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Characterization of *Staphylococcus aureus* Isolates from White-Brined Urfa Cheese

KURSAT KAV,[1]* RAMAZAN COL,[2] AND MUSTAFA ARDIC[3]

[1]*Department of Microbiology and* [2]*Department of Physiology, Faculty of Veterinary Medicine, University of Selcuk, Campus, 42075 Selcuklu, Konya, Turkey; and* [3]*Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, University of Harran, 63200 Sanliurfa, Turkey*

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ABSTRACT

The aim of this study was to investigate the presence of *Staphylococcus aureus* and staphylococcal enterotoxin (SE) genes in Urfa cheese samples and to characterize the enterotoxigenic potential of these isolates. From a total of 127 Urfa cheese samples, 53 isolates (from 41.7% of the samples) were identified by a species-specific PCR assay as *S. aureus*. Of these isolates, 40 (75.5%) gave positive PCR results for the 3′ end of the *coa* gene. The *coa*-positive *S. aureus* strains were characterized for their population levels and enterotoxigenic properties, including slime factor, β-lactamase, antibiotic susceptibilities, production of the classical SEs (SEA through SEE), in both cheese and liquid cultures by enzyme-linked immunosorbent assay (ELISA) and for the presence of specific genes, including classical SE genes (*sea* through *see*), *mecA, femA,* and *spa,* by PCR. The genetic relatedness among the *coa*-positive *S. aureus* isolates was investigated by PCR-based restriction fragment length polymorphism (RFLP) analysis and the 23S rRNA gene spacer. The 23S rRNA gene spacer and *coa* RFLP analysis using *Alu*I and *Hin*6I revealed 14 different patterns. SEB, SEC, and SEA and SEE were detected by ELISA in three cheese samples. Fourteen *S. aureus* strains harbored enterotoxin genes *sea* through *see,* and three strains carried multiple toxin genes. The most commonly detected toxin gene was *sec* (25% of tested strains). Of the 40 analyzed *S. aureus* strains, 3 (7.5%) were *mecA* positive. Based on tandem repeats, four *coa* and *spa* types were identified. The results of this study indicate that *S. aureus* and SEs are present at significant levels in Urfa cheese. These toxins can cause staphylococcal food poisoning, creating a serious hazard for public health.

Staphylococcus aureus is one of the most common pathogenic bacteria that cause food poisoning outbreaks and a range of serious human and animal infections, including pneumonia, endocarditis, and sepsis *(44)*. This organism may produce one or more staphylococcal enterotoxins (SEs), including types A, B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q, R, and U, and the corresponding genes (*sea, seb, sec, sed, see, seg, seh, sei, sej, sek, sel, sem, sen, seo, sep, seq, ser,* and *seu*) have been reported *(1, 16, 17, 30, 33, 41)*. SEA and SED in dairy products, such as raw milk and cheese, are strikingly correlated with food poisoning *(13, 33)*. Pasteurization kills *S. aureus,* and fermentation and ripening may prevent the growth of *S. aureus* in raw milk cheese. However, SEs are heat-stable proteins that are produced by approximately 25% of the *S. aureus* strains isolated from foods *(1, 8, 33)*. SEs may cause nausea, vomiting, diarrhea, abdominal cramps, and malaise 3 to 10 h after consumption *(8, 15)*.

On of the methods currently used for detection of SEs in food is the enzyme-linked immunosorbent assay (ELISA). The PCR protocol is a good molecular technique for identifying and comparing *S. aureus* subtypes *(16)*. Genotyping of *S. aureus* is important in epidemiology for determining the source of the infection and the relationships among strains. The coagulase protein of *S. aureus* is a major virulence factor and an important phenotypic determinant *(17)*. Amplification of the coagulase gene (*coa*) is a simple and accurate method of typing *S. aureus* isolated from distinct sources, and the presence of this gene has been used for identifying and discriminating among *S. aureus* strains *(16)*.

The increasing production of raw milk products around the world has spotlighted specific food safety concerns. Hand-made cheese produced from raw milk in poorly designed processing facilities by small-scale local producers may be at an elevated risk for the presence of pathogenic bacteria *(6, 45)*. Urfa cheese is a white-brined traditional dairy product and is one of the most commonly consumed cheeses in southeastern Turkey, with an annual production of 35,000 to 40,000 tons. It is mainly produced from raw ovine milk, bovine milk, or mixtures of both *(32)*. Urfa cheese is generally consumed after ripening but also can be consumed fresh *(4)*.

The occurrence of *S. aureus* and its enterotoxigenic properties in Urfa cheese has not been studied. The purpose of the present study was to characterize the phenotypes and genotypes of *coa*-positive *S. aureus* strains isolated from Urfa cheese in Turkey.

* Author for correspondence. Tel: +90 332 2232635; Fax: +90 332 2410063; E-mail: rcol@selcuk.edu.tr.

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TABLE 1. *Oligonucleotide primers and thermocycling conditions used in the amplification of staphylococcal genes*

Target gene	Primer	Oligonucleotides sequence (5′ to 3′)	Location within gene (bp)	PCR product (bp)	Reference
23S rRNA	Sau327	GGACGACATTAGACGAATCA	327–346	1,318	*35*
	Sau1645	CGG GCA CCT ATT TTC TAT CT	1,626–1,645		
coa	CoaG2	CGAGACCAAGATTCAACAAG	Variable	Variable	*12*
	CoaG3	AAAGAAAACCACTCACATCA			
spa	Spax1	CAA GCA CCA AAA GAG GAA	Variable	Variable	*10*
	Spax2	CAC CAG GTT TAA CGA CAT			
16–23S rRNA spacer	rRNA1	TTG TAC ACA CCG CCC GTC A	Variable	Variable	*19*
	rRNA2	GGT ACC TTA GAT GTT TCA GTT C			
femA	GFfemAR1	AAAAAAGCACATAACAAGCG	1,444–1,463	132	*26*
	GFfemAR2	GATAAAGAAGAAACCAGCAG	1,556–1,575		
mecA	GMecAR1	ACTGCTATCCACCCTCAAAC	1,182–1,201	163	*26*
	GMecAR2	CTGGTGAAGTTGTAATCTGG	1,325–1,344		
se	SAU	TGTATGTATGGTGGTGTAAC			*39*
sea	SA-A	ATTAACCGAAGGTTCTGT	639–657	270	*39*
seb	SA-B	ATAGTGACGAGTTAGGTA	564–582	165	*39*
sec	SA-C	AAGTACATTTTGTAAGTTCC	457–477	69	*39*
Entc	ENT-C	AATTGTGTTTCTTTTATTTTCATAA	485–510	102	*39*
sed	SA-D	TTCGGGAAAATCACCCTTAA	676–696	306	*39*
see	SA-E	GCCAAAGCTGTCTGAG	584–600	213	*39*

MATERIALS AND METHODS

Samples. A total of 127 cheese samples were collected from supermarkets and retail outlets in the Sanliurfa province of Turkey between January and March 2008. The cheeses were divided into 200- to 500-g samples and transported to the laboratory at approximately 4°C for analysis.

