Investigation of the gene carriage rates for *Staphylococcus aureus, mecA, vanA* and *nuc* genes in the nasal and milk specimens from the sheep caretakers with sheep

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SUMMARY

Methicillin-resistant *S. aureus* (MRSA) is an important pathogen that may cause serious infections in the humans and animals. The pathogenity of *Staphylococcus aureus* emerges associated with the factors such as antibiotic resistance, immune evasion, invasion capability and virulence. By the impairment of the immune system, *S. aureus*-borne skin and soft tissue infections as well as serious infections such as pneumonia, septicemia and osteomyelitis may develop in the human bodies. *S. aureus* is one of the most common causes of the intramammary infections (IMI) in the dairy ruminants. The present study aimed to identify the presence of *S. aureus*, *mecA* and *vanA* genes in humans and animals (sheep) in the rural corporations on commercial dairy sheep farms in Eastern Turkey. It was also targeted to evaluate *nuc* gene positivity of *Stapyhylococcus aureus* strains isolated from humans and animals. Totally 78 (12.7%) *S. aureus* strains were isolated and identified from 612 materials taken from the sheep caretakers (nasal swab: 204) and the sheep (204 specimens for nasal swab and 204 specimens for milk each from sheep). *S. aureus* was distributed in 27 (13.2%; 204), 16 (7.8%; 204) and 35 (17.2%; 204) of the nasal swab specimens taken from the sheep caretakers, the sheep and sheep's milk specimens, respectively. Antibiotic susceptibility testing of 78 *S. aureus* isolates performed by Vitek2 device revealed that the highest antibiotic resistance was against benzylpenicillin. Gene analysis for 12 MRSA strains isolated in the specimens of the sheep caretakers and sheep was performed by single-Polymerase Chain Reactions (sPCR) for detection of *mecA* and *vanA* genes. Twelve MRSA isolates were found positive for *mecA* gene carriage. On the other hand, 78 *S. aureus* isolates were not found to carry *vanA* gene. All of the 12 MRSA and 66 Methicilline-susceptible *S. aureus* (MSSA) isolates were found positive for *nuc* gene carriage. It was concluded that MRSA strains isolated from the sheep caretakers and sheep had impact on the public health and created at risk for food chain.

KEY WORDS

mecA, *vanA*, *nuc*, human, sheep.

INTRODUCTION

Staphylococcus aureus is an important pathogen that plays an important role in the infections that develop in the humans and animals[1]. The pathogenity of *S. aureus* emerges associated with the factors such as antibiotic resistance, immune evasion, invasion capability and virulence[2]. *S. aureus* have been reported to be colonized in the skin or nasopharyngeal regions of the healthy subjects at a rate of approximately 25-30%[3]. By the impairment of the immune system, *S. aureus*-borne skin and soft tissue infections as well as serious infections such as pneumonia, septicemia and osteomyelitis may develop in the human bodies[4]. *S. aureus* is one of the most common causes of the intramammary infections (IMI) in the dairy ruminants. The rates of clinical and subclinical mastitis infection in the sheep were identified as 5-11% and 0.22-2.06% in the sheep, respectively[5, 6]. Although, mammary is considered as an essential source of contamination with *S. aureus* for milk and dairy farm environment, other body regions (nasal cavity) may also play an important role[7].

Methicilline-resistant *Staphylococcus aureus* is defined as an important infectious agent for the hospital-acquired infections (Hospital-acquired MRSA; HA-MRSA) and an important pathogen in the community (Community-acquired MRSA; CA-MRSA)[8]. Different CA-MRSA clones (Livestock-associated MRSA; LA-MRSA) that created serious concerns in the humans exposed in the dairy environments have been identified in the different countries of the world[9]. It has been shown that LA-MRSA particularly infects dairy employers[10] and veterinary physicians[11] due to close contact. Several studies have reported the presence of MRSA in the regions of the Mediterranean countries (Greece, Spain and Italy) that sheep and goat milk as well as commercial dairy products are commonly used[12, 13]. The assessment of the risk size created by *mecA* and *vanA* gene carriage of *S. aureus* strains on public health is also considered to be important. It is particularly targeted to provide contribution to the scientific world on the fact that which treatment options should be developed in either human and veterinary medicine against particularly livestock-associated *S. aureus* strains by revealing their antibiotic resistance profiles[14]. The resistance profiles of *S. aureus* species against antibiotics

