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PCR and ELISA for staphylococcal enterotoxins and detection of some exotoxins from Staphylococcus spp. strains by PCR[1)]

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Summary

The aims of this study were to determine the existence of some staphylococcal enterotoxin (SE) (sea, seb, sec, sed, and see) proteins and genes in coagulase-positive staphylococci (CPS) by ELISA and PCR and to assess SE-like toxin (SEI) (seg, seh, sei, sej, sem, sen, and seo), exfoliative toxin (eta and etb), toxic shock syndrome toxin-1 (tst) and 16S rRNA genes in 11 different Staphylococcus strains [90 CPS and 118 coagulase-negative staphylococci (CNS)] isolated from 250 ground meat samples by either monoplex or multiplex PCR. SEs were identified in 36 (40%) out of 90 CPS isolates by both ELISA and PCR, with the following distribution: sea was identified in 7 (7.7%), seb in 5 (5.5%), sec in 3 (3.3%), sed in 4 (4.4%), and see in 17 (18.8%). In addition, a total of 90 CPS and 118 CNS isolates were investigated for the presence of 11 SE, SEI, eta-etb, tst, and 16S rRNA genes. Overall, 145 (69.7%) of the Staphylococcus spp. isolates tested positive for one or more toxin genes. These results indicate that CNS may play an important role in food poisoning and that SEI toxins must be investigated in greater detail in future studies of both CPS and CNS.

Keywords: Staphylococcus spp., enterotoxins, exotoxins, ELISA, PCR

Staphylococcus spp. has the highest prevalence in protein-rich foods of animal origin, such as meat, poultry, fish and milk products. Staphylococcal food intoxication is one of the most common types of foodborne diseases worldwide. These bacteria can be killed through the heat treatment of food; however, enterotoxins are very heat resistant. Thus, although the bacteria are eliminated, the toxins will remain (18).

Staphylococcal toxins can be characterized into the following groups: pyrogenic toxin superantigens (PTSAgs), exfoliative toxins (ETA, ETB), leukocidins and other toxins. The family of PTSAgs includes staphylococcal enterotoxins (SEs), SE-like (SEI) toxins and toxic shock syndrome toxin-1 (TSST-1) (19). TSST-1 is unique in its ability to cross mucosal surfaces, and it causes the life-threatening toxic shock syndrome in humans (28). ETA and ETB have been associated with a series of impetiginous staphylococcal diseases, referred to collectively as staphylococcal scalded skin syndrome. Although ETA and ETB have the same biological activities and similar genetics, ETA has a chromosomal origin, while ETB is produced by plasmids (16). All of these toxins are responsible for a great deal of different types of infection in humans and animals (35).

Enterotoxigenic strains of staphylococci have been extensively characterized based on their genotypic and phenotypic characteristics. Formerly, SEs were divided into 5 major serological types (SEA through SEE) based on their antigenic properties. In recent years, SEI toxins (SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER, SES, SET, SEU, SEV and SEX) have also been identified (24, 28). All of these toxins possess superantigenic activities, whereas only a few of them (SEA to SEI, SER, SES and SET) have been proven to be emetic (36). Moreover, Omoe et al. (25) recently demonstrated the emetic activities of SEK, SEL, SEM, SEN, SEO, SEP and SEQ toxins.

Many molecular studies have been conducted on enterotoxigenic *Staphylococcus* spp. for the detection of ETA and ETB (*eta-etb*) and TSST-1 (*tst*) genes (5, 6, 17, 21, 35). DNA hybridization and PCR approaches offer an alternative for the detection of staphylococ-

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cal isolates bearing the genetic sequences to produce enterotoxins, irrespective of their toxin production levels (6). PCR has been used as a simple technique for detecting exotoxigenic strains (4). However, although PCR-based methods are specific, highly sensitive and rapid, they can only detect the presence of enterotoxigenic genes and not the production of SE proteins (7). The production of these proteins can be assessed by immunological methods (ELISA, etc.), which are time-consuming and are not always able to detect toxin-producing strains (36). ELISA is the most preferred method among the immunological methods because its reagents are commercially available for both toxin screening and serotype specific identification assays (5, 10).