Reference strains. The following *S. aureus* reference strains were kindly provided by Dr. N. Ertas: R1, *S. aureus* ATCC 29213 (SEA, *sea* and *sec*); R2, *S. aureus* NCTC 10654 (SEB, *seb*); R3, *S. aureus* NCTC 10655 (SEC, *sec*); R4, *S. aureus* NCTC 10652 (SED, *sea* and *sed*); R5, *S. aureus* 27R (SEB, *seb,* methicillin-resistant *S. aureus* [MRSA]); and R6, *S. aureus* ATCC 43300 (SEC, *sea* and *sec,* MRSA).

Identification and phenotype characterization of *S. aureus*. For enumeration, isolation, identification, and phenotypic characterization of *S. aureus*, 25 g of each cheese sample was quantitatively evaluated for coagulase-positive staphylococci using standard microbiological methods (ISO 6888 1/2, International Organization for Standardization, Geneva, Switzerland) using Baird Parker medium (CM 275, Oxoid, Basingstoke, UK) with 5% egg yolk and tellurite (1.03785.0001, Merck, Darmstadt, Germany) *(2, 3, 8, 18)*. All cultures were Gram stained (Difco, BD, Sparks, MD) and tested for catalase reactivity and clumping factors. The slime production assay was performed by cultivation of *S. aureus* strains on Congo red agar using the method described by Yazdani et al. *(46)*. *S. aureus* strains were examined for the production of hemolysins by evaluating the interaction of hemolysins with β-toxin, and β-lactamase activity was determined with nitrocefin-containing identification sticks (BR0066A, Oxoid).

ELISA. The enterotoxins were detected by ELISA using a staphylococcal enterotoxin detection kit for SEA, SEB, SEC, SED, and SEE (SET-RIDASCREEN kit, R-Biopharm AG, Darmstadt, Germany), according to the manufacturer's instructions.

Molecular strain characterization. Genomic DNA was extracted from overnight cultures of *S. aureus* using the Wizard

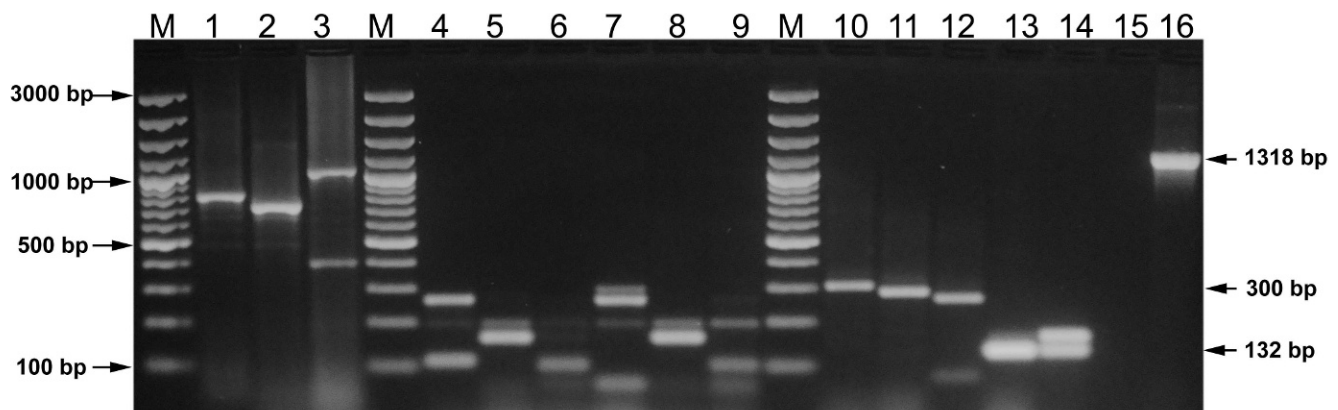

FIGURE 1. *Detection of the genes* coa, spa, sea *through* sec, femA, *and* mecA *by multiplex PCR assay of* S. aureus *reference strains. M, 100-bp molecular weight marker (SM0321, MBI Fermentas). Profiles: lines 1 through 3,* coa*; line 4, R1 (*sea *and* sec*); line 5, R2 (*seb*); line 6, R3 (*sec*); line 7, R4 (*sea *and* sed*); line 8, R5 (*seb*) and MRSA; line 9, R6 (*sea *and* sec*) and MRSA; lines 10 through 12,* spa*; line 13,* femA*; line 14,* femA *and* mecA*; line 15, negative control; line 16,* S. aureus *species-specific PCR product.*

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TABLE 2. *Characteristics of* S. aureus *reference strains and strains isolated from Urfa cheese*[a]