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create great concerns. Beta-lactams, macrolides and tetracyclines are commonly used in the treatment of *S. aureus*-associated infections[15]. However, the frequency of the multidrug resistant MRSA strains that show aminoglycoside and macrolide-lincosamide-streptogramin resistance reached very high rates[16]. The identification of medically important MRSA plays an important role in struggling with these resistance phenomena. MRSA emerges due to synthesis of PBP2a, $PBP2_{LGA}$ or PBP2 as the specific molecules determined by *mecA* gene[15, 17, 18]. Vancomycin is one of the primarily preferred antibiotics in the treatment of the MRSA infections[3]. However, the presence of Vancomycin Intermediate and Vancomycin Resistant *S. aureus* (VISA ve VRSA) clinical isolates posed serious public health concerns in the recent twenty years[3, 19].

Thermostable deoxyribonuclease (DNase) is a specific thermostable DNAse that breaks down DNA encoded by *nuc* gene. The *nuc* gene is a specific target of the PCR-based methods for the identification of *S. aureus*[20]. The *nuc* gene is one of the most commonly used indicators for differentiation of *S. aureus* from other *Staphylococcus* species and determination of its prevalence[21]. However, several PCR studies have reported that false MRSA-associated identification results due to the presence of *S. aureus* specific *nuc* gene were also obtained[22].

The present study aimed to identify presence and rate of *S. aureus* carriage detected in dairy sheep farms (nasal swab and milk specimens) and sheep caretakers (nasal swab specimens) in the Eastern Anatolia Region (Van Province) of Turkey. In this region, it is aimed to reveal the antibiotic resistance profile seen in *S. aureus* isolates isolated from humans and animals. Especially, it was aimed to reveal the prevalence of methicillin and vancomycin resistance *S. aureus* in sheep caretakers and dairy sheep farms. In addition, it was aimed to evaluate the risk posed by people associated with dairy sheep farms on public health.

MATERIALS AND METHODS

Isolation, Identification and Antibiogram Test for *S. aureus*

Totally 612 specimens were collected from the 204 different sheep caretaker and family members working in the care of sheeps (nasal swab specimens), both nasal swabs (n= 204) and milk specimens (n= 204) from the same of sheeps with mastitis out of 17 rural corporations (an average of 100 to 500 head sheeps) on commercial dairy sheep farms in Eastern Turkey. According to the anamnesis, sheep herds with previously experienced mastitis problems were included in the study. The milk specimens of the sheep were obtained by identification of clinical mastitis (38; 18.6%) and subclinical mastitis (166; 81.4%). In the flocks detected with clinical mastitis the milk specimens from the sheep with healthy appearance and positive CMT test result were evaluated. The isolation, identification and antibiotic susceptibility tests were performed in the Bacteriology Laboratory of The Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Van Yüzüncü Yıl University and the Bacteriology Laboratory of The Department of Medical Microbiology, Van Yüzüncü Yıl University Dursun Odabas Medical Center.

Swabs were used in obtaining nasal specimens from humans and sheep. Nasal swab specimens were directly taken into 5 ml Mueller-Hinton Broth. The milk specimens were also taken from the sheep simultaneously with obtaining nasal swab specimens. Nasal swab specimens were incubated at 37 °C for 18-24 hours in the incubator. The incubated swab and milk specimens were directly inoculated onto Baird Parker agar (including Egg Yolk Tellurite supplement) and incubated at 37 °C for 48 hours. The black colonies with a surrounding 2-5 mm clear field were assessed as suspected *S. aureus*. The catalase (H_2O_2) and Gram positive strains with coccus morphology that indicated β-hemolysis on blood agar were stored at -20 °C in the 10% Nutrient Broth for species identification and subsequently molecular characterization.

