Molecular Typing of Staph - Aureus MRSA Strains Isolated from Processed Fish in Port-Said Governorate
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Abstract
Fish products could be contaminated with bacterial pathogens such as Staphylococcus aureus, which could be transmitted to human causing severe illness. In order to throw the light on the presence of S. aureus in fish products as well as molecular typing of MRSA strains, a total of 200 samples of processed fish products (100 fish burger and 100 fish fillet) were collected from different frozen fish stores in Port-Said Governorate, Egypt. The collected samples were subjected to bacteriological examination. The prevalence of coagulase +ve S. aureus was (14%) in fish burger samples and (4%) in fish fillet samples. On the other hand, the prevalence of Coagulase –ve S. aureus was (4%) in fish burger samples and (1%) in fish fillet samples. Antimicrobial susceptibility for methicillin was carried out for detection of MRSA strains, where the prevalence of MRSA strains was 17% from the total isolated coagulase positive S. aureus strains, while none of the coagulase negative S. aureus strains proved to be MRSA. PCR protocol was used for detection of meca gene in MRSA strains, where all the tested MRSA strains were positive for meca gene with specific amplification size at 310 bp.

Introduction
Food processing industry was aimed to supply a healthy and palatable food for the human consumers. Contamination by highly virulent bacteria could take place resulting in food intoxication (Shena and Sanjeev 2007). S. aureus is one of the most prevalent pathogenic bacteria associated with fish, which could be transmitted to human causing severe illness (Sichewo et al. 2013). Food poisoning caused by S.aureus is mainly characterized by nausea, vomiting, severe abdominal pain and diarrhea (Llewelyn and Cohen, 2002).
Nosocomial infections have both public health importance and veterinary importance. MRSA is one of the most incriminated microorganism in nosocomial infections. MRSA strains are characterized by their ability to resist methicillin. The process of methicillin resistance is encoded by the presence of chromosomal mecA gene. The genetic information of MRSA gives it the ability to resist all penicillins, and cephalosporins (Walther et al., 2006).

Ingestion of contaminated food with MRSA strains may increase the risk of becoming carriers or infected by this m.o., So it is necessary to take the suitable measures to control or even prevent food contamination by this m.o. (EFSA, 2010).

So, this work was planned to study the prevalence of S. aureus in fish products as well as molecular typing of MRSA strains isolated from processed fish products.

**Materials and Methods**

**Samples**
A total number of 200 samples (100 fish burger samples and 100 fish fillet samples) (25g from each sample) were collected aseptically in stomacher bags from different stores in Port-Said Governorate; Samples were clearly marked and submitted to the lab. in sterile containers for bacteriological examination.

**Isolation and identification of S. aureus**

**Preparation of samples:**

Two hundred and twenty five 225ml peptone water (1%) was added to the stomacher bags containing 25 grams of each sample and mixed in the stomacher, centrifugated at 300 rpm for 2 minute (ICMSF, 1978).

**Isolation of S. aureus:**
According to Quinn et al. (2002); processed samples were inoculated in peptone water for 24 h at 37ºC and then a loopful was taken and inoculated on nutrient agar, 5% sheep Blood agar, Mannitol salt agar and Baird parker agar media. All inoculated plates were incubated at 37ºC for 24-48hrs and examined daily for bacterial growth.

**Identification of S. aureus:**
The suspected colonies were examined for their morphological characters, hemolytic activity on 5 % Sheep blood agar, microscopical examination and biochemical characters according to (Quinn et al., 2002).

**Antimicrobial susceptibility testing by disc for detection of (MRSA) strains**
The susceptibility to Methicillin antibiotic was tested according to the procedures of (NCCLS, 2007) using disc diffusion technique. The susceptibility of the strains was determined according to the diameter of inhibition zone.

**Molecular typing of MRSA strains:**
1- DNA extraction from MRSA strains according to (Van eys et al., 1989).
2- Estimation of purity and
concentration of DNA according to Sambrook and Russel, (2001)

- Spectrophotometer was used to determine the concentration and purity of the extracted DNA by estimating the optical density at wave lengths of 260 and 280 nm.
- The concentration was calculated as follows: one OD at 260nm = 50 ug /ml.

3-polymerase chain reaction (PCR):

Samples of DNA were tested [in 50 μl. Reaction volume in a 0.2 ml PCR tube , containing PCR buffer] (50 mM KCl , 10 mMtris - HCl , 1mM MgCl₂ ) each dNTPS 200 uM each ( dATP , dGTP , dCTP and dTTP ) , [ Two pairs of primers each at 50 picomol / reaction ] and 0.5 of taq DNA polymerase . After layering 40ul of mineral oil, thermal cycling was done in a programmed heating block (Coy vorporation, Grasslake, Michan, USA). A control negative PCR reaction was included with no template in this assay.

- PCR Protocol of mec gene according to (McClure et al., 2006)
  Step 1: Initial Denaturation at 94 ºC for 5 min.
  Step 2: Denaturation at 94 ºC for 45 sec.
  Step 3: Annealing at 50 ºC 45 sec.
  Step 4: Extension at 72 ºC for 45 sec.
- Cycles repeated for 35 times and proceeded by initial denaturation at 95 ºC for 5 min. and followed by final extension at 72 for 10 min.

4- Agarose gel electrophoresis:

Ten μl of amplified PCR products were analyzed by electrophoresis on 2% agarose gel stained with 0.5 μg of ethedium bromide / ml. Electrophoresis was made in 1X TAE buffer at 80 volt for 1 hour. Gels were visualized under UV transilluminator (UVP, UK) then photographed.

