ISOLATION AND IDENTIFICATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) FROM POULTRY MEAT AND POULTRY PRODUCTS.

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ABSTRACT

A total of 500 different samples were collected from poultry meat, poultry products and their human contacts in abattoirs from different Egyptian Governorates (Damietta, Dakahlia, Giza and Cairo) in 2012 and 2013. The Samples were tested for presence of S. aureus by isolation and biochemical identification, the results revealed that coagulase positive S. aureus was present in 83 samples (61.03%). The isolated strains of coagulase positive S. aureus were tested for antimicrobial sensitivity. Most strains were resistant to penicillin and ampicillin, less resistance was appeared to amoxicillin, oxacillin, Methicillin, and ceftriaxone, while all strains were sensitive to, amoxicillin clavulanic acid and vancomycin. Using PCR technique, amplification of 310 bp fragment of mecA gene from the extracted DNA of all isolated coagulase positive S. aureus strains isolated from different chicken samples and human contacts resulted in 5 samples from muscles and skin were positive. Using sequencing technique to mec A gene in three positive strains, the Phylogenetic analysis of mecA gene of these isolates were clustered together and little away from other published isolates of MRSA, Amino acid identities were 99.5% among the analyzed isolates, The three isolates shared 87.2%-
Isolation And Identification Of Methicillin-Resistant …

Salwa M. Helmy et., al.

89.2% identity with other Staphylococcus aureus isolates.

INTRODUCTION

Poultry meat is a common vehicle of food borne illness, *S.aureus* usually being one of the causes of outbreaks involving large number of peoples (*Geornaras and Von Holy, 2001*). Methicillin-resistant *S. aureus* (MRSA) included those strains that had acquired a gene giving them resistance to methicillin and essentially all other beta-lactam antibiotics. Soon after methicillin was introduced into human medicine to treat penicillin resistant staphylococci, this group of organisms had since emerged as a serious concern in human medicine. MRSA was first reported as a nosocomial pathogen in human hospitals. Although these organisms caused the same types of infections as other *S. aureus*, hospital-associated strains have become resistant to most common antibiotics, and treatment can be challenged (*Fitzgerald et al. ,2001*). The *mecA* gene which conferred resistance to methicillin encoded a variant penicillin-binding protein, PBP2a. Native PBP2 catalysed a key step in the synthesis of the bacterial peptidoglycan cell wall, and was bound and inactivated by penicillin-type antibiotics including meticillin. PBP2a was not inhibited by penicillins and could function instead of PBP2 (*Pinhoet et al.,2001*). *S.aureus* had developed resistance to most classes of antimicrobial agents. Penicillin was the first choice of antibiotics to treat staphylococcal infection. In 1944, by destroying the penicillin by penicillinase, *S.aureus* becomes resistant. More than 90% *S.aureus* strains were resistant to penicillin. However, methicillin, semi synthetic penicillin, was used to treat Penicillin Resistant *S. aureus* but resistance finally emerged. MRSA is mediated by the presence of PBP-2a which is expressed by an exogenous gene, *mecA* (*Livermore, 2001*). In Japan
however, an MRSA strain of human origin isolated from raw chicken samples appeared capable of producing enterotoxin C (Kitai et al., 2005).

**MATERIAL AND METHODS**

1. **Material:**

1.1. **Samples:**

A total of 500 different samples were collected from poultry meat, poultry products and its human contacts of poultry in abattoirs from different Egyptian Governorates (Damietta, Dakahlia, Giza and Cairo) in 2012 and 2013. All samples used were collected under aseptic condition and safety precautions. (Rodgers et al., 1999)

1.2. **Preparation of samples:**

Muscle and skin samples were collected from chicken's carcasses using sterile scissors and forceps and under aseptic condition. Each sample was collected then marked and placed in an ice box and transferred to the laboratory as soon as possible. And Cloacal swabs were collected from cloacae of living poultry by using sterile cotton swabs contain 2 ml saline.

2. **Methods:**

Isolation and identification of *Staphylococcus aureus* was done according to (Sneath et al., 1986).
Disk diffusion method applied according to (Koneman et al., 1979) Amplification of mecA gene from DNA of Staphylococcus aureus isolates was done according to (Spanu et al., 2003).

