Determination of the Important Toxin Genes of *Staphylococcus aureus* Isolated from Meat Samples, Food Handlers and Food Processing Surfaces in Turkey

Sahin, S.,[1*] Mogulkoc, M. N.,[2] Kalin, R.[2] and Karahan, M.[2,3]

[1] Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Turkey.
[2] Department of Microbiology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Turkey.
[3] Department of Microbiology, Faculty of Veterinary Medicine, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan.

* **Corresponding Author:** Assist. Prof. Dr. Seyda SAHIN, DVM, PhD, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, 58140 Sivas, Turkey. Phone: +90 346 219 10 10, Fax: +90 346 219 18 12, Email: seydasahin@cumhuriyet.edu.tr, Orcid ID: 0000-0002-8173-7818.

ABSTRACT

Staphylococcus aureus is a pathogenic microorganism causing food intoxications and producing many heat-stable toxins. In this study *S. aureus* prevalence, the presence of enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sej, sei*) together with the toxic shock syndrome toxin gene (*tsst*) were investigated by mPCR. A total of 500 samples were obtained from different animal's meat (sheep, goat, cattle and chicken), hands of staff and surfaces in contact with food. Of 500 samples, 126 (25.2%) were contaminated with *S. aureus.* One or more staphylococcal enterotoxin (*se*) gene was determined in 87 out of 126 isolates (69%). In enterotoxigenic *S. aureus* isolates, *seg* and *sei* which are newer enterotoxins were determined most frequently (39%) then followed by *seh, sea, seb, sej,* and *sed* genes. In addition, 39 out of isolates (30.9%) were determined as positive for *tsst* gene. However, *sec* and *see* toxin genes could not be determined in any of the isolates. Consequently, it was determined that recently detected genes have a higher prevalence in food borne *S. aureus* isolates positive for classical enterotoxins. This would suggest that such a condition would contribute to pathogeny of *S. aureus* that can potentially lead to food poisoning. These results revealed that *S. aureus* isolates causing food intoxications have a wider genotypic distribution. Due to the *S. aureus* contamination, employees working in processing of meat and meat products should strictly follow food safety protocols in order to prevent foodborne infections and intoxications.

Keywords: Enterotoxin genes; Foodborne pathogen; *S. aureus*; *tsst*.

INTRODUCTION

Staphylococcus aureus is one of the most frequent agent for food intoxication resulting in infections in both animals and humans (1). According to a report from the European Food Safety Authority (EFSA), bacterial toxins are taking up a higher level in foodborne outbreaks (FBO) and are responsible for about 19.5% of all occurrences. In 2015, 434 FBO caused by staphylococcal toxins were reported in members countries of the EU (2).

S. aureus has various virulence factors, such as coagulase (COA), clumping factor (CLFA), and protein A (SPA) (3). Additionally, there are extracellular toxins that contribute to the *S. aureus* pathogeny, such as staphylococcal enterotoxins (SEs), exfoliative toxins (ETs), toxic shock syndrome toxin (TSST), and Panton-Valentine leucocidin (PVL) (4). SEs (Staphylococcal enterotoxins) are mostly produced by coagulase positive *S. aureus* which are ubiquitous microorganisms and are mostly colonized in dermal and nasal mucosae

of humans. To date, 26 different SEs have been reported (5, 6). Toxins that are known as classical SEs (SEA, SEB, SEC, SED, and SEE) are composed of 5 serotypes. Other types of SEs (SE-Like) (SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, SES, SET, SEU, SEW/U2, SEV, SE/X, SE/Y) are reported in recent years (6-8). Both classical and newer types of SEs have been reported to contribute to the pathogenesis of human infections like toxic shock syndrome, pneumonia, and sepsis (6). All of the menstrual toxic shock syndromes (mTSS) and half of the all non-menstrual toxic shock syndromes were related to staphylococcal toxic shock syndrome (TSS) caused by *S. aureus* (9, 10). In addition, there are studies where *tsst* gene was detected in *S. aureus* isolates from foods (11, 12).

S. aureus can be isolated frequently from animal's meat (cattle, chicken, fish), raw milk, and ready-to-eat foods (1, 12-16). Notable efforts are evident in recent studies for molecular epidemiology of *S. aureus* related to the investigation of enterotoxins (13, 17, 18).

Epidemiological studies revealed that enterotoxigenic staphylococci play a major role in food intoxications. Almost 95% of staphylococcal intoxications have been found to be caused by classical enterotoxins of SEA, SEB, SEC, SED, and SEE (4). It was determined that the most common toxins in FBOs were SEA and SED toxins (19, 20). Nonetheless, a recent study revealed that *S. aureus* isolates from various foods have 53.3% SE-like (SEI) toxin genes (*seg, seh, sei, sej, sek, sel, sem, sen, seo, sep, seq* and *seu*) in addition to the classical enterotoxins of *sea* and *see* (12).

