

Journal of the Hellenic Veterinary Medical Society

Vol 75, No 2 (2024)



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

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

doi: [10.12681/jhvms.33318](https://doi.org/10.12681/jhvms.33318)

Copyright © 2024, H Abughonem, B Halac, AI Kekec, B Maslak, AF Bağcigil



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

To cite this article:

Abughonem, H., Halac, B., Kekec, A., Maslak, B., & Bağcigil, A. (2024). Nasal carriage, microbial resistance and genetic characterization of *Staphylococcus aureus* in cows in Turkey. *Journal of the Hellenic Veterinary Medical Society*, 75(2), 7291–7300. <https://doi.org/10.12681/jhvms.33318> (Original work published July 10, 2024)

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

H. Abughonem^{id}, B. Halac^{id}, A.I. Kecec^{id}, B. Maslak^{id}, A.F. Bağcigil^{id}

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 SCCmec 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

Date of initial submission: 30-01-2023
Date of acceptance: 20-11-2023

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- Cerrah-

paşa, 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 aerobically. After incubation period, an equal volume (400:400 μ l) 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 μ g), ceftiofur (30 μ g), cefoperazone (30 μ g), gentamicin (10 μ g), clindamycin (2 μ g), erythromycin (15 μ g), enrofloxacin (5 μ g), trimethoprim/sulfamethoxazole (1.25 / 23.75 μ g), tetracycline (30 μ g), chloramphenicol (30 μ g) and penicillin-novobiocin (10 IU /30 μ g) and doxycycline (30 μ g), quinupristin-dalfopristin (15 μ g) and linezolid (30 μ g). 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, iso-

lates 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 <i>S. aureus</i> isolates	Codes of <i>S. aureus</i> 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 30 sec; 72°C 1.5 min)	Brakstad et al., 1992
NUC R	5' AGC CAA GCC TTG ACG AAC TAA AGC 3'			
<i>mecA1</i>	5' TGC TAT CCA CCC TCA AAC AGG 3'	286 bp	35 (94°C 2 min; 57°C 1 min; 72°C 3 min)	Kondo et al., 2007
<i>mecA2</i>	5' AAC GTT GTA ACC ACC CCA AGA 3'			
<i>blaZ 1</i>	5' ACT TCA ACA CCT GCT GCT TTC 3'	173 bp	35 (94°C 30 sec; 58°C 30 sec; 72°C 30 sec)	Martineau et al., 2000
<i>blaZ 2</i>	5' TGA CCA CTT TTA TCA GCA ACC 3'			
<i>ermA F</i>	5' GTT CAA GAA CAA TCA ATA CAG AG 3'	421 bp	30 (94°C 30 sec; 52°C 30 sec; 72°C 1 min)	Lina et al., 1999
<i>ermA R</i>	5' GGA TCA GGA AAA GGA CAT TTT AC 3'			
<i>ermB F</i>	5' CCG TTT ACG AAA TTG GAA CAG GTA AAG GGC 3'	359 bp	30 (94°C 30 sec; 55°C 30 sec; 72°C 1 min)	
<i>ermB R</i>	5' GAA TCG AGA CTT GAG TGT GC 3'			
<i>ermC F</i>	5' GCT AAT ATT GTT TAA ATC GTC AAT TCC 3'	572 bp	30 (94°C 30 sec; 52°C 30 sec; 72°C 1 min)	
<i>ermC R</i>	5' GGA TCA GGA AAA GGA CAT TTT AC 3'			
<i>tetL F</i>	5' GTT GCG CGC TAT ATT CCA AA 3'	788 bp	30 (95°C 30 sec; 55°C 30 sec; 72°C 30 sec)	Kim et al., 2005
<i>tetL R</i>	5' TTA AGC AAA CTC ATT CCA GC 3'			
<i>tetM F</i>	5' GTT AAA TAG TGT TCT TGG AG 3'	647 bp	30 (95°C 30 sec; 55°C 30 sec; 72°C 30 sec)	
<i>tetM R</i>	5' CTA AGA TAT GGC TCT AAC AA 3'			
<i>tetK F</i>	5' TTA GGT GAA GGG TTA GGT CC 3'	718 bp	30 (95°C 30 sec; 55°C 30 sec; 72°C 30 sec)	
<i>tetK R</i>	5' GCA AAC TCA TTC CAG AAG CA 3'			
<i>tetO F</i>	5' GAT GGC ATA CAG GCA CAG AC 3'	643 bp	30 (95°C 30 sec; 55°C 30 sec; 72°C 30 sec)	
<i>tetO R</i>	5' CAA TAT CAC CAG AGC AGG CT 3'			
<i>pvl F</i>	5' ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A 3'	433 bp	30 (94°C 30 sec; 55°C 30 sec; 72°C 1 min)	Lina et al., 1999
<i>pvl R</i>	5' GCA TCA AST GTA TTG GAT AGC AAA AGC 3'			

