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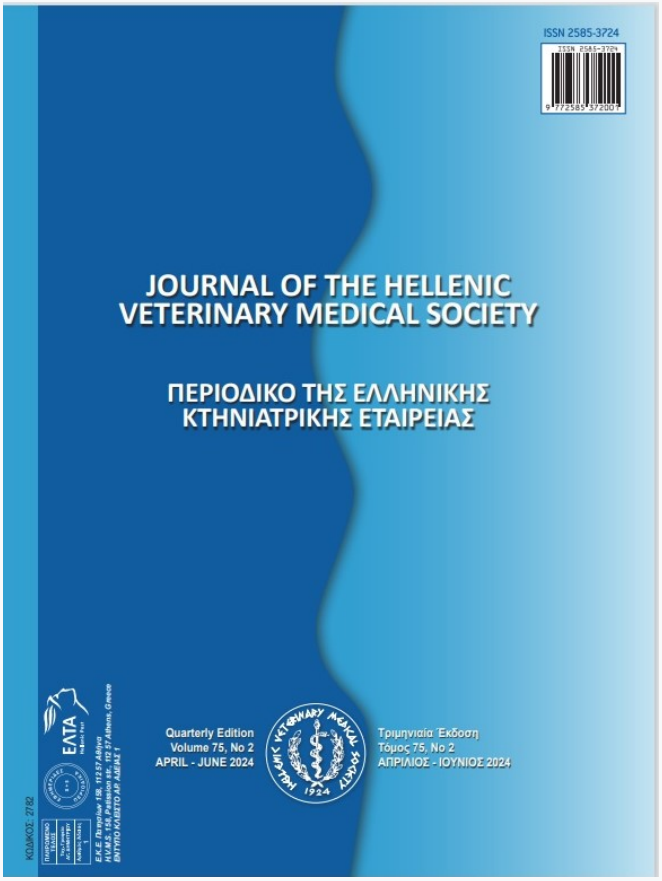

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H Abughonem, B Halac, AI Kekec, B Maslak, AF Bağcigil

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Nasal carriage, microbial resistance and genetic characterization of *Staphylococcus aureus* in cows in Turkey

H. Abughonem, B. Halac, A.I. Kekec, B. Maslak, A.F. Bağcıgil

Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul, Turkey

ABSTRACT: The aim of this study is to determine the isolation rate of *S. aureus* in nasal cavities of healthy cattle, antibiotic resistance profiles of the isolates and to investigate genes associated with antibiotic resistance. For this purpose; 250 nasal swab samples collected from healthy dairy cattle in farms located in the provinces of Istanbul, Bursa, Kırklareli and Tekirdağ), examined for the presence *S. aureus*. *S.aureus* was isolated from 7.6% (n=19) of the examined cows. Antibiotic susceptibility of isolates was determined by disc diffusion method. The isolates were resistant to penicillin (78.95%); tetracycline (68.42%); erythromycin (63.16%); doxycycline (36.84%); cefaperazone (31.58%); cefoxitin (26.32%); ceftiofur (26.32%) and clindamycin (21.05%), while all were sensitive to penicillin-novobiocin, linezolid, quinupristin-dalfopristin, chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole and enrofloxacin. Five of the 12 isolates resistant to erythromycin showed inducible clindamycin resistance. Antibiotic resistance genes and *pvl* gene were examined by Polymerase Chain Reaction method. The most common genes detected in the isolates were *blaZ* (93,33%), *mecA* (52.63%), *tetL* (23,08%), *tetK* (53,85%), *ermB* (8,33%) and *ermC* (41,67%). The *ermA*, *tetM*, *tetO* and *pvl* genes were not detected in any of the isolates. In our study, the most common type of SCC*mec* was found to be type IV 90% (n=9) and only one isolate type I 10% (n=1).