Even as low as 0.1 to 10 μg doses of enterotoxin in foods have been reported to cause food intoxication (21). Therefore, a trustworthy, swift, sensitive and simple protocol is required for *S. aureus* toxin identification. Today for this purpose various techniques, namely ELISA (Enzyme Linked Immunosorbent Assay), RIA (Radio Immuno Assay), RPLA (Reversed Passived Latex Agglutination), and VIDAS (Vitek Immuno Diagnostic Assay System) have been reported to be in use (22, 23). One of the deployed protocols for the same purpose is the multiplex Polymerase Chain Reaction (mPCR). This method is preferred over others due to its higher sensitivity, speed, trustworthiness, cost, and workload. Additionally, mPCR can be used to detect more than one *S. aureus* toxins (1, 13, 17, 24).

In this study, (i) determination of the presence of *S. aureus*; (ii) investigation of *se* genes *sea, seb, sec, sed, see, seg, seh, sej, sei* via mPCR; and (iii) determination of the *tsst* gene by PCR were aimed on isolated cultured from animal's meat (sheep, goat, cattle and chicken), hands of the food processing staff and food processing surfaces.

MATERIAL AND METHODS

Sampling and bacterial isolation

In this study a total of 400 meat samples (100 samples for each animal) from different animal's meat (sheep, goat, cattle, and chicken) were collected from food processing facilities, slaughterhouses, supermarkets, butchers, bazaars and various markets in Sivas province of Turkey. In addition, swabs were taken from hands of the staffs working in food processing plants (n: 50) and from surfaces of the food production lines (n: 50). Swab protocol was used for the staff whereas swab-rinse protocol was used for surfaces (25, 26). All samples were immediately transported to the laboratory in cold-chain procedure (+4°C refrigerated box) and both isolation and identification protocols were implemented shortly after transport to the laboratory.

S. aureus isolation from samples were done in accordance with EN ISO 6881-1 (ISO 6888-1:1999). The samples were cultured on Baird-Parker agar (Oxoid, Hampshire, United Kingdom) and incubated at 37°C for 48 hours under aerobic conditions. *S. aureus* reduces tellurite to form grey-black shiny colonies and clear zones around the colonies by proteolytic action. At the end of incubation, 3 to 5 typical and atypical suspected colonies were selected and identified by gram staining, oxidase test, catalase test and coagulase production using rabbit plasma with EDTA (BD, New Jersey, United States) (27). Obtained *S. aureus* isolates were transferred to Tryptone Soya Broth, (Oxoid, Hampshire, United Kingdom) with 20% glycerol and stored at -20°C for further analyses.

DNA extraction

Bacterial DNA was extracted using the modified method of Montanaro *et al.* (28). A few colonies from pure cultures were transferred into a 1.5 mL microcentrifuge tube containing 45 μL distilled water. Five microliters of lysostaphin was added, and the mixture was incubated at 37°C for 20 min. Then, 5 μL of proteinase K (100 mg/mL; Sigma, Steinheim, Germany) and 150 μL of 0.1M Tris/HCl (pH 7.5) were added to the suspension, which was incubated for a further 20 min. Finally, the suspension was heated at 95°C for 10 min to inactivate

proteinase K. Following DNA extraction, the genomic DNA concentration and purity were measured at A260/280 nm by a NanoDrop-DS-11 FX Spectrophotometer (DeNovix, Wilmington, USA). The value of 1.8-2.0 was considered to indicate pure DNA.

Detection of *S. aureus* isolates by PCR

PCR validation of *S. aureus* isolates were conducted by using 23S rRNA specific primer pairs described previously (29) (Table 1). PCR reactions were prepared as 50 µL [5 µL 10x PCR buffer, 5 µL 25 mM $MgCl_2$, 250 µM from each dNTP, 1.25 U Taq DNA Polymerase (MBI Fermentas, Vilnius, Lithuania), 50 ρmol for each primer, and 25 ηg genomic DNA]. The PCR protocol was carried out by using Bio-Rad T100 gradient thermal cycler device (BioRad, California, USA). The PCR protocol had the following steps: initial denaturation for 2 min at 94˚C; [denaturation for 45 s at 94˚C, hybridization for 1 min at 64˚C, extension for 2 min at 72˚C] a total of 35 cycles; final extension for 10 min at 72˚C. PCR products were run on electrophoresis in 1.5% (w/v) agarose gel and stained with ethidium bromide (10 mg/mL) for 30 min, then screened under an ultraviolet transilluminator (Vilber Lourmat Quantum ST4, Marne-la-Vallée Cedex 1, France) with using 100 bp DNA ladder (MBI, Fermentas) as reference. A band in size of 1318 bp was accepted as *S. aureus* indicator. As for the positive control, *S. aureus* ATCC25923 (Department of Microbiology, Faculty of Veterinary Medicine, Firat University, Elazig) strain was used, and distilled water was used for negative control. Sequences of the used primers are presented in Table 1. Additionally, the presence of coagulase (*coa*) gene of *S. aureus* was also determined by using the protocol defined previously (30).