Table 3: Primers and protocol used to determine SCCmec 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
a3	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'									
5RmecA	5' TAT ACC AAA CCC GAC AAC TAC 3'	359 bp	<i>mecA</i> - 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 re-

sistance 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: cefepime, QD15: quinupristin-dalfopristin, LNZ30: linezolid, DO30: doxycycline

that were phenotypically methicillin-susceptible. As a result of the SCCmec 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 SCCmec Type IV. A band at 415 bp was observed in one of 10 isolates with *mecA* gene and it was evaluated as SCCmec 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* genes 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: ceftiofur

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

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

*= 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: ceftiofur, CFP30: cefaperazone, QD15: quiniupristin 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. Tanribuyurdu (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 β -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 (Hazımoğlu, 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 *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 SCCmec 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 SCCmec 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 SCCmec Type I. In the present study, one isolate (10%) was SCCmec type I and the other isolates (90%) were SCCmec 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; ceftiofur; 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.

Acknowledgements

This study is a part of Master Thesis study titled “Characterization of *Staphylococcus aureus* Strains Isolated from Nasal Mucosa of Cows” by Veterinarian Hager ABUGHONEM

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.

REFERENCES

- Agabou A, Ouchenane Z, Ngba Essebe C, Khemissi S, Chehboub M T E, Chehboub I B, et al (2017). Emergence of nasal carriage of ST80 and ST152 *pvl*+ *Staphylococcus aureus* isolates from livestock in Algeria. *Toxins* 9(10): 303.
- Awad A, Ramadan H, Nasr S, Ateya A, Atwa S (2017). Genetic Characterization, Antimicrobial Resistance Patterns and Virulence Determinants of *Staphylococcus aureus* Isolated from Bovine Mastitis. *Pak. J. Biol. Sci. PJBS* 20(6): 298-305.
- Bagcigil FA, Moodley A, Baptiste KE, Jensen VF, Guardabassi L (2007). Occurrence, species distribution, antimicrobial resistance and clonality of methicillin- and erythromycin resistant staphylococci in the nasal cavity of domestic animals. *Vet. Microbiol.* 121 (3-4): 307-315.
- Ben Slama K, Gharsa H, Klibi N, Jouini A, Lozano C, Gómez-Sanz E, et al (2011). Nasal carriage of *Staphylococcus aureus* in healthy humans with different levels of contact with animals in Tunisia: genetic lineages, methicillin resistance, and virulence factors. *Eur. J. Clin. Microbiol. Infect. Dis.* 30(4): 499-508.
- Boubaker IBB, Abbes RB, Abdallah HB, Mamlouk K, Mahjoubi F, Kamoun A (2004). Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant *Staphylococcus aureus*. *Clin. Microbiol. Infect.* 10(8): 762-765.
- Boye K, Bartels MD, Andersen IS, Möller J A, Westh H (2007). A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I-V. *Clin. Microbiol. Infect.* 13(7): 725-727.
- Brakstad OG, Aasbakk K, Maeland JA (1992). Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. *J. Clin. Microbiol.* 30(7): 1654-1660.
- Center SI (2012). Occurrence of methicillin-resistant *Staphylococcus aureus* in milk samples from Serbian cows with subclinical mastitis. *Afr. J. Microbiol. Res.* 6(29): 5887-5889.
- Chajęcka-Wierzchowska W, Zadernowska A, Nalepa B, et al (2014). Retail ready-to-eat food as a potential vehicle for *Staphylococcus* spp. harboring antibiotic resistance genes. *J. Food Prot.* 77(6): 993-998.
- CLSI/NCCLS Document M100-ED28 (2018). Clinical and Laboratory Standards Institute/NCCLS: Performance Standards for Antimicrobial Susceptibility Testing- Nineteenth Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.
- CLSI/NCCLS Document VET01S-ED5 (2020). Clinical and Laboratory Standards Institute/NCCLS: Performance Standards for Antimicrobial Susceptibility Testing- Nineteenth Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.
- Das G, Lalnunpuia C, Sarma K, Behera SK, Dutta TK, Bandyopadhyay S (2015). Prevalence of *Staphylococcus aureus* associated sub-clinical mastitis in crossbred cows in Mizoram. *Ruminant Science.* 4(2): 167-170.
- Dastmalchi Saei H, Panahi M (2020). Genotyping and antimicrobial resistance of *Staphylococcus aureus* isolates from dairy ruminants: differences in the distribution of clonal types between cattle and small ruminants. *Arch. Microbiol.* 202(1): 115-125.