***Keywords*:** microbial resistance; cow, nasal swab; *Staphylococcus aureus*.

Corresponding Author:
Hager ABUGHONEM, Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul, Turkey
E-mail address-mhagermoha@ogr.iuc.edu.tr

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INTRODUCTION

Staphylococcus aureus is a pathogenic bacterium that can be found on the skin and mucous membranes of animals and humans, as well as causing a wide variety of diseases. Therefore, it has a significant impact on public health and the livestock industry. *S. aureus* is one of the primary causes of skin and soft tissue infections. It is an important pathogen causing mastitis in cows, sheep and goats. The isolation of *S. aureus* from the nasal mucosa of farm animals has been frequently reported (Erdem and Türkyılmaz, 2013; Gharsa et al., 2012; Hazimoğlu, 2011; Rahimi et al., 2015).

Today, increasing of antimicrobial resistance in pathogenic bacteria of animal origin and commensal bacteria is a growing concern in both veterinary and human medicine. β-lactam, macrolide and tetracyclines are antibiotics commonly used in the treatment of *S.aureus* infections and resistance to these antibiotics is increasing (Center, 2012; Diliz, 2010; Erdem and Türkyılmaz, 2013; Kaynarca, 2009; Rahimi et al, 2015). Studies revealing the presence of livestock associated MRSA (LA-MRSA) in animals and humans in contact with animals, such as veterinarians, animal caretakers, and farmers, have been reported (Bağcıgil et al., 2007; Ben Slama et al., 2011; Türkyılmaz et al., 2010). In addition, the presence of MRSA in animals and dairy products poses a potential threat to the spread of multi-drug resistant isolates in the community. With the increasing prevalence of multiple antibiotic resistant isolates, treatment of *S. aureus* infections is becoming more difficult (Rahimi et al., 2015).

In this study, it was aimed to determine the isolation rates of *Staphylococcus aureus* from nasal swabs of healthy cattle, antibiotic resistance profiles of isolates and to investigate the presence of resistance genes associated with antibiotic resistance.

MATERIALS AND METHODS

Sampling:

Between October 2020 and December 2020, a total of 250 nasal swab samples were collected from healthy dairy cattle from fifteen farms located in four different provinces (Istanbul (five farms), Bursa (four farms), Kırklareli (five farms) and Tekirdağ (One farm)). Swab samples were obtained swabbing the medial septum region of both nostrils of each cow. The samples were stored in Stuart transport medium, under aseptic conditions and refrigerated, and delivered to the laboratory of Istanbul University- Cerrahpaşa, Faculty of Veterinary Medicine, Department of Microbiology. Swabs were inoculated into Tryptone Soya Broth (TSB, Merck) containing 7.5% sodium chloride and incubated at 37°C for 24 hours. After incubation period, all cultures were streaked into Mannitol Salt Agar plates (MSA, Himedia) and incubated at 37°C for 24 hours. Presumptive *S. aureus* colonies were streaked into TSB after purification on MSA, and after 24 hours of incubation at 37°C, all isolates were stored at -20°C in TSB containing 10% glycerol until identification of *S. aureus* isolates by PCR.

S. aureus isolation:

DNA extraction was performed as described by Kariyama et al. (2000). Briefly, the isolates were streaked into TSB and incubated at 37°C for 18-24 hours aerobicaly. After incubation period, an equal volume (400:400 µl) of 5% Chelex solution and bacterial suspension was mixed in a microcentrifuge tube. Tubes were incubated at 99°C for 10 minutes. All tubes were centrifuged at 12.500 rpm for 3.5 minutes, and the supernatant was used as template DNA. All isolates were examined for *nuc* gene by PCR as described previously (Table-2) (Brakstad et al.,1992).

Antimicrobial susceptibility tests

The phenotypic antibiotic resistance profiles of the isolates were determined using the disk diffusion technique according to the Standards of the Clinical Laboratory Standard Institute (CLSI VET01S ED5:2020, CLSI M100 ED28:2018) for penicillin G (10 IU), cefoxitin (30 µg), ceftiofur (30 µg), cefoperazone (30 µg), gentamicin (10 µg), clindamycin (2 µg), erythromycin (15 µg), enrofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25 / 23.75 µg), tetracycline (30 µg), chloramphenicol (30 µg) and penicillin-novobiocin (10 IU /30 µg) and doxycycline (30 µg), quniupristin-dalfopristin (15 µg) and linezolid (30 µg) For this purpose, a suspension of 0.5 Mac Farland density in Sterile 0.9% NaCl solution was prepared from fresh cultures of the isolates. The suspensions were spread onto the Mueller Hinton Agar (MHA, Oxoid) using sterile swabs. Antibiotic discs were placed onto the agar surface, and the Petri dishes were incubated at 37 °C for 18-24 hours. Zone diameters formed at the end of the period were evaluated according to CLSI standards for veterinary pathogens (CLSI VET01S ED5:2020). D-test was performed to determine inducible clindamycin resistance. *S. aureus* ATCC 25923 was used as the reference control stra-

