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Staphylococcus aureus in animal-derived food products: the prevalence, virulence, enterotoxin-encoding genes, antibiotic resistance and PFGE profiles in northern Turkey

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ABSTRACT

The aim of this research was to investigate the prevalence of *Staphylococcus aureus* (*S. aureus*) in raw milk, Tulum cheese, and ground beef samples, and to determine their virulence, enterotoxins, antibiotic resistance, and genetic relatedness. A total of 300 food samples were purchased from public markets within different districts of Giresun, Turkey. Fifty-two (17.3%) of these food samples tested positive for *S. aureus* isolation. Fifty-two *S. aureus* isolates were further analyzed for the presence of virulence genes. The virulence genes detected were *icaA* (9.6%) and *icaD* (84.6%). Enterotoxin-encoding genes of the *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *seq*, *ser,* and *seu* groups were detected individually or in combination. Of the 52 *S. aureus* isolates, 1 methicillin-resistant *S. aureus* strain (1.9%) was isolated as having the *mecA*. The antibiotic susceptibility test of positive isolates showed resistance to cefoxitin (1.92%), tetracycline (11.5%), erythromycin (3.84%), ciprofloxacin (1.92%), gentamicin (1.92%), and fusidic acid (5.76%). Pulsed-field gel electrophoresis (PFGE) of the 52 isolates revealed 46 PFGE types, with 21 (40.3%) isolates grouped into 7 clusters. Some of the isolates from different districts showed clonal relatedness. The high occurrence of *S. aureus* strains in these products indicated a potential risk to humans. The results of this study indicated that dairy and meat products could be reservoirs of *S. aureus* strains that harbor several virulence factors and enterotoxin genes and the presence of these bacteria in foods may be a cause of concern for human health from food poisoning; therefore, hygienic measures and periodic bacteriological controls are necessary in all areas that provide these foods to the public, such as bazaars and butchers, to reduce contamination with foodborne pathogens.

Key words: *Staphylococcus aureus*; enterotoxin; food; MALDI- TOF; PFGE

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Introduction

Foodborne pathogens that spread through food are still a serious problem for food safety and international trade. Foodborne infections are a major public health problem, and bacterial toxins are considered the third most significant cause of food-borne outbreaks of disease throughout the world (EFSA, 2016; 2018). *S. aureus* is one the most notorious and common bacterial pathogens and is most likely responsible for a large number of simple skin infections and hundreds of thousands to millions of more serious, invasive infections each year worldwide (CHEUNG et al., 2021; LIU et al., 2022). In 2019, enterotoxins produced by *S. aureus* were the most frequent toxins reported at the European Union (EU) level, with a number of foodborne outbreaks (74 outbreaks, 1,400 cases, 141 hospitalizations) (EFSA, 2021).

S. aureus is a Gram-positive coccus, occurring in single, paired, or grapelike clusters that are facultative anaerobic, non-motile, non-sporing, catalase and coagulase positive bacteria (WILLEY et al., 2008). *S. aureus* owes its strong pathogenic capacities to the presence of a large number of various virulence factors, such as Panton-Valentine leukocidin (PVL), hemolysins (α, β, γ, and δ), toxic shock syndrome toxin-1 (TSST-1), exfoliative toxins (ETs), staphylococcal enterotoxins (SE), extracellular thermo-stable nuclease (nuc), blactamase (bla), staphylococcal cassette chromosome mec (SCCmec), accessory gene regulators (agr), and intercellular adhesion protein genes (ica) (WANG et al., 2018; VASUDEVAN et al., 2003). There are 24 different SEs known, and many *S. aureus* strains carry several SE genes. SEs may be divided into conventional categories (i.e., A–E) and unique types known as SEs or SE-like, on the basis of their ability to cause emesis (SEls) (GRISPOLDI et al., 2021).

Antibiotic-resistant microorganisms and antimicrobial-resistant genes can spread to humans through food (VERRAES et al., 2013). Methicillin-resistant *S. aureus* (MRSA) strains may sometimes be transferred from people to animals or animal products, highlighting the necessity for comprehensive strain characterization (PYZIK et al., 2014). It is believed that the transfer to humans of *S. aureus* strains that are resistant to antimicrobials occurs through the consumption of foods derived from animals (ÖZDEMIR, 2022).

Recent interest in the consumption of locally produced, minimally processed food has increased (EFSA BIOHAZ, 2015). Since *S. aureus* has the potential to infect most animals in the food chain, it is important to investigate the ecology of *S. aureus* in food and food-related contexts to reduce the possibility of zoonotic transmission to people (CHAALAL et al., 2018). The presence of *S. aureus* in dairy and meat products may cause significant public health concern; however, there are few data on the prevalence of *S. aureus* in the Giresun (Turkey) region. The aim of this study was to characterize *S. aureus* strains isolated from raw milk, Tulum cheese, and ground beef samples. Species identification using matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI–TOF MS) and pulsed field gel electrophoresis (PFGE) for genetic similarity analyses of isolates were conducted. The antibiotic resistance, presence of virulence genes, and presence of enterotoxin genes in the bacteria were evaluated.