Species identification and antibiotic susceptibility tests were performed in the Bacteriology Laboratory of The Department of Medical Microbiology, Van Yüzüncü Yıl University. Initially, the strains stored at -20 °C in the 10% Nutrient Broth were inoculated onto 7% human blood TSA II and incubated at 37 °C for 24-48 hours. The pure colonies that grew in the 7% human blood TSA were used to prepare 0.5 McFarland bacterial suspension. Its identification and antibiogram test were performed in the Gram positive panels using The BD Phoenix automated microbiology system (Becton Dickinson, USA). *S. aureus* isolates were stored at -20 °C in the 10% Nutrient Broth for molecular characterization.

Molecular Characterization Results of *S. aureus* Isolates

The molecular characterization procedure of the isolated and identified *S. aureus* species by sPCR was carried out in Molecular Biology Laboratory of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy. For this purpose, *nuc* gene (279bp), *mecA* gene (310bp) and *vanA* gene (1032bp) carriage rates of *S. aureus* isolates were analyzed. The reference primers of the DNA amplicons analyzed in this study were presented in Table 1. DNA extraction was carried out in accordance with the procedure described in the G-spinTM Total DNA Extraction Mini Kit (Intronbio, KOREA).

The identification of *S. aureus nuc* gene was performed ac-

Table 1 - Reference primers used in the study.

Target	Primer	Sequence (5'-3')	Product size (bp)	Reference
nuc	Forward	GCGATT GAT GGT GAT ACG GTT	279	[20]
	Reverse	AGC CAA GCC TTG ACG AAC TAA AGC		
mecA	Forward	CCA ATT CCACAT TGT TTC GGT CAT A	310	[20]
	Reverse	GTA GAA ATG ACT GAA CGT CCG ATA A		
vanA	Forward	ATG AAT AGA ATA AAA GTT GC	1032	[22]
	Reverse	TCA CCC CTT TAA CGC TAA TA		

cording to the method reported by Sahebnasagh et al.[21]. PCR amplification mixture contained 5µl template DNA, 2 µl PCR Buffer (10X), 1 µl $MgCl_2$ (50 mM), 4 µl dNTPs (1 mM), 1 µl nuc1 and nuc2 primers (10 Pmol), 0.5 µl Taq DNA polymerase (5 U/µl) and 10.5 µl double distilled water to obtain a 25 µl final solution. PCR temperature cycling conditions were performed for totally 30 cycles at 94 °C for 5 min at initial denaturation stage, following 94 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min and 72 °C for 10 min at the final stage. Then, 5 µl PCR products were run on the 1.7% agarose gel electrophoresis including ethidium bromide (0.5 µg/ml) in the 1X TBE (Tris-HCL, Boric acid, EDTA) buffer at 80 V for 120 min. The bands were analyzed by Gel Logic 2200 Imaging System as the gel imaging system. *S. aureus* ATCC 29213 and PCR water were used as positive and negative controls, respectly.

The identification of *S. aureus mecA* gene was performed according to the method reported by Sahebnasagh et al.[21]. PCR amplification mixture contained 5 µl template DNA, 2µl PCR Buffer (10X), 1 µl $MgCl_2$ (50 mM), 4 µl dNTPs (1 mM), 2 µl mecA1 and mecA2 primers (10 Pmol), 1µl Taq DNA polymerase (5 U/µl) and 8 µl double distilled water to obtain a 25 µl final solution. PCR temperature cycling conditions were performed for totally 30 cycles at 94 °C for 5 min at initial denaturation stage, following 94 °C for 30 sec, 55 °C for 1 min, 72 °C for 30 sec and 72 °C for 5 min at the final stage. Then, 5 µl PCR products were run on the 2% agarose gel electrophoresis including ethidium bromide (0.5 µg/ml) in the 1X TBE (Tris-HCL, Boric acid, EDTA) buffer at 80 V for 120 min. The bands were analyzed by Gel Logic 2200 Imaging System as the gel imaging system. *S. aureus* ATCC 25923 and PCR water were used as positive and negative controls, respectly.