Detection of *S. aureus* toxin genes

For the purposes of *se* gene investigations in *S. aureus* isolates, 9 *se* primer pairs (*sea, seb, sec, sed, see, seg, seh, sei, sej*)

Table 1: Oligonucleotide primers used for the genes encoding staphylococcal 23S rRNA and SEs genes

Primer	Gene site	Primer Sequence (5′-3′)	Product Size (bp)	References
Sau327	23S rRNA	GGACGACATTAGACGAATCA	1318	(29)
Sau1645	23S rRNA	CGGGCACCTATTTTCTATCT		
Coa-1	*coa*	CGAGACCAAGATTCAACAAG	Polymorphism	(30)
Coa-2	*coa*	AAAGAAAACCACTCACATCA		
SEA-1	*sea*	GCAGGGAACAGCTTTAGGC	521[2]	(31)
SEA-2	*sea*	GTTCTGTAGAAGTATGAAACACG		
SEB-SEC-1	*seb-sec*	ACATGTAATTTTGATATTCGCACTG	667[2]	(32)
SEB-2	*seb*	TGCAGGCATCATGTCATACCA		
SEC-1	*sec*	CTTGTATGTATGGAGGAATAACAA	284[2]	(31)
SEC-2	*sec*	TGCAGGCATCATATCATACCA		
SED-1	*sed*	GTGGTGAAATAGATAGGACTGC	385[1]	(31)
SED-2	*sed*	ATATGAAGGTGCTCTGTGG		
SEE-1	*see*	TACCAATTAACTTGTGGATAGAC	171[1]	(31)
SEE-2	*see*	CTCTTTGCACCTTACCGC		
SEG-1	*seg*	CGTCTCCACCTGTTGAAGG	328[1]	(31)
SEG-2	*seg*	CCAAGTGATTGTCTATTGTCG		
SEH-1	*seh*	CAACTGCTGATTTAGCTCAG	359[2]	(31)
SEH-2	*seh*	GTCGAATGAGTAATCTCTAGG		
SEİ-1	*sei*	CAACTCGAATTTTCAACAGGTACC	466[1]	(32)
SEİ-2	*sei*	CAGGCAGTCCATCTCCTG		
SEJ-1	*sej*	CATCAGAACTGTTGTTCCGCTAG	142[2]	(31)
SEJ-2	*sej*	CTGAATTTTACCATCAAAGGTAC		
TSST-1	*tsst*	GCTTGCGACAACTGCTACAG	559	(32)
TSST-2	*tsst*	TGGATCCGTCATTCATTGTTAT		

[1] Mix 1: *sed, see, seg* and *sei* [2] Mix 2: *sea, seb-sec, sec, seh* and *sej*
SE, staphylococcal enterotoxin; TSST, Toxic shock syndrome toxin.

proposed by Monday and Bohach (31) and Lovseth *et al.* (32) were used in multiplex PCR (mPCR) protocol (Table 1). Since the PCR product sizes of the amplified regions were very similar, the mPCR protocol was conducted using two different reaction mixtures to ease the data evaluation. Reaction mixture 1 was prepared using primer pairs for *sed, see, seg,* and *sei.* Reaction mixture 2 was prepared with using primer pairs for *sea, seb-sec, sec, seh,* and *sej.* Each reaction mixture was prepared in 50 µL [5 µL 10x PCR buffer solution (750 mM Tris-HCl at pH 8.8, 200 mM $(NH_4)_2SO_4$, and 0.1% Tween-20); 5 µL 50 mM $MgCl_2$, 400 µM for each dNTP, 2 U Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania), 20 ρmol from each primer pairs, and 5 µL (5 ηg/µL) DNA]. The PCR protocol was conducted using Bio-Rad T100 gradient thermal cycler device (BioRad, California, USA). The reaction mixtures were subjected to 35 cycles of amplification and PCR protocol has the following steps: initial denaturation for 10 min at 95˚C; [denaturation for 1 min at 95˚C, hybridization for 45 s at 68˚C, extension for 1 min at 72˚C] first 15 cycles; [denaturation for 1 min at 95˚C, hybridization for 45 s at 64˚C, extension for 1 min at 72˚C] last 20 cycles; final extension for 10 min at 72˚C. PCR products were electrophoresed in 2% (w/v) agarose gel and stained with ethidium bromide (10 mg/mL) for 30 min, and then screened under Quantum ST4 ultraviolet transilluminator (Vilber Lourmat, Marne-la-Vallée Cedex 1, France) with using 100 bp DNA ladder (MBI, Fermentas SM 0241) as reference. For the positive control, 13 different reference *S. aureus* strains (R5371/00, R5460/00, R5010/00, 4774/00, R4571/00, R4071/00, R2102/00, R963/00, FRI472, FRI913, FRI572, FRI445, and FRI3169) obtained from Norway National Institute for Veterinary Research were used, and distilled water was used for the negative control.

Determination of *tsst* gene

Investigation of the presence of *tsst* gene in *S. aureus* isolates was conducted by PCR with using specific primer pair proposed by Lovseth *et al.* (32) (Table 1). PCR mixtures were prepared as stated before and protocol for multiplex PCR was conducted (Figure 1).