Materials and methods

Sample collection and microbiological analysis. From May 2020 to May 2022, random samples of 300 animal-based foods were collected from public bazaars and butchers in Giresun Province’s coastal districts. These were 100 samples of raw cow’s milk, 100 samples of traditional Tulum cheese, and 100 samples of bovine meat. Approximately 250 g of ground beef, 250 g Tulum cheese, and 250 mL of raw milk were collected using aseptic techniques, and placed in sterile containers. All samples were transported under refrigeration to Espiye Vocational Laboratory at Giresun University, for conventional microbiological analyses within 2 h of collection. The sample preparation and additional examinations were done quickly after sampling.

Twenty-five milliliters of raw milk, 25 g of Tulum cheese, and 25 g ground beef were added and mixed using a blender (Waring, New Hartford,

CT, USA) with 225 mL of buffered peptone water (BPW) (Lab M, Lancashire, UK) and then incubated at 37°C for 24 hours while shaking. A portion of the culture was streaked onto Baird Parker Agar with Egg Yolk Tellurite Emulsion (Lab M, Lancashire, UK) and incubated at 37°C for 24-48 hours. The suspected isolates were biochemically identified using Gram staining, catalase activity, oxidase tests, coagulase tests, and thermostable DNase activity (ISO 6888-3, 2003; WANG et al., 2010).

Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) confirmation of isolates. Bacterial isolates were identified using MALDI-TOF MS (BioMérieux Inc. Marcy l'Etoile, France), according to the manufacturer's recommendations. Cultures of the suspected isolates were inoculated in Blood Agar Base and incubated at 37°C for 24 h. One or two colonies from the typical suspected colonies that were isolated from the media were spread into the slide wells on the slides of the VITEK MALDI-TOF MS equipment, after which 1 µL matrix solution (saturated cyano-4-hydroxycinnamic acid solution in 50% acetonitrile and 2.5% trifluoroacetic acid) (VITEK MS-CHCA, bioMérieux, Inc.) was pipetted into the wells and kept at room temperature until dry. The slide was then inserted into the equipment cassette and loaded into the MALDI-TOF MS device (SULAIMAN et al., 2018).

DNA extraction protocols. Templates for the polymerase chain reaction (PCR) were prepared using the boiling method using the procedure described by HOQUE et al. (2018), with some modifications. Isolated colonies were subcultured onto a plate of nutrient agar (NA) (Lab M, Lancashire, United Kingdom) and incubated at 37°C for 1 d, after which one pure colony from the NA plate was transferred into a 5-mL tube of nutrient broth (Lab M, Lancashire, United Kingdom) and incubated at 37°C with aeration using a shaker at 100 rpm. The cell pellets were recovered by centrifuging a 1.0 mL culture into a 1.5 mL microtube at 10000 rpm for 10 min, after which distilled water was used to recentrifuge the cell pellets and remove any remaining debris. Next, 200 µL of nuclease-free water was dissolved by mixing and shaking by hand. Each microtube was boiled for 10 min at 100°C, followed by cold shock on ice for a 10 min. The tubes were again centrifuged at 10000 rpm for 10 min, and the resulting supernatant (100–150 µL) was transferred into a clean microtube. The optical density of the DNA was measured at various wavelengths using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Rockland, DE, USA); and the DNA was then kept at -20°C until further use in PCR.

Genotypic characterization of virulence and enterotoxin genes. Using PCR with the primers listed in Table 1, all *S. aureus* isolates were screened for the presence of virulence genes. These were the Panton–Valentine leukocidin gene (*PVL*) (STEGGER et al., 2012), intercellular adhesion protein genes (*icaA*, *icaD*) (VASUDEVAN et al., 2003), classical enterotoxin genes (*sea, seb, sec, sed, see*) (JARRAUD et al., 2002), and other enterotoxin genes (*seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*, *seu*) (OMOE et al., 2002, 2005). The methods of PARK et al. (2011) were modified and used for genotyping the *S. aureus* isolates. PCR was conducted in a mix containing 1XPCR buffer (Thermo Scientific, Massachusetts, USA), 1.5 mM MgCl2 (Thermo Scientific, Massachusetts, USA), 0.2 mM dNTP (Thermo Scientific, Massachusetts, USA) and 1.5 U Taq-Polymerase (Thermo Scientific, Massachusetts, USA) as 1 µM from each primer and 5 µL DNA in 50 µL. The following PCR technique was used: initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at a particular temperature (Table 1), and elongation at 72°C for 90 s, and a final extension step at 72°C for 10 min. The amplified products were detected using 0.5 µg/mL ethidium bromide staining after electrophoresis at 80 V for 50 min in 1.5% agarose gel.

Detection of mecA. In the present study, PCR assays were used to detect the methicillin-resistant gene (*mecA*) in the *S. aureus* isolates. All isolates were tested for the presence of *mecA* using PCR with the specific primers as described in a previous study (MURAKAMI et al., 1991), with some modifications (Table 1).