in to validate the antibiotic susceptibility test (CLSI M100 ED28:2018).

Detection of the antibiotic resistance related genes, the *pvl* gene and SCC*mec* types

DNA extraction of all isolates was performed using the pureLink Genomic DNA Mini Kit (Thermo Fisher, Invitrogen, K1820-02). Phenotypically penicillin resistant isolates examined for *blaZ* gene, isolates resistant to erythromycin for *ermA*, *ermB* and *ermC* genes, and tetracycline resistant ones for *tetL*, *tetM*, *tetK* and *tetO* genes. All isolates were examined for the carriage of the *mecA* and *pvl* genes. The *mecA* gene positive isolates were submitted for the determination of the SCC*mec* types. All the primers and PCR protocols were shown in Table-2 and Table-3. The SCC*mec* types of isolates were determined according to the evaluation criteria summarized in Table-3.

Table 1: Isolation frequency of *S. aureus* in nasal swab samples obtained from dairy cattle

province	farm	No. of samples	No. of *S. aureus* isolates	Codes of *S. aures* isolates
Istanbul	1.	32	4 (12.5%)	23,25, 26,30
	2.	15	-	
	3.	15	-	
	4.	10	-	
	5.	10	-	
Bursa	6.	24	5 (20.8%)	71,75,76,78,79
	7.	15	-	
	8.	15	-	
	9.	15	1 (6.7%)	193
Kırklareli	10.	20	2 (10%)	59,64
	11.	15	-	
	12.	15	-	
	13.	17	-	
	14.	20	5 (25%)	138,139,140,141,142
Tekirdağ	15.	12	2 (16.7%)	33,39

Table 2: Primer sequences and PCR protocols used in the study

Primer	Primer sequences	Length	Protocol	Reference
NUC F	5' GCG ATT GAT GGT GAT ACG GTT 3'	279 bp	37 (94°C 1 min; 55°C	Brakstad et
NUC R	5' AGC CAA GCC TTG ACG AAC TAA AGC 3'		30 sec; 72°C 1.5 min)	al., 1992
***mecA*1**	5' TGC TAT CCA CCC TCA AAC AGG 3'	286 bp	35 (94°C 2 min; 57°C	Kondo et
***mecA*2**	5' AAC GTT GTA ACC ACC CCA AGA 3'		1 min; 72°C 3 min)	al., 2007
***blaZ* 1**	5' ACT TCA ACA CCT GCT GCT TTC 3'	173 bp	35 (94°C 30 sec; 58°C	Martineau
***blaZ* 2**	5' TGA CCA CTT TTA TCA GCA ACC 3'		30 sec; 72°C 30 sec)	et al., 2000
***ermA* F**	5' GTT CAA GAA CAA TCA ATA CAG AG 3'	421 bp	30 (94°C 30 sec; 52°C	Lina et al.,
***ermA* R**	5' GGA TCA GGA AAA GGA CAT TTT AC 3'		30 sec; 72°C 1 min)	1999
***ermB* F**	5' CCG TTT ACG AAA TTG GAA CAG GTA AAG GGC 3'	359 bp	30 (94°C 30 sec; 55°C	
***ermB* R**	5' GAA TCG AGA CTT GAG TGT GC 3'		30 sec; 72°C 1 min)	
***ermC* F**	5'GCT AAT ATT GTT TAA ATC GTC AAT TCC 3'	572 bp	30 (94°C 30 sec; 52°C	
***ermC* R**	5' GGA TCA GGA AAA GGA CAT TTT AC 3'		30 sec; 72°C 1 min)	
***tetL* F**	5' GTT GCG CGC TAT ATT CCA AA 3'	788 bp	30 (95°C 30 sec; 55°C	Kim et al.,
***tetL* R**	5' TTA AGC AAA CTC ATT CCA GC 3'		30 sec; 72°C 30 sec)	2005
***tetM* F**	5' GTT AAA TAG TGT TCT TGG AG 3'	647 bp	30 (95°C 30 sec; 55°C	
***tetM* R**	5' CTA AGA TAT GGC TCT AAC AA 3'		30 sec; 72°C 30 sec)	
***tetK* F**	5' TTA GGT GAA GGG TTA GGT CC 3'	718 bp	30 (95°C 30 sec; 55°C	
***tetK* R**	5' GCA AAC TCA TTC CAG AAG CA 3'		30 sec; 72°C 30 sec)	
***tetO* F**	5' GAT GGC ATA CAG GCA CAG AC 3'	643 bp	30 (95°C 30 sec; 55°C	
***tetO* R**	5' CAA TAT CAC CAG AGC AGG CT 3'		30 sec; 72°C 30 sec)	
***pvl* F**	5' ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A 3'	433 bp	30 (94°C 30 sec; 55°C	Lina et al.,
***pvl* R**	5' GCA TCA AST GTA TTG GAT AGC AAA AGC 3'		30 sec; 72°C 1 min)	1999