RESULTS

Following culture of samples collected from different animal's meat (sheep, goat, cattle, and chicken), from swabs of the

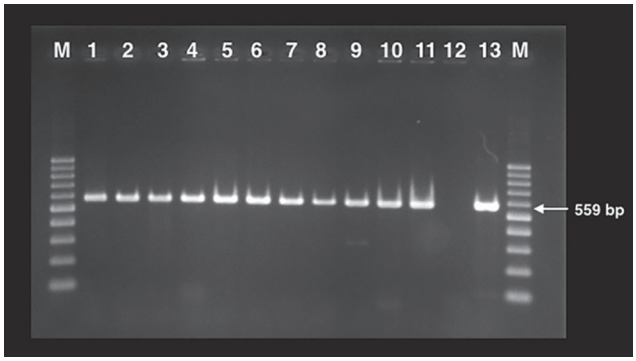

Figure 1: *tsst* genes of *S. aureus* isolates by single PCR assay. M, 100 bp DNA ladder, 1-11: *tsst*-positive isolates, 12: R4774 negative control, 13: R5371/00 positive control.

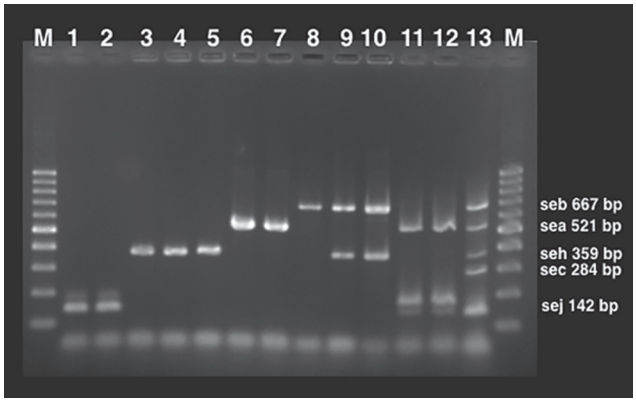

Figure 2: SE genes of *S. aureus* isolates in mPCR mixture 1. M, 100 bp DNA ladder; 1-2 *sej* positive isolates; 3-5: *seh* positive isolates 6-7: *sea* positive isolates, 8: *seb* positive isolates, 9-10: *seb* and *seh* positive isolates, 11-12: *sea* and *sej* positive isolates, 13: FR1913 +R5460/00 +R5010/00.

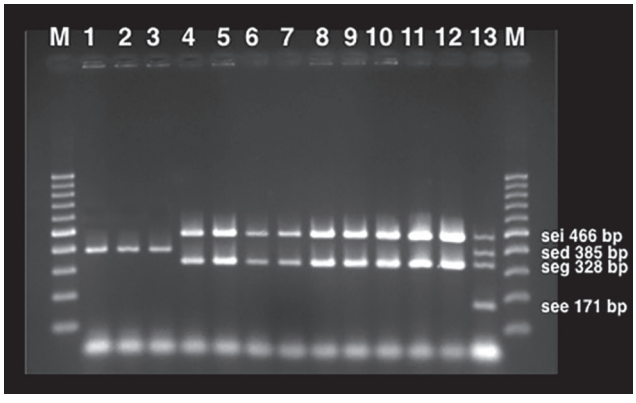

Figure 3: SE genes of *S. aureus* isolates in mPCR mixture 1. M, 100 bp DNA ladder; 1-3: *sed* positive isolates; 4-12: *seg* and *sei* positive isolates; 13: FR1913 +R5010/00.

hands of the staff, and from food processing surfaces, *S. aureus* was determined in 25.2% (126/500) of 500 samples by classical culture and biochemical methods. Of these 500 samples, 24 isolates were from sheep meat (24/100; 24%), 14 were from goat meat (14/100; 14%), 29 were from cattle meat (29/100; 29%), 32 were from chicken meat (32/100; 32%), 16 were from hands of the staff (16/50; 32%), and 11 were from surfaces (11/50; 22%).

In the study, 126 *S. aureus* isolates were investigated for *se* genes by mPCR method and 87 isolates (87/126; 69%) were found to have one or more enterotoxin genes (Table 2; Figure 2-3). Of the investigated isolates, none of them was found to have *sec* and *see* genes from the nine *se* genes. The remaining seven genes were detected from different isolates. Of these toxin genes, *seg* and *sei* genes were found together in 39 isolates (39/126; 30.9%) and determined as being the most prevalent. This was followed by *seh* gene in 15 isolates (15/126; 11.9%) where it was the sole gene in 9 isolates and coupled with *seb* in 6 isolates. Table 2 details which genes were detected in which isolates. Positive result for *tsst* gene was obtained from 39 (30.9%) isolates in total. Of these 39 isolates, both *seg* and *sei* genes were present in 20 (15.9%) isolates, only *sed* gene was present in 6 (4.8%), and *tsst* was single in 13 (10.3%) (Table 2). Besides, other 7 *se* genes were determined in 27 cattle meat (27/29; 93.1%), 31 chicken meat (31/32; 96.8%), 13 sheep meat (13/24; 54.1%), and 6 goat meat (6/14; 42.9%) (Table 2).

