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Determining the enterotoxin genes and methicillin resistance in *Staphylococcus aureus* isolated from goat milk and its products

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ABSTRACT

Milk and its products can be frequently contaminated with enterotoxigenic and methicillin-resistant *S. aureus*, and in such a case, it causes various diseases, especially staphylococcal food poisoning. In the present study, 100 sample materials (50 goat milk, 25 goat cream, and 25 goat cheese) were collected from 65 livestock farms in Erzurum. All samples were analyzed and tested selectively according to the EN ISO 6888-1 procedure standard. The obtained isolates were examined with the PCR in terms of nuc, Panton-Valentine Leukocidin (PVL), mecA, and enterotoxin genes. *S. aureus* was detected in 4 of 50 (8%) milk samples, 2 of 25 (8%) cream samples, 3 of 25 (12%) cheese samples, and 9 of the total samples (9%). While the incidence of methicillin resistance was found to be 3% by the disc diffusion method, the incidence of the mecA gene was found to be 2% by PCR. In terms of enterotoxin genes, 8% (4/50) of milk samples, 12% (3/25) of cheese samples, and 8% (2/25) of cream samples contained at least one enterotoxin gene. In total, 9% of 100 samples contained enterotoxigenic *S. aureus*. In conclusion, the consumption of enterotoxigenic and mecA positive *S. aureus*-containing raw milk, dairy products that have not been adequately heat-treated, or milk and products that are not kept in proper storage conditions pose a danger to public health.

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1. Introduction

Microorganisms have been among us for centuries because they can adapt to changing environment. The foods consumed by us contain microbial loads whose composition varies greatly. Microorganisms, which usually originate from the surrounding flora, can also originate from stages such as animal slaughter and harvesting, processing, storage, and distribution of food [1]. *Staphylococcus aureus* belongs to the *Staphylococcaceae* family and is Gram-positive, facultatively anaerobic, cocci-shaped, immobile, and non-sporing [2]. *S. aureus* is a commensal, pathogenic bacterium and is colonized by almost 30% of people [3]. It causes food poisoning as well as bacteremia, endocarditis, skin, soft tissue, and pleuropulmonary infections [4].

S. aureus is especially detected in foods that contain protein such as ham, processed or unprocessed meat, puddings, tuna, chicken, cream fillings, meat salads, milk, and dairy products (primarily those that are not pasteurized) [5]. *S. aureus* food poisoning from milk and its products constitute 26% of all *S. aureus* food poisonings. Bacteria can be transmitted to milk directly from the mammary glands of subclinically or clinically infected animals or environmentally during the processing of milk and its products [6].

Goat milk and products have an essential place in the treatment of people suffering from cow's milk allergies and gastrointestinal disorders, which are common in many countries, in meeting the gastronomic needs of consumers and in the nutrition of people who are malnourished from cow's milk in the developing world [7].

S. aureus has virulence factors causing many infections in humans and animals. These factors cause bacteria to adhere to the cell, collapse the immune mechanism, cause tissue invasion, sepsis, and toxic syndromes [8]. *S. aureus* produces staphylococcal enterotoxins (SEs) (A-E, G-I), which are potent gastrointestinal exotoxins. Food poisoning is seen as a result of the consumption of foods containing these toxins in sufficient amounts [9]. One of the characteristics of SEs is their resistance to temperature (121^{o}C for 10 minutes)[10].
A total of 25 (A-V and X, three subtypes of C) types of SEs have been defined in a recent classification [11]. SEA is the

most common staphylococcal food poisoning worldwide, followed by SED and SEB [9].

The average methicillin-resistant *S. aureus* (MRSA) rate is between 60-70% worldwide. The Centers for Disease Control and Prevention in the USA reported that an average of 100.000 MRSA-related infections occur each year and result in around 19.000 deaths, which is higher than HIV death rates [12]. Unlike the penicillinase gene, mecA, a methicillin resistance gene, is found in the *S. aureus* chromosome. The detection of the mecA gene plays an essential role in determining resistance to β-lactam antibiotics [13].

