Frequency of Enterotoxin Producing Staphylococcus aureus and Toxin Genes in Raw and Cooked Meat Samples

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C I T A T I O N    L I N K S

Introduction

*Staphylococcus aureus* is one of the most common causes of bacterial food poisoning outbreak [1, 2]. Staphylococcal food poisoning (SFP) is related to the consumption of foods containing sufficient amounts of one or more enterotoxins [2–6]. The high incidence of staphylococcal food poisoning is due to the insufficient pasteurization/decontamination of originally contaminated product source or its contamination during preparation and handling by individuals who are carriers of the organism [7]. Foods that have been frequently incriminated in staphylococcal intoxication include meat and meat products, poultry and egg products, milk and dairy products, salads, bakery products, particularly cream-filled pastries and cakes, and sandwich fillings [8]. *S. aureus* produces a wide variety of toxins belong to the fascinating family of superantigens including staphylococcal enterotoxins (SEs; SEA-SEE, SEG-SEI and SER-SET) and staphylococcal enterotoxin like (SEls) toxins, which their emetic properties have remained unconfirmed (SEIK-SEIQ and SEIU-SEIX). SEs and SEls are single chain proteins with size range from 22-29KDa and encoded by accessory genetic elements including plasmids, prophages, pathogenicity islands, genomic island vSa, or by genes located next to the staphylococcal cassette chromosome (SCC) [8–11].

The amount of SE required for establishment of typical symptoms of food poisoning including nausea, vomiting, emesis, stomach cramps and diarrhea is very low and approximately ranging from 20ng to 1μg [3, 12, 13]. Among SEs, SEA is the most common cause of staphylococcal food poisoning worldwide, but the involvement of other classical SEs has been also demonstrated [8]. Due to the stability of SEs in denaturing conditions such as heat and low pH, these toxins are not completely destroyed by mild cooking or digestion of food in the stomach [14, 15].

Although enterotoxigenic staphylococci are thermally destroyed, the cooked meat products may contain SEs because these toxins are thermostable and cannot be destroyed by heat processing. This fact represents a serious hazard to healthy consumer when ready-to-eat meat products are processed [16]. Therefore, it is essential to detect SEs-producing staphylococci and gather information about other microbial risk factors and hazards associated with raw and pre-processed meat products. Risk assessment and microbial monitoring will continue to play important role in quality assurance of meat products [16].

The detection of *S. aureus* and SEs in food is difficult. Methods currently used for detection of SEs in food are Enzyme-Linked Immunosorbent Assay (ELISA), reversed passive latex agglutination (SET-RPLA) and polymerase chain reaction (PCR) technique [14].

In this study, we determined the prevalence of *S. aureus* and frequency of SE-genes (sea, seb, sec, sed and see), in food isolates (beef, lamb and cooked meat) from Iranian markets, restaurants and other food distribution centers to determine the ability of the isolates to produce classical staphylococcal enterotoxins SEA–SEE by PCR method. In addition, a commercially available kit RIDASCREEN® SET total (SEA-SEE) was employed for the detection of Total staphylococcal enterotoxins A to E by sandwich enzyme immunoassay (ELISA) technique.

The aim of this study was to investigate the presence and the frequency of enterotoxin producing *S. aureus* and SE genes in meat samples collected from meat retail outlets and restaurants in Zanjan, Iran.

Materials and Methods

In this cross sectional study, from March to June 2015, a total of 90 individual meat samples including 23 raw beef, 22 raw lamb and 45 cocked meat samples were collected from meat retail outlets and restaurants in Zanjan, Iran. Meat samples were packed into a clean polyethylene bag then marked and transported to the laboratory of food microbiology in a cool box for analysis within 1h.

Reference strains: Reference strains of *S. aureus* ATCC 13565 (SEA), *S. aureus* ATCC 14458 (SEB), *S. aureus* ATCC 19095 (SEC), *S. aureus* ATCC 23235 (SED) and *S. aureus* ATCC 27664 (SEE) were used as positive controls in the study.

Isolation and identification of *S. aureus*: Twenty five gram of meat samples was homogenized for 90s in a stomacher (Heidolph; Schwabach; Germany) with 225mL of peptone water (PW) containing 6.5% NaCl and then incubated at 37°C for 24h. After primary enrichment, a loopful (without shaking the flask) from each of the enriched homogenates was streaked onto Baird-Parker agar (MERCK; Darmstadt; Germany) supplemented with 5% egg yolk and tellurite and incubated under aerobic conditions at 37°C for 24h. Colonies with typical grey-black appearance surrounded by a clear zone were enumerated as coagulase positive staphylococci and sub-cultured onto Mannitol salt agar (Merck; Darmstadt; Germany). The isolates were identified as *S. aureus* by further biochemical characterization using Gram stain, catalase, coagulase, oxidase, lipase, DNase and PCR targeting the *S. aureus* specific femA gene (*S. aureus* species specific).

Genomic DNA extraction: A colony of *S. aureus* (one colony per sample) was picked from nutrient agar and inoculated into 5ml of LB (Luria Bertani Broth; MERCK; Germany) and incubated with
shaking at 120rpm at 37°C. Extraction of genomic DNA was performed according to the protocol provided with the Qiagen Mini Amp kit (Qiagen Inc.; Germany).

**Detection of sea-see in S. aureus isolates by PCR:** The presence of staphylococcal enterotoxin genes; sea, seb, sec, sed and see was assessed using the primers (Table 1) [17, 18].

