DETECTION OF ENTEROTOXIGENIC STAPHYLOCOCCUS AUREUS IN SOME MEAT PRODUCTS

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ABSTRACT

Eighty four meat product samples (minced meat, sausage and beef burger 28 of each) were collected and examined for detection of Staphylococcus aureus. The mean count was 4.3x10^2, 5.1x10^3 and 8.7x10^3 cfu / gm, respectively. Multiplex Polymerase Chain Reaction (PCR) was applied for detection of genes responsible for enterotoxins production from identified coagulase positive Staphylococcus aureus. The predominant genes were A and B. They were detected in examined sausage and beef burger samples with different percentages.

Key words: Detection, Meat products, Staphylococcus aureus, Staphylococcal enterotoxins, Multiplex PCR.

INTRODUCTION

Meat and meat products are considered the most important sources of food for people in every where as they supply the consumers with the required nutritive elements such as high quality proteins, essential amino acids, B-complex vitamins and certain minerals especially iron and phosphorous and beside that the meat has high calories value.

In comparison to fresh meat, meat products retain more consumers’ palatability and easily preparation characteristics. Food safety is a global health goal and the food borne diseases take a major crisis on health. Therefore, detection of microbial pathogens in food is the solution to the prevention and recognition of problems related to health and safety (Velusamy et al., 2010). Staphylococcus aureus (S. aureus) is a major cause of food borne intoxication and its presence in food constitutes an important problem for food processors, food service workers and consumers. The main reservoirs of S. aureus are humans and animals. Healthy people carry the organism in their nose and throat (50 %), on their hands (5-30 %), and in wounds. S. aureus can also colonize food contact surfaces, and it can become a persistent organism in slaughter houses. S. aureus can contaminate foods through contact with contaminated hands, materials and surfaces, but also via the air coughing (Rao et al., 1980). Considering this hazard, meat and meat products should not be subjected to unnecessary contamination and they should be free from such serious pathogen to ensure a maximum margin of consumer safety.

S. aureus, if posses Staphylococcal enterotoxins genes may produce several enterotoxins (SEA to SEJ) (Monday and Bohach, 1999). Staphylococcal food poisoning (SFP) is one of the most prevalent causes of gastroenteritis worldwide. Symptoms of SFP have a rapid onset characterized by abdominal cramps, nausea, and vomiting, sometimes followed by diarrhea. Patients usually suffer from symptoms within 2-4 hours after ingestion of thermostable Staphylococcal enterotoxins (SEs). The approximate dose of SEs ranged from 0.1 to 1.0 mg/kg of body weight (Jorgensen et al., 2003 and Stewart et al., 2005). Since SEs are more stable than S. aureus bacteria, it is possible to test food product and obtain negative S. aureus culture results and positive SE tests. Today, up to 15 SEs are known; the last one discovered was the recently identified SEU (Lerttre et al., 2003a). Multiplex PCR assay for detection of Staphylococcal enterotoxins genes (SEA, SEB, SEC, SED and SEE) was developed and proved to be specific, sensitive, and rapid method. (Omo et al., 2002 and Zschock et al., 2005). There fore the current study was planed to through light on the incidence and count of S. aureus followed by PCR identification of enterotoxigenic strains.

MATERIALS and METHODS

Sampling:
Eighty four meat product samples (minced meats, sausages and burgers) 28 of each were collected randomly from supermarkets and butchery shops at Sharkia province, Egypt, at different levels of sanitations. The collected samples were identified, packed in sterile polyethylene bags and transferred to the laboratory as soon as possible.

Methodology:
The collected samples were examined for detection of S. aureus by ordinary culture method followed by biochemical identification and examined for coagulase enzyme production and then the detected strains were subjected to DNA extraction which was
used as template of PCR amplification for detection of \textit{S. aureus} enterotoxins genes.

**Preparation of samples, (ICMSF, 1978):**
Twenty five grams of each examined samples were transferred to a sterile polyethylene bags, and 225 ml of 0.1% of sterile buffered peptone water was aseptically added to the content of the bag. Each sample was homogenized in a blender at 2500 rpm for 1-2 minute to provide homogenate of 1/10 dilution. One ml from the original dilution was transferred to another sterile tube containing 9ml sterile buffered peptone water and mixed well to make next dilution, from which further decimal serial dilutions were prepared. Then, dilutions were subjected to the following examinations.