Sample no.[b]	Slime factor	Hemolysin type	β-Lactamase	*coa* size (bp)	*spa* size (bp)	*femA* (132 bp)	*mecA* (163 bp)	Level (CFU/g)	SEA–SEE gene(s) detected	SE production	*Alu*I profile	*Hin*6I profile	16–23S rRNA spacer genotype
R1	−	β	+	800	300	+	−		*sea, sec*	SEA	V	VII	1
R2	−	β	+	750	300	+	−		*seb*	SEB	I	XIV	1
R3	+	β	−	400–1,100	320	+	−		*sec*	SEC	X	X	1
R4	+	β	+	750	300	+	−		*sea, sed*	SED	I	XIV	1
R5	+	−	−	750	300	+	+		*seb*	SEB	I	XIV	1
R6	+	−	+	800	270	+	+		*sea, sec*	SEC	V	VII	1
1	+	β	+	800	270	+	+	4.3×10^5	*sec*	SEC[c]	I	I	3
2	+	α	+	800	320	+	−	1.3×10^3			IV	V	1
3	+	β	+	800	320	+	−	2.4×10^2			IV	IX	3
4	+	β	−	1,000	300	+	−	3.2×10^3			VI	II	3
5	+	β	+	800	320	+	−	2.8×10^3			IV	IX	3
6	+	β	+	800	320	+	−	3.4×10^2			IV	V	1
7	+	β	+	900	270	+	−	4.6×10^3	*sec*	ND	V	IX	4
8	+	β	−	1,000	270	+	−	4.4×10^5	*sec*	SEC	V	IX	4
9	+	β	−	1,000	270	+	−	2.2×10^2			V	IX	9
10	+	β	+	1,000	270	+	+	1.5×10^2			VI	X	6
11	+	β	−	600	270	+	−	5.6×10^2	*sec*	ND	VII	VI	7
12	+	−	−	900	270	+	−	3.8×10^3	*sec*	ND	V	X	2
13	+	Δ-like	+	900	270	+	−	4.3×10^3			X	III	9
14	+	−	+	900	270	+	−	6.3×10^5			V	X	1
15	+	β	+	600	240	+	−	2.2×10^5			V	IX	1
16	+	β	+	800	240	+	−	3.5×10^3	*sec*	ND	VIII	XI	10
17	+	−	+	1,000	240	+	−	2.7×10^2			IX	XII	5
18	+	β	−	800	240	+	−	1.8×10^3			XIII	X	2
19	+	−	−	800	240	+	−	4.3×10^2	*seb, sec*	ND	XIV	IV	2
20	+	−	+	1,000	240	+	−	1.0×10^3			II	VII	2
21	+	β	+	1,000	240	+	+	6.4×10^3			XII	VII	1
22	+	−	+	1,000	240	+	−	8.3×10^3			XII	VII	1
23	+	−	+	800	240	+	−	9.1×10^3	*seb, sec*	ND	XI	VII	2
24	+	Δ-like	−	1,000	240	+	−	5.3×10^3			XI	VII	11
25	+	−	+	1,000	270	+	−	3.4×10^3			XI	VII	14
26	+	β	−	900	240	+	−	2.7×10^4			I	I	8
27	+	β	−	1,000	240	+	−	3.9×10^3			XI	VII	12
28	+	β	−	900	240	+	−	5.8×10^1			VIII	VIII	12
29	+	β	+	900	240	+	−	3.9×10^1			VIII	VIII	12
30	+	β	+	900	240	+	−	3.5×10^1			VIII	VIII	12
31	+	α	−	900	270	+	−	6.3×10^1			III	XV	1
32	+	β	−	900	240	+	−	4.2×10^5			VIII	VIII	1
33	+	β	−	900	240	+	−	3.4×10^1			VIII	VIII	1

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TABLE 2. *Continued*

Sample no.[b]	Slime factor	Hemolysin type	β-Lactamase	*coa* size (bp)	*spa* size (bp)	*femA* (132 bp)	*mecA* (163 bp)	Level (CFU/g)	SEA–SEE gene(s) detected	SE production	*Alu*I profile	*Hin*6I profile	16–23S rRNA spacer genotype
34	+	β	−	900	300	+	−	3.4×10^3			IX	XIII	1
35	+	β	+	1,000	110	+	−	4.2×10^5	*sea, see*	SEA, SEE[c]	VIII	VIII	13
36	+	β	+	1,000	300	+	−	5.6×10^5	*seb*	SEB[c]	VIII	VIII	1
37	+	β	−	1,000	270	+	−	3.0×10^1	*sec*	SEC	XI	VII	1
38	+	α	−	1,000	270	+	−	3.4×10^1	*sec*	SEC	XI	VII	1
39	+	β	−	1,000	270	+	−	5.3×10^1	*sec*	SEC	XI	VII	1
40	+	β	−	1,000	300	+	−	3.4×10^1	*sec*	SEC	XI	VII	1

[a] +, positive; −, negative; ND, not detected.
[b] R1 through R6, *S. aureus* reference strains; 1 through 40, *S. aureus* strains isolated from Urfa cheese.
[c] Toxin types determined by ELISA.

Genomic DNA Purification kit (Cat. A1120, Promega, Madison, WI). DNA from all of the *S. aureus* isolates identified by the species-specific PCR assay was subjected to molecular characterization using a set of published PCR systems *(35)*. See Table 1 for amplification conditions. All isolates were analyzed for the presence of SE genes using previously published primer sequences for SE *(39)*. All of the PCRs were uniplex. PCRs were performed in a total volume of 50 μl, containing 1 μl of each primer (10 pmol/μl), 0.4 μl of each deoxynucleoside triphosphate (10 mmol; MBI Fermentas, St. Leon-Rod, Germany), 5 μl of 10× DreamTaq Green buffer, 2 to 4 mM $MgCl_2$ depending on the PCR conditions (MBI Fermentas), 0.4 μl of DreamTaq polymerase (5 U/μl; MBI Fermentas), and distilled water. A 5-μl aliquot of template DNA was added to each reaction tube. The PCR assay was performed using a master gradient thermocycler (Eppendorf, Hamburg, Germany). The PCR products (6.5 μl) were examined by electrophoresis on a 1.5% standard agarose gel (Biozym, Hessich-Oldendorf, Germany) in Tris-borate-EDTA buffer, and a Gene-Ruler 100-bp DNA ladder (SM 321/2/3, MBI Fermentas) was used as the molecular marker.

To detect polymorphisms in the coagulase gene, DNA samples from isolates identified as *S. aureus* were amplified by PCR for the *coa* gene using the primers COAG2 and COAG3 *(12)*. The PCR products that were determined to be *coa* positive by gel electrophoresis were digested with *Alu*I and *Hin*6I (MBI Fermentas) for restriction analysis. To perform this analysis, 10 μl of each PCR product was mixed with 2 μl (20 U) of enzyme and 2 μl of 10× restriction buffer and incubated at 37°C for 2 h. The digested products were separated on a 2% agarose gel, stained with ethidium bromide, and analyzed under UV light.

All fragments obtained from the 16S to 23S rRNA spacer region PCRs were compared on the same gel. Each band was treated on the gel and scored as 1 (present) or 0 (absent). These data matrices were recorded in the software program PubMLST (http://pubmlst.org/), and an unweighted pair group method with arithmetic means (UPGMA) dendogram was obtained (Fig. 3).

Antibiotic susceptibility test. An antibiotic susceptibility test was performed using the disk diffusion method on Mueller-Hinton agar (Oxoid) according to the Clinical and Laboratory Standards Institute (2006; CLSI, Wayne, PA) for the following antibiotics: penicillin-G (P; 10 U, Oxoid), ampicillin (AMP; 10 μg, Oxoid), amoxycillin (AML; 10 μg, Oxoid), ampicillin-sulbactam (SAM; 20 μg, Oxoid), amoxicillin–clavulanic acid (AMC; 30 μg, Oxoid), methicillin (MET; 5 μg, Oxoid), oxacillin (OX; 5 μg, Oxoid), cefoperazone (CFP; 75 μg, Oxoid), sulbactam-cefoperazone (SCF; 105 μg, Oxoid), erythromycin (E; 15 μg, Oxoid), gentamicin (GN; 10 μg, Oxoid), enrofloxacin (ENR; 5 μg, Oxoid), danofaloxacin (DNX; 5 μg, Pfizer, New York, NY), florfenicol (FFC; 30 μg, Oxoid), sulfamethoxazole-trimethoprim (SXT; 25 μg, Oxoid), and oxytetracycline (OT, 30 μg, Oxoid).

