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Presence of *Staphylococcus aureus* in White Cheese: Determination of Enterotoxin and Antimicrobial Resistance Genes

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ABSTRACT This study aimed to evaluate *Staphylococcus aureus* contamination, enterotoxin presence, and antibiotic resistance in 100 white cheese samples, both packaged and unpackaged. Polymerase Chain Reaction (PCR) analysis showed *S. aureus* in 16 (32%) of the 50 unpackaged samples and 8 (16%) of the 50 packaged samples. 46 *S. aureus* isolates were obtained from 24 *S. aureus* positive samples. Moreover, the *sed* gene, associated with enterotoxin production, was detected in two 46 isolates, one from packaged and one from unpackaged white cheese samples. The isolates exhibited resistance to erythromycin (6.52%), gentamicin (10.87%), chloramphenicol (6.52%), trimethoprim-sulfamethoxazole (2.37%), rifampicin (15.21%), tetracycline (28.26%), cefoxitin (43.47%), and penicillin G (34.78%). Oxacillin and vancomycin resistance among the 46 *S. aureus* isolates was examined using the minimal inhibition concentration (MIC) method. According to the MIC results, none of the isolates were resistant to oxacillin. However, 47.82% of the isolates were resistant to vancomycin. Overall, the isolates exhibited resistance to 9 out of the 10 antibiotics tested. In addition to the resistance profiles, resistance genes were also investigated. It was found that 3 out of the 46 *S. aureus* isolates (13.6%) carried the *mec*A gene, 6 (27.2%) carried the *mec*C gene, and 1 (2.17%) carried both *mec*A and *mec*C genes. In conclusion, detecting *S. aureus* in white cheese indicates poor hygiene. The high antibiotic resistance, presence of resistance genes, and enterotoxin genes in some isolates suggest poses a risk of transmitting resistant microorganisms to humans, potentially complicating disease treatment and causing foodborne illness, posing a public health concern now and in the future.

Keywords: *Antibiotic resistance, Enterotoxin, Public health, Staphylococcus aureus.*

ÖZ

Beyaz Peynirlerde *Staphylococcus aureus* Varlığı: Enterotoksin ve Antimikrobiyal Direnç Genlerinin Belirlenmesi

Bu çalışma hem ambalajlı hem de ambalajsız olmak üzere toplam 100 beyaz peynir örneğinde *Staphylococcus aureus* kontaminasyonu, enterotoksin varlığı ve antibiyotik direncini değerlendirmeyi amaçlamıştır. Polimeraz Zincir Reaksiyonu (PCR) analizi, 50 ambalajsız örneğin 16'sında (%32) ve 50 ambalajlı örneğin 8'inde (%16) *S. aureus* varlığını göstermiştir. 24 *S. aureus* pozitif örnekten 46 izolat elde edilmiştir. Ayrıca, enterotoksin üretimiyle ilişkili *sed* geni, biri ambalajlı biri ambalajsız beyaz peynir örneklerinden elde edilen iki izolatta tespit edilmiştir. İzolatlar eritromisin (%6.52), gentamisin (%10.87), kloramfenikol (%6.52), trimetoprim-sülfametoksazol (%2.37), rifampisin (%15.21), tetrasiklin (%28.26), sefoksitin (%43.47) ve penisilin G'ye (%34.78) direnç göstermiştir. 46 *S. aureus* izolatında oksasilin ve vankomisin direnci, minimal inhibisyon konsantrasyonu (MİK) yöntemi kullanılarak incelenmiştir. MİK sonuçlarına göre, izolatların hiçbirinin oksasiline dirençli olmadığı tespit edilmiştir. Ancak, izolatların %47.82'si vankomisine direnç göstermiştir. Genel olarak, izolatlar test edilen 10 antibiyotiğin 9'una direnç göstermiştir. Direnç profillerine ek olarak, direnç genleri de araştırılmıştır. 46 *S. aureus* izolatının 3'ünün (%13.6) *mec*A genini, 6'sının (%27.2) *mec*C genini ve 1'inin (%2.17) hem *mec*A hem de *mec*C genlerini taşıdığı tespit edilmiştir. Sonuç olarak, beyaz peynirde *S. aureus* tespit edilmesi, hijyen koşullarının yetersiz olduğunu göstermektedir. Yüksek antibiyotik direnci, direnç genlerinin varlığı ve bazı izolatlarda enterotoksin genlerinin bulunması, dirençli mikroorganizmaların insanlara bulaşma riskini işaret etmekte olup, bu durum, hastalık tedavisini zorlaştırabilir ve gıda kaynaklı hastalıklara neden olabilir. Bu da şimdi ve gelecekte halk sağlığı açısından endişe verici bir durum oluşturmaktadır.

Anahtar Kelimeler: *Antibiyotik dirençliliği, Enterotoksin, Halk sağlığı, Staphylococcus aureus.*

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INTRODUCTION

S. aureus, a member of the *Staphylococcaceae* family, is one of the most economically important foodborne pathogens worldwide, causing foodborne poisonings or infections (El-Jakee et al. 2013). The most common cause of food poisoning is the enterotoxins produced by *S. aureus*, which is a public health concern (Balaban and Rasooly 2000).