DISCUSSION

In various studies implemented worldwide, it is highly indicated that enterotoxigenic *S. aureus* presence in both food products and food processing facilities is the major contamination source for the staphylococcal food intoxications (33). Prevalence of enterotoxigenic *S. aureus* in foods has been reported as 85% in cattle and pork meats in the US (13), 69% in various foods in Portugal (34), 65.8% in poultry meat in Japan (22), 60.3% in various meats in Iran (23), and from 86.3% in cattle carcasses in Turkey (16). On the other hand, there are studies where the prevalence of enterotoxigenic *S. aureus* were reported as lower. For instance, it was reported as 9.3% in animal sourced foods (35), and as 34.3% in fish and shrimp samples in Iran (15), and as 46% in retail chicken meats in the US (1). The prevalence of enterotoxigenic *S. aureus* from various animal meats in this study was determined as 24.8% (99/400 samples). Differences between obtained results are thought to stem from divergence of samples, geographical regions, hygiene conditions of facilities, and different protocols used in laboratories for the isolation and identification of samples.

In this study, samples were taken from sheep, goat, cattle, and chicken meats. In addition, samples were taken from the surfaces of processing facilities and from the hands of employees to investigate the connection to humans. The staff working in food production can contaminate foods with enterotoxigenic *S. aureus* isolates during preparations,

Table 2: The presence of enterotoxin and *tsst* genes in *S. aureus* isolates.

Samples	Number of CPS isolates/ sample %	Enterotoxin genes											Number of SEs gene isolates
		Sea	*Seb*	*Sed*	*Seh*	*Sej*	*Sea+Sej*	*Seb+Seh*	*Seg+Sei*	*Sed+Tsst*	*Seg+Sei+ Tsst*	*Tsst*	
Sheep meat	%24 (24/100)	-	1	-	1	-	-	1	3	1	4	2	13
Goat meat	%14 (14/100)	-	1	-	-	-	-	-	2	1	2	1	7
Cattle meat	%29 (29/100)	2	1	-	3	2	2	2	5	2	4	4	27
Chicken meat	%32 (32/100)	2	1	1	3	2	3	2	6	2	6	3	31
Food handlers	%32 (16/50)	1	1	1	1	1	1	1	-	-	4	2	13
Food processing surfaces	%22 (11/50)	1	1	1	1	1	-	-	3	-	-	1	9
Total (n = 500)	**%25.2 (126/500)**	**6 (%4.8)**	**6 (%4.8)**	**3 (%2.4)**	**9 (%7.1)**	**6 (%4.8)**	**6 (%4.8)**	**6 (%4.8)**	**19 (%15.1)**	**6 (%4.8)**	**20 (%15.9)**	**13 (%10.3)**	**87 (%69)**

storage, and distribution stages leading to food intoxications (26, 36). Sezer *et al*. (37) conducted a study in the kitchen of a catering company and the presence of enterotoxigenic *S. aureus* was found to be 79% in the hands of the staff, and as 17% on surfaces that were in direct contact with foods. In this study, the presence of *S. aureus* was determined as 32% in the hands of employees, and as 22% on the surfaces where preparations were taking place. Aydın *et al*. (26) previously reported similar results. In their study, *S. aureus* contamination was determined as 38.7% from the staff that were on the production line, and as 25.7% in the surfaces of the line production (26). This situation poses a serious risk for both food safety and public health.

In *S. aureus* isolates, nine different enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, and *sej*) were investigated using mPCR and also *tsst* genes was investigated using single PCR assay. The results were are presented in Table 2. By investigating the isolates in terms of SE genes, 87 (69%) isolates were determined to have one or more *se* genes. In a study conducted in the US, 85% of the *S. aureus* isolates were determined to have *se* genes (13). In several studies conducted in Turkey, the presence of *se* genes in foods has been detected by utilizing conventional methods (38, 39). In some other studies in Turkey where molecular methods were used in meats and meat products, revealed 62.6% positivity of *se* genes in 1070 various foods (meat and meat products, milk and dairy products, bakery products, ready-to-consume meals) in the Marmara region (12); and revealed 86.3% (19 in 22 samples) presence of *se* genes in cattle carcasses (16).

Predominant *se* toxins in the investigated *S. aureus* isolates were *seg* and *sei* genes as found in 39 (30.9%) isolates. Since the beginning of research into new genes for enterotoxins, there have been many studies where *seg* and *sei* genes were determined as predominant (34, 40) even though their exact roles in food intoxication could not be identified as yet, but were proposed to be related. Pu *et al*. (13) determined *seg* and *sei* genes in 66% *S. aureus* isolates. Similarly, various researchers have found *seg* and *sei* genes in high frequency in similar isolates (16, 41). It has been reported that this is probable because both *seg* and *sei* genes are in the same cluster (*egc*) and have a structural similarity (42). In our study, 39 isolates that having *seg* gene also contained the *sei* gene. This result was in accordance with the other reports and therefore, it is possible to support the proposition of Jarrud *et al.* (42) where these two genes are in the same *egc* cluster and identification of either one would be sufficient to indicate others (42). It has been stated that the most frequently detected SE genes that result in staphylococcal food poisoning (SPF) are *sea, seb,* and *sed* genes (19, 43). Nevertheless, this trend is evidently changing over the course of time. In a study conducted by Aydın *et al*. (12), it was seen that newly identified toxin genes (*seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, and *seu*) in SE isolates were determined as higher (53.3%) compared to the classical toxin genes.