According to the source of the infection, *S. aureus*, which causes various human infections, is divided into two community-acquired and hospital-acquired. These two types of infections are differentiated from each other by the genetic structure of the bacteria, antibiotic susceptibility, and clinical findings [14]. Previous studies showed that MRSA is associated with human consumption, whether it is Livestock-Associated (LA-MRSA), Community-Associated (CA-MRSA), or Hospital-Associated (HA-MRSA) [15].

This study aimed to investigate nuc, PVL, mecA, and enterotoxin genes in isolates after isolating *S. aureus* in raw goat milk and dairy products by the classical method. Thus, information was obtained about the prevalence of methicillin-resistant *S. aureus* and the types of staphylococcal enterotoxins in goat milk and products in the region.

2. Materials and methods

2.1. Collection of samples

In this investigation, 100 samples were gathered in June from 65 randomly chosen goat farms in the Erzurum province, including 50 goat milk samples, 25 goat cream samples, and 25 goat cheese samples. Samples taken from the milking containers were brought to the laboratory in sterile containers with ice packs within a few hours.

2.2. Isolation and identification of S. aureus with the classical method

In the present study, EN ISO 6888-1-2:1999 was used to isolate and identify *S. aureus* from dairy products. Under aseptic conditions, 10 ml of the milk samples and 25 grams of cheese and cream samples were weighed and placed into stomacher bags. 225 ml of sterile Ringer's solution was added to the cheese and cream samples, and 90 ml was added to the milk samples. Samples were homogenized in a stomacher machine for two minutes. The resulting homogenates were diluted until a decimal dilution of 10^{-3} was produced in tubes containing 9 ml of peptone water for dilution. Prepared dilutions were inoculated on Baird Parker Agar with Egg Yolk Tellurite Emulsion added and incubated at 37^0C for 24-48 hours under aerobic conditions. At the end of the incubation, black, shiny, convex colonies with a diameter of 1-3 mm with a transparent zone around them due to lecithinase activity and atypical colonies without halos were considered suspicious for S. aureus, and 3-5 samples were taken.Then, they were inoculated in Brain Hearth Broth (BHI) and incubated at 37^oC for 24 hours under aerobic conditions to identify coagulase-positive staphylococci. The clumping factor was searched with the Staph Rapid Latex Test Kit (Mascia Brunelli) in those who were positive for coagulase in the tube. As a result of the other tests, the isolates with hemolysis ability on blood agar, positive for catalase, acetoin, DNase test and Gram stain, oxidase negative, anaerobic glucose and mannitol fermenting ability were identified as *S. aureus* [16].

2.3. Determination of phenotypic methicillin resistance by disk diffusion method

To detect methicillin-resistant *S. aureus* isolates, oxacillin, cefoxitin antibiotic discs, and Mueller-Hinton agar medium were used based on Clinical and Laboratory Standards (CLSI). [17]. For this purpose, one loopful of isolates was taken from the isolates incubated for 24 hours at 37^0C in TSB, and the turbidity of the bacterial suspension was adjusted to 0.5 McFarland standard with a densitometer in tubes containing 5 ml of 0.09% physiological saline. Then, 1 ml of suspension was taken and inoculated to the Mueller-Hinton agar medium. Antibiotic discs were placed by waiting for the agar to dry. Afterwards, the plates were incubated at 35^0C for 24-48 hours and the inhibition zones formed were measured. The diameters of the inhibition zones were interpreted according to the CLSI guidelines. Oxacillin was considered susceptible if the inhibition zone diameter was ≥ 13 mm, moderately sensitive if 11-12 mm, and resistant if ≤ 10 mm. Cefoxitin was considered susceptible if the diameter of inhibition was ≥ 22 mm, and resistant if the diameter was ≤ 21 mm. [17]

2.4. The identification of Nuc, PVL, mecA, and Enterotoxin genes

2.4.1. Primers

The nucleotide sequence of the mecA, PVL, and nuc genes of *S. aureus* and the nucleotide sequence of the enterotoxin genes were obtained from the GenBank® database and used in Table 1 design of the primer sequences that were evaluated for specificity by using the standard nucleotide comparison tool.