Table 3: Primers and protocol used to determine SCC*mec* types and evaluation criteria of PCR

Primer	Primer sequences	Length	target	I	II	III	IV	V	Protocol	Reference
B	5' ATT GCC TTG ATA ATA GCC YTC T 3'	937 bp	CCRA2-B		+		+		30 (94°C 30 sec, 55°C 30 sec, 72°C 60 sec)	Boye et al., 2007
α3	5' TAA AGG CAT CAA TGC ACA AAC ACT 3'									
CCRCF	5' CGT CTA TTA CAA GAT GTT AAG GAT AAT 3'	518 bp	CCRC			+		+		
CCRCR	5' CCT TTA TAG ACT GGA TTA TTC AAA ATA T 3'									
1272F1	5' GCC ACT CAT AAC ATA TGG AA 3'	415 bp	IS1272	+			+			
1272R1	5' CAT CCG AGT GAA ACC CAA A 3'									
5R*mecA*	5' TAT ACC AAA CCC GAC AAC TAC 3'	359 bp	*mecA*-IS431					+		
5R431	5' CGG CTA CAG TGA TAA CAT CC 3'									

RESULTS

The results of amplification of *S.aureus* specific primers revealed that 7.6% (n=19) of the isolates were confirmed as *Staphylococcus aureus*. Isolation frequency of *S. aureus* in nasal swab samples obtained from dairy cattle are shown in Table-1.

The highest resistance rates were ascribed for penicillin (n=15) and tetracycline (n=13). All strains were sensitive to penicillin-novobiocin, linezolid, quinupristin-dalfopristin, chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole and enrofloxacin. Number of antibiotic resistant isolates are shown in Table-4 and classification according to antibiotic resistance profiles are shown in Table-5. Using D test to determine inducible clindamycin resistance, five out of twelve erythromycin-resistant isolates were found to have inducible resistance to clindamycin. Of the *Staphylococcus aureus* isolates 10.53% (n=2) were resistant to one antibiotic class in, 10.53% (n=2) to two antibiotic classes in, and 63.16% (n=12) to three or more classes in. Antibiotic resistance was not detected in 15.79% (n=3) of the isolates.

The *mecA* gene was detected in 10 isolates. The *mecA* gene was found in five of the five isolates that were phenotypically methicillin-resistant, and also carried the *mecA* gene, and five of the 14 isolates

Table 4: Resistance rates to antibiotics of *S. aureus* isolates

Antibiotic	Number of resistant bacteria	(%)
P10	15	78.95
C30	0	0.00
FUR30	5	26.32
ENR5	0	0.00
TE30	13	68.42
DA2	4	21.05
SXT25	0	0.00
E15	12	63.16
CN10	0	0.00
PNV40	0	0.00
FOX30	5	26.32
CFP30	6	31.58
QD15	0	0.00
LNZ30	0	0.00
DO30	7	36.84