The identification of *S. aureus vanA* gene was performed according to the method reported by Saadat et al.[23]. PCR amplification mixture contained 2 µl template DNA, 2µl PCR Buffer (10X), 1 µl $MgCl_2$ (50 mM), 4 µl dNTPs (1 mM), 4 µl Forward and Reverse primers (10 Pmol), 0.25 µl Taq DNA polymerase (5 U/µl) and 2.75 µl double distilled water to obtain a 20 µl final solution. PCR temperature cycling conditions were performed for totally 30 cycles at 98 °C for 2 min at initial denaturation stage, following 98 °C for 10 sec, 50 °C for 1 min, 72 °C for 90 sec and 72 °C for 10 min at the final stage. Then, 5 µl PCR products were run on the 1.5% agarose gel electrophoresis including ethidium bromide (0.5 µg/ml) in the 1X TBE (Tris-HCL, Boric acid, EDTA) buffer at 100 V for 100 min. The bands were analyzed by Gel Logic 2200 Imaging System as the gel imaging system. Vancomycin-resistant Enterococcus ATCC 51299 and PCR water were used as positive and negative controls, respectly.

Statistical Analysis

Descriptive statistics for the studied variables (characteristics) were presented as count and percent. Proportions were compared with Z test or Fisher's Exact test for two proportions. Statistical significance levels was considered as 5% and MINITAB for windows (ver: 14) statistical program was used for all statistical computations.

Ethics Committee Approval

The human part of the study was carried out due to Approval of Clinical Researches Ethics Committee of Van Yüzüncü Yıl University Medical Faculty, Dated on 16th February 2018 and Decision Number: 2018/19. Animal part of the study was performed due to the Approval of Animal Experiments Local Ethics Committee of Van Yüzüncü Yıl University (decision number: 2018/01).

RESULTS

Isolation, Identification and Antibiogram Results of *S. aureus*

Totally 78 (12.7%) *S. aureus* strains were isolated from 612 materials taken from the sheep caretakers and sheep. *S. aureus* was identified in 27 (13.2%) of 204 nasal swab specimens taken from the sheep caretakers. Nasal swabs belonging to sheep caretakers from 17 different farms showed positivity in 6 (35.3%) different dairy sheep farms and 35 [17.2%; 11 (64.7%) out of 17 dairy sheep farms are positive] of the nasal swab specimens (n=204) and milk specimens (n=204) from the sheep, respectively. *S. aureus* was isolated in 5 [2.5%; 2 (11.8%) out of 17 dairy sheep farms are positive] of the 38 milk specimens from the sheep with clinical mastitis. On the other hand, *S. aureus* was identified in 30 [14.7%; 11 (64.7%) out of 17 dairy sheep farms are positive] of the 166 milk specimens from the sheep with subclinical mastitis (Table 2). *S. aureus* was found positive in both nasal and milk samples of three sheep with subclinical mastitis in a farm. MRSA colonization determined in both nasal and milk samples of a sheep. In addition, nasal MRSA presence was detected in one of the sheep caretakers at this farm. The antibiotic resistance profiles of the MRSA isolates obtained in the specimens of the sheep caretakers and sheeps were presented in Table 3. The 4 MRSA strains obtained from the sheep caretakers were found to manifest the highest antibiotic resistance against cefoxitin, benzylpenicillin, ampicillin, oxacillin and clindamycin (100%). These MRSA isolates were found to be susceptible to quinopristin/ dalfopristin, tigecycline and rifampin. It was exhibited that 2 MRSA strains isolated from the nasal specimens of the sheep demonstrated the highest antibiotic resistance against cefoxitin, benzylpenicillin, ampicillin, oxacillin, erythromycin and clindamycin (100%). It was identified that one MRSA isolated from the milk specimens of the sheep with clinical mastitis indicated the highest

Table 2 - The distribution of *S. aureus* and MRSA isolates in the specimens taken from the sheep caretakers and sheep.