Statistical analysis. Study results were evaluated by chi-square test. Results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Turkish law stipulates 10^1 to 10^2 CFU/g as the legal limit for *S. aureus* in cheese *(43)*. Our results indicate that *S. aureus* strains carrying enterotoxin genes are widely distributed in cheese samples from the investigated area. The prevalence of *coa*-positive *S. aureus* contamination in Urfa cheese was 31.5% (40 of 127 samples) at levels of 3.0×10^1 to 6.3×10^5 CFU/g. In 23 of the 40 *S. aureus*–

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TABLE 3. *Antibiotic susceptibility of* S. aureus *reference strains and strains isolated from Urfa cheese*[a]

Antibiotic disc	*S. aureus* cheese isolates ($n = 40$)						*S. aureus* reference strains						Zone diameter (mm)		
	S		MS		R										
	n	%	*n*	%	*n*	%	R1	R2	R3	R4	R5	R6	S	Interpretive standard for MS	R
P	16	40	0		24	60	R	S	S	R	R	R	≤28		≥29
AMP	20	50	0		20	50	R	R	S	R	R	R	≤28		≥29
AML	33	82.5	0		7	17.5	R	R	R	R	R	R	≤19		≥20
SAM	39	97.5	1	2.5	0		S	S	S	S	S	S	≤11	12–14	≥15
AMC	36	90	0		4	10	S	S	S	S	S	S	≤19		≥20
MET	34	85	3	7.5	3	7.5	S	S	S	S	R	MS	≤9	13	≥14
OX	37	92.5	0		3	7.5	S	S	S	S	R	R	≤10	11–12	≥13
CFP	32	80	6	15	2	5	S	MR	S	S	R	MR	≤15	16–20	≥21
SCF	39	97.5	1	2.5	0		S	S	S	S	R	S	≤15	16–20	≥21
E	29	72.5	4	10	7	17.5	MR	MR	MR	S	R	R	≤13	14–22	≥23
GN	33	82.5	0		7	17.5	S	S	S	S	S	S	≤12	13–14	≥15
ENR	40	100	0		0		S	S	S	S	S	S	≤15	16–20	≥21
DNX	33	82.5	5	12.5	2	5	S	S	S	S	S	S	≤15	16–20	≥21
FFC	40	100	0		0		S	S	S	S	S	S	≤12	17	≥18
SXT	35	87.5	0		5	12.5	S	R	S	S	MR	S	≤15	16–18	≥19
OT	40	100	0		0		S	R	S	S	R	S	≤14	15–18	≥19

[a] S, susceptible; MS, moderately susceptible; R, resistant; MR, moderately resistant.

contaminated samples, the level of *S. aureus* was above the legal Turkish limit. In seven cheese samples, *S. aureus* levels were above 10^4 CFU/g. The prevalence of *S. aureus* contamination in these cheese samples was lower than that previously reported by Yalcin et al. *(45)*, Sahan et al. *(37)*, and Yetismeyen and Yildiz *(47)*, who found *S. aureus* in Urfa cheese samples at levels of $<10^1$ to 6.4×10^4 CFU/g. These differences in findings may be associated with the fact that Urfa cheeses vary widely in microbiological and chemical quality *(45)*.

Of the 40 isolates found in this study, 3 were positive for α-hemolysis only, 27 were positive for β-hemolysis only, and 2 were positive for Δ-like hemolysis (Table 2). The other eight isolates were nonhemolytic based on cultivation on sheep blood agar. Slime production and β-lactamase activity were found in 40 (100%) and 21 (52.5%) of the isolates, respectively. MRSA has emerged as an important cause of hospital-acquired, community-acquired, and foodborne infections *(29)*. MRSA may have arisen because of the extensive therapeutic use of antibiotics and their administration as growth promoters in food animal production *(5)*. The transmission of MRSA through foods also may be involved in food poisoning outbreaks, as reported by Jones et al. *(14)* and Spanu et al. *(40)*. All *S. aureus* strains isolated in our study were susceptible to enrofloxacin, florfenicol, and oxytetracycline and were resistant at a significant level to penicillin-G (60% of strains) and ampicillin (50% of strains). The other antimicrobial susceptibility tests revealed that the isolates generally had multidrug resistance (Table 3). The antimicrobial susceptibility testing revealed the presence of MRSA; 3 (7.5%) of the 40 tested *S. aureus* isolates carried the *mecA* gene and were consistently resistant to methicillin. One MRSA strain harbored the *sec* gene and produced SEC, as determined by the PCR assay and ELISA, respectively (Table 4). Various authors have investigated the *mecA* and *femA* and *B* genes in MRSA isolated from food samples of animal origin *(27, 30, 40)*. Lee *(22)* reported that 15 of 421

TABLE 4. *Characteristics of* S. aureus *strains isolated from Urfa cheese*[a]

Slime factor ($n = 40$)	Hemolysin type ($n = 40$)	β-Lactamase ($n = 40$)	*coa* size (bp) ($n = 53$)	*spa* size (bp) ($n = 40$)	*femA* ($n = 53$)	*mecA* ($n = 40$)	Population (CFU/g) ($n = 40$)	SEA–SEE gene(s) detected ($n = 40$)	SE production ($n = 14$)	SE production in cheese ($n = 127$)
+ (40)	α (3) B	+ (21)	1,000 (17) A	320 (4) B	+ (53)	+ (3)	10^2–10^4 (33)	*seb* (1) B	SEB (1) B	SEB (1)
	β (27) A	ND (19)	900 (12) A	300 (4) B		ND (37)	10^5–10^6 (7)	*sec* (10) A	SEB (6) A	SEC (1)
	Δ-like (2) B		800 (9) A	270 (14) A				*sec*+*seb* (2) A	SEA+SEE (1) B	SEA+SEE (1)
	− (8)		600 (2) B	240 (17) A				*sea*+*see* (1) B	ND (6)	ND (124)
			ND (13) A	110 (1) B				ND (26)		

[a] +, positive; ND, not detected; −, no hemolysis. Number of strains with each characteristic is given in parentheses. Within the same column, entries followed by different letters are significantly different ($P < 0.05$, chi-square test).