- Dilsiz B (2010). subklinik mastitisli inek sütlerinden izole edilen staphylococcus aureus suşlarında metisilin direncinin fenotipik ve genotipik araştırılması. Yüksek lisans tezi, Mustafa Kemal Üniversitesi, Sağlık Bilimleri Enstitüsü, Hatay.
- Duyuk M (2018). Çiğ inek sütlerinde *Staphylococcus aureus* etkeninin izolasyonu, identifikasyonu ve antibiyotik duyarlılığının belirlenmesi. Yüksek lisans tezi, Adnan Menderes Üniversitesi, Sağlık Bilimleri Enstitüsü, Aydın.
- Erdem Z, Türkyılmaz S (2013). Molecular typing of methicillin resistant *Staphylococcus aureus* strains isolated from cows and farm workers. *Kafkas Univ Vet Fak Derg* 19(6): 963-968.
- Feßler A, Scott C, Kadlec K, Ehrlich R, Monecke S, Schwarz S (2010). Characterization of methicillin-resistant *Staphylococcus aureus* ST398 from cases of bovine mastitis. *J. Antimicrob. Chemother.* 65(4): 619-625.
- Feng YANG, Qi WANG, Wang XR, Ling WANG, et al (2016). Genetic characterization of antimicrobial resistance in *Staphylococcus aureus* isolated from bovine mastitis cases in Northwest China. *J. Integr. Agric.* 15(12): 2842-2847.
- Gao J, Ferreri M, Yu F, Liu X, Chen L, Su J, Han B (2012). Molecular types and antibiotic resistance of *Staphylococcus aureus* isolates from bovine mastitis in a single herd in China. *Vet. J.* 192(3): 550-552.
- Gharsa H, Sallem RB, Slama KB, Gómez-Sanz E, Lozano C, et al (2012). High diversity of genetic lineages and virulence genes in nasal *Staphylococcus aureus* isolates from donkeys destined to food consumption in Tunisia with predominance of the ruminant associated CC133 lineage. *BMC Vet. Res.* 8(1): 203.
- Gharsa H, Ben Slama K, Gomez-Sanz E, Lozano C, Zarazaga M, et al (2015). Molecular characterization of *Staphylococcus aureus* from nasal samples of healthy farm animals and pets in Tunisia. *Vector Borne Zoonotic Dis.* 15:109-115.
- Güler L, Ok Ü, Gündüz K, Gülcü Y Hadimli HH (2005). Antimicrobial susceptibility and coagulase gene typing of *Staphylococcus aureus* isolated from bovine clinical mastitis cases in Turkey. *J. Dairy Sci.* 88(9): 3149-3154.
- Hazımoğlu Ş, (2011). *Staphylococcus aureus* suşlarında Pantón-Valentine Lökositiden (*pvl*) genlerinin araştırılması. doktora tezi, Adnan Menderes Üniversitesi Sağlık Bilimleri Enstitüsü, Aydın.
- Huber H, Koller S, Giezendanner N, Stephan R, Zweifel C (2010). Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. *Euro Surveill* 15(16): 19542.
- Kariyama R, Mitsuhashi R, Chow JW, Clewell DB, Kumon H (2000). Simple and reliable Multiplex-PCR assay for surveillance isolates of vancomycin-resistant enterococci. *J. Clin. Microbiol.* 38: 3092-3095.
- Kaynarca S (2009). sığır mastitislerinden izole edilen stafillokoklarda metisilin direnci ve slaym pozitifliği. Yüksek lisans tezi. Aydın Adnan Menderes Üniversitesi, Sağlık Bilimleri Enstitüsü, Aydın.
- Khan A, Firyal S, Khan I, Awan AR, Tayyab M, et al (2020). Phenotypic and genotypic characterization of beta-lactams resistant *Staphylococcus aureus* isolates from bovine mastitis and its zoonotic implications. *Pak. Vet. J.* 40(4): 523-526.
- Kim TJ, Na YR, Lee JI (2005). Investigations into the basis of chloramphenicol and tetracycline resistance in *Staphylococcus intermedius* isolates from cases of pyoderma in dogs. *J. Vet. Med Series B* 52(3): 119-124.
- Kondo Y, Ito T, Ma X X, Watanabe S, Kreiswirth B N, Etienne J, Hiramatsu K (2007). Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrob. Agents Chemother.* 51(1): 264-274.
- Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J (1999). Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. *Antimicrob. Agents Chemother.* 43(5): 1062-1066.
- Lina G, Piémont Y, Godail-Gamot F, et al (1999). Involvement of Pantón-Valentine Leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin. Infect. Dis.* 29:1128-32.
- Luini M, Cremonesi P, Magro G, Bianchini V, Minozzi G, Castiglioni B, Piccinini R (2015). Methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with low within-herd prevalence of intra-mammary infections in dairy cows: Genotyping of isolates. *Vet. Microbiol.* 178(3-4): 270-274.
- Martineau F, Picard FJ, Grenier L, Roy PH, Ouellette M, Bergeron M G (2000). Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in staphylococci isolated from patients infected after cardiac surgery. *J. Antimicrob. Chemother.* 46(4): 527-534.
- Martini CL, Lange CC, Brito MA, Ribeiro JB, Mendonça LC, Vaz E K (2017). Characterisation of penicillin and tetracycline resistance in *Staphylococcus aureus* isolated from bovine milk samples in Minas Gerais, Brazil. *J. Dairy Res.* 84(2): 202-205.