RESULTS

Table (1): Isolation rate of Staphylococcus aureus from different samples of poultry and its products

<table>
<thead>
<tr>
<th></th>
<th>No. of tested samples</th>
<th>positive</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Muscles &amp; skin</td>
<td>100</td>
<td>46</td>
<td>54</td>
</tr>
<tr>
<td>Cloacal swabs</td>
<td>100</td>
<td>32</td>
<td>68</td>
</tr>
<tr>
<td>Burger products</td>
<td>23</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>Pane products</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Human samples</td>
<td>200</td>
<td>54</td>
<td>146</td>
</tr>
<tr>
<td>total</td>
<td>500</td>
<td>136</td>
<td>364</td>
</tr>
</tbody>
</table>

Table (2): percentages of coagulase positive Staphylococcus aureus obtained from different samples of poultry and its products

<table>
<thead>
<tr>
<th></th>
<th>No. of tested samples</th>
<th>positive</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Muscles &amp; skin</td>
<td>46</td>
<td>63.04</td>
<td>17</td>
</tr>
<tr>
<td>Cloacal swabs</td>
<td>32</td>
<td>43.75</td>
<td>18</td>
</tr>
<tr>
<td>Burger products</td>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Pane products</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Human samples</td>
<td>54</td>
<td>68.52</td>
<td>17</td>
</tr>
<tr>
<td>total</td>
<td>136</td>
<td>61.03</td>
<td>53</td>
</tr>
</tbody>
</table>

Table (3): Interpretation of antimicrobial sensitivity testing for all coagulase positive S. aureus isolates.

<table>
<thead>
<tr>
<th>Antimicrobial disk</th>
<th>Antibiotic sensitivity of Coagulase positive S. aureus</th>
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<tbody>
<tr>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>P</td>
<td>64</td>
</tr>
<tr>
<td>MET</td>
<td>16</td>
</tr>
<tr>
<td>OX</td>
<td>25</td>
</tr>
<tr>
<td>AMP</td>
<td>61</td>
</tr>
<tr>
<td>AMOXY</td>
<td>23</td>
</tr>
</tbody>
</table>
Isolation And Identification Of Methicillin-Resistant ...  Salwa M. Helmy et., al.

<table>
<thead>
<tr>
<th></th>
<th>AM+CL</th>
<th>VA</th>
<th>KF</th>
<th>CRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM+CL</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VA</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>KF</td>
<td>7</td>
<td>8.43</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>CRO</td>
<td>6</td>
<td>7.2</td>
<td>34</td>
<td>0</td>
</tr>
</tbody>
</table>

(\(P\) (penicillin), \(MET\) (methicillin), \(OX\) (oxacillin), \(AMP\) (ampicillin), \(AMOXY\) (amoxicillin), \(AM+CL\) (amoxicillin + clavulanic acid), \(VA\) (vancomycin), \(KF\) (cephalothin), and \(CRO\) (ceftriaxone),}

**Amplification of 310 bp fragment of mecA gene from the extracted DNA of all isolated coagulase positive \(S.aureus\) strains**

**Photo (1):** showed the agarose gel electrophoresis with positive PCR amplification of 310 bp fragment of mecA gene from DNA of coagulase positive \(S. aureus\) isolates from different samples. Lanes (5, 6, 7, 8).

**Lanes (1, 2, 3):** Negative samples

**Lane 4:** the DNA molecular weight marker (Gelpilot 100bp ladder).

**Lane 9:** positive control
**Isolation And Identification Of Methicillin-Resistant ...**

**Salwa M. Helmy et., al.**

**Lane 10: negative control**

Purified and sequenced *mecA* gene of three isolates were revealed 87.2%-89.2% identity with other *Staphylococcus aureus* isolates.

### DISCUSSION

Today, poultry meat industry has become the predominant source of protein from meat in the diet of the population of most developing countries (*Robert, 1990*). But during conventional slaughter procedures and further processing, microorganisms are introduced into and onto carcasses (*Holder et al., 1997*).
During processing, contamination of carcasses with *S. aureus* increased to > $10^3$ CFU/g of skin. Plucking and evisceration appeared to be the main stages at which contamination of carcasses with *S. aureus* occurred (Notermans et al., 1982).

