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The prevalence, enterotoxigenic properties and antimicrobial susceptibility of *Staphylococcus aureus* isolated from various foods of animal origin

Pelin Kocak Kizanlik*and Ergun Omer Goksoy

Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Bati Kampusu, Aydın, Türkiye

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ABSTRACT

This study aimed to determine the prevalence, enterotoxigenic properties and antimicrobial resistance profile of *Staphylococcus aureus* isolated from 850 food samples, including bulk tank milk, Tulum cheese, chicken meat and beef carcasses, in Türkiye. *S. aureus* contamination rates and the mean contamination levels of 86 positive samples from bulk tank milk, Tulum cheese, chicken meat and beef carcasses were 10.8% (n:49) and 3.01±0.48 log cfu/ml, 17% (n:17) and 3.08±0.42 log cfu/g, 12% (n:12) and 2.89±0.27 log cfu/g, and 4% (n:8) and 1.28±0.54 log cfu/cm^2, respectively. 39 out of 86 isolates (45.3%) had one or more enterotoxin genes (*sea*-*see*, *seg*-s*elj*, *sep*). *sed* was the most common classical enterotoxin gene, whereas *sei* was the most common new enterotoxin gene. Antimicrobial susceptibility testing against 16 antibiotics performed by VITEK 2 showed that 61 isolates (70.9%) were resistant to various antibiotics. 32.8% of the resistant isolates were multidrug resistant (MDR) and the mean multiple antimicrobial resistance (MAR) index was 0.301. The isolates were mostly resistant to penicillin (63.9%), followed by oxacillin (24.4%), clindamycin (19.7%) and erythromycin (12.7%). *mecA* was detected in 13 isolates (15.1%), but no *mecC* was found. It was concluded that most of the *S. aureus* isolates had enterotoxin genes which might cause foodborne intoxications. The high antibiotic resistance rates observed in these strains, including MRSA, may also result in some public health hazards.

Key words: *Staphylococcus aureus*; virulence factors; antibiotic resistance; public health

Introduction

S. aureus is considered to be one of the most common bacterial pathogens reported in foodborne outbreaks worldwide (BIANCHI et al., 2013; CDC, 2018). Some strains of *S. aureus* are able to produce Staphylococcal enterotoxins (SEs) in foodstuffs. At first, *S. aureus* enterotoxins (SEs) were divided into 5 serological "classical types" (SEA-SEE), however, new types of SEs and SE-like (SE*l*) toxins have also been described (AYDIN et al., 2011a). Foods that have been frequently incriminated in Staphylococcal food poisoning (SFP) mainly include foods of animal origin (such as meat and meat products, poultry and egg products, milk and dairy products) (DOYLE et al.,

*Corresponding author:
Pelin Kocak Kizanlik, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Bati Kampusu, 09016, Aydın, Türkiye, phone: +90 256 2206000, phone: +90 505 7168973, fax: +90 256 2206299, e-mail: pelin.kocak@adu.edu.tr

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2012; PAPADOPOULOS et al., 2019; MAHROS et al., 2021). In general, food contamination may directly occur from livestock, or may result from unhygienic conditions during production processes, or retail and storage of foods, since personnel and equipment may carry *S. aureus* (DOYLE et al., 2012). The acceptable levels for *S. aureus* in raw milk, cheese, poultry meat and beef carcasses etc. are given in Commission Regulations (EC) No. 2073/2005 (2005). In the same regulations, it is also stated that no enterotoxin should be present in milk and milk products.

Antimicrobial resistance has been considered to be a major problem for public health. Antibiotic resistance profiles of bacteria are rapidly changing because of the extended use and misuse of antibiotics (PESAVENTO et al., 2007; CAN and ÇELIK, 2012). In relation to the intensity of resistance, bacteria can be categorized as multi-drug resistant (MDR), acquired nonsusceptibility to at least one agent in three or more antimicrobial categories; extensive-drug resistant (XDR), remaining susceptible to only one or two antimicrobial categories; and pan-drug resistant (PDR), that is, non-susceptible to all agents in all antimicrobial categories (MAGIORAKOS et al., 2012).