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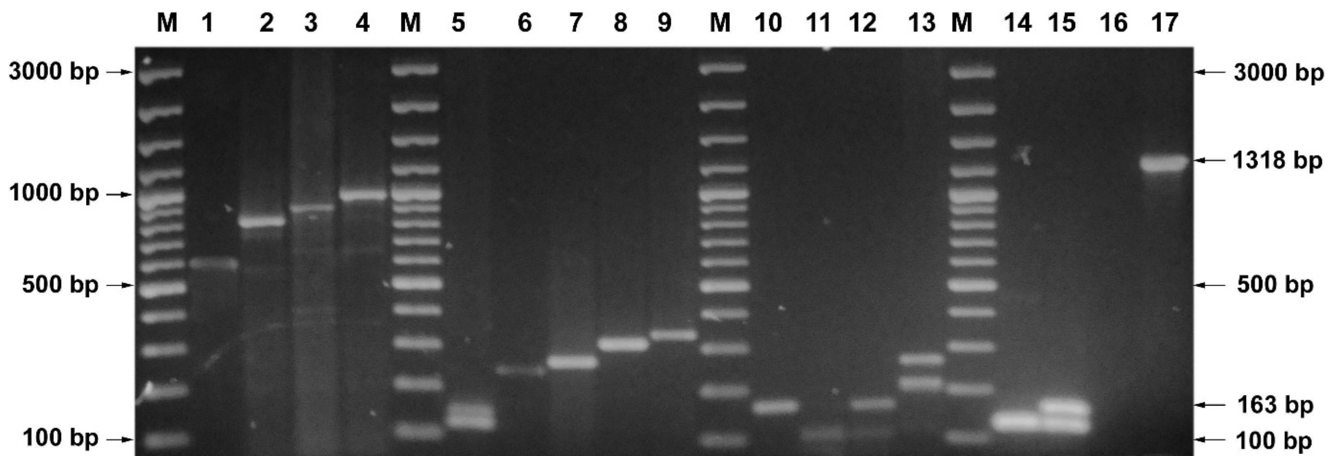

FIGURE 2. *Detection of the genes* coa, spa, sea *through* see, femA, *and* mecA *by uniplex and multiplex PCR assay of* S. aureus *strains isolated from Urfa cheese. M, 100-bp molecular weight marker (SM0321, MBI Fermentas). Profiles: lines 1 through 4,* coa*; lines 5 through 9,* spa*; line 10,* seb-*positive field isolates; line 11,* sec-*positive field isolates; line 12,* seb- *and* sec-*positive field isolates; line 13,* sea- *and* see-*positive field isolates; line 14,* femA-*positive field isolates; line 15,* femA- *and* mecA-*positive field isolates (MRSA); line 16, negative control; line 17,* S. aureus *species-specific PCR product.*

S. aureus isolates carried the *mecA* gene, as determined by PCR analysis, and that based on antimicrobial susceptibility tests of *mecA*-positive MRSA strains using the disk diffusion method all of the isolates were resistant to members of the penicillin family, as also found in our study. Our findings also are in agreement with those of Normanno et al. *(29),* who determined that 6 (3.75%) of 160 *S. aureus* strains analyzed were *mecA* positive; these strains were isolated from food samples of animal origin such as bovine milk, mozzarella cheese, and pecorino cheese in Italy. In *S. aureus* strains, *femA* and *mecA* are the genetic determinants of methicillin resistance. A multiplex PCR assay for *femA* can be used to differentiate *S. aureus* (*femA* positive) from coagulase-negative staphylococci (*femA* negative). In our study, all of the strains were positive for the *S. aureus*–specific marker gene *femA* (Figs. 1 and 2). Pelisser et al. *(33)* evaluated meat and milk-derived products for the presence of coagulase-positive staphylococci and found that 91 of 102 coagulase-positive *Staphylococcus* isolates were positive for *femA*. In the present study, 53 coagulase-positive isolates were found in Urfa cheese, 40 of which were also positive for *femA*. The animal MRSA isolates may have originated from humans, considering the rate of methicillin resistance among human *S. aureus* isolates. The detection of MRSA in animal products indicates the need for improved hygiene in food production processes.

In the present study, amplification of *coa* by the PCR method resulted in a single band for each strain, which appeared at one of four different sizes from 600 to 1,000 bp (Fig. 2 and Table 4). In *S. aureus* isolates from goat milk cheese, Akineden et al. *(1)* found that PCR amplification of the *coa* gene resulted in a single amplicon for all isolates, but size polymorphisms (four to eight repeats) were observed in a number of isolates. Katsuda et al. *(18)* reported found three to nine tandem repeats in the *coa* gene; five tandem repeats was the most common form in bovine *S. aureus* strains. Mašlanková et al. *(24)* found the *coa* gene in *S. aureus* strains isolated from ovine products, including cheese and raw milk. They identified five *coa* types, based on the tandem repeats in the *coa* gene. Karahan and Çetinkaya *(17)* found that most isolates (83.9%) produced a single band for *coa* after PCR amplification, with sizes of 500 to 1,400 bp, whereas a small number of isolates (16.1%) yielded two amplification products. These authors suggested that milking personnel may play a role in the transmission of *S. aureus,* and *coa* restriction fragment length polymorphism (RFLP) analysis using *Alu*I and *Hin*6I revealed 23- and 22-band patterns, respectively. In our study, the *coa* RFLP analysis using *Alu*I and *Hin*6I produced 14 different banding patterns (Tables 2 and 5 and Fig. 3).

The number and sequence of individual repeats may differ among strains, and these repeat codes are used in bacterial typing *(9, 20, 25)*. Frénay et al. *(10)* investigated whether this region could be used to discriminate between epidemic and nonepidemic MRSA strains. These authors found a correlation between the number of repeats and the epidemic character of MRSA strains. Strains with more than seven repeats in the X region tended to be epidemic, whereas strains with seven or fewer repeats were usually nonepidemic. In the present study, a single *spa* band was observed for each isolate after PCR amplification, and four amplicon sizes were observed: 240 bp for 17 strains (42.5%), 270 bp for 14 strains (35%), 300 bp for 4 strains (10%), and 320 bp for 4 strains (10%) (Fig. 2). When studying *S. aureus* strains isolated from goat milk cheese, Akineden et al. *(1)* found that amplification of the X region of *spa* resulted in 10 sizes of amplicons, corresponding to 3 to 14 repeats, and amplification of the immunoglobulin G binding region of *spa* revealed 3 to 5 repeats. In this study, we evaluated the natural polymorphisms of the genomic 16S to 23S rRNA spacer regions of *S. aureus* so it could be used in genotyping (Fig. 3).