In the present work, all *S. aureus* isolates showed yellow colonies surrounded by yellow halo on mannitol salt agar that agree with *Howard and Kloss, 1993* who mentioned that the selectivity of MSA is based on its high concentrations of salt. Also after 18 to 24 hrs incubation, Staphylococci appear as opaque, smooth, circular colonies with a butyrous consistency in blood agar.

In this study, Table (1) showed that the total prevalence of coagulase positive *S. aureus* from chickens and human contacts samples were 61.03 % of the samples (83/136), while, 38.97 % were coagulase negative Staphylococci (53/136).

Out of 46 muscles and skin samples, 29 samples were coagulase positive with the percentage of 63.04%, that nearly agree with the results of *Kawano et al. (1996)* who isolated *S. aureus* strains from the skin of 103 chickens with percentage of 79.2% of the 130 chickens.

Regarding to the current study 32 cloacal swabs subjected for isolation of *S. aureus*, The overall isolated coagulase positive *S. aureus* was 14 with the percentage of 43.75 %, On the other hands *Kinsman et al. (1981)* concluded that the natural populations of *S. aureus* found on the skin and respiratory tract of healthy birds were probably contributing to the source of infection when the clinical disease develops.

Studying of 83 strains of coagulase positive *S. aureus* against 9 antimicrobial discs revealed different degree of sensitivity, those results
coincide with many authors as Gardiniet al. (2003) who found that Staphylococci were generally susceptible to beta-lactams, but 12 strains were resistant to methicillin, 8 were resistant to oxacillin, and 9 were resistant to penicillin G.

Archer and Niemeyer (1994) determined that The S. aureus had acquired a gene (mecA) coding for the altered penicillin-binding protein 2A, allowing the organism to grow in the presence not only of methicillin but also all new β-lactams. While Strommengeret al. (2006) confirmed that all isolated S.aureus that carrying the mecA gene mediated resistance to β-lactam antibiotics.

Few studies were planned for detection of mecA among chickens (Perez-Roth et al., 2001). In present study 5 from 83 samples were containing mecA gene which are lower than that recorded by Lee (2003) who found only three (10%) from chickens (6%).

There are also concerns about MRSA as a possible zoonosis. Both human-to-animal and animal-to-human transmission are known to be possible; however, it has not yet been determined whether animals are an important primary source of MRSA infections for humans, or if most animals are colonized after contact with human carriers (Baptiste et al., 2005; Duquette and Nuttall, 2004; Weese et al., 2006). In contrary, some authors conclude that, currently the risk to human health from zoonotic MRSA seems to be very small (Duquette and Nuttall, 2004).

the Phylogenetic analysis of mecA gene of three Staphylococcus aureus isolates with other staph isolates based on amino acid sequence showed that all isolates under study clustered together and little away from other published isolates of MRSA, Amino acid identities is 99.5% among the analyzed isolates, The three isolates shared 87.2%-89.2%
identity with other *Staphylococcus aureus* isolates. Nucleotide similarity report of 195 base and amino acid similarity report of 66 amino acids of three isolates with other reference staph isolates showed that Sequenced part of the mecA gene showing partial homology to other *Staphylococcus aureus* strains.

REFERENCES


- **Pinho M.G., Filipe S.R., de Lencastre H. and Tomasz A. (2001):** Complementation of the essential peptidoglycan transpeptidase function of penicillin-binding protein 2 (PBP2) by the drug resistance


Salwa M. Helmy et al.

Isolation And Identification Of Methicillin-Resistant ... Salwa M. Helmy et., al.

تفاعل البممرة المتسلسل 10.9% (5/46) عينة إيجابية من عينات الجلد والعضلات من عينات الدجاج. بينما العينات إيجابية التخثر للميكروب العقدي الذهبي المعزولة من مسحات المجمع ومنتجات الدواجن ومن العاملين المخالطين كانت لا تحتوي على جين mecA. وعمل تحليل جيني لثلاثة من هذه المعزولات وإسناد النتائج لعيرات المكورات العقدية الذهبيه المرجعية الموجودة في بنك الجينات وجد أنها تتشابه فيما بينها بنسبة 99.5% ونسبة التماثل علي مستوي الأحماض الأمينية بين المعزولات الأخرى كانت 87.2%.