Table 1. *Primer list*

Gen	Primer	Sequence
mecA	mecA fw	CAATGCCAAAATCTCAGGTAAAGTG
	mecA rev	AACCATCGTTACGGATTGCTTC
PVL	pvl fw	AAATGCTTTGCAGCGTTTTGTTTTCG
	pvl rev	TGGACAAAACTTCTTGG
Nuc	nuc fw	GGCATATGTATGGCAATTGTTTC
	nuc rev	CGTATTGCCCTTTCGAAACATT
SEA	SEA fw	TTGGAAACGGTTAAAACGAA
	SEA rev	GAACCTTCCCATCAAAAACA
SEB	SEB fw	TCGCATCAAACTGACAAACG
	SEB rev	GCAGGTACTCTATAAGTGCC
SEC	SEC fw	GACATAAAAGCTAGGAATTT
	SEC rev	AAATCGGATTAACATTATCC
SED	SED fw	CTAGTTTGGTAATATCTCCT
	SED rev	TAATGCTATATCTTATAGGG

2.4.2. Total RNA Isolation

In total RNA isolation, 1 mL Qiazol (Qiagen, Germany) was added to the bacteria samples that were stored in a deep freezer at -80°C, left for 5 minutes at room temperature, and centrifuged at 12.000 x g 4°C for 15 minutes. Then, the supernatant was taken into a new Eppendorf, 500 µl chloroform was added, mixed for 1 minute, and centrifuged at 12.000 x g 4°C for 15 minutes. The supernatant was taken again to a new Eppendorf, 200 µl isopropanol was added and centrifuged at 12.000 x g 4°C for 10 minutes. The supernatant was then discarded and 500 µl 75% ethanol was added to the pellet and centrifuged at 7.500 x g 4°C for 10 minutes. Finally, after the removal of the ethanol, the RNA pellet was dissolved with an appropriate volume of water without RNA-degrading enzymes (DEPC-treated RNase free).

The purity and amount of the resulting RNA were determined with 260-280 nm absorbance measurements in the spectrometer. The integrity and amount of RNA were checked by loading 1 µg RNA, which was quantified according to 260 nm absorbance, on a 1.5% agarose gel.

2.4.3. DNase I application and cDNA translation

DNase I (Thermo Scientific) was used against DNA contamination in the isolated total RNA samples.

Table 2. *Nuc, mecA, and PVL prevalence on isolate basis*

Samples	Classic Culture Method		PCR		
	BP Isolate	**OT**	***S. aureus* (nuc)**	***S. aureus* (PVL)**	**Methicillin Resistance Gene (mecA)**
Milk (n=50)	43	5	5	5	-
Cream (n=25)	34	2	2	2	-
Cheese (n=25)	79	6	6	6	2
Total (n=100)	156	13	13	13	2

OT: Result of other biochemical tests

The application was made according to the protocol provided with the product. Then, 2-5 µg of these RNAs were translated into cDNA by using the miScript Reverse Transcription Kit (Qiagen) in line with the given protocol. The purity and amount of the resulting cDNA were determined with 260-280 nm absorbance measurements in the spectrophotometer and diluted at the same rates. It was stored at -20°C for later use in Real-Time PCR studies.

2.4.4. Quantitative Real-time PCR (qRT-PCR)

In the present study, the purpose was to determine the expressions of selected genes. The master mix content created in Real-Time PCR experiments (Qiagen Rotor-Gene HRM+) is as follows: Syber Green 2X Rox Dye Master Mix (Qiagen), forward and reverse primers, cDNAs as templates, and nuclease-free water. The samples were placed in the Real-Time Device after the master mixes were prepared, and the results were evaluated. This way, the CT/CQ values of the genes were obtained. Melting Curve Analysis was performed in all reactions. The primer sequences of the relevant genes are given in Table 1.

2.5. Statistical Analysis

Statistical analyses were performed using SPSS software (Version 25.0, SPSS 167 Inc., Chicago, IL, USA) to evaluate *Staphylococcus aureus* in milk, cream and cheese. One-way ANOVA with *post hoc* Tukey's test was used to determine

significant differences. All statistical comparisons were performed at the significance level of P<0.05.