Staphylococcal food poisoning results from the ingestion of food containing preformed SEs produced by enterotoxigenic CPS, mainly *Staphylococcus aureus* (*S. aureus*), in addition to *S. intermedius*, *S. hyicus* and *S. epidermidis* (8, 9, 24, 26). Both CPS and CNS carry genes for the production of these enterotoxins (5, 6, 11, 12, 36). However, although *S. aureus* is a strong enterotoxin producer, CNS have been determined to produce these enterotoxins at a low level (8, 26, 37).

Little is known about the occurrence of virulence factors in retail ground beef and lamb staphylococcal strains. The aims of this study were to determine the prevalence of SE genes (*sea*, *seb*, *sec*, *sed* and *see*), several SEI toxin genes (*seg*, *seh*, *sei*, *sej*, *sel*, *sem*, and *seo*), exfoliative toxin genes (*eta* and *etb*) and the toxic shock syndrome 1 toxin (*tst*) gene, each of which has a potent effect on cells of the immune system, in *Staphylococcus* spp. strains by PCR and also to detect the phenotypic presence of SE proteins by ELISA using coagulase-positive *S. aureus* and *S. intermedius* strains.

Material and methods

Bacterial strains. Isolates were obtained from ground beef and lamb meat in the Diyarbakır region of Turkey. Eighty-five *S. aureus*, 5 *S. intermedius* (CPS) and 118 CNS isolates have been identified at the species level, and some of the genes of these species (*mecA*, *pvl* etc.) have been detected by multiplex PCR in a previous study (13). Reference *Staphylococcus aureus* strains in Table 1 were used as positive controls in this study. Also *S. epidermidis* 12228, *Escherichia coli* 0157:H7 ATCC 43895, *Salmonella* Typhimurium ATCC 14028, *Enterococcus feacalis* WHO 3, and *Listeria monocytogenes* ATCC 7644 were used as negative controls (all of them provided by the Ünal N. Department of Microbiology, School of Veterinary Medicine, University of Kırıkkale, Kırıkkale, Turkey). Before DNA extraction, cultures were streaked on tryptic soy broth (TSB) agar plates and grown overnight at 37°C.

ELISA. SEs from 90 CPS isolates were assessed using commercially available kits (Ridascreen SET A, B, C, D, E, R-biopharm, Germany, Art no: R1101). Briefly; we took an appropriate portion of bacterial stock culture (chilled or frozen storage) and inoculated it into 10 ml sterile BHI broth, then incubated for 16 to 18 h (overnight) at 37°C. Streak out a loop of the pre-culture on BHI plates, incubated it for 24 h at 37°C, and we took one or two (depending on size) of single colonies with a loop and transferred the material to 10 ml sterile BHI broth, then incubated overnight (16-18 h) at 37°C. Pure cultures were used for ELISA, following the manufacturer's protocol (2).

Primers. The primers used for the PCR assays are listed in Tab. 2. Each SE primer pair was unique to an SE gene, with the exception of *seb-sec*, for which one pair of *sec* primers was included to differentiate between them.

DNA isolation. DNA was extracted from the isolates using an InstaGene Matrix DNA Extraction Kit (Bio-Rad Laboratories, Hercules, CA, USA, Cat No.: 7326030), following the manufacturer's recommendations.

Detection of SE, SEI, *eta-etb*, *tst* and 16S rRNA genes by PCR. Six different mixtures were prepared for monoplex and multiplex PCR analyses. After optimization with the mentioned negative controls, for the negative control, sterile water was added instead of template DNA. Mixture 1 included the *sed*, *see*, *seg*, *sei* and *tst* primers, while mixture 2 contained the *sea*, *seb-sec*, *sec*, *seh*, *sej* and 16S rRNA primers. These mixtures were prepared in a total volume 50 µl, containing 5 µl 10X PCR buffer (750 mmol l^{-1}

Tab. 1. *Staphylococcus aureus* reference strains used in this study and evaluation of the specificity of the PCR amplification