Staphylococcal enterotoxins (SEs) are globular, heterogeneous proteins produced by enterotoxigenic Staphylococcus species. They are water-soluble, single-chain, proteins weighing between 28000-35000 Da, highly resistant to heat, and cause foodborne intoxications by affecting the digestive and nervous systems (Jay et al. 2005).

Food poisoning caused by staphylococci occurs through the oral ingestion of enterotoxins, which are produced when enterotoxigenic staphylococci are present in foods at concentrations of 10^5 cfu/g or higher. The most important enterotoxigenic staphylococcal species is *S. aureus* (EFSA and ECDC 2019).

SEs are a group of highly heat-resistant toxins, serologically classified into five basic types: SEA, SEB, SEC (SEC1, SEC2, SEC3), SED, and SEE. Recent studies have identified new SE types, including SEG, SEH, SEI, SEJ, SEK SEL, SEM, SEN, SEO, SER, and SEU. However, it remains unclear whether these newly detected enterotoxins play a significant role food poisoning (Thomas et al. 2007).

Classically named enterotoxins SEA, SEB, SEC, and SED are responsible fore more than 90% of staphylococcal food poisoning (SFP) cases. Milk and dairy products can serve as sources of enterotoxic staphylococci (Asao et al. 2003).

In addition to these, newly identified SE-like (Sel) enterotoxin sequences have been discovered. These include SelJ, SelK, SelL, SelM, SelN, SelO, SelP, SelQ, SelU, SelU2, SelV, and SelX. However, the emetic activity of these Sels has not yet been confirmed (Omoe et al. 2005; Thomas et al. 2007).

Since *S. aureus* can rapidly and easily develop resistance to antibiotics through various mechanisms, it poses a significant threat to public health today and in the near future (Jevons et al. 1963).

In determining the genotype of methicillin-resistant *Staphylococcus aureus* (MRSA) strains, the *mec*A gene, which encodes the synthesis of penicillin-binding protein (PBP), is used as a potential biomarker. The *mec*A gene is located on the Staphylococcal Cassette Chromosome *mec* (SCC*mec*), a mobile genetic element. Additionally, this element can be integrated with plasmids and transposons, which encode resistance to various antibiotics. (Sundsfjord et al. 2004).

The mobile genetic element SCC*mec* consists of the *mec* gene complex (including the *mec*A gene and its regulators) and recombinase genes (*ccr*A, *ccr*B, *ccr*C). The *mec*A gene, along with the *mec*I and *mec*R1 genes that regulate it, forms the *mec*A gene complex (*mec*I-*mec*R1-*mec*A) (Sundsfjord et al. 2004).

The phenotypic expression of methicillin resistance varies. Although some *S. aureus* strains do not carry the *mec*A gene, their phenotypic sensitivity to methicillin has been observed. It has been suggested that this sensitivity occurs because *mec*I represses *mec*A transcription, and most ß-lactam antibiotics cannot activate *mec*R1 (Lee 2006).

S. aureus isolates lacking the mecA gene but testing positive for methicillin sensitivity with oxacillin and cefoxitin assay may still exhibit phenotypic resistance. Possible explanations include the presence of the *mec*C gene, a *mec*A homolog, and other factors contributing to β-lactam resistance. These factors include excessive production of β-lactamase and mutations in penicillin-binding proteins (PBP) (Petersen et al. 2013).

*The mec*C gene was detected in *S. aureus* isolates from animal and human infection in 2011. Isolates carrying the *mec*C gene, a homolog of *mec*A, are rarely detected and have primarily been reported in European countries. Susceptibility testing studies have shown that MRSA strains with the *mec*C gene are typically resistant to cefoxitin but susceptible to oxacillin. Therefore, cefoxitin is considered an important test for detecting MRSA isolates carrying the *mec*C gene (Palavecino 2020).

MRSA strains often exhibit resistant to wide range of commonly used antimicrobial agents, such as aminoglycosides, macrolides, chloramphenicol, tetracycline, and fluoroquinolones (Lee 2003).

The aim of this study was to determine the presence of *S. aureus* and enterotoxins that pose a risk to food hygiene and public health in white cheeses consumed in the Van province market, as well as and assess the antimicrobial resistance of these isolates.

MATERIAL AND METHODS

Approval was obtained from the Van Yuzuncu Yil University (Türkiye) Animal Researches Local Ethic Committee with the number No: 2021/07-01 dated 29.07.2021.

Reference strains

S. aureus ATCC 25923 was used as a positive control in this study. The *S. aureus* positive strains for sea, seb, sec and sed enterotoxin genes obtained from the culture collection of Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Van Yuzuncu Yil University.

Sample Collection

A total of 100 white cheese samples (50 packaged and 50 unpackaged) were used as the study material from Van province in February and March 2021. The samples were obtained from the sales points under aseptic conditions, placed in sterile sample containers with a minimum weight of 200 grams, transported to the laboratory at +4 °C, and analyzed immediately.