**Count and isolation of \textit{Staphylococcus aureus}, (APHA, 1992):**
From the previously prepared decimal dilutions of the examined samples 0.1 ml was transferred and evenly spread on dry surface of Baird-Parker agar medium plates (Oxoid, 1998). Inoculated and control plates were incubated at 37° C for 48 hours and Staphylococci count was calculated and recorded. Each suspected colony (black shiny with narrow white margin and surrounded by clear hallow-zone extended into the opaque medium) was picked up and cultivated on slope agar for further biochemical identification according to (APHA, 1992).

**Identification of isolated \textit{Staphylococci} organisms:**
- **Morphological characters:**
  - **Staining (microscopical examination):**
    Films were made from the pure culture of isolated organisms stained by Gram's stain and examined microscopically. Gram +ve, grapes shape, cocci were considered as positive Staphylococci.

- **Biochemical reactions:**
  1. Catalase test.
  2. Oxidation-fermentation test.
  3. Coagulate test.

**PCR detection of \textit{Staphylococcal enterotoxins} genes:**

**Extraction of the DNA from Bacterial culture:**
Bacterial DNA was extracted using GeneJET Genomic DNA Purification kit (Fermentase, USA # k0722) following the manufacture's protocols.

**PCR assays:**
Oligonucleotide sequences of SE genes' were listed in table (1). PCR amplification was performed in a thermal cycler (Thermal cycler: PTC-100™ programmable thermal controller, Peltier-Effect cycling, MJ, RESEARCH, INC.) with an initial denaturation of 94° C for 5 minutes; 35 cycles of amplification (denaturation at 94° C for 2 minutes, annealing at 57° C for 2 minutes, and extension at 72° C for 1 minute), with a final extension at 72° C for 7 minutes. Electrophoration was done using 1.5% agarose gel in 0.5% Trisacetate- EDTA (TEA) buffer, stained with ethidium bromide. The amplified products then were visualized using ultra violet lamp wherein, the images were taken.

**Multiplex PCR for detection of selected \textit{Staphylococcal} genes:**
The reaction conditions for the multiplex PCR assay were optimized to ensure that all of the target gene sequences were satisfactorily amplified. The primers were designed to target the coding regions of the genes; care was taken to avoid areas of homology within the structural genes for the enterotoxins. The primers used in each set had almost equal annealing temperatures, which reduced the possibility of occurrence of unwanted bands originating from nonspecific amplification.

**Table 1:** Nucleotide sequences, gene locations, and anticipated sizes of PCR products for the \textit{S. aureus} gene-specific oligonucleotide primers used in this study (Mehrotra et al., 2000).

<table>
<thead>
<tr>
<th>Gene</th>
<th>primer</th>
<th>Location within gene</th>
<th>Sequence (5’ – 3’)</th>
<th>Size of amplified product</th>
</tr>
</thead>
<tbody>
<tr>
<td>sea</td>
<td>GSEAR-1</td>
<td>349–368</td>
<td>GGTATCATATGGTCGGG</td>
<td>102 bp</td>
</tr>
<tr>
<td></td>
<td>GSEAR-2</td>
<td>431–450</td>
<td>CGGCACATTTTTTTTCATCGG</td>
<td></td>
</tr>
<tr>
<td>seb</td>
<td>GSEBR-1</td>
<td>666–685</td>
<td>GTATGATGATGATGAACTGA</td>
<td>164 bp</td>
</tr>
<tr>
<td></td>
<td>GSEBR-2</td>
<td>810–829</td>
<td>CCAAATATGACGAGGTTAGG</td>
<td></td>
</tr>
<tr>
<td>sec</td>
<td>GSEC-1</td>
<td>432–455</td>
<td>AGATCGATATGATGATGATGATGG</td>
<td>451 bp</td>
</tr>
<tr>
<td></td>
<td>GSEC-2</td>
<td>863–882</td>
<td>CACACTTTTATAATCCCCCG</td>
<td></td>
</tr>
<tr>
<td>sed</td>
<td>GSEDR-1</td>
<td>492–514</td>
<td>CAATATAGAGGAAAAATAAAAG</td>
<td>278 bp</td>
</tr>
<tr>
<td></td>
<td>GSEDR-2</td>
<td>750–769</td>
<td>ATTTGGATTATTTTTCTGTTT</td>
<td></td>
</tr>
<tr>
<td>see</td>
<td>GSEE-1</td>
<td>237–257</td>
<td>AGGTTTTTTTCACAGGTCATCC</td>
<td>209 bp</td>
</tr>
<tr>
<td></td>
<td>GSEE-2</td>
<td>425–445</td>
<td>CTTTTTTTTCTCGGTCAATC</td>
<td></td>
</tr>
</tbody>
</table>
RESULTS

Table 2: Incidence and count of S. aureus in examined meat product samples and its percentage (n = 28).