Table 1. The oligonucleotide primers of virulence factors, enterotoxin genes and *mecA* gene

Target gene	Primer sequence (5'-3')	Fragment size (pb)	$T_{annealing}$ (°C)
PVL	F: GCT GGA CAA AAC TTC TTG GAA TAT R: GAT AGG ACA CCA ATA AAT TCT GGA TTG	83	59
icaA	F: CCT AAC TAA CGA AAG GTA G R: AAG ATA TAG CGA TAA GTG C	1315	49
icaD	F: AAA CGT AAG AGA GGT GG R: GGC AAT ATG ATC AAG ATA C	381	62
sea	F: GAA AAA AGT CTG AAT TGC AGG GAA CA R: CAA ATA AAT CGT AAT TAA CCG AAG GTT C	560	55
seb	F: ATT CTA TTA AGG ACA CTA AGT TAG GGA R: ATC CCG TTT CAT AAG GCG AGT	404	55
sec	F: GTA AAG TTA CAG GTG GCA AAA CTT G R: CAT ATC ATA CCA AAA AGT ATT GCC GT	297	55
sed	F: GAA TTA AGT AGT ACC GCG CTA AAT AAT ATG R: GCT GTA TTT TTC CTC CGA GAG T	492	55
see	CAA AGA AAT GCT TTA AGC AAT CTT AGG C CAC CTT ACC GCC AAA GCT G	482	55
seg	AAG TAG ACA TTT TTG GCG TTC C AGA ACC ATC AAA CTC GTA TAG C	287	57
seh	F: GTC TAT ATG GAG GTA CAA CACT R: GAC CTT TAC TTA TTT CGC TGTC	213	57
sei	F: GGT GAT ATT GGT GTA GGT AAC R: ATC CAT ATT CTT TGC CTT TAC CAG	454	57
sej	F: ATA GCA TCA GAA CTG TTG TTC CG R: CTT TCT GAA TTT TAC CAC CAA AGG	152	55
sek	F: TAG GTG TCT CTA ATA ATG CCA RTAG ATA TTC GTT AGT AGC TG	293	57
sel	F: TAA CGG CGA TGT AGG TCC AGG R: CAT CTA TTT CTT GTG CGG TAA C	383	56
sem	F: GGA TAA TTC GAC AGT AAC AG R: TCC TGC ATT AAA TCC AGA AC	379	57
sen	F: CAT CAT GCT TAT ACG GAG GAG R: CCC ACT GAA CCT TTT ACG TT	301	53
seo	F: TCG CCT GTG TAT TAT CTC CC R: TCT TTA GAA ATC GCT GAT GA	214	57
sep	F: TGA TTT ATT AGT AGA CCT TGG R: ATA ACC AAC CGA ATC ACC AG	381	57
seq	F: TCA AGG AGT TAG TTC TGG AAA TT R: GCT TAC CAT TGA CCC AGA GA	251	53
ser	F: GGA TAA AGC GGT AAT AGC AG R: GTA TTC CAA ACA CAT CTA AC	166	57
seu	F: ATC AGA AAC AAA CAT TAA AGC CCA R: TGA CCA TTT CCT TCG ATA AAC TTT AT	500	53

Table 1. The oligonucleotide primers of virulence factors, enterotoxin genes and *mecA* gene (continued)

Target gene	Primer sequence (5’-3’)	Fragment size (pb)	$T_{annealing}$ (°C)
mecA	F: AAA ATC GAT GGT AAA GGT TGG C R: AGT TCT GCA GTA CCG GAT TTG C	533	55

$T_{annealing}$: annealing temperature; sea, staphylococcal enterotoxin a gene; seb, staphylococcal enterotoxin b gene; sec, staphylococcal enterotoxin c gene; sed, staphylococcal enterotoxin d gene; see, staphylococcal enterotoxin e gene; seg, staphylococcal enterotoxin g gene; seh, staphylococcal enterotoxin h gene; sei, staphylococcal enterotoxin i gene; sej, staphylococcal enterotoxin j gene; sek, staphylococcal enterotoxin k gene, sel, staphylococcal enterotoxin l gene; sem, staphylococcal enterotoxin m gene; sel, staphylococcal enterotoxin l gene; sem, staphylococcal enterotoxin m gene; sen, staphylococcal enterotoxin n gene; seo, staphylococcal enterotoxin o gene; sep, staphylococcal enterotoxin p gene; seq, staphylococcal enterotoxin q gene; ser, staphylococcal enterotoxin r gene; seu, staphylococcal enterotoxin u gene.

Antimicrobial susceptibility testing. The antibiotic susceptibilities of *S. aureus* isolates were investigated using the disc diffusion method, according to the protocols of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2022). To determine antibiotic resistance in the isolates, 30 µg Amikacin (AK), 5 µg ciprofloxacin (CIP), 30 µg chloramphenicol (C), 2 µg clindamycin (CD), 15 µg erythromycin (E), 10 µg gentamicin (CN), 10 µg fusidic acid (FC), 10 µg linezolid (LNZ), 30 µg tetracycline (TE), 15 µg tigecycline (TGC), and 1.25/23.75 µg trimethoprim/sulfamethoxazole (SXT) antibiotic discs were used (all purchased from Liofilchem, Roseto degli Abruzzi, Italy). After incubation, the resulting diameters of the inhibition zones that formed around the discs of CIP, C, CD, E, CN, LNZ, TE and SXT were classified as susceptible, intermediate, or resistant, according to the diameters and the breakpoints available in CLSI documents (CLSI, 2022). For the remaining antimicrobial agents (AK, FC and TGC), the critical values were evaluated according to the zone table described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) document (EUCAST, 2022). For quality control purpose*s, S. aureus* ATCC 25923 was used as a control strain.