3. Results

3.1 Prevalence of S. aureus

A total of 156 isolates were obtained from the BP agar in 100 samples, and 13 (8.3%) of them were found to be *S. aureus* as a result of the classical culture method and biochemical tests. The nuc and PVL genes were detected with PCR from those 13 samples (Table 2). *S. aureus* was detected in 4 of 50 milk samples, 2 of 25 cream samples, and 3 of 25 cheese samples. Statistical analysis revealed that there were no significant differences in the presence of *S. aureus* in the milk, cream, and cheese samples (P=0.612).

3.2. Phenotypic Methicillin Resistance

In goat milk and dairy products, Cefoxitin and oxacillin resistance was observed in 1 of 5 isolates (20%) obtained from milk, 2 of 6 isolates (33.3%) obtained from cheese, and 3 (23%) of 13 isolates in total. No resistance was observed in 2 isolates obtained from cream samples.

3.3. MecA Gene Prevalence

In the *S. aureus* isolates, genotypic methicillin resistance is determined by the presence of the mecA gene. Also, methicillin resistance may be caused by the newly identified mecA homolog, the mecC gene, or other factors that provide resistance. There are studies in which mecA and mecC genes are absent, but methicillin resistance is seen [15]. It was observed with the PCR that 2 of 13 (15.3%) *S. aureus* isolates had the mec A gene, and they were obtained from cheese samples but not in isolates obtained from milk and cream samples (Table 2).

Table 3. *Allocation of staphylococcal enterotoxin types*

SEA/CT value	SEB/CT value	SEC/CT value	SED/Ct Value	Samples	İzolat No
14.12	Negative	24.17	25.26	Goat mik	130a -2
14.16	**Negative**	**24.16**	**25.38**	Goat mik	114a-2
14.25	**Negative**	**24.18**	**25.46**	Goat cream	163b-2
14.28	**Negative**	**24.65**	**25.15**	Goat cheese	199b-2
14.01	**20.15**	**Negative**	**25.19**	Goat cheese	199c-3
14.95	**Negative**	**24.56**	**25.32**	Goat mik	114a-3
14.25	**Negative**	**24.02**	**Negative**	Goat mik	145e--2
14.26	**Negative**	**24.05**	**Negative**	Goat cheese	184d-2
14.8	**Negative**	**24.12**	**Negative**	Goat mik	112a-2
14.13	**Negative**	**Negative**	**Negative**	Goat cheese	184b-2
14.23	**Negative**	**Negative**	**25.01**	Goat cream	168a-2
14.58	**20.67**	**Negative**	**25.26**	Goat cheese	178b-3
15.01	**Negative**	**24.04**	**Negative**	Goat cheese	199f-2

3.4. Staphylococcal Enterotoxin Gene Prevalence

Food poisoning caused by enterotoxigenic *S. aureus* has been reported previously due to consuming contaminated dairy products [18]. In this study, 8% (4/50) of goat milk samples, 12% (3/25) of cheese samples, and 8% (2/25) of cream samples contained at least one enterotoxin gene. In total, 9% of 100 samples contained enterotoxigenic *S. aureus* (Table 3, 4).

When the isolates were examined, it was found that one isolate had one enterotoxin gene, five isolates had two enterotoxin genes, and seven isolates had three enterotoxin genes.

4. Discussion

In the present study, the incidence of *S. aureus* in goat milk and dairy products was 9%. This rate is close to the rate of 4.65% reported in the study of Günday [19] on 86 goat milk and the rate of 6.2% obtained by Mørk et al. from 5761 goat milk samples [20].

However, there are also studies reporting the presence of *S. aureus* at less than 3% in goat products [21]. Unlike these,

there are also studies reporting positive *S. aureus* rates in cow and goat milk samples, ranging from 75% to 96.2% [15].

These differences may be due to the collection of samples from animals with mastitis, milking hygiene conditions, milking equipment, storage, and transportation conditions.