In the present study, the other most frequent *se* genes were *seh* in 15 isolates (single gene in 9 isolates and coupled with *seb* in 6 isolates); *sea* in 12 isolates (single genes in 6 isolates and coupled with *sej* in 6 isolates); *seb* in 12 isolates (single gene in 6 isolates and coupled with sea in 6 isolates); and single gene *sed* in 9 isolates (Table 2). Similar to present study, most frequently *seh* (20%), *sed* (15%), *sej* (13%), and *sea* (1%) genes were isolated after the predominant genes of *seg* and *sai* in USA from *S. aureus* isolates obtained from cattle and pork meats (13). In Turkey, it was determined that enterotoxigenic *Staphylococcus* strains isolated from cattle carcasses have newly identified enterotoxin genes in following percentages: *seh* (36.3%), *seg*+*sei* (9%), and *sei* (4.5%) (16). However in Iran, Rahimi *et al*. (23) were reported that 86.7% of isolates of enterotoxigenic *Staphylococcus* strains obtained from different animal's meats (cattle, sheep, goats, and camels) have the classical enterotoxin genes (14 SEA, 1 SEB, 6 SEC, and 5 SED) while the remaining 13.3% have more than one toxins (1 SEA+SEC, 3 SEA+SED). SEE toxin could not be found in any of the isolates (23). In a study, it was reported that *seh* gene has emetic activity and thus would lead to potential food intoxication (44). Indeed, Aydın *et al*. (12) isolated the *seh* gene in 15 isolates (16.3%) and reported that only 8 isolates were included the *seh* gene. In the present study, *seh* gene was detected together with *seb* gene in 15 enterotoxigenic *S. aureus* isolates (11.9%). Similar to the data presented by Aydın *et al*. (12), the great majority of the isolates contain only the *seh* gene (Table 2).

In the single PCR process performed in this study, a total of 39 (30.9%) isolates were positive in terms of the *tsst* gene. Of these 39 isolates, 20 were found to carry both *seg* and *sei* genes, 6 were found to carry *sed* gene, and remaining 13 were found to carry this gene alone. The importance of this toxin, which causes toxic shock syndrome in humans, in animal health has not yet been fully explained. The detection of the presence of the *tsst* gene in *S. aureus* isolates deserves

more detailed studies. At the same time, the fact that the vast majority of isolates that have toxic shock syndrome toxins were found together with *seg, sei,* and *sed* genes suggests that exposures can be more serious in humans. In their study, Pu *et al.* (13) stated that none of the 152 *S. aureus* isolated contained the *tst* gene. Sur and Turkyilmaz (18) reported that the *tst* gene was present 6.6% of cow milk. However, Oh *et al.* (45) detected 13.5% *tst* gene in ready-to-eat foods. A study conducted at restaurants in Kuwait found that 4% of the 8 *S. aureus* isolates obtained from food processors contained *tst* gene (46).

As a result of our findings, different animal meats were found to have 25.2% of *S. aureus* prevalence due to food processing staff and food processing, and 69% of the isolates contained multiple enterotoxin genes. It was found that some *se* genes (*seg, seh, sei* and *sej*) have high prevalence, especially in the isolates that were positive in terms of classical enterotoxins (*sea, seb, sec, sed* and *see*). This may potentially contribute to the pathogenicity of the isolates that can cause food poisoning. This study found that *S. aureus* isolates responsible for food poisoning showed a wide genotypic distribution. On the other hand, with the mPCR process applied, a large number of samples can be examined more quickly in determining the production of enterotoxins in *S. aureus* isolates obtained from meat samples for epidemiological studies. The detection of the presence of the *tsst* gene, which causes toxic shock syndrome in humans in particular, has shown that caution should be exercised when consuming raw or undercooked meats or converting them into processed food.

ACKNOWLEDGEMENTS

We are gratefully thanks to Dr. Astrid Lovseth from the Department of Feed and Food Microbiology, Norwegian National Veterinary Institute, for kindly providing the reference isolates. This research was supported by the Scientific Research Project Fund of Sivas Cumhuriyet University (Project Number: CUBAP V-014).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