In three samples of the 127 fresh Urfa cheeses analyzed, enterotoxins SEB, SEC, and SEA plus SEE were detected by ELISA. Fourteen of the 40 *S. aureus* strains isolated from Urfa cheese harbored at least one enterotoxin

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TABLE 5. *Distribution of RFLP profiles among 40* coa*-positive* S. aureus *isolates from Urfa cheese*

*Alu*I			*Hin*6I			16–23S rRNA spacer regions	
Profile	No. (%) of isolates[a]	*coa* PCR product size(s) (bp)	Profile	No. (%) of isolates[a]	*coa* PCR product size(s) (bp)	Profile	No. (%) of isolates[a]
I	2 (5) B	800, 900	I	2 (5) CB	800, 900	1	15 (37.5) A
II	1 (2.5) B	1,000	II	1 (2.5) C	1,000	2	5 (12.5) B
III	1 (2.5) B	900	III	1 (2.5) C	900	3	4 (10) B
IV	4 (10) AB	800, 1,000	IV	2 (5) CB	800	4	2 (5) B
V	6 (15) AB	600, 900, 1,000	V	1 (2.5) C	800	5	1 (2.5) B
VI	2 (5) B	1,000	VI	11 (27.5) A	600	6	1 (2.5) B
VII	1 (2.5) B	600	VII	7 (17.5) AB	1,000	7	1 (2.5) B
VIII	8 (20) A	800, 900, 1,000	VIII	5 (12.5) AB	900, 1,000	8	1 (2.5) B
IX	2 (5) B	900, 1,000	IX	5 (12.5) AB	800, 900, 1,000	9	2 (5) B
X	1 (2.5) B	900	X	1 (2.5) C	800, 900, 1,000	10	1 (2.5) B
XI	8 (20) A	1,000	XI	1 (2.5) C	800	11	1 (2.5) B
XII	2 (5) B	1,000	XII	1 (2.5) C	1,000	12	4 (10) B
XIII	1 (2.5) B	800	XIII	1 (2.5) C	900	13	1 (2.5) B
XIV	1 (2.5) B	800	XV	1 (2.5) C	900	14	1 (2.5) B

[a] Within the same column, values followed by different letters are significantly different ($P < 0.05$, chi-square test).

gene, as indicated by multiplex PCR analysis, for an incidence of 35%. The SE genes were distributed as follows: 10 strains had *sec* (25% of cheese strains), 2 had both *seb* and *sec,* 1 had both *sea* and *see,* 1 had *seb* alone, and none had *sed*. These rates are lower than those detected by other authors, who reported that *S. aureus* strains frequently carried enterotoxin genes (55 to 94% of strains examined) *(7, 15, 38)*. The production of enterotoxin by the isolates carrying these genes was confirmed on brain heart infusion broth. Not all of the isolates harboring the SE genes were capable of producing toxin in the broth culture. SEs and their genes were closely correlated with the *S. aureus* strain origin; a higher ratio of strains isolated from cheese produced SEA and SEE. Of the 14 *S. aureus* strains examined, 8 produced enterotoxins, and 6 strains harboring enterotoxin genes were nonenterotoxigenic. The detection of enterotoxin genes in samples does not necessarily indicate that the toxins will be present at significant levels in the sampled food. *S. aureus* isolates from dairy products harboring enterotoxin genes may not produce enterotoxins *(33)*.

Our finding that *sec* was the most common SE gene is in agreement with the findings of Normanno et al. *(30),* who reported that 298 (55.5%) of the *S. aureus* strains examined produced one or more SEs. Our results also are similar to those from other studies indicating that SEC is the enterotoxin most frequently produced in raw milk and raw milk products *(15, 23, 41)*. However, according to some authors, SEA is the enterotoxin most frequently produced by enterotoxigenic strains of *S. aureus,* and the source of contamination was most likely a food handler who used a wounded hand to prepare the food *(3, 8, 33, 34)*.

The observed variations in enterotoxin prevalence may be due to geographical differences. The prevalence of an enterotoxin probably is affected by the origin of the strain (food, human, or animal) and the numbers and types of samples examined *(11, 26)*. SEC is an important cause of staphylococcal food poisoning associated with the con-

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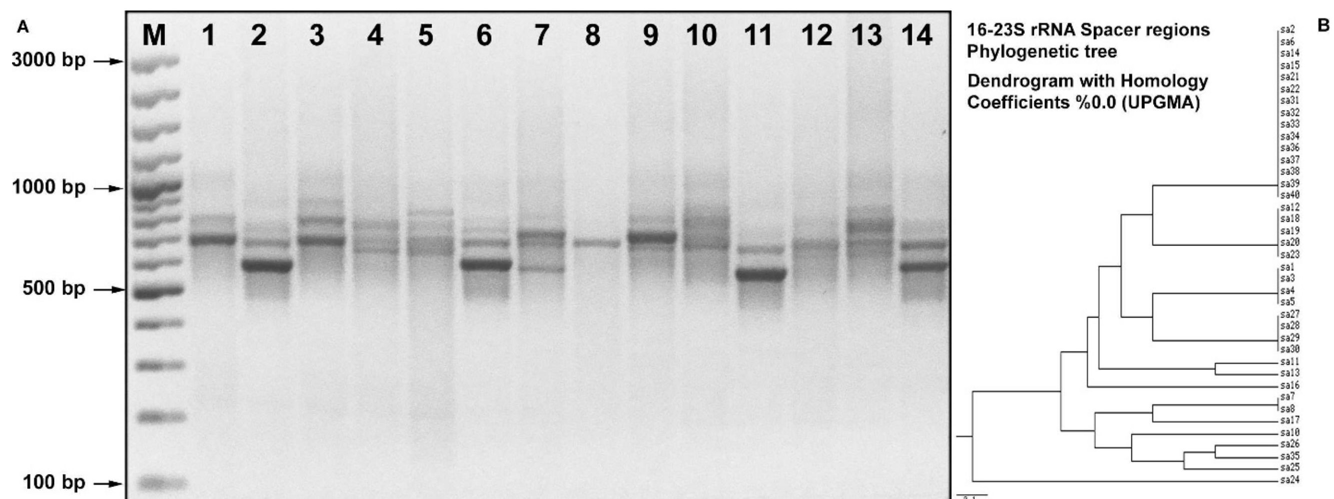

FIGURE 3. *Example of agarose gel electrophoresis of 16S to 23S rRNA spacer PCR amplification products (A) and a dendrogram of the* S. aureus *strains (B). M, 100-bp molecular weight marker (SM0321, MBI Fermentas).*

sumption of dairy products *(42)*. Normanno et al. *(29)* stated that human biotype strains found in handled foods, such as cheeses, are present probably because of poor hygiene during the production processes. Other authors have found conflicting results for the frequency of SEs among staphylococcal strains. SEA is the most common enterotoxin among strains from human sources, including strains that cause food poisoning *(26, 28)*, and SEC was the most common toxin produced by strains of bovine origin *(21, 39)*. However, in other studies *(11, 36)* foodborne human *S. aureus* strains were usually enterotoxigenic and SEC was the predominant enterotoxin. Ostyn et al. *(31)* found SEE associated with six food poisoning outbreaks caused by staphylococci in France. We determined that *S. aureus* strains can harbor and produce both SEA and SEE.