Food, especially from animal origin, is recognized as an important vector for the transfer of antibiotic resistance, including Methicillin resistant *S. aureus* (MRSA). *S. aureus* resistance to methicillin is related to the *mecA* or *mecC* genes, that encode the penicillin-binding protein 2a (PBP2a) (CAN and ÇELIK, 2012; BASANISI et al., 2017).

S. aureus contamination in various foods of animal origin is of major importance for food safety and public health, with implications for the regional economy. Therefore, this study aimed to evaluate the prevalence of *S. aureus* in foods of animal origin, to determine the antimicrobial resistance patterns of *S. aureus* isolates, and to analyze the distribution of SEs genes and methicillin resistance.

Materials and methods

Sample collection. A total of 850 samples (450 bulk tank milk, 100 Tulum cheese, 100 chicken meat, and 200 beef carcass) were collected from large cities (Aydın, İzmir, Muğla), located in the Western Aegean region of Türkiye. Bulk tank milk samples were collected from various districts of Aydın, and cheese and chicken meat samples were purchased from some conventional markets, supermarkets and butchers in Aydın and İzmir. In addition, carcass samples were randomly selected from a municipal slaughterhouse in Muğla.

Isolation, enumeration and identification of S. aureus. 10 grams (ml) of each sample, except carcass samples, were homogenized in 90 ml buffered peptone water (CM0509, Oxoid, Hampshire, UK) using a stomacher (Bagmixer, Interscience, Mourjou, France) for 2 min. A non-destructive sponge sampling method was used for carcasses, following the instructions stated in ISO 17604 (2015). Sampling was carried out from the rump, flank, brisket and neck areas of half of the randomly selected carcasses (total of 400 cm^2 for each carcass) using a 10 cm×10 cm sterile square template and sterile swab sponges (World Bioproduct SRDRY-G, Washington, USA). Each swab was homogenized for at least 2 minutes and all the homogenates from one of the carcasses were put into a new stomacher bag for repeated homogenization. After this, serial dilutions of all samples were prepared.

The isolation and enumeration of *S. aureus* were carried out as stated in the ISO method 6888-1 (ISO, 2001). Five suspected colonies on selective agar were subcultured and identified by Gram staining, the catalase test, coagulase tests, DNase activity, mannitol fermentation and the latex agglutination test (Dryspot staphytect test plus, Oxoid, DR0100, Oxoid, Hampshire, UK). One *S. aureus* isolate per positive sample was selected, and confirmation was carried out by PCR.

DNA extraction was performed according to the manufacturer's instructions (Thermo Scientific Fisher, Waltham, MA, USA). *16S rRNA*, *nuc* and *coa* were screened by PCR in order to confirm the identification of *S. aureus* isolates. The detection of *16S rRNA* and *nuc* genes were carried out using multiplex-PCR assay (KEYVAN and OZDEMIR, 2016). The *coa* gene was detected as described by HOOKEY et al. (1998).

Table 1. Primers and multiplex PCR sets used in this study

Gene*	Primer	Primer sequence (5'-3')	Product size	Multiplex PCR set
16S rRNA	16SrRNA-for 16SrRNA-rev	GTAGGTGGCAAGCGTTATCC CGCACATCAGCGTCAG	228	A
nuc	NUC-for NUC-rev	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	270	
coa	COA-for COA-rev	ATAGAGATGCTGGTACAGG GCTTCCGATTGTTCGATGC	500-650	-
sea	SEA-for SEA-rev	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102	B
seb	SEB-for SEB-rev	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164	
sec	SEC-for SEC-rev	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	451	
sed	SED-for SED-rev	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278	
see	SEE-for SEE-rev	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC	209	
seg	SEG-for SEG-rev	GTTAGAGGAGGTTTTATG TTCCTTCAACAGGTGGAGA	198	C
sei	SEI-for SEI-rev	GGCCACTTTATCAGGACA AACTTACAGGCAGTCCA	328	
selj	SE*l*J-for SE*l*J-rev	GTTCTGGTGGTAAACCA GCGGAACAACAGTTCTGA	131	
seh	SEH-for SEH-rev	CAACTGCTGATTTAGCTCAG CCCAAACATTAGCACCA	173	D
sep	SEP-for SEP-rev	TCAAAAGACACCGCCAA ATTGTCCTTGAGCACCA	396	
mecA	MECA-for MECA-rev	ACTGCTATCCACCCTCAAAC CTGGTGAAGTTGTAATCTGG	163	-
mecC	MECC-for MECC-rev	GCTCCTAATGCTAATGCA TAAGCAATAATGACTACC	304	-