Strains	*Se* genotype	PCR Targets												
		sea	seb	sec	sed	see	seg	seh	sei	sej	sem	sen	seo	tst
ATCC 27664*	*see*$^+$	–	–	–	–	–	–	–	–	–	–	–	–	–
D4508#	*sea*$^+$, *seh*$^+$	+	–	–	–	–	–	+	–	–	–	–	–	–
DSM 20231†	–	–	–	–	–	–	–	+	–	–	–	–	–	–
ATCC 14458*	*seb*$^+$	–	+	–	–	–	–	–	–	–	–	–	–	–
ATCC 25923*	egc$^+$ (*seg*, *sei*, *sem*, *sen*, *seo*, *sec*, *seh*)	–	–	+	–	–	+	+	+	–	+	+	+	–
ATCC 19095*	*sec*$^+$, *seh*$^+$, egc$^+$ (*seg*, *sei*, *sem*, *sen*, *seo*)	–	–	–	–	–	+	–	+	–	+	+	+	–
A900322#	egc$^+$ (*seg*, *sel*, *sem*, *sen*, *seo*)	–	–	–	–	–	+	–	+	–	+	+	+	–
NCTC 9393+	*sed*$^+$, *sej*$^+$, egc$^+$ (*seg*, *sei*, *sem*, *sen*, *seo*)	–	–	–	+	–	+	–	+	+	+	+	+	–
RIMD 31092#	*seb*$^+$, *sec*$^+$, egc$^+$ (*seg*, *sei*, *sem*, *sen*, *seo*), *tst*	–	+	+	–	–	+	–	+	–	+	+	+	+

Explanations: *ATCC, American Type Culture Collection (Rockville, Maryland, USA); # Strains provided from Asos. Prof. Dr. Nilgün Unal, Kırıkkale University Departmant of Microbiology, Turkey; † DSM, Deutsche Sammlung von Microorganism und Zellkulturen, Gmbh, Braunschweig, Germany; + NCTC, National Collection of Type Cultures, PHLS Central Public Health Laboratory (London, UK).

Tab. 2. Target gene, references and expected product length amplified by primers

Gene	Sequence (5'-3')	References	Product (bp)
sea	GCA GGG AAC AGC TTT AGG C GTT CTG TAG AAG TAT GAA ACA CG	Monday and Bohach 1999	520
seb-sec	ACA TGT AAT TTT GAT ATT CGC ACT G TGC AGG CAT CAT GTC ATA CCA	Lovseth et al. 2004	667
sec	CTT GTA TGT ATG GAG GAA TAA CAA TGC AGG CAT CAT ATC ATA CCA	Monday and Bohach 1999	284
sed	GTG GTG AAA TAG ATA GGA CTG C ATA TGA AGG TGC TCT GTG G	Monday and Bohach 1999	385
see	TAC CAA TTA ACT TGT GGA TAG AC CTC TTT GCA CCT TAC CGC	Monday and Bohach 1999	171
seg	CGT CTC CAC CTG TTG AAG G CCA AGT GAT TGT CTA TTG TCG	Monday and Bohach 1999	328
seh	CAA CTG CTG ATT TAG CTC AG GTC GAA TGA GTA ATC TCT AGG	Monday and Bohach 1999	359
sei	CAA CTC GAA TTT TCA ACA GGT ACC CAG GCA GTC CAT CTC CTG	Monday and Bohach 1999	466
sej	CAT CAG AAC TGT TGT TCC GCT AG CTG AAT TTT ACC ATC AAA GGT AC	Monday and Bohach 1999	142
tst	GCT TGC GAC AAC TGC TAC AG TGG ATC CGT CAT TCA TTG TTA T	Lovseth et al. 2004	559
eta	CTA GTG CAT TTG TTA TTC AA TGC ATT GAC ACC ATA GTA CT	Johnson et al. 1991	119
etb	ACG GCT ATA TAC ATT CAA TT TCC ATC GAT AAT ATA CCT AA	Johnson et al. 1991	200
sem	CCAATTGAAGACCACCAAAG CTTGTCCTGTTCCAGTATCA	Blaiotta et al. 2004	517
sen	ATTGTTCTACATAGCTGCAA TTGAAAAAACTCTGCTCCCA	Blaiotta et al. 2004	682
seo	AGTCAAGTGTAGACCCTATT TATGCTCCGAATGAGAATGA	Blaiotta et al. 2004	534
16S rRNA	GCAAGCGTTATCCGGATTT CTTAATGATGGCAACTAAGC	Al-Talib et al. 2009	597