Phenotypic detection of methicillin-resistant S. aureus. Methicillin resistance in *S. aureus* isolates was investigated using the disc diffusion method according to CLSI protocols (CLSI 2022). Susceptibility tests using 30 µg of cefoxitin (FOX) (Liofilchem, Roseto degli Abruzzi, Italy) were used to differentiate MRSA strains from *S. aureus* isolates in the food samples. Strains with cefoxitin zone diameter ≤21 mm were identified as methicillin resistant. CLSI standards were followed for all tests. *S. aureus* ATCC 43300 was used as a control strain (CLSI, 2022).

Pulsed-field gel electrophoresis analysis. PFGE of *Sma*I-digested (Takara Bio Inc., Shiga, Japan) chromosomal DNA samples of 52 *S. aureus* isolates were conducted according to the standard procedure used in the PulseNet program by the Center for Disease Control and Prevention (CDC) with minor changes, as described by GOLDING et al. (2015). DNA fragments were separated on 1% w/v agarose gels in 0.5× TBE buffer using a CHEF DR-II electrophoresis chamber (Bio-Rad, Nazareth, Belgium) with 6 V/cm^2 for 20 h at 14°C, with an initial switch time of 5.3 s and a final switch time 34.9 s. The gels were stained with 1 mg/mL ethidium bromide in 0.5x TBE for 30 min. Band profiles obtained by agarose gel electrophoresis were photographed under an ultraviolet (UV) transducer, and stored electronically for analyses. The TIFF images obtained using PFGE were analyzed using Gel Compar ver. 6.6 (Applied Maths, Kourtrai, Belgium). Cluster analysis of Dice similarity indices based on the unweighted pair-group method with arithmetic mean (UPGMA) was used to create the dendrogram that illustrated the relationship among the PFGE profiles. In the analysis, position tolerance and optimization were used as 1.0%. Isolates with a Dice similarity index of ≥90% were classified into the same PFGE cluster. PFGE was conducted using *Salmonell*a Braenderup H9812 as the molecular weight marker. Under UV light, the gel was dyed with ethidium bromide and photographed. TENOVER et al. (1995) classified

the isolates as indistinguishable (cluster), closely related, perhaps related, or distinct.

Results

The overall prevalence of *S. aureus* in the commercially available raw milk, Tulum cheese and ground beef samples from eight coastal districts in Giresun, Turkey, was 17.3% (52/300). Raw milk had the highest prevalence of *S. aureus* (40%); Tulum cheese (6%) and ground beef (6%) had the lowest (Table 2).

The incidences of the virulence gene among *S. aureus* isolates in the milk, cheese and meat are shown in Table 3. The most common virulence factor profiles, *icaA* and *icaD,* were found in 44 isolates (84.6%) and in 5 (9.6%) isolates, respectively. None of the isolates investigated harbored the Panton–Valentine leukocidin gene (*pvl*). The specific genotypes of the *S. aureus* strains, with respect to the enterotoxin genes tested (*sea, seb, sec, sed, see, seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*, *seu*) are shown in Table 3. The enterotoxin-coding genes *sem* (9.6%, 5/52), *seg* (7.6%, 4/52), *sea* (5.7%, 3/52), *sec* (5.7%, 3/52), *sen* (5.7%, 3/52), and *seo* (5.7%, 3/52) were the most prevalent, followed by *seh* (two strains), *sei* (two strains), *seb* (one strain) and *seq* (one strain) in raw milk samples. In ground beef samples, *sea* (7.6%, 4/52), *seg* (7.6%, 4/52), *sec* (3.8%, 2/52), *sem* (3.8%, 2/52), *sen* (3.8%, 2/52), *seo* (3.8%, 2/52), *seb* (1.9%, 1/52), and *sei* (1.9%, 1/52) were detected. *seb* (1.9%, 1/52) and *sep* (1.9%, 1/52) were detected in cheese samples.

Table 5 shows the results of the susceptibility of the isolated 52 *S. aureus* strains to 12 antibiotics. The highest prevalence of resistance, at 11.5%, was recorded for tetracycline, followed by fusidic acid at 5.76%,, erythromycin at 3.84%, ciprofloxacin at 1.92%, gentamicin at 1.92%, and cefoxitin at 1.92%. The intermediate resistance profiles of the *S. aureus* isolates are as follows: erythromycin, 5.76%; ciprofloxacin, 3.84% and clindamycin, 1.92%. No resistance to amikacin, chloramphenicol, linezolid, tigecycline, and trimethoprim-sulfamethoxazole was observed in the *S. aureus* isolates. One isolate (1.92%) was observed to contain the methicillin-resistant encoding gene and methicillin resistance.