Bacteria	Nasal swap of sheep caretakers (n=204) 1	Nasal swap of sheep (n=204) 2	Sheep milk (Clinical Mastitis) (n=38) 3	Sheep milk (Subclinical Mastitis) (n=166) 4	Sheep milk (Mastitis) (N=204) 5	$p_{(1-2)}$	$p_{(3-4)}$	$p_{(3-5)}$	$p_{(4-5)}$	$p_{(1-5)}$	$p_{(2-5)}$
S. aureus	27 (13,2%)	16 (7,8%)	5 (13.2%)	30 (18.1%)	35 (17.2%)	0,075	0,431	0,511	0,818	0,269	**0,004**
MRSA	4 (2%)	2 (1%)	1 (2,6%)	5 (3%)	6 (2.9%)	0,410	0,896	0,914	0,968	0,522	0,152

Table 3 - Antibiotic resistance profiles of MRSA isolates.

	Nasal S. aureus of the sheep caretaker (n=4)			Nasal S. aureus of sheep (n=2)			S. aureus of the sheep milk with Clinical mastitis (n=1)			S. aureus of the sheep milk with subclinical mastitis (n=5)		
Antibiotics	S	I	R	S	I	R	S	I	R	S	I	R
Cefoxitin Screening	-	-	4	-	-	2	-	-	1	-	-	5
Benzylpenicillin	-	-	4	-	-	2	-	-	1	-	-	5
Ampicillin	-	-	4	-	-	2	-	-	1	-	-	5
Oxacillin	-	-	4	-	-	2	-	-	1	-	-	5
Gentamycin	-	-	4	-	-	2	-	-	1	-	-	5
Ciprofloxacin	1	-	3	1	-	1	1	-	-	-	-	5
Levofloxacin	1	-	3	-	-	2	1	-	-	4	-	1
Moxifloxacin	2	-	2	1	-	1	1	-	-	1	-	4
Inducible Clindamycin resistance	-	-	4	-	-	2	-	-	1	-	-	5
Erythromycin	1	-	3	-	-	2	-	-	1	-	-	5
Clindamycin	-	-	4	-	-	2	-	-	1	-	-	5
Q/D*	4	-	-	1	-	1	1	-	-	2	-	3
Linezolid	2	-	2	1	-	1	1	-	-	-	-	5
Vancomycin	4	-	-	2	-	-	1	-	-	5	-	-
Tetracycline	2	-	2	2	-	-	-	-	1	-	-	5
Tigecycline	4	-	-	2	-	-	1	-	-	5	-	-
Nitrofurantoin	1	-	3	1	-	1	1	-	-	5	-	-
Rifampin	4	-	-	2	-	-	1	-	-	5	-	-
SXT*	1	-	3	2	-	-	1	-	-	-	-	5

*Q/D: Quinupristin/ Dalfopristin; SXT: Sulfamethoxazole/Trimethoprim

antibiotic resistance to cefoxitin, benzylpenicillin, ampicillin, oxacillin, gentamycin, erythromycin and clindamycin. This MRSA isolate was found to be susceptible to the other antibiotics. The 5 MRSA isolates of the sheep with subclinical mastitis indicated the highest antibiotic susceptible to tigecycline, nitrofurantoin and rifampin. The antibiotic resistance profiles of the *S. aureus* isolates obtained in the specimens of the sheep caretakers and sheep were presented in Table 4, respectively. The 27 *S. aureus* strains obtained from the sheep caretakers were found to manifest the highest antibiotic resistance against benzylpenicillin (17; 63%), ampicillin (13; 48%) and erythromycin (13; 48%). All the *S. aureus* isolates were found to be susceptible to vancomycin. It was exhibited that 16 *S. aureus* strains isolated from the nasal specimens of the sheep demonstrated the highest antibiotic resistance against benzylpenicillin (14; 87.5%) and ampicillin (11; 69%). All the *S. aureus* isolates were found be susceptible to tetracycline, tigecycline and rifampin. It was identified that 5 *S. aureus* isolates isolated from the milk specimens of the sheep with clinical mastitis indicated the highest antibiotic resistance to benzylpenicillin (5; 100%). It was also determined that 5 *S. aureus* isolates were highly susceptible to the other antibiotics. The 30 *S. aureus* isolates of the sheep with subclinical mastitis indicated the highest antibiotic resistance to benzylpenicillin (26; 87%) and gentamicin (19; 63%). All the 30 *S. aureus* isolates were detected to be susceptible to tigecycline and rifampin.