REFERENCES

1. Li, S.X., Wang, P.P., Zhao, J.L., Zhou, L.H., Zhang, P.F., Fu, C.Y., Meng, J. and Wang, X.: Characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* from retail raw chicken meat. J. Food Prot. 81:528-533, 2018.
2. European Food Safety Authority (EFSA). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA J. 14(12):4634, pp:231, 2016.
3. Jenul, C. and Horswill, A.R.: Regulation of *Staphylococcus aureus* virulence. Microbiol. Spectr. 6:1, 2018.
4. Dinges, M.M., Orwin, P.M. and Schlievert, P.M.: Exotoxins of *Staphylococcus aureus*. Clin. Microbiol. Rev. 13:16-34, 2000.
5. Ono, H.K., Sato'o, Y., Narita, K., Naito, I., Hirose, S., Hisatsune, J., Asano, K., Hu, D.L., Omoe, K., Sugai, M. and Nakane, A.: Identification and characterization of a novel staphylococcal emetic toxin. Appl. Environ. Microbiol. 81:7034-7040, 2015.
6. Fisher, E.L., Otto, M. and Cheung, G.Y.C.: Basis of virulence in enterotoxin-mediated staphylococcal food poisoning. Front. Microbiol. 9:436, 2018.
7. Bania, J., Dabrowska, A., Bystron, J., Korzekwa, K., Chrzanowska, J. and Molenda, J.: Distribution of newly described enterotoxin-like genes in *Staphylococcus aureus* from food. Int. J. Food Microbiol. 108:36-41, 2006.
8. Argudín, M.A., Mendoza, M.C. and Rodicio, M.R.: Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins. 2:1751-1773, 2010.
9. Bohach, G.A., Fast, D.J., Nelson, R.D. and Schlievert, P.M.: Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit. Rev. Microbiol. 17:251-272, 1990.
10. Sharma, H., Turner, C.E., Siggins, M.K., El-Bahrawy, M., Pichon, B., Kearns, A. and Sriskandan, S.: Toxic shock syndrome toxin 1 evaluation and antibiotic impact in a transgenic model of staphylococcal soft tissue infection. mSphere. 4:e00665-19, 2019.
11. Cha, J.O., Lee, J.K., Jung, Y.H., Yoo, J.I., Park, Y.K., Kim, B.S. and Lee, Y.S.: Molecular analysis of *Staphylococcus aureus* isolates associated with staphylococcal food poisoning in South Korea. J. Appl. Microbiol. 101:864-871, 2006.
12. Aydın, A., Sudagıdan, M. and Muratoglu, K.: Prevalance of staphylococcal enterotoxins, toxin genes and genetic-relatdeness of foodborne *Staphylococcus aureus* strains isolated in the Marmara Region of Turkey. Int. J. Food Microbiol. 148:99-106, 2011.
13. Pu, S., Wang, F. and Ge, B.: Characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* isolates from Louisiana retail meats. Foodborne Pathog. Dis. 8:299-306, 2011.
14. Gucukoglu, A., Kevenk, T.O., Uyanık, T., Cadırcı, O., Terzi, G. and Alisarli, M.: Detection of enterotoxigenic *Staphylococcus aureus* in raw milk and dairy products by multiplex PCR. J. Food Sci. 7:620-623, 2012.
15. Arfatahery, N., Davoodabadi. A. and Abedimohtasab, T.: Characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* isolates in fishery products in Iran. Sci. Rep. 6:34216, 2016.
16. Keyvan, E. and Özdemir, H.: Occurrence, enterotoxigenic properties and antimicrobial resistance of *Staphylococcus aureus* on beef carcasses. Ankara Univ. Vet. Fak. Derg. 63: 17-23, 2016
17. Karahan, M., Acik, M.N. and Cetinkaya, B.: Investigation of toxin genes by polymerase chain reaction in *Staphylococcus aureus* strains isolated from bovine mastitis in Turkey. Foodborne Pathog. Dis. 6:1029-1035, 2009.