PFGE typing of the 52 *S. aureus* strains yielded 46 PFGE patterns (Fig. 1). Five of these patterns (1, 1a, 10, 10a and 33) were indistinguishable, including 21 strains (grouping rate, 40.3%). PFGE type 1 was the predominate type, including six clonally related strains. Twenty-one (40.3%) genotyped strains showed clonal relationships**.** Among the 52 *S. aureus* isolates, clustered isolates were collected in 7 clusters, referred to as clusters A to G (tolerance 1.0, optimization 1.0, cutoff 90%). Isolates from different food types and different districts were significantly highly similar (100%), such as 73 and 81 from cheese (Bulancak) and milk (Tirebolu), respectively, in cluster C. There were isolates in 1, 3, 10, and 12 from different districts (Tirebolu, Espiye, Bulancak, Piraziz, Görele). Cluster A comprised most of the strains (N = 6), followed by clusters C (N = 4) and G (N = 3). Cluster group G was dominated by enterotoxin gene *sec*, *seg*, *seh*, *sem*, *sen* and *seo* (14.2%).

Table 2. The presence of *Staphylococcus aureus* in milk, cheese and meat samples

Food Samples	*S. aureus*	Districts								
		Total	Piraziz n=24	Bulancak n=58	Central n=57	Keşap n=27	Espiye n=45	Tirebolu n=32	Görele n=33	Eynesil n=24
Raw milk n=100	40	40	3	10	0	1	9	6	8	3
Cheese n=100	6	6	0	1	0	0	2	3	0	0
Ground beef n=100	6	6	0	5	0	0	0	1	0	0
Total N=300	52	52	3	16	0	1	11	10	8	3

Table 3. The prevalence of virulence and enterotoxin gene profiles among 52 *Staphylococcus aureus* isolated from milk, cheese, and meat samples

Virulence genes and enterotoxin genes	Number of *S. aureus*			Total
	Raw milk n=40	Cheese n=6	Ground beef n=6	
sea	3	-	4	7
seb	1	1	1	3
sec	3	-	2	5
sed	-	-	-	-
see	-	-	-	-
seg	4	-	4	8
seh	2	-	-	2
sei	2	-	1	3
sej	-	-	-	-
sek	-	-	-	-
sel	-	-	-	-
sem	5	-	2	7
sen	3	-	2	5
seo	3	-	2	5
sep	-	1	-	1
seq	1	-	-	1
ser	-	-	-	-
seu	-	-	-	-
pvl	-	-	-	-
icaA	5	-	-	5
icaD	35	4	5	44

Table 4. Distribution of enterotoxin gene profiles among 52 *Staphylococcus aureus* isolated from milk, cheese, and meat samples

SE genotypes	Number (%) of staphylococcal enterotoxin genotypes from food samples			
	Milk (n = 100) n(%)	Cheese (n = 100) n(%)	Ground beef (n = 100) n(%)	Total (n = 300) n(%)
sea	1(1)		1(1)	2(0,6)
sem	1(1)			1(0.3)
sea, seb	1(1)		1(1)	2(0,6)
sea, seg			1(1)	1(0.3)
seb, sep		1(1)		1(0.3)
sea, seh, seq	1(1)			1(0.3)
seg, sei, sem	1(1)			1(0.3)
seg, sem, seo			1(1)	1(0.3)
sea, sec, seg, sen			1(1)	1(0.3)
sec, seg, seh, sem, sen, seo	2(2)			2(0,6)
sec, seg, sei, sem, sen, seo	1(1)		1(1)	2(0,6)

