (Oxoid, England) and Nutrient agar (Oxoid, England) were used for isolation, identification and determination of antibiotic sensitivity.

All these media were prepared according to manufacturer instructions, sterilized by autoclaving at 121°C for 15 minutes.[16] *S. aureus* strains were isolated from food handlers working in Samara city restaurants during the period from January to April 2015. They consisted of 43 isolates from 200 nasal cavities from 100 adult male workers in Samara city. One swab was taken from each nare. After sampling, swabs were immediately transferred into mannitol salt agar which considered as selective and differential medium for the isolation, purification and identification of Staphylococci, and for detecting the ability of each isolate to ferment mannitol Then transferred and streaked on the blood agar , The isolates were examined for their shape, size, colour, pigments, and haemolytic activity. All plates were incubated at 37°C for 24 hours then a single pure isolated colony was transferred to Nutrient agar medium for the preservation and to carry out other biochemical tests that confirmed the identification of isolates.
Antimicrobial Susceptibility Test\textsuperscript{[17]}

This test performed by modified Kirby-Bauer method as the following

1- From an overnight culture plate, 4-5 colonies of bacterial isolate were picked up by sterilized inoculating loop and emulsified in 5ml of sterile normal saline until the turbidity is approximately equivalent to that of the McFarland No. 0.5 turbidity standard.

2- A sterile swab was dipped into the bacterial suspension, any excess fluid was expressed against the side of the tube.

3- The surface of a Mueller-Hinton agar plate was inoculated by bacterial isolate as follows:
   The whole surface of the plate was streaked with the swab, then the plate was rotated through a 45º angle and streaked the whole surface again; finally the plate was rotated another 90º and streaked once more.

4- By a sterile forceps the antimicrobial disc was picked up and placed on the surface of the inoculated plate. The disc was pressed gently into full contact with the agar.

5- The step (4) was repeated to all antimicrobial discs under the test, spaced evenly a way from each other.

6- The plates were incubated at 35ºC for 18-24 hours.

7- After incubation, the plates were examined for the presence of inhibition zone of bacterial growth (clear rings) around the antimicrobial discs, if there was no inhibition zone the organism was reported as resistant to the antimicrobial agent in that disc. If a zone of inhibition surrounded the disc, the diameter of the zone of inhibition was measured (by millimetres) and compared their sizes with values listed in a standard chart, (Table 1):

RESULTS & DISCUSSION

Table (1): zone diameter interpretation standards\textsuperscript{[17]}

<table>
<thead>
<tr>
<th>No.</th>
<th>Antimicrobial Agents</th>
<th>Disk Potency</th>
<th>Diameter of Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>1</td>
<td>Methicillin</td>
<td>10</td>
<td>≤17</td>
</tr>
<tr>
<td>2</td>
<td>Cefotaxim</td>
<td>30</td>
<td>≤25</td>
</tr>
<tr>
<td>3</td>
<td>Trimethoprim</td>
<td>10</td>
<td>≤19</td>
</tr>
<tr>
<td>4</td>
<td>Vancomycin</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Ceftriaxone</td>
<td>30</td>
<td>≤22</td>
</tr>
<tr>
<td>6</td>
<td>Ceftazidim</td>
<td>10</td>
<td>≤16</td>
</tr>
<tr>
<td>7</td>
<td>Imipenem</td>
<td>10</td>
<td>≤13</td>
</tr>
<tr>
<td>8</td>
<td>Ciprofloxacin</td>
<td>5</td>
<td>≤15</td>
</tr>
<tr>
<td>9</td>
<td>Gentamycin</td>
<td>10</td>
<td>≤19</td>
</tr>
<tr>
<td>10</td>
<td>Oxacillin</td>
<td>10</td>
<td>≤18</td>
</tr>
<tr>
<td>11</td>
<td>Cefoxitin</td>
<td>30</td>
<td>≤23</td>
</tr>
<tr>
<td>12</td>
<td>Azthromycin</td>
<td>15</td>
<td>≤21</td>
</tr>
</tbody>
</table>
MRSA carrier rate

The carrier rate of S. aureus was 28% (28 isolates from 100 individuals), in food handlers in Samara restaurants. Unfortunately, all the isolates were resistant to methicillin, Table 2. Thus this study indicated a high rate (100%) of MRSA in food handlers, which represent a health problem with impact on health care.

Table (2): Prevalence of Staphylococcus aureus among restaurant workers

<table>
<thead>
<tr>
<th>NO.</th>
<th>Index</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>MRSA</td>
<td>28</td>
<td>28</td>
</tr>
</tbody>
</table>

From the total 200 swabs (both nares), 43 (21.5 %) of food handlers were positive for S. aureus, Table 3.

