Enterotoxin Gene Profiles of Staphylococcus aureus and Other Staphylococcal Isolates from Various Foods and Food Ingredients*

Yılmaz Emre GENCAY1, Naim Deniz AYAZ1, Aylin KASIMOGLU DOĞRU1
1 Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Kırıkkale-TURKEY.

Summary: This study was aimed to identify the toxin gene profiles of 107 staphylococcal isolates from a variety of foods. Firstly, 102 CPS were analyzed by PCR and 70 (68.6%) of them were identified as S. aureus. Then, all isolates (70 S. aureus, 32 CPS and 5 CNS) were investigated for the presence of SE genes (sea, seb, sec, sed, see, seg, seh, sei, and sej) by using multiplex PCR. Among 70 S. aureus, only 2 (2.9%) which were isolated from poultry meat shown to be positive for sea gene. None of the other isolates harboured any of the SE genes ascertained. Though these results of toxin gene positivity are too low, enterotoxin genes of enterotoxigenic staphylococci constitute a potential risk for consumers' health.

Key Words: Enterotoxin genes, food, Staphylococcus aureus

Coagulase positive staphylococci (CPS) and especially S. aureus are known as the primary causes of SFPs (10, 12). Therefore, the coagulase negative staphylococci (CNS) are not detected by standard microbiological methods (18). However, it was shown in previous studies that some strains of both CPS and CNS could produce enterotoxins and might involve in staphylococcal food poisonings (5, 10, 24, 25).

Nine major antigenic types of SE’s have been reported comprising the five classical (SEA, SEB, SEC, SED and SEE) and four newly described (SEG, SEH, SEI and SEJ) (23). Genes encoding SEs have different genetic supports, most of which are mobile genetic elements such as; phages (sea, see), transposons (seb), plasmids (sec, sed and sej), pathogenicity islands (seb, sec) or chromosomal genes (sec, see, sed, seh and sej) (2, 3, 10) which enables horizontal spread within staphylococci populations (4).

The SE production by staphylococcal strains has been studied in Turkey (7, 13, 14, 15, 20). However, there is a lack of information on foodborne staphylococcal SE genes and there are a few molecular studies on this subject in Turkey. The objectives of this study were to identify the S.
Staphylococcal toxin gene profile...

Y. E. GENCAY, N. D. AYAZ, A. KASIMOGLU DOĞRU

**Material and Method**

**Bacterial strains**

There was a total of 102 CPS and 5 CNS that were previously isolated from various kinds of foods. The number of the CPS and CNS isolates related to the food samples were as follows: 33 from beef (32 CPS, 1 CNS), 22 from poultry meat (20 CPS: 9 from chicken and 11 from turkey meat, 2 CNS from chicken), 24 from dairy products (23 CPS, 1 CNS), 19 from ready to eat foods (18 CPS, 1 CNS), and 9 CPS from some food ingredients. All of the isolates were kindly provided by Çağatay Çelik (Headquarters of 2nd Armoured Brigade of Turkish General Staff, Maltepe, Istanbul, Turkey) that was not published previously.

Reference strains of *S. aureus* D4508 (sea, seh), RIMD31092 (seb, sec, seg, sei), NCTC9393 (sed, seh, seg, sei), FRI326 (ATCC 27664) (see) and A900322 (seg, sei) were kindly and generously provided by G. Blaiotta, Dipartimento di Scienza degli Alimenti, Sezione di Microbiologia Agraria, Alimentare, Ambientale e di Igiene, Stazione di Microbiologia Industriale, Università degli Studi di Napoli “Federico II”, Via Universita, Portici, Italy.

**Identification of *S. aureus* by PCR analysis**

In the study, for the identification of *S. aureus*, 102 CPS were analyzed by PCR assay. For this purpose 16S rRNA specific primers (Integrated DNA Technologies- IDT, Leuven, Belgium) were used (17, 19) (Table 1).