18. Sur, E. and Turkyilmaz, S.: Investigation of the toxin genes and antibiotic resistance in *Staphylococcus aureus* isolates from subclinical mastitic cow milk. Isr. J. Vet. Med. 75:35-42, 2020.
19. Balaban, N. and Rasooly, A.: Staphylococcal enterotoxins. Int. J. Food Microbiol. 61:1-10, 2000.
20. Chen, Q. and Xie, S.: Genotypes, enterotoxin gene profiles, and antimicrobial resistance of *Staphylococcus aureus* associated with foodborne outbreaks in Hangzhou, China. Toxins. 11:307, 2019.
21. Erol, İ.: *Staphylococcus aureus*. In: Erol, İ. (Eds.). Gıda Hijyeni ve Mikrobiyolojisi Kitabı. Ankara, ISBN 978-975-00131-0-9, s:135-144. 2007.
22. Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Uji, T. and Kitagawa, H.: Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. J. Vet. Med. Sci. 67:107-110, 2005.
23. Rahimi, E., Nonahal, F. and Salehi, E.A.: Detection of classical enterotoxins of *Staphylococcus aureus* strains isolated from raw meat in Esfahan, Iran. Health Scope. 2:95-98, 2013.
24. Saka, E. and Gulel, G.T.: Detection of enterotoxin genes and methicillin-resistance in *Staphylococcus aureus* isolated from water buffalo milk and dairy products. J. Food Sci. 83:1716-1722, 2018.
25. Legnani, P., Leoni, E., Berveglieri, M., Mirolo, G. and Alvaro, N.: Hygienic control of mass catering establishments, microbiological monitoring of food and equipment. Food Control. 15:205-211, 2004.
26. Aydın, A., Aksu, H. and Arun, O. O.: Evaluation of hygienic properties of food handlers and equipment in food production and sales units. Med. Weter. 63: 1067-1070, 2007.
27. ISO. EN ISO 6888-1 Microbiology of food and animal feeding stuffs: Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker agar medium. ISO, Geneva, Switzerland, 1999.
28. Montanaro, L., Arciola, CR., Baldassarri, L., Borsetti, E.: Presence and expression of collagen adhesin gene *(cna)* and slime production in *Staphylococcus aureus* strains from orthopaedic prosthesis infections. Biomaterials. 20:1945-1949, 1999.
29. Riffon, R., Sayasith, K., Khalil, H., Dubreuil, P., Drolet, M. and Lagace, J.: Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. J. Clin. Microbiol. 39:2584-2589, 2001.
30. Goh, S., Byrne, S.K., Zhang, J.L. and Chow, A.W.: Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphisms. J. Clin. Microbiol. 30:1642-1645, 1992.
31. Monday, S.R. and Bohach, G.A.: Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. J. Clin. Microbiol. 37:3411-3444, 1999.
32. Lovseth, A., Loncarevic, S. and Berdal, K.G.: Modified multiplex PCR method for detection of pyrogenic exotoxin genes in staphylococcal isolates. J. Clin. Microbiol. 42:3869-3872, 2004.
33. Seo, K.S. and Bohach, G.A.: *Staphylococcus aureus.* In: Doyle, M.P. and Beuchat, L.R. (Eds.). Food Microbiology: Fundamentals and Frontiers. ASM Press, Washington DC, pp. 493-518, 2007.
34. Pereira, V., Lopes, C., Castro, A., Silva, J., Gibbs, P. and Teixeira, P.: Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. Food Microbiol. 26:278-282, 2009.
35. Mashouf, R.Y., Hosseini, S.M., Mousavi, S.M. and Arabestani, M.R.: Prevalence of enterotoxin genes and antibacterial susceptibility pattern of *Staphylococcus aureus* strains isolated from animal originated foods in West of Iran. Oman Med. J. 30:283-290, 2015.
36. Ayçiçek, H., Aydoğan, H., Küçükkaraslan, A., Baysallar, M. and Başustaoğlu, A.C.: Assesment of the bacterial contamination on hands of hospital food handlers. Food Control. 15:253-259, 2004.
37. Sezer, Ç., Çelebi, Ö., Aksoy, A. and Vatansever, L.: Food Handlers: a bridge in the journey of enterotoxigenic MRSA in Food. J. Verbr. Lebensm. 10:123-129, 2015.
38. Küplülü, Ö., Sarımehmetoğlu, B. and Çelik, T.H.: Determination of the enterotoxigenicity of coagulase positive staphylococci isolated from cheese by ELISA. Milchwissenschaft. 59:17-19, 2004.
39. Kaya, H., Ertaş Onmaz, N., Gönülalan, Z. and Al, S.: Detection of *Staphylococcus aureus* and their enterotoxins in poultry meats consumed in Kayseri. Erciyes Üniv. Vet. Fak. Derg. 12:93-98, 2015.
40. Kwon, N.H., Kim, S.H., Park, K.T., Bae, W.K., Kim, J.Y., Lim, J.Y., Ahn, J.S., Lyoo KS, Kim, J.M., Jung, W.K., Noh, K.M., Bohach, G.A. and Park, H.Y.: Application of extended single-reaction multiplex polymerase chain reaction for toxin typing of *Staphylococcus aureus* isolates in Korea. Int. J. Food Microbiol. 97:137-145, 2004.
41. Omoe, K., Ishikawa, M., Shimoda, Y., Hu, D.L., Ueda, S. and Shinagawa, K.: Detection of seg, seh, and sei genes in *Staphylococcus aureus* isolates and determination of the enterotoxin productivities of S. aureus isolates harboring *seg, seh*, or *sei* genes. J. Clin. Microbiol. 40:857-862, 2002.
42. Jarraud, S., Peyrat, M.A., Lim, A., Tristan, A., Bes, M., Mougel, C., Etienne, J., Vandenesch, F., Bonneville, M. and Lina, G.: egc, a highly prevalent operon of enterotoxin gene, forms a putative nursery of superantigens in *Staphylococcus aureus*. J. Immunol. 66:669-677, 2001.
43. Kerouanton, A., Hennekinne, J.A., Letertre, C., Petit, L., Chesneau, O., Brisabois, A. and De Buyser, M.L.: Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. Int. J. Food Microbiol. 115:369-375, 2007.
44. Su, Y.C. and Wong, A.C.: Identification and purification of a new staphylococcal enterotoxin, H. Appl. Environ. Microbiol. 61:1438-1443, 1995.
45. Oh, S.K., Lee, N., Cho, Y.S., Shi,n D.B., Choi, S.Y. and Koo, M.: Occurrence of toxigenic *Staphylococcus aureus* in ready-to-eat food in Korea. J. Food Prot. 70:1153-1158, 2007.
46. Udo, E.E., Al-Mufti, S. and Albert, M.J.: The prevalence of antimicrobial resistance and carriage of virulence genes in *Staphylococcus aureus* isolated from food handlers in Kuwait City restaurants. BMC Res. Notes. 2:108, 2009.