Table (3): Prevalence of Staphylococcus aureus from total samples

<table>
<thead>
<tr>
<th>NO.</th>
<th>Index</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>43</td>
<td>21.5</td>
</tr>
<tr>
<td>3</td>
<td>Nasal Right</td>
<td>28</td>
<td>65.1</td>
</tr>
<tr>
<td>4</td>
<td>Nasal left</td>
<td>15</td>
<td>34.9</td>
</tr>
</tbody>
</table>

This results agreed with many other studies, that was reported a result of (32.4%)[18], (23.4%)[19], and (29%)[20], also the present study finding was in consistent with Vanderbergh[21] who reported that the isolation of S. aureus could vary from 20 to 55% in a healthy adult population and higher than that reported by Asghar (22.4%) [22] in Makkah. and other study in the same city ( 40.3 % ).[23]

Prevalence rates of MRSA in the samara city restaurants (food handlers) in this study were 21.5 %. Higher S. aureus prevalence among food handlers, of 44.6%, 53.2% and 23.1% was noted in Botswana, Kuwait, and South-eastern Anatolia, respectively.[24]

These high results may be due to the transmission mode of S. aureus and MRSA through hands, which may become contaminated by contact with colonized or infected individuals or through contact with colonized or infected body sites of other persons. Other factors contributing to transmission include close skin to-skin contact, crowded conditions, and poor hygiene. Ordinary food handlers are subjected to medical examination before assignment to
work. However, they are mostly lacking proper training in food handling operations, mass feeding, and sanitary practices.[25]

**Antibiotic susceptibility**

This study indicated a high resistant rate of MRSA to the tested antibiotics, Table 4 and Figure 1. Unfortunately this finding may lead to increased risk of MRSA infections as a result of indiscriminate using of multiple broad spectrum antibiotic.[26] This can be attributed to the fact that, antibiotics may have revolutionized the treatment of common bacterial infections.[27] The results revealed that all bacterial isolates showed high resistance (100%), to Methicillin, Ceftazadim and Oxacillin. The resistance to Ceftriaxone in the present study was 97.68%, while it was 93.02% to Cefotaxim.

In the present study all bacterial isolates exhibited high sensitivity to imipenem (Figure1). Imipenem is a broad-spectrum antibiotic and the beta-lactum ring of these antibiotic are resistant to hydrolysis by most β-lactamases.[28] However, the result was in accordance with those results being reported before.[29] In addition, high resistance ratio was found in the present MRSA nasal isolates to Cefotaxim (93.2%), Azthromycin (83.7) , and Trimethoprim (79.06%). The high resistance rate of MRSA in the nasal of food handlers to Trimethoprim is of clinical importance and with health hazard impact since this agent is recommended for treatment of such isolate infections. Furthermore, the MRSA isolates rate of resistance to Gentamycin was (25.58%), however, a high rate of intermediate sensitivity (69.7%) was demonstrated in this study, which are prone to become resistant to gentamicin. MRSA clinical isolates from the food handlers were with low resistance rate to Ciprofloxacin (6.97%).

**Table (5): The Susceptibility of isolates to antimicrobial agents.**

<table>
<thead>
<tr>
<th>No</th>
<th>Antimicrobial discs</th>
<th>Sensitive %</th>
<th>Intermediate %</th>
<th>Resistant %</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methicillin</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>Cefotaxim</td>
<td>0</td>
<td>6.98</td>
<td>93.02</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>Trimethoprim</td>
<td>2.32</td>
<td>18.62</td>
<td>79.06</td>
<td>34</td>
</tr>
<tr>
<td>4</td>
<td>Vancomycin</td>
<td>81.39</td>
<td>18.61</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>Ceftriaxone</td>
<td>0</td>
<td>2.32</td>
<td>97.68</td>
<td>42</td>
</tr>
<tr>
<td>6</td>
<td>Ceftazadim</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>43</td>
</tr>
<tr>
<td>7</td>
<td>Imipenem</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>8</td>
<td>Ciprofloxacin</td>
<td>90.39</td>
<td>2.33</td>
<td>6.97</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>Gentamycin</td>
<td>4.66</td>
<td>69.76</td>
<td>25.58</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>Oxacillin</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>43</td>
</tr>
</tbody>
</table>
This study indicated that multidrug resistance in MRSA clinical isolates from food handlers is very common and this represent a health problem since the infections that are caused by such strains is difficult to treat. Our MRSA cohort were demonstrated one isolate that was resistant to 5 antibiotics, two isolate were resistances to 6 antibiotics, and the remaining isolates were resistance to (7-10) antibiotics.

All isolates (100%) were resistant to Penicillin group antibiotic and this result was agreed with many others studies that demonstrated the increasing in the rate of Penicillin resistance in all Staphylococci isolates from restaurant workers specially in the Methicillin resistance strains. This result was agreed with the local studies who showed that Penicillin resistance rates in the S. aureus were (90.5%)\textsuperscript{30}, (97.5%)\textsuperscript{31} and (87.8%).\textsuperscript{32}

This increased in the Penicillin resistance isolates among Staphylococci strains can be explained in most cases to the production of β-lactamase enzyme that destroyed the β-lactam ring and inactivated the Penicillin antibiotic and this enzyme was encoded by plasmid that easy to transfer among strains.\textsuperscript{33} The carrier state of MRSA and their multidrug resistance in food handlers as this study indicated must considered as problem with health impact. Intervention treatment to such carrier state is warranted.

In conclusion, MRSA represent a health care problem in food handlers in Samara City.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Antibiotics} & \textbf{Cefoxitin} & \textbf{Azthromycin} & \textbf{Mean} & \textbf{SD} & \textbf{Min} & \textbf{Max} & \\
\hline
\textbf{Rate} & 0 & 0 & 2.32 & 1 & 97.68 & 42 & 43 \\
\hline
\end{tabular}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure1.png}
\caption{The Susceptibility of isolates to antimicrobial agents.}
\end{figure}
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