### Table 1. PCR Primers and expected product sizes for the identification of *S. aureus* and detection of enterotoxin genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Oligonucleotide sequence (5’-3’)</th>
<th>Product size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>sea</td>
<td>F- GCA GGG AAC AGC TTT AGG C</td>
<td>521 bp</td>
<td>(19)</td>
</tr>
<tr>
<td></td>
<td>R- GTT CTG TAG AAG TAT GAA ACA CG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>seb</td>
<td>F- ACA TGT AAT TTT GAT ATT CGC ACT G</td>
<td>667 bp</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>R- TGC AGG CAT CAT GTC ATA CCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sec</td>
<td>F- CTT GTA TGT ATG GAG GAA TAA CAA</td>
<td>284 bp</td>
<td>(19)</td>
</tr>
<tr>
<td></td>
<td>R- TGC AGG CAT CAT ATC ATA CCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sed</td>
<td>F- GTG GTG AAA TAG ATA GGA CTG C</td>
<td>385 bp</td>
<td>(19)</td>
</tr>
<tr>
<td></td>
<td>R- ATA TGA AGG TGC TCT GTG G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>see</td>
<td>F- TAC CAA TTA ACT TGT GGA TAG AC</td>
<td>171 bp</td>
<td>(19)</td>
</tr>
<tr>
<td></td>
<td>R- CTC TTT GCA CCT TAC CGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>seg</td>
<td>F- CGT CTC CAC CTG TTG AAG G</td>
<td>328 bp</td>
<td>(19)</td>
</tr>
<tr>
<td></td>
<td>R- CCA AGT GAT TGT CTA TTG TCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>seh</td>
<td>F- CAA CTC CTG ATT TAG CTC AG</td>
<td>359 bp</td>
<td>(19)</td>
</tr>
<tr>
<td></td>
<td>R- GTC GAA TGA GTA ATC TCT AGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sei</td>
<td>F- CAA CTC GAA TTT TCA ACA GGT ACC</td>
<td>466 bp</td>
<td>(17, 19)</td>
</tr>
<tr>
<td></td>
<td>R- CAG GCA GTG CAT CTC CTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sej</td>
<td>F- CAT CAG AAC TGT TGT TCC GCT AG</td>
<td>142 bp</td>
<td>(19)</td>
</tr>
<tr>
<td>16S</td>
<td>F- GTA GGT GGC AAG CTG TAT CC</td>
<td>228 bp</td>
<td>(19)</td>
</tr>
<tr>
<td>rRNA</td>
<td>R- CGC ACA TCA GCG TCA G</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
One ml overnight culture at 37°C in 5ml brain-heart infusion (BHI, Oxoid CM0225B, Hampshire, UK) of each strain was transferred into an eppendorf tube and centrifuged (Beckman Coulter Microfuge 22R, Fullerton, USA) at 12000g for 3 min. After the removal of the supernatant, pellet was suspended with 1 ml of sterile ultra pure water and mixed thoroughly. The washing step repeated. Two 2 µl of proteinase K [70 mg/ml (AppliChem GmbH, Darmstadt, Germany)] and 200 l of Chelex 100 [5 % (Bio-Rad, Hercules, CA, USA)] were added on the remaining pellet and mixed. Mixtures were floated in a water bath (Memmert, Schwabagh, Germany) at 95°C for 10 min after the former incubation at 55°C for 40 min. Consequently, centrifugation with the same parameters was done and 10 µl of the supernatant used as the template DNA.

PCR was performed in a total volume of 50 l containing the following: 5 l of 10×PCR buffer (Bioron GmbH, Ludwigshafen, Germany), 3 l of 25 mM MgCl2 (Bioron), 2 l of 10 mM dNTP mixture (Bioron), 0.5 l of primer which contains 100 pmol/ l, 0.5 l of 5 U Taq DNA polymerase (Bioron). The volume of the master mix was adjusted to 40 l with sterile ultra pure water and 10 l of template DNA was added. DNA amplification was carried out in a thermocycler (Eppendorf mastercycler gradient, Hamburg, Germany) as described previously (19). After an incubation at 95°C for 10 min, continued as follows: 30 cycles of denaturation at 94°C for 1 min, annealing at 64°C for 1 min, extension at 72°C C for 1 min, and finally a terminating incubation at 72°C for 10 min.