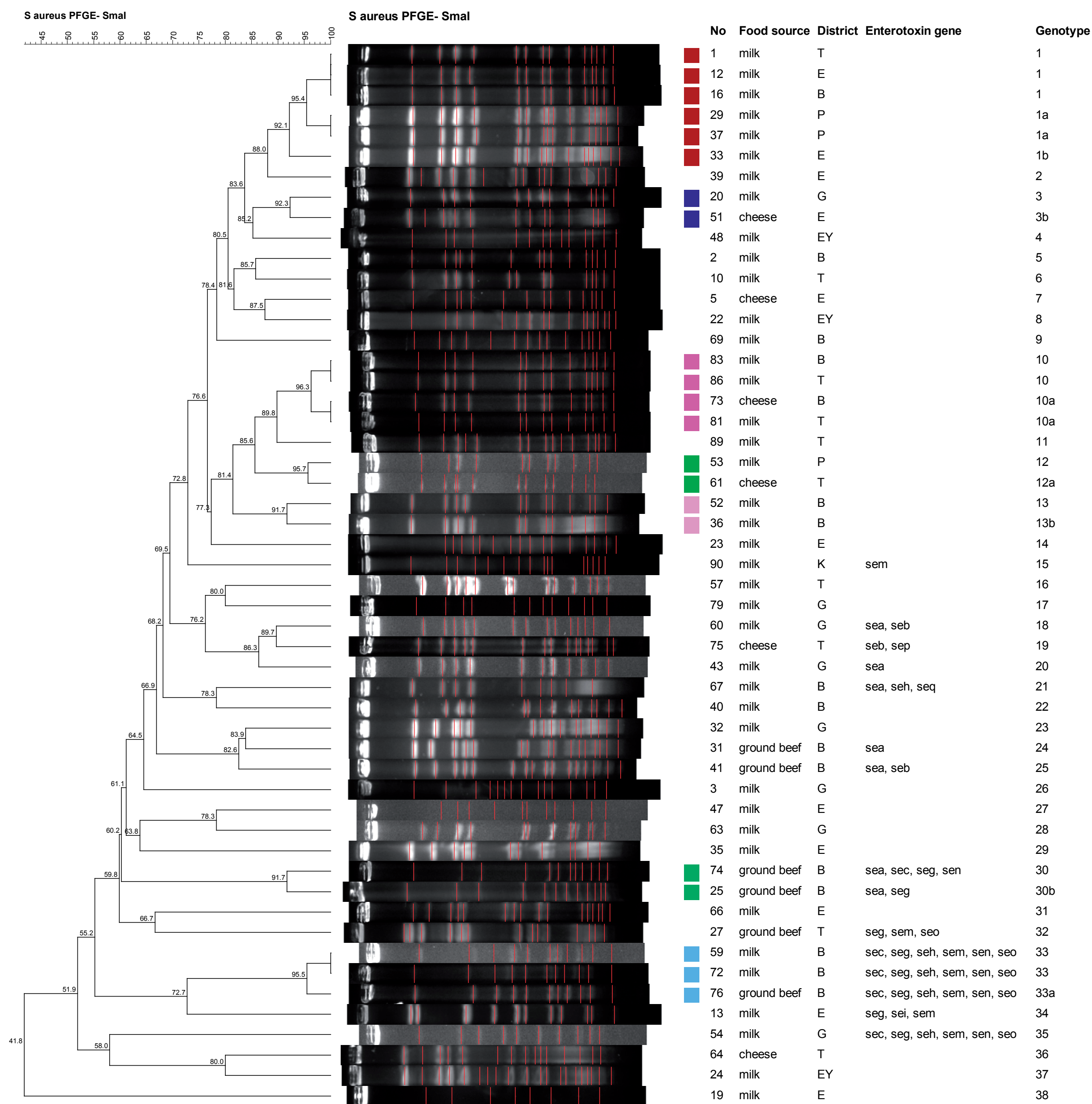

Fig. 1. *Sma*I-PFGE dendrogram based on DICE coefficient of similarity for *Staphylococcus aureus* strains isolated from milk, cheese and ground beef. Clusters (genotype), A (1, 1a, 1b), B (3, 3b), C (10, 10a), D (12, 12a), E (13, 13b), F (30,30b), G (33, 33a), T, Tirebolu; E, Espiye; B, Bulancak; P, Piraziz; G, Görele; EY, Eynesil; K, Keşap

Table 5. Antimicrobial susceptibility pattern of 52 *Staphylococcus aureus* isolated from milk, cheese and meat samples

Antibiotics	Sensitivity of the total isolates		
	S (%)	I (%)	R (%)
AK	100	0	0
C	100	0	0
CD	98	1.92	0
CIP	94.2	3.84	1.92
CN	98	0	1.92
E	90.3	5.76	3.84
FC	94.2	0	5.76
FOX	98	0	1.92
LNZ	100	0	0
TE	88.4	0	11.5
TGC	100	0	0
SXT	100	0	0

S: sensitive; R: resistant; I: intermediate, AK: amikacin; C: chloramphenicol; CD: clindamycin; CIP: ciprofloxacin; CN: gentamicin; E: erythromycin; FC: fusidic acid; FOX: cefoxitin; LNZ: linezolid; TE: tetracycline; TGC: tigecycline; SXT: trimethoprim-sulfamethoxazole

Discussion

S. aureus is widely found in a variety of foods, particularly those of animal origin, and is a globally significant foodborne pathogen (WANG et al., 2012). The present study focused in particular on the variety of antimicrobial resistance, molecular lineages, virulence factors, and enterotoxin genes in *S. aureus* isolated in Giresun from raw milk, traditional Tulum cheese, and ground beef. The current study results indicated that ingestion of these products could be a potential source of infection. We assessed the potential spread of *S. aureus* through milk, cheese and meat in Giresun, Turkey, given that raw milk, Tulum cheese and ground beef are consumed by a large share of the population within this area. The overall prevalence of *S. aureus* in the commercially available milk, cheese and meat samples from eight coastal districts in Giresun was found to be 16.6% (52/300). *S. aureus* was most common in milk (40/100 samples, 40%); a much lower prevalence was found in cheese (6/100 samples, 6%) and meat (6/100 samples, 6%) (Table 2). Incidences of *S. aureus* contamination (6.08–60%) have been reported for milk, cheese and meat in retail sales in different studies from various countries, such as Portugal (PEREIRA et al., 2009), Poland (KORPYSA-DZIRBA and OSEK, 2019), Turkey (ÖZDEMİR, 2022) and Egypt (GHABBOUR et al., 2022). Differences in manufacturing, transportation, and sanitary conditions during food handling may be to blame for the varying prevalence rates of *S. aureus* found in milk, cheese, and meat. There are several other aspects that need to be considered, such as the sample size, detection technique, and food origin.