Isolation and identification of *S. aureus*

The isolation of *S. aureus* isolats from raw milk was performed according to TS EN ISO 6888-1 and TS 6582-2 EN ISO 6888-2. Typical and atypical colonies on Baird-Parker Agar (Oxoid CM275, UK) containing Egg yolk tellurite (Oxoid SR 0054, UK) were subcultured and identified by Gram staining, catalase test, coagulase test, DNase activity and mannitol fermentation (TS, 2021). The isolates that were identified to be *S. aureus* were confirmed using PCR.

A commercial kit (Hibrigen, MG-BGDNA-01, Türkiye) and master mix (A.B.T™ 2X PCR Mastermix, Türkiye) were used for DNA extraction and PCR amplification from *S. aureus* colonies isolated from white cheese samples. The specific primer pair (5'-GGACGACATATTAGACGAATCA-3'; 5'-CGGGCACCTATTTTCTATCT-3', 1318 bp), described by Riffon et al. (2001), was used for the PCR confirmation of the *S. aureus* isolates. For the preparation of the PCR

mixture, 10 µL mastermix, 1.5 µL (10 µM) of each primer, and 5 µL genomic Deoxyribose Nucleic Acid (DNA) were added and the total volume was completed to 25 µL using PCR water. After keeping the mixture at 94 °C for 5 min for pre-denaturation, a 35 cycles amplification procedure was employed comprising denaturation at 94 °C for 60 s, annealing at 51 °C for 60 s, extension at 72 °C for 60 s, and final extension at 72 °C for 10 min. The gel electrophoresis of the amplicons was carried out using 1.5% agarose gel (Bioshop, Canada) in a horizontal tank (Thermo Scientific, OwlR EasyCastTM B1, USA) at 70 volts electric current (Hoefer Scientific PS 500X power, United States) for 90 min.

Identification of staphylococcal enterotoxin genes

Specific primer pairs developed by the researchers, as listed in Table 1, were used to determine enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sej*) in *S. aureus* isolates. To prepare the PCR mixture, 10 µL mastermix (A.B.T™ 2X PCR Mastermix, Türkiye), 1.5 µL of each primer (10 µM) and 5 µL genomic DNA were added and the total volume was completed to 25 µL with PCR water. The PCR protocol applied in the *analyses* is presented in Table 2. Then, agarose gel electrophoresis was performed and amplicons that gave bands in appropriate places were detected as positive.

Table 1: Oligonucleotide sequences used in the PCR.

	Gene	Primer Sequences	Amplicon (bp)	Reference
Oxacillin	mecA-1	5'-CCTAGTAAAGCTCCGGAA-3'	314	Çiftçi et al. 2009
	mecA-2	5'-CTAGTCCATTCGGTCCA-3'		
	mecC-1	5'-TGTTGTAGCAATGTTCACAC-3'	138	Monecke et al. 2013
	mecC-2	5'-CAAGCACTTAATATCAACGC-3'		
Glycopeptides	vanA vanA	5'-ATGAATAGAATAAAAGTTGC-3'	1032	Saha et al. 2008
		5'-TCACCCCTTTAACGCTAATA-3'		
Enterotoxin Genes	sea-1	5'-ATTAACCGAAGGTTCTGTAGA-3'	582	Shanehbandi et al. 2014
	sea-2	5'-TTGCGTAAAAAGTCTGAATT -3'		
	seb-1	5'-CCTAAACCAGATGAGTTGCAC-3'	592	Wongboot et al. 2013
	seb-2	5'-CAGGCATCATGTCATACCAAA-3'		
	sec-1	5'-AGATGAAGTAGTTGATGTGTATGG-3'	454	Wongboot et al. 2013
	sec-2	5'-CTTCACACTTTTAGAATCAACCG-3'		
	sed-1	5'-GCTTGTACATATGGAGGTGTCA-3'	263	Wongboot et al. 2013
	sed-2	5'-GACCCATCAGAAGAATCAAACT-3'		
	see-1	5'-AGGTTTTTTCACAGGTCATCC -3'	209	Mehrotra et al. 2000
	see-2	5'-CTTTTTTTTCTTCGGTCAATC-3'		
	seg-1	5'-AATTATGTGAATGCTCAACCCGATC -3'	642	Jarraud et al. 1999
	seg-2	5'AAACTTATATGGAACAAAAGGTACTAGTTC-3'		
	seh-1	5'-CACATCATATGCGAAAGCAGA-3'	567	Srinivasan et al. 2006
	seh-2	5'-CCTTTTAAATCATAAATGTCGAATGA-3'		
	sej-1	5'-CATCAGAACTGTTGTTCCGCTAG-3'	142	Monday et al. 1999
	sej-2	5'-CTGAATTTTACCATCAAAGGTAC-3'		

Determination of antibiotic resistance

The antibiotic resistance of the isolates was determined based on the Kirby-Bauer disk diffusion method recommended by EUCAST (European Committee on Antimicrobial Susceptibility Testing). For this purpose, erythromycin (E, 15 µg) , gentamicin (G, 10 µg), chloramphenicol (C, 30 µg), rifampicin (RD, 5 µg), trimethoprim-sulfamethoxazole (SXT, 1.25-23.75 µg), tetracycline (TE, 30 µg), cefoxitin (CTX, 30 µg), and penicillin G (P, 1 U) antibiotic resistance of the isolates obtained were determined by disk diffusion method and methicillin and vancomycin resistance were determined by MIC method. Oxacillin resistance was interpreted according to the CLSI standards. For this purpose, oxacillin was used to evaluate methicillin resistance in line with the recommendation of EUCAST (CLSI 2020; EUCAST 2021).