To our knowledge, this is the first investigation to assess the properties of enterotoxigenic *S. aureus* in Urfa cheeses produced for human consumption in Turkey. These results indicate that *S. aureus* is a serious hazard for cheese consumers, especially in the absence of strict hygienic and preventive measures to avoid the production of SEs in foods.

REFERENCES

1. Akineden, O., A. A. Hassan, E. Schneider, and E. Usleber. 2008. Enterotoxigenic properties of *Staphylococcus aureus* isolated from goats' milk cheese. *Int. J. Food Microbiol.* 124:211–216.
2. Anonymous. 1997. Microbiology of food and animal feeding stuffs: horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). ISO/DIS 6888-1. International Organization for Standardization, Geneva.
3. Aragon-Alegro, L. C., E. M. Konta, K. Suzuki, M. G. Silva, A. Fernandes Júnior, R. Raal, and V. L. M. Rall. 2007. Occurrence of coagulase-positive *Staphylococcus* in various food products commercialized in Botucatu, SP, Brazil and detection of toxins from food and isolated strains. *Food Control* 18:630–634.
4. Ardic, M., M. Atasever, G. Adiguzel, M. Atasever, Y. Karakaya, C. Unsal, and H. Durmaz. 2008. A survey on the presence of aflatoxin M1 in Urfa cheese. *Int. J. Food Saf.* 10:13–17.
5. Barber, D. A., G. Y. Miller, and P. E. McNamara. 2003. Models of antimicrobial resistance and foodborne illness: examining assumptions and practical applications. *J. Food Prot.* 66:700–709.
6. Boynukara, B., T. Gulhan, M. Alisarli, K. Gurturk, and H. Solmaz. 2008. Classical enterotoxigenic characteristics of *Staphylococcus aureus* strains isolated from bovine subclinical mastitis in Van, Turkey. *Int. J. Food Microbiol.* 125:209–211.
7. Chiang, Y. C., W. W. Liao, C. M. Fan, W. Y. Pai, C. S. Chiou, and H. Y. Tsen. 2008. PCR detection of staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in *Staphylococcus aureus* isolates from food-poisoning cases in Taiwan. *Int. J. Food Microbiol.* 121:66–73.
8. Ertas, N., Z. Gonulalan, Y. Yildirim, and E. Kum. 2010. Detection of *Staphylococcus aureus* enterotoxins in sheep cheese and dairy desserts by multiplex PCR technique. *Int. J. Food Microbiol.* 142: 74–77.
9. Frénay, H., A. Bunschoten, L. Schouls, W. van Leeuwen, C. Vandenbroucke-Grauls, J. Verhoef, and F. Mooi. 1996. Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphism. *Eur. J. Clin. Microbiol. Infect. Dis.* 15:60–64.
10. Frénay, H. M., J. P. Theelen, L. M. Schouls, C. M. Vandenbroucke-Grauls, J. Verhoef, W. J. van Leeuwen, and F. R. Mooi. 1994. Discrimination of epidemic and nonepidemic methicillin-resistant *Staphylococcus aureus* strains on the basis of protein A gene polymorphism. *J. Clin. Microbiol.* 32:846–847.
11. Fueyo, J. M., M. C. Martin, M. A. Gonzalez-Hevia, and M. C. Mendoza. 2001. Enterotoxin production and DNA fingerprinting in *Staphylococcus aureus* isolated from human and food samples. Relations between genetic types and enterotoxins. *Int. J. Food Microbiol.* 67:139–145.
12. Goh, S. H., S. K. Byrne, J. L. Zhang, and A. W. Chow. 1992. Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphism. *J. Clin. Microbiol.* 30:1642–1645.
13. Hwang, S. Y., S. H. Kim, E. J. Jang, N. H. Kwon, Y. K. Park, H. C. Koo, W. K. Jung, J. M. Kim, and Y. H. Park. 2007. Novel multiplex PCR for the detection of the *Staphylococcus aureus* superantigen and its application to raw meat isolates in Korea. *Int. J. Food Microbiol.* 117:99–105.
14. Jones, T. F., M. E. Kellum, S. S. Porter, M. Bell, and W. Schaffner. 2002. An outbreak of community-acquired foodborne illness caused by methicillin resistant *Staphylococcus aureus*. *Emerg. Infect. Dis.* 8: 82–84.
15. Jørgensen, H. J., T. Mørk, H. R. Høgåsen, and L. M. Rørvik. 2005. Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. *J. Appl. Microbiol.* 99:158–166.
16. Karahan, M., M. N. Acik, and B. Cetinkaya. 2009. Investigation of toxin genes by polymerase chain reaction in *Staphylococcus aureus* strains isolated from bovine mastitis in Turkey. *Foodborne Pathog. Dis.* 6:1029–1035.
17. Karahan, M., and B. Çetinkaya. 2007. Coagulase gene polymorphisms detected by PCR in *Staphylococcus aureus* isolated from subclinical bovine mastitis in Turkey. *Vet. J.* 174:428–431.
18. Katsuda, K., E. Hata, H. Kobayashi, M. Kohmoto, K. Kawashima, H. Tsunemitsu, and M. Eguchi. 2005. Molecular typing of *Staphylococcus aureus* isolated from bovine mastitic milk on the basis of toxin genes and coagulase gene polymorphisms. *Vet. Microbiol.* 105:301–305.
19. Kostman, J. R., M. B. Alden, M. Mair, T. D. Edlind, J. J. LiPuma, and T. L. Stull. 1995. A universal approach to bacterial molecular epidemiology by polymerase chain reaction ribotyping. *J. Infect. Dis.* 171:204–208.
20. Kuźma, K., E. Malinowski, H. Lassa, and A. Kłossowska. 2005. Analysis of protein A gene polymorphism in *Staphylococcus aureus* isolates from bovine mastitis. *Bull. Vet. Inst. Pulawy* 49:41–44.
21. Larsen, H. D., F. M. Aarestrup, and N. E. Jensen. 2002. Geographical variation in the presence of genes encoding superantigenic exotoxins and beta-hemolysin among *Staphylococcus aureus* isolated from bovine mastitis in Europe and USA. *Vet. Microbiol.* 85:61–70.
22. Lee, J. H. 2003. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Appl. Environ. Microbiol.* 69:6489–6494.
23. Loncarevic, S., H. J. Jørgensen, A. Løvseth, T. Mathisen, and L. M. Rørvik. 2005. Diversity of *Staphylococcus aureus* enterotoxin types within single samples of raw milk and raw milk products. *J. Appl. Microbiol.* 98:344–350.
24. Mašlanková, J., I. Pilipčincová, and L. Tkáčiková. 2009. Pheno- and genotyping of *Staphylococcus aureus* isolates of sheep origin. *Acta Vet. Brno* 78:345–352.
25. Mehndiratta, P. L., P. Bhalla, A. Ahmed, and Y. D. Sharma. 2009. Molecular typing of methicillin-resistant *Staphylococcus aureus* strains by PCR-RFLP of *spa* gene: a reference laboratory perspective. *Indian J. Med. Microbiol.* 27:116–122.
26. Mehrotra, M., G. Wang, and W. M. Johnson. 2000. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J. Clin. Microbiol.* 38:1032–1035.
27. Murphy, B. P., E. O'Mahony, J. F. Buckley, S. O'Brien, and S. Fanning. 2010. Characterization of *Staphylococcus aureus* isolated from dairy animals in Ireland. *Zoon. Public Health* 57:249–257.
28. Naffa, R. G., S. M. Bdour, H. M. Migdadi, and A. A. Shehabi. 2006. Enterotoxicity and genetic variation among clinical *Staphylococcus aureus* isolates in Jordan. *J. Med. Microbiol.* 55:183–187.
29. Normanno, G., M. Corrente, G. La Salandra, A. Dambrosio, N. C. Quaglia, A. Parisi, G. Greco, A. L. Bellacicco, S. Virgilio, and G. V.