P10: penicillin G, C30: chloramphenicol, FUR30: ceftiofur, ENR5: enrofloxacin, TE30: tetracycline, DA2: clindamycin, SXT25: trimethoprim-sulfamethoxazole, E15: erythromycin, CN10: gentamicin, PNV40: penicillin-novobiocin, FOX30: cefoxitin, CFP30: cefaperazone, QD15: quniupristin-dalfopristin, LNZ30: linezolid, DO30: doxycycline

that were phenotypically methicillin-susceptible. As a result of the SCC*mec* typing, bands at 937 bp and 415 bp were observed in nine of the 10 isolates carrying the *mecA* gene, and it was evaluated as SCC*mec* Type IV. A band at 415 bp was observed in one of 10 isolates with *mecA* gene and it was evaluated as SCC*mec* Type I. The *blaZ* gene was detected in 14 of the 15 isolates that were phenotypically resistant to penicillin. The *tetL* gene was found in three of 13 isolates that were phenotypically resistant to tetracycline; the *tetK* gene was detected in seven of them. The *tetM* and *tetO* genes were not detected in any of the isolates. One of the 12 isolates found phenotypically resistant to erythromycin carrying also the *ermB* gene; the *ermC* gene was detected in five of them. The *ermA* and the *pvl* gens were not detected in any of those isolates. Phenotypic resistance patterns among *S. aureus* strains and PCR results of *S. aureus* isolates are shown in Table-6.

Table 5: Distribution of isolates according to antibiotic resistance profiles

Antibiotic	Number of isolates	(%)
P	2	10.53
P/ TE	1	5.26
P/ CFP	1	5.26
P/ TE/ E	1	5.26
P/ TE/ DO/ E	2	10.53
TE/ DO/ DA/ E	1	5.26
P/TE/ DO/ DA/ E	3	15.79
P/ TE/ E/ FOX/ CFP / FUR	4	21.05
P/ TE/ DO/ E/ FOX/ CFP/ FUR	1	5.26

P: penicillin G, FUR: ceftiofur, CFP: cefaperazone; TE: tetracycline; DO: doxycycline, DA: clindamycin, E: erythromycin, FOX: cefoxitin

Table 6: Phenotypic resistance patterns among *S. aureus* strains and PCR results

İzolat No	P10	C30	Fur 30	ENR 5	TE 30	DA2	SXT 25	E15	CN 10	PNV 40	FOX 30	CFP 30	QD 15	LNZ 30	DO 30	*blaZ*	*mecA*	*Sccmec tip*	*erm A/B/C*	*Tet K/L/M/O*	*Pvl*
23	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	*	-	-	*	*	-
25	R	S	R	S	R	S	S	R	S	S	R	R	S	S	S	+	+	I	*ermC*	-	-
26	R	S	R	S	R	S	S	R	S	S	R	R	S	S	İ	-	+	IV	*ermC*	-	-
30	R	S	R	İ	R	S	S	R	S	S	R	R	S	S	İ	+	+	IV	*ermC*	*tetK*	-
33	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S	+	-	-	*	*	-
39	R	S	S	S	S	S	S	S	S	S	S	İ	S	S	S	+	-	-	*	*	-
59	R	S	R	S	R	S	S	R	S	S	R	R	S	S	R	+	+	IV	-	*tetK*	-
64	R	S	R	S	R	S	S	R	S	S	R	R	S	S	İ	+	+	IV	*ermC*	*tetK*	-
71	R	S	S	S	R	R	S	R	S	S	S	İ	S	S	R	+	-	-	*ermB*	*tetL*	-
75	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	*	-	-	*	*	-
76	R	S	S	S	R	S	S	S	S	S	S	İ	S	S	İ	+	-	-	*	*tetL*	-
78	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	*	-	-	*	*	-
79	S	S	S	S	R	R	S	R	S	S	S	S	S	S	R	*	-	-	***ermC***	*tetL*	-
138	R	S	S	S	R	S	S	R	S	S	S	S	S	S	S	+	+	IV	-	*tet K*	-
139	R	S	S	S	R	S	S	R	S	S	S	İ	S	S	R	+	+	IV	-	-	-
140	R	S	S	S	R	S	S	R	S	S	S	İ	S	S	R	+	+	IV	-	*tet K*	-
141	R	S	S	S	R	R	S	R	S	S	S	İ	S	S	R	+	+	IV	-	*tetK*	-
142	R	S	S	S	R	R	S	R	S	S	S	İ	S	S	R	+	+	IV	-	*tetK*	-
193	R	S	S	S	S	S	S	S	S	S	S	İ	S	S	S	+	-	-	*	*	-