Genotypic characterization of antibiotic resistance

The primer pairs and their properties used in the determination of methicillin (*mec*A and *mec*C) and vancomycin (*van*A) resistance genes in *S. aureus* isolates confirmed by PCR method are given in Table 1.

For the PCR mix of each gene, 10 µL mastermix (A.B.T™ 2X PCR Mastermix, Türkiye), 1.5 µL of each primer (10 µM) and 5 µL genomic DNA were added and the total volume was completed to 25 µL with PCR water. The PCR protocol applied in the analyses is presented in Table 2. The amplicons obtained from the PCR process were subjected to electrophoresis for 90 minutes, as previously described.

Statistical analysis

Statistical analysis was performed using Pearson chi-square and independent sample T test. For statistical significance, the error level was chosen as $p<0.05$ (SPSS, 2006).

Table 2: PCR protocol applied for antibiotic resistance and enterotoxin gene presence.

	Gene	Pre-Denaturation (°C/min)	Amplification (35 Cycles) (denaturation/binding/extension)	Final Extension (°C/min)
Methicillin	mecA	95 °C/10 min	95 °C, 45 s/48 °C, 45 s/72 °C, 45 s	72 °C/5 min
	mecC	95 °C/10 min	95 °C, 45 s/48 °C, 45 s/72 °C, 45 s	72 °C/5 min
Vancomycin	VanA	94 °C/10 min	94 °C, 60 s/46 °C, 60 s/ 72 °C, 60 s	72 °C/10 min
Enterotoxin	sea-1	94 °C/10 min	94 °C, 60 s/56 °C, 60 s/ 72 °C, 60 s	72 °C/10 min
	sea-2	94 °C/10 min		
	seb-1	94 °C/10 min	94 °C, 60 s/51 °C, 60 s/ 72 °C, 60 s	72 °C/10 min
	seb-2	94 °C/10 min		
	sec-1	94 °C/10 min	94 °C, 60 s/52 °C, 60 s/ 72 °C, 60 s	72 °C/10 min
	sec-2	94 °C/10 min		
	sed-1	94 °C/10 min	94 °C, 60 s/52 °C, 60 s/ 72 °C, 60 s	72 °C/10 min
	sed-2	94 °C/10 min		
	see-1	94 °C/10 min	94 °C, 60 s/48.5 °C, 60 s/ 72 °C, 60 s	72 °C/10 min
	see-2	94 °C/10 min		
	seg-1	94 °C/10 min	94 °C, 60 s/55 °C, 60 s/ 72 °C, 60 s	72 °C/10 min
	seg-2	94 °C/10 min		
	seh-1	94 °C/10 min	94 °C, 60 s/50 °C, 60 s/ 72 °C, 60 s	72 °C/10 min
	seh-2	94 °C/10 min		
	sej-1	94 °C/10 min	94 °C, 60 s/ 53 °C, 60 s/ 72 °C, 60 s	72 °C/10 min
	sej-2	94 °C/10 min		

Table 3: Levels of *S. aureus* and presence of enterotoxin genes in cheese samples.

Sample No	Packaged/Unpackaged	Presence of Enterotoxin Gene	Log cfu/gr	n	Min	Max	Mean	Std. Error
1		-	2.66					
11		-	2.08					
26		-	3.20					
38	Packaged	*sed*	4.67	8	1.30	4.67	**2.68**	**0.38**
37		-	1.60					
36		-	1.30					
33		-	3.17					
87		-	2.78					
17		-	1.60					
7		-	3.11					
12		-	4.59					
97		-	2.38					
20		-	3.33					
10		-	4.77					
13		-	4.74					
24	Unpackaged	-	3.96	16	1.30	4.95	**2.85**	**0.34**
14		-	1.30					
16		*Sed*	1.30					
93		-	2.30					
92		-	2.30					
100		-	4.95					
81		-	1.60					
84		-	2.08					
74		-	1.30					
			Total	24	1.30	4.95	**2.79**	**0.26**

RESULTS

Isolation of *S. aureus*

A total of 100 white cheese samples (50 packaged and 50 unpackaged (sold in the open) were analyzed in Van province between February and March 2021. Of these samples, 24 (24%) were positive for *S. aureus*, including 8 (16%) packaged samples and 16 (32%) unpackaged samples. The average *S. aureus* count in the white cheese samples was determined to be 2.79 ± 0.26 log cfu/g (Table 3).