*Nucleotide sequence and locations were used from the published sequences for *16S rRNA* and *nuc* (KEYVAN and ÖZDEMİR, 2016), *coa* (HOOKEY et al., 1998), *sea*, *seb*, *sec*, *sed*, *see* and *mecA* (MEHROTRA et al., 2000), *seg*, *seh, sei*, *selj* and *sep* (BANİA et al., 2006), and *mecC* (CUNY et al., 2011)

Detection of staphylococcal enterotoxin genes. The detection of 10 genes encoding staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *selj* and *sep*), were performed using ten specific primer sets combined in three multiplex-PCR assays, according to the protocols described by MEHROTRA et al. (2000) and BANIA et al. (2006).

Antimicrobial susceptibility test. Susceptibility testing using a Vitek 2 system was performed with AST P640 (BioMerieux, 418579, Lyon, France) cards according to the manufacturer's instructions, and susceptibility breakpoints of *S. aureus* were interpreted per CLSI (2017) and EUCAST (2021). The AST-P640 card contains

16 antimicrobial agents (penicillin, cefoxitin, oxacillin, ciprofloxacin, clindamycin, daptomycin, erythromycin, fosfomycin, fusidic acid, gentamicin, linezolid, teicoplanin, tetracycline, tigecycline, trimethoprim-sulfamethoxazole and vancomycin) with different concentrations. MDR was defined as showing resistance to at least three of the antimicrobials used.

Detection of mecA and mecC genes. All *S. aureus* isolates were tested for the presence of *mecA* and *mecC* by PCR assays using the primers and protocols described by MEHROTRA et al. (2000) and CUNY et al. (2011).

The amplicons were resolved by electrophoresis on 1.5% (w/v) agarose gel in 1xTAE buffer. The primer sequences used in the monoplex/multiplex PCRs are described in Table 1. *S. aureus* ATCC 25923 was used as a positive control for targeting *16S rRNA*, *nuc* and *coa* genes. *S. aureus* ATCC 43300 was used as a positive control for MRSA. The *S. aureus* positive strains for enterotoxin genes and the *mecC* gene were kindly supplied by Prof. Ali Aydın from the Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul, Türkiye.

Statistical analysis. All data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to express the mean log, and standard deviation of the *S. aureus* counts.

Results

S. aureus was present in 86 (10.1%) of 850 food samples of animal origin. The distributions of the isolates according to the food types were 49 (10.8%), 17 (17%), 12 (12%) and 8 (4%) from bulk tank milk, Tulum cheese, chicken meat and beef carcasses, respectively. The mean contamination levels of *S. aureus* for bulk tank milk, Tulum cheese, chicken meat and beef carcass samples were 3.01±0.48 log cfu/ml, 3.08±0.42 log cfu/g, 2.89±0.27 log cfu/g and 1.28±0.54 log cfu/cm^2, respectively (Table 2).

39 of 86 *S. aureus* (45.3%) isolates had at least one of the enterotoxin genes (*sea-see, seg-sei, selj, sep*). The SEs genes were detected in 67.3%, 33.3% and 1% of *S. aureus* isolated from bulk tank milk, chicken meat and beef carcass samples, respectively. The bulk tank milk isolates showed higher SE gene incidence when compared with the others. None of the SEs/SEls genes examined were determined in Tulum cheese isolates. 13 distinct genotypes of the toxin genes were observed (Table 3). Classical SE genes (*sea-see*) were found alone or in combination in 11 (28.2%) of the enterotoxigenic isolates: nine from bulk tank milk and two from beef carcasses. Among the classical SE genes, *sed* was the most frequently detected gene (six isolates), followed by *sec* (five isolates), *seb* (four isolates), *sea* (two isolates) and *see* (two isolates). The recently identified SE genes (*seg, seh, sei* and *sep*) were observed in 28 enterotoxigenic isolates, 21 of which carried *sei* alone or in combination with other enterotoxin genes. It was observed that 17 isolates carried *seg* and *sei* together. *selj* was not found in any of the isolates.