It was detected that 12 *S. aureus* [15.4%; 9 (52.9%) out of 17 dairy sheep farms are positive isolates] obtained in the specimens of the sheep caretakers and sheep were phenotypically MRSA. Of these 12 *S. aureus* isolates; 4 [33.3%; (4/ 17, 23.5%)], 2 [16.7%; (2/17, 11.8%)], and 6 [50%; (3/17, 17.6%)] were distributed in the nasal swab specimens of the sheep caretakers, nasal swabs and milk specimens of the sheeps, respectively.

Molecular Characterization Results of the *S. aureus* Isolates

Gene analysis for phenotypically 12 MRSA and 66 Methicillin-susceptible *S. aureus* (MSSA) strains isolated in the specimens of the sheep caretakers and sheep was performed by single-Polymerase Chain Reactions (sPCR) for detection of *mecA* and *vanA* genes. Twelve of the 78 *S. aureus* isolates were found positive for *mecA* gene carriage (Figure 1). No *mecA* gene was detected in 66 MSSA isolates. It was also revealed that 78 *S. aureus* isolates were negative for *vanA* gene carriage. All the 12 MRSA and 66 Methicilline-susceptible *S. aureus* (MSSA) isolates were found positive for *nuc* gene carriage (Figure 2).

The Statistical Analysis Results

As a result of statistical analysis, a significant difference was found between sheep nasal samples and *S. aureus* isolated from mastitis, but no difference was found between MRSA rates (Table 2). No statistically significant difference was detected between the rates of *S. aureus* and MRSA isolated from the nasal swab specimens of the sheep caretakers and sheep. No difference was found between the sheep with clinical and subclinical mastitis with respect to carriage of *S. aureus* and MRSA strains. It has been revealed that humans and animals carry the same risk

Table 4 - Antibiotic resistance profiles of *S. aureus* isolates.

	Nasal S. aureus of the sheep caretaker (n=27)			Nasal S. aureus of sheep (n=16)			S. aureus of the sheep milk with Clinical mastitis (n=5)			S. aureus of the sheep milk with subclinical mastitis (n=30)		
Antibiotics	S	I	R	S	I	R	S	I	R	S	I	R
Cefoxitin Screening	-	-	4	-	-	2	-	-	1	25	-	5
Benzylpenicillin	7	3	17	2	-	14	-	-	5	4	-	26
Ampicillin	14	-	13	5	-	11	3	-	2	13	-	17
Oxacillin	23	-	4	9	-	6	4	-	1	25	-	5
Gentamycin	15	-	12	12	-	4	3	-	2	11	-	19
Ciprofloxacin	21	-	6	13	-	3	5	-	-	14	-	16
Levofloxacin	20	-	7	14	-	2	5	-	-	16	-	14
Moxifloxacin	19	-	8	15	-	1	5	-	-	23	-	7
Inducible Clindamycin resistance	-	-	4	-	-	2	-	-	1	-	-	5
Erythromycin	14	-	13	11	-	5	4	-	1	13	-	17
Clindamycin	23	-	4	10	-	6	4	-	1	25	-	5
Q/D*	22	-	5	14	-	2	5	-	-	23	-	7
Linezolid	18	-	9	14	-	2	5	-	-	25	-	5
Vancomycin	27	-	-	16	-	-	5	-	-	30	-	-
Tetracycline	16	-	11	16	-	-	4	-	1	6	-	24
Tigecycline	24	-	3	16	-	-	5	-	-	30	-	-
Nitrofurantoin	22	-	5	13	-	3	5	-	-	30	-	-
Rifampin	27	-	-	16	-	-	5	-	-	30	-	-
SXT*	24	-	3	14	-	2	5	-	-	25	-	5

*Q/D: Quinupristin/Dalfopristin; SXT: Sulfamethoxazole/Trimethoprim

for exposure to *S. aureus* and MRSA in the sheep farms in Van Province.