When the samples were examined, it was seen that goat cheeses had the highest prevalence of *S. aureus*, which was isolated in 3 of 25 (12%) kind of goat cheese. Findings with similar rates were found as 20.2% in the study conducted by Yücel and Anıl [22] on 79 cheese samples, and as 16.7% in the study of Rahimi [23] conducted on 60 traditional cheese samples.

Table 4. *Prevalence of the enterotoxin genes*

Samples	Enterotoxin Genes PCR				
	***S. aureus* Positive**	**sea**	**seb**	**sec**	**sed**
Milk (n=50)	5	5	-	5	3
Cream (n=25)	2	2	-	1	2
Cheese (n=25)	6	6	2	3	3
Total (n=100)	13	13	2	9	8

Basanisi et al. [24] reported this rate to be 41.1% in a study conducted on sheep and goat cheeses in Italy, which was higher than the rates found in this study, and as 61% in the study of Özpınar and Gümüşsoy [25] on 100 kinds of Erzincan Tulum cheese.

These differences occur because of improper processing of foods, not applying enough heat to homemade cheese, obtaining raw milk from unhealthy animals, or contamination of production tools and equipment.

The prevalence of *S. aureus* was 8% in cream samples. One of the reasons for this low rate was the heat treatment (95°C) applied in obtaining cream from raw milk. This application is effective in the inactivation of bacteria present in raw milk. For this reason, no *S. aureus* was found in some cream studies [15].

The MRSA rate varies in disk diffusion studies on milk and dairy products. Turkyilmaz et al. [26] in their research conducted in Aydın province, 99 *S. aureus* isolates were obtained from milk collected from cows, and 16 (17%) were found to be MRSA by disc diffusion method. Can and Çelik [27] obtained 12 *S. aureus* isolates in a study of 200 Tulum and feta cheese samples, and they determined that 2 (16.6%) of them were MRSA by disc diffusion method. In their investigation of 400 samples (200 milk, 200 nasal swabs) taken from sheep and goats in Nigeria, Omoshaba et al. [28] isolated *S. aureus* in 72 samples and found MRSA in 52 (72.2%) of them phenotypically

In the study, the presence of he mecA gene was 2% (2/100) based on all samples and 15.3% (3/13) based on the isolates obtained. Similar to these rates, Saka and Gulel [15] found the mecA gene in 9 of 99 (9%) *S. aureus* isolates (eight isolate milk, one isolate cheese) obtained from 200 buffalo milk and products collected in the study that was conducted in Samsun. Basanisi et al. [24] obtained 37 *S. aureus* isolates from 90 sheep and goat cheeses and found the mecA gene in only 1 of them (2.7%). In another study, Obaidat et al. [29] found the mecA gene ratio to be 11.9% in the samples that were collected from 42 goat milk tanks in Jordan.

Methicillin resistance is classified as homogeneous and heterogeneous resistance. Homogeneous resistant bacteria have the mecA gene, and it is active. These bacteria can reproduce by resisting the presence of high levels of methicillin. On the other hand, Heterogeneous resistant bacteria have the mecA gene, but resistance is seen only in some. [30]

In the present study, Genotypic resistance was detected in 2 of 13 *S. aureus* isolates and phenotypic resistance in 3 (Table 5). In similar studies, it has been reported that phenotypic resistance is not only caused by the mecA gene but also by changes in PBP2a based on the production of beta-lactamase and methicillinase enzymes. In addition, it has been stated that strains that have the mecA gene but do not show resistance