A 10 µl aliquot of each resultant PCR product was further analyzed by agarose gel (1.5 % Agarose-Basica LE, Prona, Spain) electrophoresis (CSL MSMini-Duo, Corston, UK), stained with 0.1 µg ml−1 ethidium bromide (BioChemica GmbH, Darmstadt, Germany), at 100 V for 1 h and visualized by a gel documentation and analysis system (Sygene Ingenius, Cambridge, UK).

**Multiplex PCR assay for the detection of staphylococcal enterotoxin genes**

The nine oligonucleotide primer pairs (Integrated DNA Technologies- IDT, Leuven, Belgium) used to amplify the genes sea, seb, sec, sed, see, seg, seh, sei, sej and the expected amplicon sizes are listed in Table 1 (17, 19). For DNA extraction, method described above was used.

Two sets of primer mixtures were used. Mixture A consisted of the primer pairs; sea, seb, sec, seh and sej, while the mixture B contained primer pairs; sed, see, seg and sei. Multiplex PCR was performed in a total volume of 50 l containing the following: 5 l of 10×PCR buffer (Bioron GmbH, Ludwigshafen, Germany), 8 l of 25 mM MgCl2 (Bioron), 3 l of 10 mM dNTP mixture (Bioron), 0.5 l of each primer which contains 100 pmol/ l, 0.5 l of 5 U Taq DNA polymerase (Bioron) and 10 l of template DNA. The volume of the master mix was adjusted to 40 l with sterile ultra pure water. Reference strains were included as positive controls on every reaction depending on the mixture’s primer contents. DNA amplification was carried out in a thermocycler (Eppendorf mastercycler gradient, Hamburg, Germany) as described previously (19) with a slight decrease in the annealing temperature. After an incubation at 95°C for 10 min, continued as follows: 30 cycles of denaturation at 94°C for 1 min, annealing at 64°C for 1 min, extension at 72°C for 1 min, and finally a terminating incubation at 72°C for 10 min. Electrophoresis of the resultant PCR products were performed as described above.

**Results**

In the present study, a total of 102 CPS were analyzed by PCR and 70 (68.6%) of them were identified as S. aureus. According to the analysis, 65.6% of the beef isolates (21/32), 55.0% of the poultry meat isolates (11/20; 5/9 for chicken meat, 6/11 for turkey meat), 73.9% of the dairy products (17/23), 77.7% of the ready to eat foods (14/18), and 77.7% of the food ingredients (7/9) were identified as S. aureus.

Then, detection of enterotoxin gene profiles of 70 S. aureus, 32 CPS and 5 CNS was performed with multiplex PCR assay. Although in every multiplex PCR reaction, reference positive control strains were shown to be positive for the targeted genes, only 2 (2.9 %) of the tested 70 S. aureus strains belonging to turkey meat were shown to be positive for sea gene (Fig. 1). None of the CPS or CNS isolates harboured any of the SE genes (sea-see, seg-sej).