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Celano. 2007. Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. *Int. J. Food Microbiol.* 117: 219–222.

30. Normanno, G., A. Firinu, S. Virgilio, G. Mula, A. Dambrosio, A. Poggiu, L. Decastelli, R. Mioni, S. Scuota, G. Bolzoni, E. Di Giannatale, A. P. Salinetti, G. La Salandra, M. Bartoli, F. Zuccon, T. Pirino, S. Sias, A. Parisi, N. C. Quaglia, and G. V. Celano. 2005. Coagulase-positive staphylococci and *Staphylococcus aureus* in food products marketed in Italy. *Int. J. Food Microbiol.* 98:73–79.
31. Ostyn, A., M. L. De Buyser, F. Guillier, J. Groult, B. Félix, S. Salah, G. Delmas, and J. A. Hennekinne. 2010. First evidence of a food poisoning outbreak due to staphylococcal enterotoxin type E, France, 2009. *Euro Surveill.* 15:1–4.
32. Ozer, B. H., A. F. Atasoy, and M. S. Akin. 2002. Some properties of Urfa cheese (a traditional white-brined Turkish cheese) made from bovine's and ovine's milk. *Int. J. Dairy Technol.* 55:94–99.
33. Pelisser, M. R., C. S. Klein, K. R. Ascoli, T. R. Zotti, and C. M. Arisi. 2009. Ocurrence [sic] of *Staphylococcus aureus* and multiplex PCR detection of classic enterotoxin genes in cheese and meat products. *Braz. J. Microbiol.* 40:145–148.
34. Rall, V. L., F. P. Vieira, R. Rall, R. L. Vieitis, A. Fernandes, J. M. Candeias, K. F. Cardoso, and J. P. Araújo. 2008. PCR detection of staphylococcal enterotoxin genes in *Staphylococcus aureus* strains isolated from raw and pasteurized milk. *Vet. Microbiol.* 132:408–413.
35. Riffon, R., K. Sayasith, H. Khalil, P. Dubreuil, M. Drolet, and J. Lagace. 2001. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. *J. Clin. Microbiol.* 39:2584–2589.
36. Rosec, J. P., J. P. Guiraud, C. Dalet, and N. Richard. 1997. Enterotoxin production by staphylococci isolated from foods in France. *Int. J. Food Microbiol.* 35:213–221.
37. Sahan, N., V. Isil, and M. S. Akin. 1998. Taze urfa peynirlerinin mikrobiyolojik ozellikleri ve bazi patojen bakterilerin aranmasi, p. 315–327. *In* M. Demirci (ed.), Fifth symposium of milk and milk products, Tekirdag, Turkey, 21 to 22 May 1998.
38. Scherrer, D., S. Corti, J. E. Muehlherr, C. Zweifel, and R. Stephan. 2004. Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from raw bulk-tank milk samples of goats and sheep. *Vet. Microbiol.* 101:101–107.
39. Sharma, N. K., C. E. D. Rees, and C. E. R. Dodd. 2000. Development of a single reaction multiplex PCR toxin typing assay for *S. aureus* strains. *Appl. Environ. Microbiol.* 66:1347–1353.
40. Spanu, V., S. Virdis, C. Scarano, F. Cossu, E. P. De Santis, and A. M. Cosseddu. 2010. Antibiotic resistance assessment in *S. aureus* strains isolated from raw sheep's milk cheese. *Vet. Res. Commun.* 34:87–90.
41. Stephen, R., C. Annemuller, A. A. Hassan, and C. H. Lammler. 2001. Characterization of enterotoxigenic *Staphylococcus aureus* strains isolated from bovine mastitis in north-east Switzerland. *Vet. Microbiol.* 78:373–382.
42. Tamarapu, S., J. L. Mckillip, and M. Drake. 2001. Development of a multiplex polymerase chain reaction assay for detection and differentiation of *Staphylococcus aureus* in dairy products. *J. Food Prot.* 64:664–668.
43. Turkish Food Codex. 23 Eylül 2002. Gida Maddelerinde Belirli bulasanlarin maksimum seviyelerinin belirlenmesi hakkinda teblig. Resmi Gazete, Basbakanlik Basimevi, Ankara. Sayı 24885.
44. Wallin-Carlquist, N., D. Márta, E. Borch, and P. Radström. 2010. Prolonged expression and production of *Staphylococcus aureus* enterotoxin A in processed pork meat. *Int. J. Food Microbiol.* 141: 69–74.
45. Yalcin, S., M. Ardic, and M. Nizamlioglu. 2007. Urfa Peynirinin Bazı Kalite Nitelikleri. *Atatürk Üniv. Vet. Bil. Derg.* 2:90–95.
46. Yazdani, R., M. Oshaghi, A. Havayi, E. Pishva, R. Salehi, M. Sadeghizadeh, and H. Foroohesh. 2006. Detection of *icaAD* gene and biofilm formation in *Staphylococcus aureus* isolates from wound infections. *Iran. J. Public Health* 35:25–28.
47. Yetismeyen, A., and F. Yildiz. 2003. Urfa Peynirlerinin Mikrobiyolojik, Kimyasal ve Duyusal Niteliklerinin Saptanması. *Gıda* 28:287–294.

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