As a result of the analysis, although there was a difference between packaged and unpackaged white cheese samples, no statistically significant relationship was found between the type of product and the bacterial counts ($p<0.05$).

Enterotoxin genes in S. aureus isolates

A total of 46 S. aureus isolates obtained from 24 white cheese samples were examined for the presence of the sea, seb, sec, sed, see, seg, she, and sej enterotoxin genes using the PCR method. The sed gene was detected in two of 46 isolates; one of them is in the packaged White cheese sample and the other is in the unpackaged sample. (Table 3).

Antibiotic susceptibility testing

In this study, the antibiotic resistance of 46 S. aureus isolates obtained from white cheese samples was analyzed using the disk diffusion antibiogram test. The results are presented in Table 4.

Table 4: Antibiotic resistance of *S. aureus* isolates obtained from white cheese samples.

		Antibiotics										Resistance Genes	
		E	**CN**	**C**	**SXT**	**RA**	**TE**	**CTX**	**P**	**OX**	**V**	**mecA**	**mecC**
ISOLATES	**Isolate 1**	S	S	S	S	S	S	R	S	S	R	-	+
	Isolate 2	S	S	S	S	S	S	R	S	S	R	-	-
	Isolate 3	R	R	R	R	R	R	R	R	S	S	-	-
	Isolate 4	S	S	S	S	S	S	R	R	S	S	-	-
	Isolate 5	R	R	R	S	R	R	R	R	S	R	+	-
	Isolate 6	S	S	S	S	S	S	S	S	S	S	-	-
	Isolate 7	S	S	S	S	S	S	S	S	S	R	-	-
	Isolate 8	S	S	S	S	S	S	S	S	S	S	-	-
	Isolate 9	S	S	S	S	S	S	S	S	S	R	-	-
	Isolate 10	S	S	S	S	S	S	S	S	S	R	-	-
	Isolate 11	S	S	S	S	S	S	R	R	S	R	-	+
	Isolate 12	S	S	S	S	R	R	R	R	S	R	-	-
	Isolate 13	S	R	S	S	R	S	R	R	S	S	-	-
	Isolate 14	S	S	S	S	S	S	S	S	S	R	-	-
	Isolate 15	S	S	S	S	R	S	R	R	S	R	-	-
	Isolate 16	S	S	S	S	S	R	R	R	S	R	-	-
	Isolate 17	S	S	S	S	S	R	R	R	S	R	-	+
	Isolate 18	S	S	S	S	S	S	S	S	S	S	-	-
	Isolate 19	S	S	S	S	S	S	S	S	S	R	-	-
	Isolate 20	S	S	S	S	S	S	S	S	S	R	-	-
	Isolate 21	S	S	S	S	S	S	R	S	S	S	-	+
	Isolate 22	S	S	S	S	S	S	R	S	S	S	-	-
	Isolate 23	S	S	S	S	S	R	R	S	S	S	+	-
	Isolate 24	S	S	S	S	S	S	S	S	S	S	-	-
	Isolate 25	S	S	S	S	S	S	S	S	S	S	-	-
	Isolate 26	S	S	S	S	S	R	R	S	S	S	+	+
	Isolate 27	S	S	S	S	S	R	R	S	S	S	-	+
	Isolate 28	S	S	S	S	S	S	S	S	S	S	-	-
	Isolate 29	R	R	R	S	R	R	R	R	S	S	-	-
	Isolate 30	S	S	S	S	S	S	S	S	S	S	-	-
	Isolate 31	S	S	S	S	S	S	S	S	S	R	-	-
	Isolate 32	S	S	S	S	S	S	S	S	S	R	-	-
	Isolate 33	S	S	S	S	S	S	S	S	S	S	+	-
	Isolate 34	S	S	S	S	S	S	S	S	S	S	-	-
	Isolate 35	S	S	S	S	S	R	S	R	S	S	-	-
	Isolate 36	S	S	S	S	S	S	S	R	S	R	-	-
	Isolate 37	S	S	S	S	S	R	S	R	S	S	-	-
	Isolate 38	S	S	S	S	S	S	S	R	S	R	-	-
	Isolate 39	S	S	S	S	S	S	R	S	S	S	-	-
	Isolate 40	S	S	S	S	S	R	S	S	S	R	-	-
	Isolate 41	S	S	S	S	S	S	R	S	S	S	-	+
	Isolate 42	S	S	S	S	S	S	S	R	S	R	-	-
	Isolate 43	S	R	S	S	R	R	R	R	S	R	-	-
	Isolate 44	S	S	S	S	S	S	S	S	S	S	-	-
	Isolate 45	S	S	S	S	S	S	S	S	S	R	-	-
	Isolate 46	S	S	S	S	S	S	S	S	S	S	-	-

R: Resistance, S: Susceptible, E: Erythromycin, CN: Gentamicin, C: Chloramphenicol, SXT: Trimethoprim-sulfamethoxazole, RA: Rifampicin, TE: Tetracycline, CTX: Cefoxitin, P: Penicillin G, OX: Oxacillin, V: Vancomycin.