- Mistry H, Sharma P, Mahato S, Saravanan R, Kumar P A, Bhandari V (2016). Prevalence and characterization of oxacillin susceptible *mecA*-positive clinical isolates of *Staphylococcus aureus* causing bovine mastitis in India. *PLoS One*. 11(9): e0162256.
- Mourabit N, Arakrak A, Bakkali M, Zian Z, Bakkach J, Laglaoui A (2020). Nasal carriage of *Staphylococcus aureus* in farm animals and breeders in north of Morocco. *BMC Infect. Dis.* 20(1): 1-6.
- Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S (1991). Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J. Clin. Microbiol.* 29(10): 2240-2244.
- Nunes SF, Bexiga R, Cavaco LM, Vilela CL (2007). Antimicrobial susceptibility of Portuguese isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* in subclinical bovine mastitis. *J. Dairy Sci.* 90(7): 3242-3246.
- Papadopoulos P, Angelidis AS, Papadopoulos T, Kotzamanidis C, et al (2019). *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in bulk tank milk, livestock and dairy-farm personnel in north-central and north-eastern Greece: Prevalence, characterization and genetic relatedness. *Food Microbiol.* 84: 103249.
- Prashanth K, Rao KR (2011). Genotypic characterisation of *S. aureus* obtained from humans and bovine mastitis samples in India. *J. Glob. Infect. Dis.* 3(2):115-22.
- Rahimi H, Saei HD, Ahmadi M (2015). Nasal carriage of *Staphylococcus aureus*: Frequency and antibiotic resistance in healthy ruminants. *Jundishapur J. Microbiol.* 8(10).
- Rossi BF, Bonsaglia ECR, Castilho IG, Dantas STA, et al (2019) Genotyping of long term persistent *Staphylococcus aureus* in bovine subclinical mastitis. *Microb. Pathog.* 132: 45-50.
- Russi NB, Bantar C, Calvino LF (2008). Antimicrobial susceptibility of *Staphylococcus aureus* causing bovine mastitis in Argentine dairy herds. *Rev. Argent. Microbiol.* 40: 116-119.
- Saidi R, Cantekin Z, Khelef D, Ergün Y, Solmaz H, Kaidi R (2015). Antibiotic susceptibility and molecular identification of antibiotic resistance genes of staphylococci isolated from bovine mastitis in Algeria. *Kafkas Univ Vet Fak Derg* 21 (4): 513-520.
- Santos RP, Souza FN, Oliveira ACD, De Souza Filho AF, et al (2020). Molecular typing and antimicrobial susceptibility profile of *Staphylococcus aureus* isolates recovered from bovine mastitis and nasal samples. *Animals*, 10(11), 2143.
- Tanrıbuğurdu E (2014). Sığır mastitislerinden izole edilen *staphylococcus aureus* suşlarında biofilm oluşumu ve antibiyotiklere dirençliliğinin belirlenmesi. Yüksek lisans tezi, Aydın Adnan Menderes Üniversitesi, Aydın.
- Türkyılmaz S, Tekbıyık S, Oryasin E, Bozdoğan B (2010). Molecular epidemiology and antimicrobial resistance mechanisms of methicillin-resistant *Staphylococcus aureus* isolated from bovine milk. *Zoonoses Public Health* 57:197-203
- Ünal N, Yıldırım M (2010). İneklerin süt, meme başı derisi ve burun mukozalarından izole edilen stafilkok türlerinin antibiyotik direnç profilleri. *Kafkas Univ Vet Fak Derg* 16: 389-396.
- Ünal N (2013). Subklinik mastitisli inek sütlerinden izole edilen *Staphylococcus aureus* izolatlarında bazı toksin genleri ve metisilin direnç geninin araştırılması. *Ank. Univ. Vet. Fak. Derg.* 60: 21-26.
- Yang F, Wang Q, Wang X, Wang L, et al (2015). Prevalence of *blaZ* gene and other virulence genes in penicillin-resistant *Staphylococcus aureus* isolated from bovine mastitis cases in Gansu, China. *Turkish J. Vet. Anim. Sci.* 39(5): 634-636.
- Zehra A, Singh R, Kaur S, Gill JPS (2017). Molecular characterization of antibiotic-resistant *Staphylococcus aureus* from livestock (bovine and swine). *Vet. World* 10(6): 598.