Table 5. *Methicillin resistance comparison*

Nuc/CT value	*MecA/CT value*	*Samples*	*İzolat No*	*Oxacillin*	*Cefoxitin*
24.01	**Negative**	Goat milk	130a -2	**Susceptible**	**Susceptible**
23.12	**Negative**	Goat milk	114a-2	**Susceptible**	**Susceptible**
23.58	**Negative**	Goat cream	163b-2	**Susceptible**	**Susceptible**
23.16	**Negative**	Goat cheese	199b-2	**Susceptible**	**Susceptible**
22.16	**26.15**	Goat cheese	199c-3	**Resistant**	**Resistant**
23.17	**Negative**	Goat milk	114a-3	**Susceptible**	**Susceptible**
23.18	**Negative**	Goat milk	145e--2	**Susceptible**	**Susceptible**
23.16	**Negative**	Goat cheese	184d-2	**Resistant**	**Resistant**
23.18	**Negative**	Goat milk	112a-2	**Resistant**	**Resistant**
23.17	**Negative**	Goat cheese	184b-2	**Susceptible**	**Susceptible**
22.15	**Negative**	Goat cream	168a-2	**Susceptible**	**Susceptible**
22.02	**26.15**	Goat cheese	178b-3	**Susceptible**	**Susceptible**
23.14	**Negative**	Goat cheese	199f-2	**Susceptible**	**Susceptible**

phenotypically can cause serious health problems and are clinically significant. [31, 32] It is reported that many factors such as incubation time, temperature, amount of inoculum and pH of the medium can affect the analysis methods for determining phenotypic resistance. [33] Since detecting heterogeneous resistant MRSA strains does not provide accurate results by phenotypic methods, mecA gene detection is accepted as the gold standard in determining methicillin resistance [34].

Considering the studies on enterotoxin genes, in a study conducted in Norway, Jørgensen et al. [35] examined enterotoxin genes in 220 cattle, 213 goat tank milk, and 82 raw milk samples. They found enterotoxigenic *S. aureus* 52.5% in 101 cattle isolates, 55.8% in 95 goat isolates, and 72.4% in 29 raw milk isolates. Mørk et al. [20] found the rate of enterotoxigenic *S. aureus* as 71.9% in their study on 153 isolates obtained from dairy products. Rahimi [23] found this rate at 35% in 347 dairy products.

When the total number of samples was considered, the seb gene was detected only in 2 kinds of goat cheese. Less than synthetic chemicals are sufficient for this toxin, the strongest SE, to have a toxic effect. Even at low concentrations, death is observed because of multi-organ failure [36].

Although some studies on cow and goat milk only reported isolates with the seb gene, no isolates with the seb gene were reported in some other studies [15]. The ability of *S. aureus* to form toxins in foods can vary according to many factors, some of which are pH, salt content, contents of foods, competitive microflora, and the a_w value [37].As can be seen, the prevalence of enterotoxin-producing *S. aureus* in milk and its products varies among studies. This may be because of the source of the samples and geographical differences

5. Conclusion

The present study shows that goat milk and its products can be contaminated with enterotoxigenic and methicillin-resistant *S. aureus*. The presence of the seb gene, which produces high toxic SEB secretion, poses a danger to human health in milk and dairy products. Since the PVL gene was isolated from all samples, the possibility of human-to-animal transmission of S. aureus is high. Therefore, consuming milk and milk products that have not undergone adequate heat treatment or are stored under appropriate storage conditions must be avoided.

References

[1]. Bezirtzoglou E., Maipa V., Voidarou C., Tsiotsias A., Papapetropoulou M., “Food-borne intestinal bacterial pathogens”, Microb. Ecol., 12(2), (2000), 96-104.

[2]. Wieland B., Feil C., Gloria-Maercker E., Thumm G., Lechner M., Bravo J. M., Götz F., “Genetic and biochemical analyses of the biosynthesis of the yellow carotenoid 4, 4'-diaponeurosporene of Staphylococcus aureus”, J. Bacteriol, 176(24), (1994). 7719-7726.

[3]. Wertheim, H. F., Melles, D. C., Vos, M. C., van Leeuwen W., Van Belkum A., Verbrugh H. A., Nouwen J. L., "The role of nasal carriage in Staphylococcus aureus infections", Lancet Infect. Dis., 5(12), (2005), 751-762.

[4]. Stryjewski M.E., G.R. Corey, "Methicillin-resistant Staphylococcus aureus: an evolving pathogen". Clin. Infect. Dis., 58, (2014), 10-19.