Table (1): list of primers used for PCR assay

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence (5'-3')</th>
<th>Size of amplified product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA-F.</td>
<td>GTA GAA ATG ACT GAA CGT CCG ATA A</td>
<td></td>
</tr>
<tr>
<td>mecA-R.</td>
<td>CCA ATT CCA CAT TGT TTC GGT CTA A</td>
<td>310 bp</td>
</tr>
</tbody>
</table>

Results

Table (2): Number and percentage of S. aureus strains isolated from fish products:

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>No of examined samples</th>
<th>No of Coagulase +ve S. aureus</th>
<th>%</th>
<th>No of Coagulase –ve S. aureus</th>
<th>%</th>
<th>Total No of S.aureus strains</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish burger</td>
<td>100</td>
<td>14</td>
<td>14</td>
<td>4</td>
<td>4</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>
| Fish fillet     | 100                    | 4                             | 4 | 1                             | 1 | 5                             | 5 }
Table (3): Number and percentage of MRSA strains isolated from fish products (based on antibiotic sensitivity):

<table>
<thead>
<tr>
<th>Types of samples</th>
<th>Number of Coagulase +ve S. aureus</th>
<th>MRSA strains</th>
<th>Number of Coagulase -ve S. aureus</th>
<th>MRSA strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Fish burger</td>
<td>14</td>
<td>21%</td>
<td>4</td>
<td>0%</td>
</tr>
<tr>
<td>Fish fillet</td>
<td>4</td>
<td>0%</td>
<td>1</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>17%</td>
<td>5</td>
<td>0%</td>
</tr>
</tbody>
</table>

Discussion

*S. aureus* is believed to be the third main causative pathogen of foodborne diseases transmitted by fish and fish products in the European Union (*EFSA, 2009*). In the present study, the prevalence of coagulase +ve *S. aureus* was (14%) in fish burger samples and (4%) in fish fillet samples. On the other hand, the prevalence of Coagulase –ve *S. aureus* was (4%) in fish burger samples and (1%) in fish fillet samples as shown in Table (2). These results agreed with that obtained by Daniel et al., (2012) and Sergelidis et al., (2014). The higher prevalence of *S. aureus* in fish burger was due to the human handling during processing.

During PCR assay

L: (100 bp DNA ladder)
Lane 1: Control positive *S. aureus* for mecA gene
Lanes 2, 3, 4: showed positive *S. aureus* strains for mecA gene (310 bp)
Lane 5: control negative (E. coli)
of fish products. Contamination may occur due to poor sanitary conditions, or bad storage conditions (Normanno et al., 2007). As regards to the results of antibiotic susceptibility testing as shown in Tables (3), the prevalence of MRSA strains was 17% from the total isolated coagulase positive S. aureus strains, while none of the coagulase negative S. aureus strains proved to be MRSA. Resistance to methicillin occurred mostly due to the presence of mecA gene on S. aureus chromosome which is responsible for the production of Penicillin binding protein PBP2a. (Ito et al., 2003).

In this work, PCR protocol was used for amplification of 310 bp fragment of mecA gene in the isolated MRSA strains, as shown in Fig. (1), all the tested MRSA strains were positive for mecA gene. These results agreed with those obtained by John (2003; Sajith et al. (2012); Rania et al. (2013).

In conclusion, S. aureus is a very important bacterial agents causing food-borne illness in human due to contamination of fish products. The combination of phenotypic and genotypic characterization of S. aureus is an effective epidemiological tool for identification of the isolates. PCR is a fast and accurate technique can be used for the detection of MRSA strains as compared with traditional methods.

References


Staphylococcus aureus in fishery products and fish processing factory workers, Food Control, 18:1565-1568.


التصنيف الجزيئي لمياء المكور الープكوى الذهبً مبسبى من المنتجات المصنعة في محافظة بورسعود

في هذه الدراسة تم إقابة الضوء على تواجد المكور الープكوى الذهبى في عدد 200 عينة عشوائية من الأسماك المصنعة (100 عينة بجر أسماك و 100 عينة فيليب أسماك). وظف أظهرت نتائج الفحص البكتيريولوجي والتعريف البيولوجي للعينات المستخدمة إيجابية 23 عينة (11.5%) من عينات الأسماك المصنعة المستخدمة في هذه الدراسة (200 عينة)، وكانت نتيجة اختبار التجلط إيجابية 18 معزولة من المكور الープكوى الذهبى بنسبة (9%) بينما كانت 5 معزولات سالبة لاختبار التجلط.

وحذ وجد ان عينات المعزولة الموجبة لاختبار التجلط من بجر الأسماك 14 معزولة بنسبة (14%) بينما وجد ان عينات المعزولة السالبة لاختبار التجلط من فيليب الأسماك يمثل 4 مزعولات فقط بنسبة (4%). وكانت المعزولات الموجبة لاختبار التجلط من بجر الأسماك يمثل 4 مزعولات بنسبة (4%) أما المعزولات السالبة لاختبار التجلط فقد كانت مزعولات واحدة فقط بنسبة (1%).

وبدراسة حساسية جميع عسرات المكور الープكوى الذهبى المعزولة وعدها الامثل 23 عزة من عينات الأسماك المصنعة للميشلين واظهرت نتائج الدراسة التالي: ثلاثة معزولات أظهرت مقاومة للميشلين من اجمالى 23 معزولة موجبة للميكروب الープكوى الذهبى من عدد 200 عينة من الأسماك المصنعة. وكانت الثلاث معزولات المقاومة للميشلين جمعها من (mec A gene) عند الأربعة عشر عينة بجر الأسماك موجبة التجلط. وللتأكيد على وجود (mec A gene) شمال الثلاث معزولات المقاومة للميشلين، تم إجراء تفاعل أنزيم البكرة المتسائل للثلاث معزولات و تم بيهم. اثبات تواجد (mec A gene)