<table>
<thead>
<tr>
<th>Product</th>
<th>No of +ve samples</th>
<th>percentage</th>
<th>Min</th>
<th>Max</th>
<th>Mean **</th>
<th>± S.E*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mince meat</td>
<td>6</td>
<td>21.4 %</td>
<td>&lt;100</td>
<td>10x10³</td>
<td>4.3x10²b</td>
<td>3.6x10²</td>
</tr>
<tr>
<td>Sausage</td>
<td>11</td>
<td>39.3 %</td>
<td>&lt;100</td>
<td>40x10³</td>
<td>5.1x10³a</td>
<td>2.1x10³</td>
</tr>
<tr>
<td>Burger</td>
<td>17</td>
<td>60.7 %</td>
<td>&lt;100</td>
<td>30x10³</td>
<td>8.7x10³a</td>
<td>1.8x10³</td>
</tr>
</tbody>
</table>

* Min: minimum.
** Max: maximum.
***Means carrying the different letter were significant; (P < 0.05).
* S.E: Standard error of mean.

Table 3: Incidence of enterotoxins of coagulase positive S. aureus detected in examined meat product samples and its percentage.

<table>
<thead>
<tr>
<th>Product</th>
<th>No of +ve samples</th>
<th>% of +ve samples</th>
<th>Enterotoxin A</th>
<th>Enterotoxin B</th>
<th>Enterotoxins A&amp;B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mince meat</td>
<td>0</td>
<td>0 %</td>
<td>0 %</td>
<td>0 %</td>
<td>0 %</td>
</tr>
<tr>
<td>Sausage</td>
<td>9</td>
<td>32.1 %</td>
<td>8 %</td>
<td>11.1 %</td>
<td>0 %</td>
</tr>
<tr>
<td>Burger</td>
<td>8</td>
<td>28.6 %</td>
<td>6 %</td>
<td>12.5 %</td>
<td>1 %</td>
</tr>
</tbody>
</table>
DISCUSSION

Staphylococcus aureus count:
Staphylococcal food-poisoning (SFP) is caused by Staphylococcal enterotoxins (SEs) produced during massive growth of S. aureus in food. SFP is a prevalent cause of food-borne disease worldwide, (Jablonski and Bohach, 2001).

The results showed in table (2) revealed that, the incidence of S. aureus in minced meat was 6 (21.4 %) similar result was recorded by Vorster et al. (1994) while lower incidence was detected by El-Said (2005). Higher incidences were recorded by Depourcq and Poucke, (1991); El-Gohary (1993); Ramasastry (1999); Salek (2000); Huffman (2002) and Tavakoli and Riazipour (2008). The mean count was $4.3 \times 10^2 \pm 3.6 \times 10^2$ cfu/gm and the count ranged from $<10^2$ to $10 \times 10^3$ cfu/gm. Such results were lower than that recorded by Tharwat (2008) and Nossier (2010).

In sausage samples, the incidence was 11 (39.3 %). This incidence was similar to that obtained by EL-Sherbeeny et al. (1990) who detect S. aureus in 43.1% of examined sausage samples. On contrary lower incidences were reported by Abd El-Aziz et al. (1996); Soutos et al. (2003); El-Said (2005) and Gehad et al. (2006). Higher incidences were recorded by Rashad (1990), El-Gohary (1993) in the examined sausage samples.

The mean value was $5.1 \times 10^3 \pm 2.1 \times 10^3$ cfu/gm with a minimum of $<10^2$ and a maximum of $40 \times 10^3$ cfu/gm. Lower results were obtained by El-Mossalami, (2002) who found that S. aureus count was $<50$ cfu/gm and Gehad et al. (2006) whom failed to detect S. aureus in the examined sausage samples.