Tris-HCl (pH 8.8; 25°C), 200 mmol l^{-1} $(NH4)_2SO_4$ and 0.1% Tween 20), 5 µl 40 nM $MgCl_2$, 400 µM of each deoxynucleotide triphosphate, 2 U of *Taq* DNA polymerase (MBI Fermentas, Hannover, MD), 20 pmol of the SE and 16S rRNA primer pairs and 5 µl of template DNA (5 ng/µL). Amplification was performed as follows: an initial denaturation step at 95°C for 10 min, 30 cycles of denaturation at 95°C for 1 min, annealing at 64°C for 45 sec, extension at 72°C for 1 min, and a final extension at 72°C for 10 min.

Mixture 3 was prepared for the *eta* and *etb* primers as described above. After the initial denaturation step at 94°C for 5 min, a total of 30 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 1 min were performed, followed by a final extension at 72°C for 5 min.

Single PCRs were performed for the *sem*, *sen* and *seo* primers. Therefore, 3 different PCRs were performed in a total volume of 25 µl, containing 3 µl of template DNA, 1 U of *Taq* DNA polymerase, 10X PCR buffer (750 mmol l^{-1} Tris-HCl (pH 8.8; 25°C), 200 mmol l^{-1} $(NH_4)_2SO_4$ and 0.1% Tween 20), 1.25 µl of 50 mmol $MgCl_2$, 0.25 µl of dNTP mix (25 mmol each) (Roche Diagnostics, Mannheim, Germany), and 20 pmol of primer. These single PCRs were performed as follows: an initial denaturation for 5 min at 95°C, 30 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 75 sec, and extension at 72°C for 30 sec, as well as a final extension at 72°C for 10 min.

All PCRs were performed with an ABI Veriti Thermal Cycler (Applied Biosystems Asia Pte Ltd., Singapore). PCR products were separated in a 1.5% agarose gel, stained with ethidium bromide and then visualized using a gel imaging system (Quantum ST4, Vilber Louma, Germany).

Results and discussion

ELISA. The ability to synthesize enterotoxins was determined in 36 (40%) of the 90 CPS isolates (*S. aureus* and *S. intermedius*) using ELISA. These SEs were determined to be distributed as follows: SEA was detected in 7 (7.7%) isolates, SEB in 5 (5.5%), SEC in 3 (3.3%), SED in 4 (4.4%), and SEE in 17 (18.8%). Five *S. intermedius* isolates did not produce SEs, and positive results were determined only for 36 *S. aureus* isolates.

Detection of SE, SEI, *eta-etb*, *tst*, and 16S rRNA genes by PCR. The prevalence of toxin genes among the 208 isolates as determined by PCR is shown in Tab. 3. One hundred forty-five isolates (69.7%) were found to be positive for one or more toxin genes. The *Staphylococcus* strains that tested positive for at least one toxin gene included *S. aureus* (86.6%), *S. intermedius* (20%), *S. hominis* (65.2%), *S. lentus* (37.5%), *S. pasteuri* (12.5%), *S. warneri* (60%), and *S. saprophyticus* (73.3%). The *S. kloosi*, *S. vitulinus*, *S. chromogenes*, and *S. equorum* isolates were positive for any of the toxin genes. Among the SE genes, *see* (9.1%) was the most widespread, followed by *seb* (7.2%), *sea* (6.7%), *sed* (4.3%) and *sec* (2.9%). Among the SEI toxin genes, *seg* (16.3%) was the most predominant, followed by *sem* (15.9%), *seo* (11.5%), *sen* (10.6%), *seh* (8.6%), and *sei* (7.7%). None of the isolates harbored the gene encoding *sej*.