The ability of *S. aureus* clonal complexes to colonize host tissues and cause severe illness varies, which may be related to the absence or presence of certain virulence factors as well as the amounts at which they are generated. Staphylococcal enterotoxins are produced by enterotoxigenic *S. aureus*, and are responsible for staphylococcal food poisoning (LIAO et al., 2022). There is a strong correlation between *S. aureus* and foodborne illness because of the presence of enterotoxins in contaminated food supply chains worldwide (WANG et al., 2017). SEs, particularly

SEA, SEB, SEC, SED, SEG, SEH, SEI, and SEM, have been found in a wide variety of foods, the most common of which are milk and dairy products, raw meat (poultry and livestock) and meat products, eggs and egg products, fermented foods, vegetables, fish products, salted foods (such as ham), baked products (especially cream-filled pastries and cakes), sandwich fillings, and other ready-to-eat foods (NIA et al., 2021). In the present study, overall, 52 isolates tested positive for one or more of the virulence-factor genes. Among the 52 isolates, the prevalence of individual virulence-factor genes (biofilm-related genes) were *icaA* (9.6%) and *icaD* (84.6%). The present study found that *seg* (15.3%) was the most frequent gene, followed by *sea* (13.4%), *seb* (5.7%), *sec* (9.6%), *seh* (5.7%), *sei* (5.7%), *sem* (13.4%), *sen* (9.6%) and *seo* (9.6%) whereas *sep* (1.9%) and *seq* (1.9%) were the least frequent genes (Tab 3, 4). Our results agree with a study by LIAO et al. (2022) in China in which the *sea, seb, sec, sed, seg,* and *sei* gene were observed in 5.6%, 19.8%, 40.8%, 1.4%, 49.3%, and 30.9% of *S. aureus* isolates respectively. Similarly, a study reported by FILIPELLO et al. (2019) in Italy detected *sea, seb, sec, seg, seh* and *sep* in 53%, 0.9%, 8.1%, 6.3% and 0.9% of *S. aureus* isolates respectively. In contrast, in another study conducted by KORPYSA-DZIRBA and OSEK et al. (2019), *sed* was detected in nine isolates of *S. aureus*. The diversity in *S. aureus* virulence genes reported in other studies might be attributable to various sampling techniques, sample types, isolation processes, environmental conditions, or geographic regions.

Antimicrobial resistance in bacteria constitutes a significant danger to food safety and public health, and is developing rapidly (NELSON et al., 2019). Antibiotic resistance may spread across the food chain through direct or indirect contact (MARSHALL and LEVY, 2011). *S. aureus* is a well-known bacterium that is capable of developing resistance to antibiotics as a result of its capacity to acquire a range of resistance mechanisms against antimicrobial drugs. One example is the organism's resistance to methicillin. The transmission of MRSA might be through contact with infected meals or by ingesting such foods. As a result of their potential to contaminate food and to colonize and infect both people and animals, *S. aureus* and MRSA are regarded as a serious public health risk (MEKHLOUFI et al., 2021). In the present study, *S. aureus* strains were tested for susceptibility to 12 antimicrobial drugs of veterinary and human health significance. *S. aureus* strains were examined for the antimicrobial susceptibility, and it was determined that the isolates were resistant to various drugs, particularly the tetracyclines (TE), fusidic acid (FC), macrolides (E), fluoroquinolones (CIP), aminoglycosides (CN) and cephalosporins (FOX) (Table 5). Only one isolate was resistant to methicillin and showed the presence of *mecA*. Very high resistance to penicillin G (67.11%), and TE (27.63%) was noted by CASTRO et al. (2020) in strains of *S. aureus* isolated from cheese samples from Brazil. ÖZDEMİR (2022) described 100% resistance to penicillin G and sulphamethoxazole, and 41.7% resistance to TE in 17 strains of *S. aureus* isolated from ground beef in Turkey. By contrast, GHABBOUR et al. (2022) demonstrated resistance to nalidixic acid, ampicillin, sulfamethoxazole-trimethoprim, cefuroxime, azithromycin, oxacillin, rifampin, vancomycin, neomycin, streptomycin, amoxicillin and levofloxacin cephalosporin in *S. aureus* isolated from food samples from Egypt. These fluctuations in resistance frequency among isolates may be the result of a variety of factors, such as the geographical regions studied, the origins of the isolates, and the use of various antibiotics.