Table 2. The prevalance and levels of *S. aureus* in food samples of animal origin

Samples	No. of samples tested	No. (%) of positive samples	Levels of *S. aureus* (log cfu/ml, log cfu/g, log cfu/cm^2)		
			Minimum	Maximum	Mean±SD
Bulk tank milk	450	49 (10.8)	2	4.17	3.01±0.48
Tulum cheese	100	17 (17)	2.3	3.77	3.08±0.42
Chicken meat	100	12 (12)	2.3	3.27	2.89±0.27
Beef carcass	200	8 (4)	0.47	2.23	1.28±0.54

Table 3. Enterotoxin gene distribution among *S. aureus* isolates

Enterotoxin genes	Bulk tank milk (n=33) (%)	Chicken meat (n=4) (%)	Beef carcass (n=2) (%)	Total (n=39) (%)
sea	1 (3)			1 (2.5)
seg	2 (6)			2 (5.1)
seh	12 (36.3)			12 (30.7)
sei		1 (25)		1 (2.5)
seb, sed			2 (100)	2 (5.1)
seg, sei	8 (24.2)	3 (75)		11 (28.2)
sea, seg, sei	1 (3)			1 (2.5)
sec, seh, sei	3 (9.1)			3 (7.6)
seg, seh, sei	2 (6)			2 (5.1)
sed, see, seh, sep	1 (3)			1 (2.5)
seb, sed, seg, sei, sep	1 (3)			1 (2.5)
seb, sec, sed, seg, seh, sei	1 (3)			1 (2.5)
sec, sed, see, seg, seh, sei, sep	1 (3)			1 (2.5)

Table 4. Antimicrobial resistance distribution among *S. aureus* isolates

	No. of *S. aureus* isolates (n=86)									
	Bulk tank milk (49)		Tulum cheese (17)		Chicken meat (12)		Beef carcass (8)		Total	
	n	%	n	%	n	%	n	%	n	%
Penicillin	24	48.9	15	88.3	8	66.7	8	100	55	63.9
Oxacillin	9	18.3	9	47.1	2	16.6	1	12.5	21	24.4
Clindamycin	6	12.2	7	41.1	2	16.6	2	25	17	19.7
Erythromycin	4	8.1	4	23.6	2	16.6	1	12.5	11	12.8
Cefoxitin	5	10.2	2	11.7	2	16.6	0	0	9	10.4
Ciprofloxacin	3	6.1	3	17.6	3	25	0	0	9	10.4
Fusidic Acid	6	12.2	1	5.8	1	8.3	1	12.5	9	10.4
Daptomycin	3	6.1	3	17.6	1	8.3	0	0	7	8.1
Fosfomycin	4	8.1	2	11.7	0	0	0	0	6	6.9
Tetracycline	4	8.1	2	11.7	0	0	0	0	6	6.9
Linezolid	1	2	3	17.6	0	0	0	0	4	4.6
Teicoplanin	1	2	2	11.7	0	0	0	0	3	3.5
Trimethoprim-Sulfamethoxazole	1	2	2	11.7	0	0	0	0	3	3.5
Vancomycin	1	2	2	11.7	0	0	0	0	3	3.5
Gentamicin	1	2	0	0	0	0	0	0	1	1.1
Tigecycline	0	0	0	0	0	0	0	0	0	0

61 of 86 (70.9%) isolates were found to be resistant to one or more antibiotics. The highest resistance was determined to penicillin (63.9%), followed by oxacillin (24.4%), clindamycin (19.7%) and erythromycin (12.8%) (Table 4). No resistance to tigecycline was found. The antibiotic resistance of isolates obtained from bulk tank milk and chicken meat were observed as 53% (n:26) and 88.3% (n:10), respectively. It was determined that all of the Tulum cheese and beef carcass isolates were resistant to at least one antibiotic. The antibiotic resistance profiles of the isolates are shown in Table 5.