**Discussion and Conclusion**

It is widely accepted that SE production is characteristic of CPS, and most studies have dealt with S. aureus (8). Geniogeorgis (9) concluded that there is no prevailing type of SEs, apart from the strains isolated from foods involved in staphylococcal gastroenteritis, where SEA is the main type of SE recovered. In an epidemiological analysis, it has been reported that a total of 100 to
200 ng of SEA in food was sufficient to cause food poisoning (8). Previous data also indicate that SEA is mostly involved in outbreaks of staphylococcal food poisoning (16). In addition to \( \text{sea, sec,} \) and \( \text{sed} \) genes, the occurrence of \( \text{seg, seh, sei,} \) and \( \text{sej} \) genes in food-borne \( \text{S. aureus} \) strains was evaluated by some researchers (1, 23). Rosec and Gigaud (23) determined that the frequency of the strains harbouring \( \text{seg, seh, sei,} \) and \( \text{sej} \) genes was very high (57%) and greater than that of the strains harbouring “classical” SE genes (\( \text{sea, seh, sec, sed,} \) and \( \text{see} \)). Akineden et al. (1) found that 75 of 103 \( \text{S. aureus} \) isolates had one or more toxin genes which were \( \text{sea, sec,} \) \( \text{sed,} \) \( \text{seg, sei,} \) and \( \text{sej} \). Omoe et al. (22) determined all of the 146 \( \text{S. aureus} \) isolates harboured SE genes and 36 of them (17.7%) had \( \text{sea} \) genes. Also, they reported that the most commonly detected SE genotypes were \( \text{seg and sei} \) (38.9%). However, Lim et al. (16) determined 37 of 166 \( \text{S. aureus} \) isolates harboured \( \text{sea, seb and sec} \) genes while \( \text{sea} \) (32 of 37 the SE positive isolates) was the most frequent SE gene. While the results of the present study confirms previous reports of high isolation rates of foodborne \( \text{S. aureus} \) (68.6%), the low incidence of SE gene positive isolates (2 of the 70 \( \text{S. aureus} \) isolates) of the present results is in disagreement with the results of some researchers (1, 22, 23).

For \( \text{S. aureus} \), a large spectrum of different exotoxins which are responsible for toxin-mediated diseases has been described but only a little and conflicting data is available regarding the toxin production in all other staphylococcal species (5). The results of the previous studies indicate that the occurrence of SE genes in CNS is rare (6, 11). Our results about CNS are in agreement with some of the studies carried out on CNS (6, 11, 23). Valle et al. (26) isolated high percentages of enterotoxigenic CPS from milk. On the contrary, our results showed that none of the CPS other than \( \text{S. aureus} \) isolates harboured SE genes.

The results concluded that the incidence of \( \text{S. aureus} \) is high while the incidence of SE genes (\( \text{sea-see, seg-sej} \)) among foodborne \( \text{S. aureus} \) in Turkey is very low. Though the toxin gene positivity is too low, enterotoxin genes in enterotoxigenic staphylococci continue to constitute a potential risk for consumers’ health. Therefore, not only enterotoxin production, but also detection of the enterotoxin genes must be taken into account for all food-borne staphylococcal isolates.
The results of the present study confirm that the most frequent SE gene positive isolates was the sea gene. In addition to sea gene, determined 37 of 166 (22.4%) isolates harboured SE genes. While the results of the present study confirms that the frequency of the sea gene positive isolates (2 of the 70 foodborne isolates) was the most frequent SE gene.

The results concluded that the incidence of SE genes in CNS is rare (6, 11). Our results about CNS are in agreement with the occurrence of SE genes in CNS is rare (6, 11). The results of the previous studies indicate that production in all other staphylococcal species (5).

In addition to sea gene, determined 37 of 166 (22.4%) isolates harboured SE genes. While the results of the present study confirms that the frequency of the sea gene positive isolates (2 of the 70 foodborne isolates) was the most frequent SE gene.

The results of the previous studies indicate that production in all other staphylococcal species (5).

For staphylococcal intoxication, a large spectrum of different enterotoxins which are responsible for toxin production in all other staphylococcal species (5).

References


Yazışma Adresi :
Doç. Dr. Aylin KASIMOĞLU DOĞRU
Kırıkkale Üniversitesi Veteriner Fakültesi
Besin Hijyen ve Teknolojisi Anabilim Dalı
71450 Yahşihan-KIRIKKALE
E-mail: akasimoglu@superonline.com
Tel: (318) 357 42 42 / 3161
Fax: (318) 357 33 04