According to EUCAST (2021), of the 46 isolates, 3 (6.52%) were resistant to erythromycin, 5 (10.87%) to gentamicin, 3 (6.52%) to chloramphenicol, 1 (2.17%) to trimethoprim-sulfamethoxazole, 7 (15. 21%) to rifampicin, 13 (28.26%) to tetracycline, 20 (43.47%) to cefoxitin and 16 (34.78%) to penicillin G. 16 of the isolates (34.78%) were found to be resistant to at least two antibiotics. Of the isolates, 11 (23.91%) showed no resistance to any of the 10 antibiotics, 13 (28.26%) were resistant to 1 antibiotic, 12 (26.08%) to 2 antibiotics, 1 (2.17%) to 3 antibiotics, 4 (8. 69%) to 4 antibiotics, 1 (2.17%) to 5 antibiotics, 1 (2.17%) to 6 antibiotics, 1 (2.17%) to 7 antibiotics, and 2 (4.34%) to 8 antibiotics (Table 5).

Table 5: Resistance and susceptible levels of *S. aureus* isolates isolated from cheese samples to antibiotics.

Antibiotics	Resistant n (%)	Susceptible n (%)
Erythromycin	3 (6.52)	43 (93.48)
Gentamicin	5(10.87)	41 (89.13)
Chloramphenicol	3 (6.52)	43 (93.48)
Trimethoprim-sulfamethoxazole	1 (2.17)	45 (97.83)
Rifampicin	7 (15.21)	39 (84.79)
Tetracycline	13 (28.26)	33 (71.74)
Cefoxitin	20 (43.47)	26 (56.53)
Penicillin G	16 (34.78)	30 (65.22)
Oxacillin	0 (0)	46 (100)
Vancomycin	22 (47.82)	24 (52.18)

n: number of positive isolates.

Antibiotic resistance genes in S. aureus isolates

22 (47.83%) isolates were phenotypically determined to be resistant to vancomycin, although none were found to carry the vanA gene genotypically. Additionally, among all strains that were not phenotypically resistant to oxacillin, 3 (6.52%) carried the mecA gene, 6 (13.04%) carried the mecC gene, and 1 (2.17%) carried both the mecA and mecC genes (Figure 1).

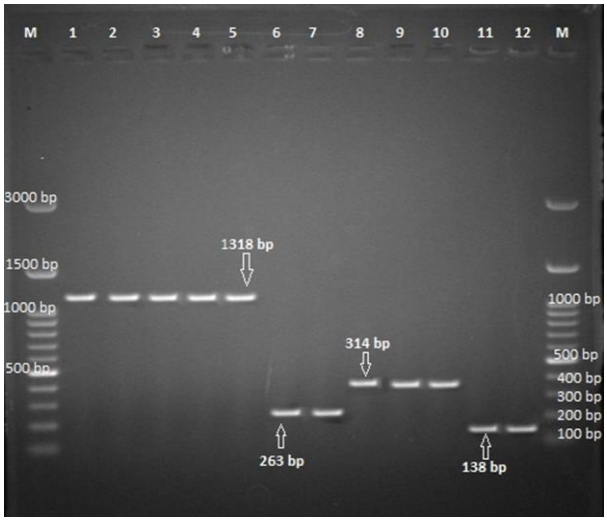

Figure 1: M: Marker (100 bp); 1-5: S. aureus isolates (1318 bp); 6-7: Enterotoxin gene sed (263 bp); 8-9: Methicillin resistance gene (mecA) (314 bp); 11-12: Methicillin resistance gene (mecC) (138 bp).

DISCUSSION AND CONCLUSION

S. aureus is capable of causing toxin-mediated diseases such as food poisoning, toxic shock syndrome, and scalded skin syndrome (Omoe et al. 2005). Additionally, it is a major pathogen responsible for various infections in the community, including pneumonia, postoperative wound infections, endocarditis, and bacteremia, as well as being a leading cause of hospital-acquired infections (Lindsay and Holden 2004). Additionally, *S. aureus i*s the causative agent of staphylococcal food poisoning (SFP), one of the most common foodborne illnesses (Hennekinne et al. 2012). The most frequent cause of poisoning is due to enterotoxins produced by *S. aureus* (Balaban and Rasooly 2000). The majority of food poisoning outbreaks are caused by staphylococcal enterotoxin type A (SEA) (Balaban and Rasooly 2000). Foodborne illnesses caused by *S. aureus* are considered one of the most economically significant foodborne problems worldwide (El-Jakee et al. 2013).

There are variations between our study and the results of other studies (Kocak 2014; Godek et al. 2021).