*= PCR not performed (relevant gene region only in isolates with phenotypical resistance tested); R= resistant; S= sensitive; I= intermediate sensitive; P10: penicillin G, C30: chloramphenicol, FUR30: ceftiofur, ENR5: enrofloxacin, TE30: tetracycline, DA2: clindamycin, SXT25: trimethoprim-sulfamethoxazole, E15: erythromycin, CN10: gentamicin, PNV40: penicillin-novobiocin, FOX30: cefoxitin, CFP30: cefaperazone, QD15: quniupristin dalfopristin, LNZ30: linezolid, DO30: doxycycline

DISCUSSION

S. aureus is one of the bacteria frequently isolated from bovine mastitis cases and is therefore important for veterinary microbiology. Santos et al. (2020), associated the nasal-borne *S. aureus* strain (ST89) with a transient intramammary infection in another cow on the farm. The aim of this study was to investigate the presence *S. aureus* in the nasal cavity of healthy cows. The study showed that the prevalence of *S.aureus* isolated from the nasal mucosa of cows was 7.6%. In Turkey, Ünal and Yıldırım (2010) found the isolation rate of *S. aureus* in nasal mucosa was 6.3% and 0.0% in small and medium-sized enterprises respectively. In the literature different isolation rates have been reported. Isolation rate of 5.66% has been reported in Brazil (Santos et al., 2020); 36.2% in India (Zehra et al., 2017),5.06% and 5.1% in two different studies conducted in Iran, (Rahimi et al., 2015; Dastmalchi Saei and Panahi, 2020), 15% in Algeria (Agabou et al., 2017), 24.7% in Greece (Papadopoulos et al., 2019) and 1.3% in Tunisia (Gharsa et al., 2015).

In the present study fifteen (78.95%) of 19 isolates were resistant to penicillin G. Tanrıbuyurdu (2014) reported that 74% of *S. aureus* isolates from cattle milk with mastitis in Turkey were resistant to penicillin G. Saidi et al. (2015) reported that all *S. aureus* isolates from bovine mastitis in Algeria were resistant to penicillin. Resistance was detected in *S. aureus* isolates from bovine mastitis at a rate of 40% in Pakistan (Khan et al., 2020), 83.3% in Egypt (Awad et al., 2017) and 91.3% in Iran (Dastmalchi Saei and Panahi, 2020) and from bovine raw milk samples at a rate of 83% in Brazil (Martini et al., 2017) and from cow nasal swabs at a rate of 33.3% in Iran (Dastmalchi Saei and Panahi, 2020). In the present study the *blaZ* gene, which encodes the production of ß-lactamase, was detected in 14 (93.33%) of 15 phenotypically penicillin-resistant *S. aureus* isolates. Interestingly, one isolate carrying the *mecA* gene was found negative for the *blaZ*. In studies conducted with different molecular methods in different countries, the detection rates of the *blaZ* gene in penicillin-resistant *S. aureus* isolates vary between 40% and 100% (Awad et al., 2017; Gao et al., 2012; Khan et al. 2020; Yang et al., 2015). The development of penicillin resistance adversely affects the treatment of mastitis cases.