Multiple toxin gene combinations were the most commonly observed, including *seg-seh*, *seg-sei,* and *sem-sen*, which were detected at the same rate (3.4%), followed by *see-seo* (2.4%), *seg-sem-seo* (2.4%), *see-seg* (1.9%), *sem-seo* (1.9%), *sea-sem* (1.4%), *see-seh* (1.4%), *sea-sed-see* (1%), *seb-sec-see* (1%), *seh-sem-seo* (1%), *see-sei* (0.5%), and *sei-sem-seo* (0.5%) (Tab. 4).

Compared with the CPS and CNS isolates, the prevalence of toxin genes in the 90 CPS isolates was 87.7% (79/90), while that in the 118 CNS isolates was 55.9% (66/118). In addition, the PCR results showed that 73.5% of the isolates contained SEI toxin genes (*seg*, *seh*, *sei*, *sej*, *sem*, *sen* and *seo*), which were more frequently observed compared with the SE genes (*sea* to *see*) (31.7%).

Tab. 3. Prevelance of toxin genes in 208 *Staphylococcus* spp. isolates from ground beef and lamb meat

Strains		Total number of strains	No. of toxigenic strains	PCR Target														
				Eta	Etb	sea	seb	sec	sed	see	seg	seh	sei	sej	sem	sen	seo	tst
S. aureus	Beef	35	33	–	–	2	3	–	1	9	11	7	–	–	11	7	9	1
	Lamb	50	45	–	–	5	2	3	3	8	8	10	1	–	9	6	12	2
S. intermedius	Beef	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Lamb	5	1	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–
S. hominis	Beef	18	14	–	–	–	3	1	2	–	2	–	3	–	3	1	3	–
	Lamb	5	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–
S. lentus	Beef	8	3	–	–	1	–	–	1	–	–	–	–	–	2	–	–	–
	Lamb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. pasteuri	Beef	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Lamb	5	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–
S. warneri	Beef	5	3	–	–	–	–	–	–	2	–	–	1	–	1	–	–	–
	Lamb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. saprophyticus	Beef	40	31	–	–	4	5	2	1	–	10	–	8	–	5	5	–	–
	Lamb	20	13	–	–	1	2	–	–	–	3	–	3	–	2	3	–	–
S. kloosi	Beef	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Lamb	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. vitulinus	Beef	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Lamb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. chromogenes	Beef	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Lamb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. equorum	Beef	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Lamb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total (%)		208	145 (69.7)	0 (0)	0 (0)	14 (6.7)	15 (7.2)	6 (2.8)	9 (4.3)	19 (9.1)	34 (16.3)	18 (8.6)	16 (7.7)	0 (0)	33 (15.9)	22 (10.6)	24 (11.5)	3 (3.3)

Tab. 4. Frequency of *S. aureus* and coagulase-negative *S. aureus* species carrying two and more enterotoxin genes

Staphylococcal Species	a, m*	e, g*	e, h*	e, i*	e, o*	g, h*	g, i*	m, n*	m, o*	a, d, e*	b, c, e*	g, m, o*	h, m, o*	i, m, o*	Total
S. aureus	2	4	3	–	5	7	1	5	3	2	2	4	2	–	40
Other *Staphylococcus* spp.	1	–	–	1	–	–	6	2	1	–	–	1	–	1	13
Total (%)	3 (1.4)	4 (1.9)	3 (1.4)	1 (0.5)	5 (2.4)	7 (3.4)	7 (3.4)	7 (3.4)	4 (1.9)	2 (1)	2 (1)	5 (2.4)	2 (1)	1 (0.5)	53

Explanation: * staphylococcal exotoxin genes

Three *S. aureus* isolates (3.3%) showed positive results for the *tst* gene. Two of these isolates have also been found to be *mecA*-positive in a previous study (13). Further, one of these two isolates carried the *sec* gene, and none harbored the gene encoding *eta* or *etb*.