According to the analysis results of this study, the number of *S. aureus* in white cheeses ranged from 1.30-4.95 log cfu/g, with an average of 2.79±0.26 log cfu/g. This value was lower than those reported by Bostan et al. (2006) and Kocak (2014), but higher than those reported by Sağun et al. (2001) and Godek et al. (2021). In the study, *S. aureus* was detected in 24% of the samples, which was higher than the rates found by Yücel and Anıl (2011), Gücükoğlu et al. (2012), and Kayılı (2020), and lower than those reported by Alghizzi and Shami (2021), and Güngören et al. (2022). The varying presence of *S. aureus* in different numbers and proportions among the samples is believed to be due to factors such as the microbial quality of the milk used for cheese production, whether the milk was pasteurized or not, different practices during the cheese production stages and duration, whether the cheese is ripened or not, adequacy of sanitation, hygiene of personnel at production and sales points, and cleanliness of the equipment and tools used, as well as the number of samples examined.

In the study, it was determined that 10% of the cheese samples exceeded the limits (10^2-10^3 cfu/gr) specified in the Turkish Food Codex Microbiological Criteria Regulation (2011), with 6% of those being packaged cheeses and 14% being unpackaged cheeses (Table 3).

The most important species of staphylococci with enterotoxigenic properties is *S. aureus*. Food poisoning occurs as a result of oral ingestion of enterotoxin, which are exotoxins synthesized when the bacterial count reaches 10^5 cfu/g or higher in foods (EFSA and ECDC 2019).

In our study, it was observed that a total of 4 (4%) cheese samples, including 1 packaged and 3 unpackaged, approached the enterotoxin formation limit (5 log cfu/g) (Table 3). It is suggested that if proper storage, preservation and hygiene conditions are not maintained for these cheeses, enterotoxin may form, posing a risk to public health.

In our study, the s*ea, seb, sec, sed, see, seg, she,* and *sej* enterotoxin genes were searched in the isolates. The *sed* gene was detected in 2% (2 isolates), one from a packaged and one from an unpackaged white cheese sample. Since SED enterotoxin may be produced in isolates carrying this gene under suitable conditions and in sufficient quantities, its could pose a health risk. Our findings regarding the *sed* gene are consistent with the results of study by Ertas et al. (2010) and Gücükoğlu et al. (2012).

In the study, it was found that 3 of the isolates examined (6.52%) were resistant to erythromycin. This resistance rate was lower than those reported by Bendahou et al. (2008) (10%), Zayda et al. (2020) (12%), Kayılı (2020) (22%), Cai et al. (2021) (27.4%), Prabakusuma et al. (2022) (34.78%), Güngören et al. (2022) (40.4%), and Kızanlık and Göksoy (2024) (23.6%). However, it was higher than those reported by Spanu et al. (2010) (no resistance), Yücel and Anıl (2011) (3.7%), and Titouche et al. (2019) (2.9%).

In this study, 5 of the *S. aureus* isolates (10.87%) were resistant to gentamicin. The resistance rate was lower than those reported by Zayda et al. (2020) (36%) and Kayılı (2020) (32%). However, it was higher than those reported by Bendahou et al. (2008) (5%), Yücel and Anıl (2011) (5%), Titouche et al. (2019) (no resistance), Prabakusuma et al. (2022) (no resistance), and Kızanlık and Göksoy (2024) (no resistance).

In the study, 3 of the isolates obtained from cheese samples (6.52%) were found to be resistant to chloramphenicol, a rate similar to that reported by Bendahou et al. (2008) (5%). However, it was higher than the rate found in the studies by Titouche et al. (2019) (no resistance) and Kayılı (2020).

In the study, 1 of the *S. aureus* isolates (2.17%) was found to be resistant to trimethoprim-sulfamethoxazole. This rate was higher than those reported by Bendahou et al. (2008) (no resistance), Yücel and Anıl (2011) (1.2%), and Titouche et al. (2019) (no resistance). However, it was lower than the rates reported by Cai et al. (2021) (3.2%), Prabakusuma et al. (2022) (34.78%) and Kızanlık and Göksoy (2024) (11.7%).

7 of the *S. aureus* isolates (15.21%) resistant to rifampicin. This rate was lower than those reported by Kayılı (2020)

(21%) and Cai et al. (2021) (16.1%), but higher than the rate reported by Bendahou et al. (2008).

In the study, 13 of the *S. aureus* isolates (28.26%) were found to be resistant to tetracycline. This rate was higher than those reported by Bendahou et al. (2008) (25%), Spanu et al. (2010) (11.1%), Yücel and Anıl (2011) (11.3%), Kayılı (2020) (6%), Prabakusuma et al. (2022) (21.74%), Cai et al. (2021) (22.6%), and Kızanlık and Göksoy (2024) (11.7%), but lower than the rate reported by Titouche et al. (2019) (47.8%).

In the study, 20 of the *S. aureus* isolates (43.47%) were found to be resistant to cefoxitin. This rate was higher than those reported by Özpınar (2011) (13.11%), Titouche et al. (2019) (15.9%), and Prabakusuma et al. (2022) (21.74%), but lower than the rate reported by Zayda et al. (2020) (64%).

In the study, 16 of the *S. aureus* isolates (34.78%) were found to be resistant to penicillin G. This rate was lower than those reported by Titouche et al. (2019) (91.3%), Kayılı (2020) (85%), and Kızanlık and Göksoy (2024) (88.3%).