**Table 1**) Primers used in the study

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer sequence (5’→3’)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>femA</td>
<td>AAAAAAGECATAAAGCAGGATAGAAAAGAACAGCAG</td>
<td>132</td>
</tr>
<tr>
<td>sea</td>
<td>CTCTTGGAAGGTTAAACGTCGAACTGCTTTTCATCCTAAACAC</td>
<td>127</td>
</tr>
<tr>
<td>seb</td>
<td>TCGCATGACGACAAAGGGGACGCTACCTATAAGTGGC</td>
<td>477</td>
</tr>
<tr>
<td>sec</td>
<td>CTCAAGAAGCTAGCATAAAAACTTCTTTTACATTATTTCC</td>
<td>271</td>
</tr>
<tr>
<td>sed</td>
<td>CAGTATGTCATAATGCTATCATATTATAATAGGTTAAAGACCTC</td>
<td>319</td>
</tr>
<tr>
<td>see</td>
<td>CTAATTGACCTGGAACCTC</td>
<td>178</td>
</tr>
</tbody>
</table>

Single PCR was performed using DreamTaq PCR Master Mix (Thermo Fisher Scientific), which contains Taq polymerase, dNTPs, MgCl2 and the appropriate buffer. Each PCR tube contained 25µl reaction mixture composed of 12.5µl of the master mix, 2.5µl of each forward and reverse primer solution (in a final concentration of 200nM), 2µl of DNA with concentration of 400ng and nuclease-free water to complete the final volume. PCR was performed using the Gene Atlas 322 system (ASTEC) with the same cycling conditions for sea-see genes.

Amplification involved an initial denaturation at 94°C, 5min followed by 30 cycles of denaturation (94°C, 1.5min), annealing (55°C, 1.5min) and extension (72°C, 1.5min), with a final extension step (72°C, 8min). The amplified DNA was separated by submatine gel electrophoresis on 1.5% agarose, stained with etidium bromide and visualized under UV transillumination.

**Detection of SEA-SEE enterotoxins:** Staphylococcal enterotoxins (SEA, SEB, SEC, SEE and SEF) were determined in homogenized meat samples (Table 2) by ELISA technique (Thermo; Finland) with commercially available kit (Ridascreen® SET total; R-Biopharm AG; Darmstadt; Germany, Art. No. R4105).

**Findings**

**Frequency of S. aureus in meat samples:** A total of 90 individual meat samples were studied for the presence of S. aureus. Conventional cultural method based on appearance of grey-black colonies surrounded by a clear zone on Baird Parker agar plates were detected coagulase positive staphylococci in 43 (47.8%) out of the 90 samples. However, the biochemical tests and molecular analysis of femA in coagulase positive staphylococci indicated that 34.4% (31/90) of samples were positive for S. aureus: 12 (13.3%) isolates from raw lamb, 9 (10.0%) isolates from raw beef and 10 (11.1%) isolates from cooked meat samples (Table 2).

**Frequency of total enterotoxins (SEA-SEE) in meat samples by ELISA technique:** Of 18 enterotoxin positive samples, 10 (11.1%) were homogenized lamb and 8 (8.9%) beef samples. Total classical enterotoxins were not found in cooked meat samples (Table 2).

**Distribution of enterotoxin genes (sea, seb, sec, sed and see) in S. aureus isolates:** Overall, 58.1% (18/31) of isolates were positive for the presence of at least one or more SE genes: 10 isolates (32.3%) from lamb and 8 isolates (25.8%) from beef samples. Comparison of SE genes frequency among beef and lamb isolates showed different distribution of these genes. The most prevalent SE gene among beef and lamb isolates was sea (38.7%), followed by see (22.6%), sec (16.1%) and seb (12.9%). SE genes were not found in strains isolated from cooked meat samples (Table 3).

The presence of multiple SE genes with different combinations was found among isolates. Of 18 S. aureus isolates carrying enterotoxin genes, 10 (55.5%) isolates had two or more SE genes simultaneously. The frequent combination of SE genes was sea+see (16.7%), followed by sea+seb+see (11.1%). Furthermore, one isolate (5.5%) of lamb samples carried sea, seb, sec and see simultaneously (Table 4).

**Table 2** Frequency of S. aureus and total enterotoxins (SEA-SEE) in meat samples

<table>
<thead>
<tr>
<th>Meat type</th>
<th>No.</th>
<th>Samples containing S. aureus</th>
<th>Samples containing total enterotoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive samples</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Raw beef</td>
<td>23</td>
<td>B1, B2, B3, B6, B8, B9, B10, B12, B13</td>
<td>9 (10.0)</td>
</tr>
<tr>
<td>Cooked meat</td>
<td>45</td>
<td>C5, C13, C16, C24, C26, C32, C34, C35, C39, C45</td>
<td>10 (11.1)</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>A2, A3, A5, A7, A8, A9, A10, A14, A16, A18, A19, A21, B1, B2, B3, B6, B8, B9, B10, B12, B13, C5, C13, C16, C24, C26, C32, C34, C35, C39, C45</td>
<td>31 (34.4)</td>
</tr>
</tbody>
</table>
Detection of enterotoxin producing *S. aureus* has been strongly recommended in order to evaluate the human health risk arising from food consumption. The limitation of our study was unavailability of ELISA kit and the number of samples.

**Conclusion**

Detection of enterotoxin producing *S. aureus* in raw meat marketed in Zanjan, Iran shows a probable risk for public health.

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Ethical Permissions: All procedures followed were in accordance with the ethical standards of the responsible committee (ZUMS.REC.1394.69).

Conflicts of Interests: The authors declare that there is no conflict of interests.

Authors’ Contribution: Asgarpoor D. (First author), Introduction author/Methodologist/Original researcher (30%); Haghi F. (Second author), Introduction author/Methodologist/Assistant/Statistical analyst/Discussion author (35%); Zeighami H. (Third author), Methodologist/Original researcher/Statistical analyst/Discussion author (35%).

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