Table 5. Antimicrobial resistance profiles of *S. aureus* strains isolated from food samples of animal origin (n=61)

Antimicrobial resistance profile	No (%) isolates	*MAR index	Classification of isolates	
			Type of resistance	No (%) isolates
P, OXSF, OX, CIP, E, CM, LNZ, DAP, TEC, VAN, TE, FSA, STX	1 (1.6)	0.812	Extensive-drug resistant	1 (1.6)
P,OX, CIP, E, CM, LNZ, DAP, TEC, VAN, TE, STX	1 (1.6)	0.687	Multi-drug resistant	20 (32.8)
P, OX, E, CM, LNZ, DAP, TEC, VAN, FOS, FSA	1 (1.6)	0.625		
P, OXSF, OX, GM, E, CM, TE, FOS, FSA	1 (1.6)	0.562		
P, CIP, E, CM, LNZ, DAP, TE, STX	1 (1.6)	0.5		
P, OXSF, OX, CIP, E, LNZ	1 (1.6)	0.375		
P, OX, CM, DAP, VAN, FSA	1 (1.6)	0.375		
P, OX, CM, DAP, FOS, FSA	1 (1.6)	0.375		
P, OX, CM, FOS, FSA	1 (1.6)	0.312		
P, E, CM, FSA	1 (1.6)	0.25		
OXSF, OX, CIP, DAP	1 (1.6)	0.25		
P, OX, CM, FOS	1 (1.6)	0.25		
P, E, TE, FSA	1 (1.6)	0.25		
OX, CIP, E, FOS	1 (1.6)	0.25		
P, OXSF, OX	3 (4.9)	0.187		
P, OX, CM	2 (3.2)	0.187		
E, CM, FSA	1 (1.6)	0.187		
CIP, E, CM	1 (1.6)	0.187		
P, OX	3 (4.9)	0.125	Low-drug resistant	40 (65.6)
OXSF, OX	2 (3.2)	0.125		
P, CM	2 (3.2)	0.125		
P, CIP	1 (1.6)	0.125		
P	31 (50.8)	0.062		
CIP	1 (1.6)	0.062		

MAR Index=number of resistance antibiotics/total number of antibiotics tested (KRUMPERMAN, 1983). The mean multiple antibiotic resistance (MAR) index is 0.301

65.6%, 32.8%, and 1.6% of all isolates were classified as low-drug resistant (LDR), MDR and XDR, respectively. The most MDR isolates were obtained from bulk tank milk and Tulum cheese samples, with 20 isolates. Furthermore, the multiple antimicrobial resistance (MAR) index rate for all isolates was 0.301. 23.3% (14/61) of the resistant isolates showed MAR indexes >0.2.

13 out of 86 *S. aureus* isolates were found to be *mecA* positive. *mecA* was detected in seven (14.2%), three (17.6%), two (16.6%) and one (12.5%) of *S. aureus* isolates recovered from bulk tank milk, Tulum cheese, chicken meat and beef carcass samples, respectively. Nine out of 13 MRSA isolates were also found to be phenotypically resistant to cefoxitin and oxacillin. Eight MRSA isolates showed MDR. Moreover, six MRSA isolates were also found to be enterotoxigenic. Although the presence of *mecC* was investigated in *S. aureus* isolates, no *mecC* was detected.

Discussion

The results of several studies on the prevalence of enterotoxigenic staphylococci and the distribution of enterotoxin genes isolated from food vary from one report to another (BANIA et al., 2006; ARCURI et al., 2010; BIANCHI et al., 2013; KEYVAN and OZDEMIR, 2016). In the current study the prevalence of *S. aureus* in bulk tank milk, Tulum cheese, chicken meat and beef carcasses were 10.8%, 17%, 12% and 4%, respectively. The prevalence of newly described SEs were higher than classical enterotoxins, which was in agreement with other studies (BANIA et al., 2006; ARCURI et al., 2010; AYDIN et al., 2011a). *sea* was found in only 5.1% of isolates, in contrast with previous studies reporting that *sea* was predominant (SALLAM et al., 2015; KEYVAN and OZDEMIR, 2016). All of the *sec* and *seh* were also found in isolates of bulk tank milk. In milk and dairy products, *sec* and *seh* generally coexist, and their expression is suppressed under similar conditions (JORGENSEN et al., 2005). However, no relationship between the *agr*-dependent *sec* and the *agr*-independent *seh* has yet been proven (VALIHRACH et al., 2014). The results of the study were in agreement with the findings of previous studies that *seg* and *sei* coexist together (AYDIN et al., 2011a; BASANISI et al., 2017). A similar situation was also reported for *sed* and *selj* (JORGENSEN et al., 2005). However, our results did not concur with this since although *sed* was the most common classical enterotoxin gene, *selj* was not detected in any of the isolates.