In a study conducted in Turkey, the MRSA was isolated at a rate of 7.1% from the nasal swabs of cows and 3.3% from their milk (Hazimoğlu, 2011). Erdem and Türkyılmaz (2013) similarly isolated the MRSA from 7.14% of cow nasal swabs. The MRSA nasal carriage rate in cows has been reported as 1.1% in Greece (Papadopoulos et al., 2019). Zehra et al (2017) in India; Agabou et al. (2017) reported that the *mecA* gene was not found in bovine nasal *S.aureus* isolates in Algeria. It is difficult to determine methicillin resistance only phenotypically by the disk diffusion method, so it is very important to confirm the obtained findings by PCR as detection of *mecA* by PCR is considered the gold standard method. (Erdem and Türkyılmaz, 2013; Hazımoğlu, 2011; Martineau et al., 2000). In this study, MRSA was identified phenotypically based on the detection of cefoxitin resistance and genotypically by the presence of the *mecA* gene. From the total of 19 *S. aureus* isolates in this study, 10 (52,63%) of them were MRSA. Of these, five isolates (50%) were both phenotypically resistant to cefoxitin and carrying the *mecA* gene. In addition, it was observed that five isolates were sensitive to cefoxitin even though they carried the the *mecA* gene. The different results between these phenotypic and the genotypic ones may be caused by the differences in the regulation mechanisms of the *mecA* gene (Boubaker et al., 2004; Dilsiz, 2010) may be due to their inability to produce adequate PBP2' (Murakami et al., 1991).

Turkyilmaz et al. (2010) mentioned that 14 of the 16 MRSA strains isolated from bovine milk in Turkey, were hospital-acquired MRSA SCC*mec* type III (87.5%), which may be the result of a transmission from humans to animals. Some authors were reported that MRSA strains isolated from milk of cows with mastitis were SCC*mec* type IV or type V (Huber et al., 2010; Feßler, 2010; Center, 2012; Luini et al., 2015; Dastmalchi Saei and Panahi, 2020). Unlike these results, in Brazil, Rossi et al. (2019) reported that all MRSA isolates from bovine subclinical mastitis were classified as SCC*mec* Type I. In the present study, one isolate (10%) was SCC*mec* type I and the other isolates (90%) were SCC*mec* type IV. Based on these results, one of the isolates was interpreted as hospital-acquired MRSA (HA-MRSA) and 9 isolates as Community-acquired MRSA (CA-MRSA). CA-MRSA strains can cause foodborne illness as well as clinical cases ranging from skin infections to severe pneumoniae and sepsis (Mistry et al., 2016).

In this study, 13 *S. aureus* isolates were phenotypically tetracycline resistant. This resistance was determined to be 68.42%. Papadopoulos et al. (2019) in Greece and Mourabit et al. (2020) in Morocco, found tetracycline resistance with rates of 81.8% and 16.7%

respectively in *S. aureus* isolates from the nasal mucosa of ruminants. In Brazil, Santos et al. (2020) reported tetracycline resistance rate of 43.96% of *S. aureus* isolates from milk samples, while the same authors did not detect this resistance in nasal isolates. Tetracycline resistance has also been reported between 12.1% and 100% in cattle milk samples in Iran, Turkey, China, Brazil and Algeria respectively (Dastmalchi Sari and Panahi, 2020; Duyuk , 2018; Feng et al., 2016; Martini et al., 2017; Rossi et al., 2019; Saidi et al., 2015; Tanrıbuyurdu, 2014).

In the current study, the *tetK* gene was found in seven (53.85%) of 13 phenotypically tetracycline resistant isolates and *tetL* gene was detected in three (23.08%) isolates. The *tetM* and *tetO* genes were not detected in any of the 13 isolates. Rahimi et al. (2015) detected the *tetK* and *tetM* genes in two of four *S.aureus* isolates from nasal mucosa of cattle. In India, Zehra et al. (2017) found that the *tetK* gene was common among bovine nasal *S. aureus* strains. Martini et al. (2017) in Brazil, found the *tetK*, *tetL*, *tetM* and *tetO* genes with rates of 84%, 9%, 2% and 1% respectively in *S. aureus* isolates from bovine milk samples. In Poland, Chajęcka-Wierzchowska et al. (2014) reported the *tetM* and *tetL* genes, but less the *tetK* gene were found in most *S. aureus* isolates obtained from ready-to-eat foods.