Burger samples showed a high incidence of 17 (60.7 %), while lower incidences were reported by Ranucci et al. (2004) they isolated S. aureus from 21.1% of the examined hamburgers also El-Said (2005) recorded that S. aureus was isolated from frozen beef burger with an incidence of 2 (4%) and Shahraz et al. (2012) isolated 64 (24%) S. aureus strains from 256 samples of packaged hamburger. The mean value was $8.7 \times 10^3 \pm 1.8 \times 10^3$ organisms/gm with a minimum of $<10^2$ and a maximum of $30 \times 10^3$ organisms/gm. Lower count was recorded by Essa and Makar, (2003).

Comparatively, the obtained results declared that burger samples gave a higher incidence of S. aureus followed by sausage then minced meat. This indicates that the unsanitary handling of the prepared meat products during processing, transportation and marketing. Also, was due to using a low quality meat and meat additives. The shape of burger may explain the higher incidence than that of sausage. Statistical analysis revealed that both sausage and burger were significantly higher than minced meat at p value < 0.05.

The United Kingdom Public Health Laboratory Service in 2000 classified meat products to four categories satisfactory, acceptable, unsatisfactory and unacceptable according to number of S. aureus as $<20$, 20 to $<100$, 100 to $<10^4$ and $\geq 10^4$ CFU/g, respectively. We found that, all examined minced
meat samples were within the permissible limit and considered acceptable except only one sample that show unsatisfactory level of contamination (10x10^3 organisms/ gm). The examined samples of sausage and burger showed unsatisfactory level of contamination and were 11(39.3 %) and 17 (60.7%) for sausage and burger.

PCR assays used to identify the pathogen and its enterotoxin genes in food samples. The PCR assays could be made in hours rather than days, with a high sensitivity and method accuracy, allowing for the detection of very low concentrations of micro-organisms (Najera-Sanchez et al., 2003).

Data in table (3) revealed that the incidence of identified enterotoxigenic S. aureus isolated from meat product samples by PCR technique was 9 (32.1 %) from sausage and 8 (28.6 %) from burger, while it couldn’t be detected from minced meat. Nearly similar result was detected by Pinto et al. (2005). They found that forty out of 131 isolates (31%) tested were positive for enterotoxins production. Lower incidence of enterotoxins was detected by Kevin et al. (2009). They examined 155 samples of fermented pork product, and found that 39.35% of the samples were positive for S. aureus, but none were positive for the Staphylococcal enterotoxins. Higher incidence of enterotoxigenic S. aureus was detected by Oh et al. (2007) they found that 47% of the isolated S. aureus strains from the contaminated meat product were enterotoxigenic S. aureus. Also, El-Shater (2010) detect enterotoxigenic S. aureus with an incidence of (55.6 %) in sausage samples and Aydin et al. (2011) found that out of 147 S. aureus isolated from different foods including sausage, 92(62.6%) were enterotoxigenic S. aureus.

Enterotoxins are a group of serologically distinct proteins (A, B, C1–3, D, E and F) that are the causative agents of Staphylococcal food poisoning. Although their exact mode of action has yet to be fully elucidated, they are believed to stimulate an enteric-vagus nerve reflex triggering the vomiting centers of the brain (Sears and Kaper, 1996; Arbuthnott et al., 1990). The enterotoxins can also act as super antigens, stimulating T lymphocytes to release cytokines and T-cell proliferation (Balaban and Rasooly, 2000; Krakauer, 1999).

The predominant enterotoxins isolated from examined sausage samples were enterotoxin A 8 (88.9 %) figures (1, 2, 3 and 4) detected at 102 bp then enterotoxins B 1 (11.1 %) figure (2) detected at 164 bp. Application of PCR assays for detection of enterotoxin production from coagulate positive S. aureus isolated from beef burger samples revealed that 6 (75 %) enterotoxin A detected at 102 bp figures (3, 4, 5 and 6), followed by enterotoxin B figure (5) detected at 164 bp. With a percentage of 1 (12.5%) and enterotoxins A and B figure (3) detected in one sample at 102 and 164 bp. With a percentage of (12.5%). Such results substantiate what have been reported by Hwang et al. (2007); Ruzickova et al. (2008); El-Shater (2010) and Aydin et al. (2011).

Finally we conclude that:
Not all coagulate positive S. aureus are enterotoxigenic, since minced meat samples were negative, only nine sausage samples from eleven coagulate positive were enterotoxigenic (82 %) and only eight burger samples from seventeen coagulate positive were enterotoxigenic (47 %).

REFERENCES


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