Milk and milk products are often incriminated in SFP. Several studies conducted on cheese samples, including Tulum cheese, showed variable results in the presence of enterotoxin genes (AYDIN et al., 2011a; CAN and ÇELIK, 2012). However, in this study, the isolates obtained from Tulum cheese did not carry any of the enterotoxin genes examined. The reason why no enterotoxin gene was found could be due to the production methodology of İzmir Tulum cheese, in which first dry curing with salt, and then a brine ripening period are used (KARABEY et al., 2018). Expression of enterotoxin genes depends on many factors, such as the phase in which the agent is present (logarithmic phase, stationary phase), the structure of the food, the pH of the environment, the NaCl concentration, and the competitive flora. It has been reported that the expression of enterotoxin genes may be suppressed especially in ripened cheeses (CRETENET et al., 2011).

Penicillin resistance was reported in *S. aureus* isolates from raw milk, cheese, poultry meat and beef carcasses (AYDIN et al., 2011b; CAN et al., 2017; HIZLISOY et al., 2018). The high penicillin resistance observed in this study might be explained by the wide use of penicillin for therapeutic and prophylactic purposes in livestock, the presence of β-lactamase producing strains in *S. aureus* isolates, and plasmid penicillin resistance.

Broad-spectrum tetracyclines are extensively used both in the treatment of bacterial infections in animals and humans, and as growth factors in livestock (AYDIN et al., 2011b). Tetracycline resistance in *S. aureus* isolates in several countries were previously reported to be between 7.9% and 24.7% (PESAVENTO et al., 2007; GOWDA et al., 2017; PAPADOPOULOS et al., 2019), and in Türkiye between 15.6% and 30% (AYDIN et al., 2011b; CAN et al., 2017; HIZLISOY et al., 2018). In this study, it was found to be 6.9%, which is lower than in previous studies. The differences in

the reported antibiotic resistance patterns may be attributed to differences in hygiene levels, the types of samples tested (geographical origin, manufacturing technology, slaughtering process, sample storage, and handling), and/or the sensitivity of the detection methods used (PAPADOPOULOS et al., 2019).

S. aureus has developed MDR worldwide, with a broad prevalence spectrum in different regions (PAPADOPOULOS et al., 2019; MEKHLOUFI et al., 2021). The results of this study show that the most MDR isolates were obtained from bulk tank milk and Tulum cheese samples, indicating a higher incidence of MDR *S. aureus* present on dairy farms. The mean MAR index for all antibiotic resistant *S. aureus* isolates was 0.301. The MAR index is a good risk evaluation mark, and a MAR value of 0.2 indicates high-risk contamination, and overuse or misuse of antimicrobials in the relevant region (KRUMPERMAN, 1983; MAHROS et al., 2021). *S. aureus* isolates with various MAR values were previously determined by several researchers (HACHEMI et al., 2019; KIŠ et al., 2021; MAHROS et al., 2021; URMI et al., 2021).

In the present study, cefoxitin and oxacillin resistance was evaluated for determination of phenotypic methicillin resistance, and the presence of *mecA* and *mecC* was investigated in all isolates according to CLSI (2017). Although nine out of 13 MRSA isolates were found to be phenotypically resistant to cefoxitin and oxacillin, the remaining four isolates did not show any phenotypical resistance to either antibiotic. Phenotypical oxacillin resistance without *mecA* in 12 isolates could be explained by excessive synthesis of β-lactamase (borderline resistance), established biofilm formation, or mutations occurring in the structural components of the PBPs (MCCALLUM et al., 2010). It was considered that four isolates, phenotypically susceptible to cefoxitin and oxacillin, with *mecA*, possessed heterogenic methicillin resistance. *fem* genes located outside of the *mec* region or regulatory resistance genes are considered to be responsible for this situation. The inactivation of these genes or any mutations in these genes may result in some changes to the cell wall structure, therefore alterations may occur in methicillin resistance. This type of resistance may be affected by environmental factors, such as pH, temperature, NaCl concentration etc. and it is seen frequently in strains isolated from the field (MCCALLUM et al., 2010; FOSTER, 2017).