It is generally acknowledged that SE production is characteristic of CPS, and most studies have evaluated *S. aureus* in this regard (5, 7, 10, 17, 21, 27, 28, 32). Despite reports demonstrating the presence of toxin genes in CNS species (6, 11, 12, 36), using this genotypic technique (especially PCR), which is important for the confirmation of the toxicity of CNS species, many investigators still question their toxigenic potentials. In fact, both CPS and CNS carry genes for the production of enterotoxins (5). Therefore, this study aimed to investigate the presence of SE, SEI, *tst* and *eta-etb* genes in both CPS and CNS isolates by PCR and to detect the phenotypic presence of SEs by ELISA in coagulase-positive *S. aureus* and *S. intermedius* isolates.

PCR is as a useful, rapid and reliable tool for the detection of exotoxin and enterotoxin genes (11, 12, 16, 17, 21, 27, 31, 34). Defects in toxin expression have been shown to be due to point mutations, which convert toxin-encoding genes to silent genes (23). These genes can be reactivated by a single mutational event and subsequently be expressed under appropriate conditions (13). Consequently, not only enterotoxin production but also the detection of enterotoxin genes must be considered for all staphylococcal strains (30); however, PCR is only able to demonstrate the presence or absence of a certain gene in a strain, it cannot detect the production of enterotoxins. Thus, this study also aimed to use both the commercially available ELISA and PCR for the detection of SE production by CPS isolates.

One hundred forty-five *Staphylococcus* spp. isolates (69.7%) tested positive for one or more toxin genes. These results are similar to those of other food-based studies. For example, Maslankova et al. (21) found that 75.9% of *S. aureus* isolates collected from sheep were positive for one or more toxin genes. In addition, Aydın et al. (5) analyzed 1070 food samples obtained from retail markets and dairy farms in Turkey and found that out of 147 *S. aureus* isolates, 92 (62.6%) were enterotoxigenic. However, Gencay et al. (11) reported that only 2.9% of poultry meat *S. aureus* isolates from Turkey were positive for SE genes. The differences between the findings of this study and previous results may be due to the fact that all of the previous studies evaluated only *S. aureus* isolates; conversely, we assessed 11 different *Staphylococcus* species.

A comparison of the CPS and CNS isolates revealed that the prevalence of toxin genes was 87.7% (79/90) in the 90 CPS isolates and 55.9% (66/118) in the 118 CNS isolates. These results are in agreement with those of previous studies (6, 12), indicating that SE genes are frequently present in CNS; however, our findings are in contrast with those of other studies (31, 34) reporting that the presence of SE genes in CNS is very rare. This discrepancy may be due to differences in the sample types, detection methods, detected genes and number of samples evaluated in these studies.

The *Staphylococcus* strains that tested positive for at least one toxin gene included *S. aureus* (86.6%), *S. intermedius* (20%), *S. hominis* (65.2%), *S. lentus* (37.5%), *S. pasteuri* (12.5%), *S. warneri* (60%), and *S. saprophyticus* (73.3%). The *S. kloosi*, *S. vitulinus*, *S. chromogenes*, and *S. equorum* isolates were positive for only one toxin gene. Few studies evaluate all of these *Staphylococcus* strains. Park et al. (26) investigated a total of 263 CNS representing 11 different *Staphylococcus* spp., which were examined from bovine intramammary infections. Another study that has indicated that *S. warneri* possesses the highest number of toxin genes within its genome compared with other evaluated strains (36).

Among the SE genes, we determined that *see* (9.1%) was the most widespread, followed by *seb* (7.2%), *sea* (6.7%), *sed* (4.3%) and *sec* (2.9%). These results differ from those of other studies (10, 11, 21, 27, 31). For example, Maslankova et al. (21) reported a higher frequency of the *sec* gene (24.1%), followed by *tst* (22.8%), *seb* (13.9%), *sed* (10.1%) and *sea* (5.1%), in *S. aureus* isolates from sheep. This difference may have been due to the fact that this study (21) used sheep milk and cheese, but not meat, isolates.