When erythromycin resistance was examined in *S. aureus* strains isolated from nasal mucosa of animals, in Morocco, Mourabit et al (2020) were reported a resistance rate of 11.9% to erythromycin. The same results were reported in Iran (Dastmalchi Saei and Panahi, 2020) and in Greece (Papadopoulos et al., 2019), with rates of 11.1% and 18.2%, respectively. In contrast to other studies, in this study, erythromycin resistance was observed in the third place after penicillin and tetracycline with a rate of 63.16%. In this study, clindamycin resistance was found in 21.05% of *S. aureus* isolates. Using the D-test, five out of the 12 isolates resistant to erythromycin were found to have inducible clindamycin resistance. In this study, the *ermA*, *ermB* and *ermC* genes, that confer resistance to Macrolide-lincosamide-streptogramin B (MLSB) by changing the target region of the ribosome were investigated. The *ermC* gene was detected in five isolates (41.67%) of the twelve phenotypically erythromycin-resistant isolates and the *ermB* gene was detected in one isolate (8.33%) while the *ermA* gene was not detected. Different combinations of the *erm* genes were not detected in any of the *S. aureus* isolates. In Turkey, Türkyılmaz et al. (2010) found the *ermA* genes in nine of the erythromycin-resistant strains isolated from bovine milk, and both the *ermA* and *ermB* genes in seven, while the *ermC* gene was not detected in any of the isolates. Saidi et al. (2015) reported that all erythromycin-resistant *S.aureus* isolates from bovine mastitis in Algeria were negative for the *ermA* and *ermC* genes. In Iran, Rahimi et al. (2015) did not detect the *ermA* and *ermC* genes in *S. aureus* isolates from nasal swabs from cattle. In India, Zehra et al. (2017) showed the presence of the *ermB* gene among bovine nasal *S.aureus* isolates, while the *ermA* or the *ermC* genes were not detected in any of them.

In previous studies, *S. aureus* strains isolated from bovine mastitis were found to be susceptible to enrofloxacin (Güler et al., 2005; Nunes et al., 2007). In India, Das et al. (2015) reported that *S.aureus* isolates from bovine milk with subclinical mastitis showed the highest sensitivity (64%) to enrofloxacin. In this study, all isolates were found to be susceptible to enrofloxacin. These high levels of susceptibility to enrofloxacin may be due to its infrequent use in cattle for intramammary or systemic administration for the treatment of mastitis (Nunes et al., 2007; Russi et al., 2008).

Panton-Valentine leucocidin, a potent cytotoxin of *S. aureus*, is a virulence factor commonly found in community-acquired CA- MRSA. In this study, the *pvl* gene was not detected in any of the MSSA and MRSA isolates. Ünal (2013) detected the *pvl* gene in 6.6% of *S.aureus* strains isolated from the milk of cows with subclinical mastitis. Hazimoğlu (2011) found that one MRSA strain from bovine milk was *pvl* positive. The *pvl* gene was not detected in any of the *S.aureus* strains isolated from nasal swabs and mastitis milk samples of cows in studies conducted in Iran (Dastmalchi Saei and Panahi, 2020), from bovine Mastitis (Prashanth and Rao, 2011) and bovine nares (Zehra et al., 2017) in India and from raw milk of dairy cattle in Greece (Papadopoulos et al., 2019). The *pvl* gene was not detected in any of the MRSA strains isolated from cow's milk and cow's nasal swabs in Turkey (Erdem and Türkyılmaz, 2013) and mastitis milk in Switzerland (Huber et al., 2010) and Germany (Feßler, 2010).

CONCLUSION

In this study, *S. aureus* was isolated from nasal mucosa from 7.6% of healthy cattle. The isolates showed high resistance to penicillin; tetracycline;

erythromycin; doxycycline; cefaperazone; cefoxitin; ceftiofur and clindamycin. The most common genes that detected in the isolates were the *blaZ*, *mecA*, *tetL*, *tetK, ermC* and *ermB* genes. Methicillin resistance was detected in 52.63% of the isolates despite the low rate of *S.aureus* isolation and this result should not be ignored in terms of MRSA carriage in healthy animals. This situation highlights that healthy cows may harbor *S.aureus* isolates resistant to antibiotics and which may pose a significant risk for public health.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest that could have appeared to influence the work reported in this paper.

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