[5]. Xu Z., Peters B. M., Li B., Li L., Shirtliff M. E., "Staphylococcal Food Poisoning and Novel Perspectives in Food Safety", in Prevention and Control of Food Related Diseases, vol. 1, M.H. Anthony, Eds. Croatia, İnTech, 2016, 159.

[6]. Di Giannatale E., Prencipe V., Tonelli A., Marfoglia C., Migliorati G., "Characterisation of Staphylococcus aureus strains isolated from food for human consumption", Veterinaria Italiana, 47(2), (2011), 165-173.

[7]. Haenlein G., "Goat milk in human nutrition. Small ruminant research", 51(2), (2004), 155-163.

[8]. Kim H.K., Falugi F., Missiakas D.M., Schneewind, O., "Peptidoglycan-linked protein A promotes T cell-dependent antibody expansion during Staphylococcus aureus infection", Proc. Natl. Acad. Sci., 113(20), (2016), 5718-5723.

[9]. Argudín M.Á., Mendoza M.C., Rodicio M.R., "Food poisoning and Staphylococcus aureus enterotoxins. Toxins", 2(7), (2010), 1751-1773.

[10]. Tsutsuura S., Shimamura Y., Murata M., "Temperature dependence of the production of staphylococcal enterotoxin A by Staphylococcus aureus", Biosci. Biotechnol. Biochem., 77, (2013) 30–37.

[11]. Omoe K., Hu D. L., Ono H. K., Shimizu S., Takahashi-Omoe H., Nakane A., Imanishi K. I., "Emetic potentials of newly identified staphylococcal enterotoxin-like toxins", Infect. Immun., 81(10), (2013), 3627-3631.

[12]. Klevens R. M., Morrison M. A., Nadle J., Petit S., Gershman K., Ray S., "Invasive methicillin-resistant Staphylococcus aureus infections in the United States", Jama, 298(15), (2007), 1763-1771.

[13]. Ito T., Katayama Y., Asada K., Mori N., Tsutsumimoto K., Tiensasitorn C., Hiramatsu K., "Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant Staphylococcus aureus", Antimicrob. Agents Chemother., 45(5), (2001), 1323-1336.

[14]. Archer, G.L., "Staphylococcus aureus: a well-armed pathogen" Rev. Infect. Dis., 26(5), (1998),1179-1181.

[15]. Saka, E., G. Terzi Gulel, "Detection of enterotoxin genes and methicillin-resistance in Staphylococcus aureus isolated from water buffalo milk and dairy products", J. Food Sci., 83(6), (2018), 1716-1722.

[16]. Wald R., Hess C., Urbantke V., Wittek T., Baumgartner M., "Characterization of Staphylococcus Species Isolated from Bovine Quarter Milk Samples", Animals, 9(5), (2019), 200.

[17]. Wayne, P.A., "Performance standards for antimicrobial susceptibility testing", Clinical Laboratory Standard Institute, 1, (2009), 19.

[18]. Oliver S.P., Jayarao B.M., Almeida R.A., "Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications", Foodbourne Pathogens & Disease, 2(2), (2005), 115-129.

[19]. Günday H., "Aydın bölgesinde ürüne işlenecek çiğ sütlerde staphylococcus aureus ve enterotoksin varlığının araştırılması," M.S. thesis, Institute of Health Sciences, Department of Microbiology, Adnan Menderes Üniversitesi, Aydın,Türkiye, 2017.

[20]. Mørk T., Kvitle B., Mathisen T., Jørgensen H. J., "Bacteriological and molecular investigations of Staphylococcus aureus in dairy goats", Vet. Microbiol., 141(1-2), (2010), 134-141.

[21]. Menzies, P.I., Ramanoon S.Z., "Mastitis of sheep and goats", Vet. Clin. North Am. Food Anim., 17(2), (2001), 333-358.

[22]. Yücel N., Anıl Y., "Çiğ süt ve peynir örneklerinden Staphylococcus aureus ve koagülaz negatif stafilokokların identifikasyonu ve antibiyotik duyarlılığı", Türk Hijyen ve Deneysel Biyoloji Dergisi, 68(2), (2011), 73-78.