In the study, all of the examined *S. aureus* isolates (100%) were susceptible to oxacillin. This result aligns with the findings of Spanu et al. (2010), where no resistant isolates were reported. However, it is higher than the resistance rates reported by Bendahou et al. (2008) (15%), Özpınar (2011) (14.75%), Titouche et al. (2019) (15.9%), Zayda et al. (2020) (60%), Kayılı (2020) (64%), Cai et al. (2021) (11.3%), and Prabakusuma et al. (2022) (21.74%).

It was determined that 22 isolates (47.82%) were resistant to vancomycin in this study. This rate was higher than that reported by Bendahou et al. (2008) (no resistance), Spanu et al. (2010) (no resistance), Özpınar (2011) (no resistance), Kayılı (2020) (19%), Zayda et al. (2020) (no resistance), Cai et al. (2021) (1.6%), Güngören et al. (2022) (no resistance), Prabakusuma et al. (2022) (no resistance), and Kızanlık and Göksoy (2024) (11.7%).

In the study, 16 isolates (34.78%) were resistant to two or more antibiotics. This value was lower than the rates reported by Normanno et al. (2007) (68.8%) and Kayılı (2020) (75.29%) for multidrug resistance. However, it was higher than the rate determined by Kızanlık and Göksoy (2024) (32.8%).

It is postulated that the discrepancies between the antibiotic sensitivity and resistance values obtained in the present study and those reported in other studies may be attributed to serotype variations of the S. aureus isolates, differences in geographical location, the disease history of the animals from which the milk used in cheese making was obtained, and the uncontrolled and inappropriate use of antibiotics.

In this study, the *mec*A gene was identified in 3 out of 46 isolates (6.52%) (Table 3). Some studies have reported the inability to detect the *mec*A gene in *S. aureus* isolates (Bulajic et al. 2017), while others (Giacinti et al. 2017) reported a lower detection rate, and in some studies (Özpınar 2011; Obaidat et al. 2018), the detection rate was higher.

This study determined that 6 of the isolates (13.04%) carried the *mec*C gene (Table 3). Some researchers (Bulajic et al. 2017; Obaidat et al. 2018; Alghizzi and Shami (2021) reported that they were unable to detect the *mec*C gene in *S. aureus* isolates, while others (Giacinti et al. 2017; Taban et al. 2021) reported a lower detection rate of the *mec*C gene compared to the rate found in our study.

In our study, both the *mec*A and *mec*C genes were detected in one isolate (2.17%). This rate is similar to the findings of Taban et al. (2021).

It was concluded that the differences between the MRSA values and the number of resistance genes detected in this study and those in other studies may be attributed to serotype variations of the *S. aureus* isolates, the disease history, nutrition, growing region, environmental factors of the sample sources, and the uncontrolled or inappropriate use of antibiotics at incorrect dosages.

Vancomycin is considered the standard treatment for serious Methicillin resistant *S. aureus* (MRSA) infections. As a key antibiotic for treating MRSA infections, the emergence of vancomycin-resistant *S. aureus* (VRSA) raises serious concerns for the treatment of patients infected with multiantibiotic-resistant *S. aureus* (Spanu et al. 2014).

Although 43.83% of the isolates in the study were phenotypically resistant to vancomycin, none of the isolates carried the *van*A gene. The results obtained from Özpınar's (2011) study are consistent with our findings. Additionally, Cai et al. (2021) reported that although they detected the *van*A gene in their isolates, these isolates were not phenotypically resistant to vancomycin.

The absence of resistance genes for the same antibiotic in isolates with phenotypic antibiotic resistance suggests that different resistance mechanisms (such as integrons, plasmids and transposons) may be responsible for resistance to ampicillin, tetracycline and sulfamethoxazole. In the study, *mec*A and *mec*C genes were detected in isolates that were phenotypically sensitive to oxacillin. *S. aureus* exhibits both homogeneous and heterogeneous resistance to methicillin. Homogeneous resistance occurs when the *mec*A gene is active in *S. aureus* strains that possess it, resulting in phenotypic resistance in antibiotic tests. In contrast, heterogeneous resistant *S. aureus* strains that do not express the *mec*A gene may be detected as sensitive in antibiotic tests, despite being inherently resistant (Çiftçi et al. 2009). Considering this situation, we believe that when applying antibiotics to livestock, it is important to check not only phenotypic analysis but also the presence of resistance genes.

As a result, it is crucial to pay attention to udder health and milking hygiene in milk-producing animals, ensure the use of pasteurized milk, and adhere to hygiene standards in cheese production along with personnel hygiene training. Additionally, it is essential to prevent the proliferation and toxin formation of staphylococci through appropriate processes in food technology, control the antibiotic content of the milk intended for cheese processing, raise awareness to prevent the development of antibiotic resistance, and effectively implement regular antibiotic residue control programs and food safety systems.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: RMT, SNAA
Supervision / Consultancy: RMT
Data Collection and / or Processing: RMT, SNAA
Analysis and / or Interpretation: RMT, SNAA
Writing the Article: RMT, SNAA
Critical Review: RMT

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