Among the SEI toxin genes, we found that *seg* (16.3%) was the most widespread, followed by *sem* (15.9%), *seo* (11.5%), *sen* (10.6%), *seh* (8.6%), and *sei* (7.7%). None of the isolates harbored the gene encoding *sej*. Previous studies reported that *sed*, *sej* and *ser* were encoded adjoiningly by the same plasmid, such as pIB485-like plasmid, pUO-Sa-SED2 (3, 25). And so, in spite of the certain exceptions (e.g. Fukuoka 5 strain), *sed*, *sej*, *ser* always occur together (25). When we were evaluated our primers which were only *sed*, *sej*, our results were in disagreement with this case. It was could be the result of primer design or sentences, because of the company's erroneous. Also, we detected multiple "incomplete egc" in our isolates. This was possibly the result of corresponding to evolutionary intermediates (33) or the wrong primers. These cases can be worked as molecularly in another study in the near future.

The PCR results were showed that 73.5% of the CPS and CNS isolates contained SEI toxin genes (*seg*, *seh*, *sei*, *sej*, *sem*, *sen* and *seo*) and that they were more frequent than the SE genes (*sea* to *see*) (31.7%). These results are in accordance with those of Aydın et al. (5), who performed PCR and found that 53.3% of the isolates studied contained 8 SEI toxin genes (*seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq and seu*), which were more frequently detected compared with the SE genes. Furthermore, this group reported rates of 37.0%, 32.7%, 30.4%, 29.3% and 27.2% for *seo*, *sei*, *sem*, *seg*, *seu* and *sec*, respectively, for the *Staphylococcus* spp. isolates assessed.

Multiple toxin gene combinations were most commonly observed in this study, including *seg-seh*, *seg--sei*, and *sem-sen*, which were detected at the same rate (3.3%), followed by *see-seo* (2.4%), *seg-sem--seo* (2.4%), *see-seg* (1.9%), *sem-seo* (1.9%), *sea-sem* (1.4%), *see-seh* (1.4%), *sea-sed-see* (1%), *seb-sec-see* (1%), *seh-sem-seo* (1%), *see-sei* (0.5%), and *sei-sem--seo* (0.5%). Similar results have been reported, particularly for the *seg* and *sei* combination in *S. aureus* and the CNS isolates (15, 36). The presence of this combination indicates the high toxigenic potential of CNS strains that are often overlooked and only considered to be contaminants (36).

Three *S. aureus* isolates (3.3%) have tested positive for the *tst* gene and two have also tested positive for *mecA* in our previous study (13). This rate of 3.3% is lower than that reported by Maslonkova et al. (20), who found 22.8% *tst*-positive *S. aureus* strains of sheep origin, but it is consistent with those reported by other studies (16, 17, 21, 35, 38). In addition, one of the *tst*-positive isolates carried the *sec* gene, which is in agreement with previous studies reporting a positive correlation between *sec* and *tst* (21, 38).

All of the CPS and CNS isolates tested positive for the 16S rRNA gene. However, none of them harbored the gene encoding *eta* or *etb*. These results are consistent with those of other studies (17, 21, 35). Thus it can be concluded that ET genes are generally harbored by *S. aureus* isolates of human origin (17).

A PCR assay was used to investigate the presence of important exotoxins (enterotoxins, *eta-etb*, and *tst*) in CNS and CPS strains isolated from ground beef and lamb meat for the first time in Turkey. We determined that SEs and SEIs are widespread among not only *S. aureus* but also other CPS and CNS strains. ET genes

were not detected in the meat isolates. Multiplex PCR is a good alternative in terms of its reliability for SE gene detection and time and labor efficiencies. The determination of SEI toxin genes in staphylococcal isolates at high levels indicates that new serologic detection methods can be developed for SEI toxins in the future. In addition, the results of the commercially available ELISA results were in 100% agreement (100% sensitivity and specificity) with the PCR results for the detection of SEs in this study. This study also showed that CPS play significant roles in many food poisoning cases. Additional studies of CNS and SEIs should be conducted in the near future.

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