PFGE is a method that is regarded as being highly selective, and it is used to assess the genetic diversity of several bacterial pathogens, one of which is *S. aureus* (TANG, 2009). SmaI-PFGE (Fig. 1) revealed that these *S. aureus* strains have been classified into 46 genotypes, which indicates clonal transmission. On the basis of a similarity coefficient higher than >90%, seven PFGE groups (Cluster A-G) were identified, the largest of which comprised 16 strains (isolates no 1, 12, 16, 29, 37 and 33). PFGE typing indicated that there is high genetic diversity in *S. aureus* isolate collection (for the 52 isolates, 46 pulsotypes were identified). Previous studies have reported genetic heterogeneity among the strains isolated from food samples. Our results show similar high genetic diversity to that found

among *S. aureus* isolates from foods in Xinjiang, China (CAI et al., 2021), and the PFGE analysis generated 28 PFGE pulsotypes for 43 *S. aureus* isolates sampled from Kazak cheese. GHARSA et al. (2019) published results in Tunisia, where 16 PFGE pulsotypes were distinguished among 26 *S. aureus* isolates from dairy products. In contrast, low genetic diversity was also found among *S. aureus* isolated from raw milk, cheese, minced meat, and chicken meat samples from Turkey with 9 PFGE pulsotypes (40 isolates) (CAN et al., 2017). The high diversity of isolates in the present study may be the result of the different geographical origins of the samples; however, our PFGE results confirmed that the enterotoxigenic *S. aureus* strains isolated from cheese samples in pulsotype 10a (isolate 73) were identical to milk isolate 81 (pulsotype 10a), all belonging to cluster C. The occurrence of the same profile from different manufacturers, different food types in the same years and different districts suggests that the isolate is persistent and spreading within the studied region. Pulsotype 1a (isolates 29 and 37) and pulsotype 33 (isolates 59 and 72) were found in Piraziz and Bulancak, respectively, but samples from different manufacturers may share the same supplier and be the presumed source of contamination. In addition, the relationship seen in the DNA profiles of *S. aureus* isolates, independent of the food type, district, or manufacturer from whom the samples were obtained, show the circulation and transmission of clones with high genetic diversity within our area.

Conclusions

This present study was the first to present a detailed description of the possible contamination of dairy and meat products in public markets with *S. aureus* in Giresun, Turkey. The results of the present study reveal that staphylococci are common contaminants in Giresun's public markets, with raw milk and ground meat being the most contaminated. PFGE results show that isolates from different districts presented clonal relatedness. Molecular typing revealed the circulation of 46 different PFGE types, of which type 1(6) accounted for 11.5% of the isolates examined. Most of the PFGE patterns showed enterotoxin and biofilm genes (*icaA*, *icaD*). The presence and expression of virulence factors and antibiotic resistance in these strains are an interesting result in terms of public health protection, and demonstrate the existence of harmful staphylococci in food. The results emphasize the importance of surveillance studies and the necessity for constant monitoring of the food chain of animal-derived foods for the prevalence and spread of drug-resistant zoonotic *S. aureus*. Findings from these analyses may provide additional information for the development of control strategies that can be implemented to ensure the safety of food supplies, and help track the spread of such strains during epidemiological investigations. Future studies might include urban, rural, and other food sources to assess the prevalence and characteristics of *S. aureus* in Turkey.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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SAŽETAK

Cilj je rada bio istražiti prevalenciju bakterije *Staphylococcus aureus* (*S. aureus*) u sirovom mlijeku, u siru Tulum i uzorcima mljevene govedine. U izoliranih bakterija analizirani su virulencija, enterotoksini, antibiotska rezistencija i genetska srodnost. Ukupno je 300 uzoraka hrane kupljeno na javnim tržnicama u različitim okruzima Giresuna u Turskoj. Među njima su 52 uzorka (17,3%) bila pozitivna na *S. aureus*. U izolatima bakterije otkriveni geni virulencije bili su *icaA* (9,6%) i *icaD* (84,6%). Geni koji kodiraju enterotoksin *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *seq*, *ser* i *seu* otkriveni su zasebno ili u kombinaciji. Među 52 izolata bakterije *S. aureus* jedan je izolirani meticilin-rezistentni soj *S. aureus* (1,9%) otkriven kao *mecA*. Test osjetljivosti pozitivnih izolata na antibiotike pokazao je rezistenciju na cefoksitin (1,92%), tetraciklin (11,5%), eritromicin (3,84%), ciprofloksacin (1,92%), gentamicin (1,92%) i fusidatnu kiselinu (5,76%). Elektroforeza u gelu s pulsirajućim poljem (PFGE) među 52 izolata pokazala je 46 PFGE tipova, s 21 izolatom (40,3%) grupiranim u 7 skupina/klastera. Neki su izolati iz različitih okruga pokazali klonsku srodnost. Visoka pojavnost sojeva *S. aureus* u prehrambenim proizvodima upućuje na potencijalan rizik za ljude. Rezultati ovog istraživanja pokazali su da bi mliječni i mesni proizvodi mogli biti rezervoar sojeva *S. aureus* koji su nositelji nekoliko čimbenika virulencije i gena enterotoksina, a njihova prisutnost u hrani mogla bi biti zabrinjavajuća za zdravlje ljudi s obzirom na mogućnost otrovanja. Zbog toga je u svim subjektima koji posluju s hranom za ljude, kao što su tržnice i mesnice, nužna primjena higijenskih mjera i periodičnih bakterioloških kontrola kako bi se smanjila kontaminacija patogenima koji se prenose hranom.

Ključne riječi: *Staphylococcus aureus*; enterotoksin; hrana; MALDI-TOF; PFGE