The first foodborne gastrointestinal outbreak caused by MRSA strains was described in 2002 (JONES et al., 2002). However, to date, the actual MRSA involvement in SFP (cases or outbreaks) has not been precisely elucidated, and a general underestimation is suggested. Six MRSA isolates have harbored *sea*, *seb*, *sed*, *seh*, *seg*, and *sei* presenting their potential role as SFP agents. The association of *seh* and *mecA* genes has been previously described (NOTO and ARCHER, 2006). The results were in agreement with previous studies stating that SE genes were detected in MRSA isolated from various food products (TITOUCHE et al., 2020; MEKHLOUFI et al., 2021). The presence of MRSA in foods of animal origin may constitute a risk for consumers, especially for immunocompromised individuals (BASANISI et al., 2017; FOSTER, 2017; TITOUCHE et al., 2020). WHO has listed MRSA as one of the high-priority antimicrobial-resistant pathogens globally (WHO, 2017).

It was concluded that most of the *S. aureus* isolates obtained from food of animal origin possessed enterotoxin genes which might have the possibility of causing foodborne intoxication, and the high antibiotic resistance profiles of these strains, including MRSA, might also cause a public health hazard.

Governments and health authorities should consider MRSA as a high-priority antimicrobial-resistant pathogen, and place it on the list of foodborne pathogens. The improvement of hygienic practices at all stages of the food production chain, on the principle of 'from farm to fork', and the reduction in the pathogen load in foodstuffs of animal origin seem to be the only realistic measures for the prevention of SFP dissemination and antimicrobial-resistant bacteria.

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SAŽETAK

Cilj je istraživanja bio odrediti prevalenciju, enterotoksigena svojstva i rezistenciju na antimikrobne lijekove bakterije *Staphilococcus aureus* izolirane iz 850 uzoraka hrane u Turskoj. Navedeno je uključivalo spremnike za mlijeko, sir Tulum, pileće meso i govođe trupove. Stopa kontaminacije bakterijom *S. aureus* i srednja stopa kontaminacije u 86 pozitivnih uzoraka iznosile su: 10,8% (n:49) i 3,01±0,48 log cfu/mL za spremnike mlijeka, 17% (n:17) i 3,08±0,42 log cfu/g za sir Tulum, 12% (n:12) i 2,89±0,27 log cfu/g za pileće meso te 4% (n:8) i 1.28±0.54 log cfu/cm^2 za govođe trupove. Trideset i devet od 86 izolata (45,3%) imalo je jedan ili više gena za enterotoksin (*sea*-*see*, *seg*-s*elj*, *sep*). Najčešći je gen za klasične, poznate enterotoksine bio *sed*, dok je *sei* bio najčešći gen za nove enterotoksine. Uporabom VITEK 2 uređaja za 16 antibiotika analizirana je osjetljivost na antimikrobne lijekove a rezultati su pokazali da je 61 izolat (70,9%) bio otporan na antibiotike. Ukupno je 32,8% pozitivnih izolata bilo otporno na više lijekova (engl. *multidrug resistant*, MDR), a indeks prosječne višestruke otpornosti na antimikrobne lijekove (MAR) bio je 0,301. Izolati su najčešće bili otporni na penicilin (63,9%), zatim na oksacilin (24,4%), klindamicin (19,7%) i eritromicin (12,7%). Gen *mecA* pronađen je u 13 izolata (15,1%), dok gen *mecC* nije pronađen. Zaključeno je da je većina izolata bakterije *S. aureus* imala gene za enterotoksine koji mogu uzrokovati trovanja hranom. Uz navedeno, visoka stopa otpornosti na antibiotike kod izoliranih sojeva, uključujući MRSA-u, upućuje i na javnozdravstveni rizik.

Ključne riječi: *Staphylococcus aureus*; čimbenici virulencije; otpornost na antibiotike; javno zdravstvo