[23]. Rahimi E., "Enterotoxigenicity of Staphylococcus aureus isolated from traditional and commercial dairy products marketed in Iran", Brazilian Journal of Microbiology, 44, (2013), 393-399.

[24]. Basanisi M. G., Nobil, G., La Bella G., Russo R., Spano G., Normanno G., La Salandra G., "Molecular characterization of Staphylococcus aureus isolated from sheep and goat cheeses in southern Italy", Small Rumin. Res., 135, (2016), 17-19.

[25]. Özpınar N., Gümüşsoy K.S., "Phenotypic and genotypic determination of antibiotic resistant and biofilm forming Staphylococcus aureus isolated in Erzincan tulum cheese", Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 19(3), (2013), 517-521.

[26]. Türkyılmaz S., Tekbıyık S., Oryasin E., Bozdogan B., "Molecular epidemiology and antimicrobial resistance mechanisms of methicillin-resistant Staphylococcus

aureus isolated from bovine milk", Zoonoses and Public Health, 57(3), (2010),197-203.

[27]. Can H.Y., Celik T.H., "Detection of enterotoxigenic and antimicrobial resistant S. aureus in Turkish cheeses", Food Control, 24(1-2), (2012), 100-103.

[28]. Omoshaba E.O., Ojo O.E., Oyekunle M.A., Sonibare A. O., Adebayo A. O., "Methicillin-resistant Staphylococcus aureus (MRSA) isolated from raw milk and nasal swabs of small ruminants in Abeokuta", Nigeria. Trop. Anim. Health Prod., 52(5), (2020), 2599-2608.

[29]. Obaidat M.M., Bani Salman A.E., Roess A.A., "High prevalence and antimicrobial resistance of mecA Staphylococcus aureus in dairy cattle, sheep, and goat bulk tank milk in Jordan", Trop. Anim. Health Prod., 50(2), (2018), 405-412.

[30]. Jacoby G.A., Archer G.L., "New mechanisms of bacterial resistance to antimicrobial agents", N. Engl. J. Med., 324(9), (1991), 601-612.

[31]. Oliveira D.C., Lencastre H.N.D., "Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant Staphylococcus aureus", Antimicrob. Agents Chemother., 46(7), (2002), 2155-2161.

[32]. Swenson J.M., Lonsway D., McAllister S., Thompson A., Jevitt L., Zhu W., Patel J.B., "Detection of mecA-mediated resistance using reference and commercial testing methods in a collection of Staphylococcus aureus expressing borderline oxacillin MICs", Diagn. Microbiol. Infect. Dis., 58(1), (2007), 33-39.

[33]. Cauwelier B., Gordts B., Descheemaecker P., Van Landuyt H., "Evaluation of a disk diffusion method with cefoxitin (30 µg) for detection of methicillin-resistant Staphylococcus aureus", Eur. J. Clin. Microbiol. Infect. Dis., 23(5), (2004), 389-392.

[34]. Tomasz A., Drugeon H.B., De Lencastre H.M., Jabes D., McDougall L., Bille, J., "New mechanism for methicillin resistance in Staphylococcus aureus: clinical isolates that lack the PBP 2a gene and contain normal penicillin-binding proteins with modified penicillin-binding capacity", Antimicrob. Agents Chemother., 33(11), (1989), 1869-1874.

[35]. Jørgensen H.J., Mørk T., Rørvik L.M., "The occurrence of Staphylococcus aureus on a farm with small-scale production of raw milk cheese", J. Dairy Sci., 88(11), (2005), 3810-3817.

[36]. Kashiwada T., Kikuchi K., Abe S., Kato H., Hayashi H., Morimoto T., Gemma A, "Staphylococcal enterotoxin B toxic shock syndrome induced by community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA)", Internal Medicine, 51(21), (2012), 3085-3088.

[37]. Lyra D.G., Sousa F.G., Borges M.F., Givisiez P.E., Queiroga R.C., Souza E.L., Oliveira C.J., "Enterotoxin-encoding genes in Staphylococcus spp. from bulk goat milk. Foodborne Pathog". Dis., 10(2), (2013), 126-130.