DISCUSSION

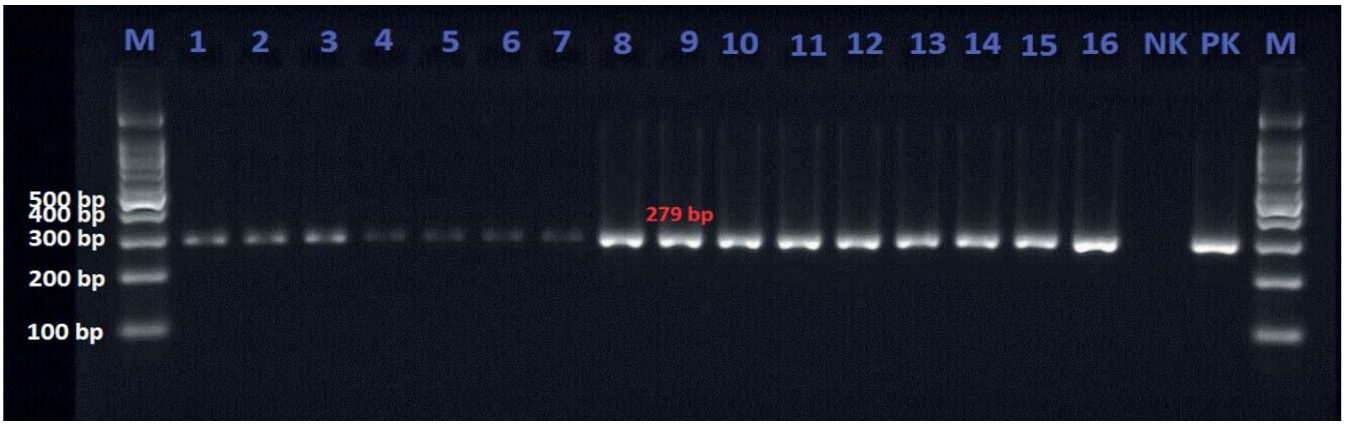
S. aureus may also cause severe invasive diseases (osteomyelitis and septic endocarditis), soft tissue and skin infections as well as it is a member of normal flora (nasal region) in humans[24]. Several studies have addressed livestock-associated nasal carriage of *S.aureus*[25, 26]. It has been exhibited in a study carried out in Latium (Italy) that 5 MRSA out of 12 *S. aureus* strains were isolated from the 14 swab specimens (nasal, oral and skin) taken from the sheep caretakers, between 2013 and 2015[25]. Carfora et al.[26] have determined three MRSA strains in the nasal specimen from the farm owners and from the hands and nasal of milker in Rome. In the present study, 4 MRSA out of 27 *S. aureus* strains were detected in 204 nasal swab specimens from the sheep caretakers. The rate of nasal *S. aureus* and MRSA was lower compared with the other studies[26, 27]. This difference was considered resulting from *S. aureus* colonization in the exposed environment, racial features, geographical residence and differences between the isolation methods.

Staphylococcus species are isolated from various body regions (nasal and mammary region) of the healthy ruminants. Mastitis, impetigo, abscess, vaginal infections, abort, osteomyelitis and rhinosinusitis may develop due to Staphylococcus[28]. Researchers have reported that nasal region is the most available site for the initial colonization of *S. aureus* in the small ruminants (sheep) and functions as the primary source in the infections[7]. The nasal swab specimens (n=204) of the sheep from the 17 different sheep farms of our regions indicated 16 [7.8%; 4 (23.5%) out of 17 dairy sheep farms are positive] *S. aureus* strains.

A study carried out in Algeria has reported that 45 (%36.6) *S. aureus* strains were isolated in the 123 milk specimens obtained from the animals with clinical mastitis[29]. Vasileiou et al.[28] have reported 26% subclinical mastitis caused by *S. aureus* in the sheep flocks. Compared with the other researches, the isolation rate of *S. aureus* from subclinical mastitis was lower in our region [28, 30]. While the rate of clinical mastitis is reported to be less than 5% in average sheep every year in the USA, clinical mastitis was detected in sheep at the same rate in our Region [31, 32]. This result was considered to be associated with *S. aureus* colonization in the sheep population, milking techniques (mechanical milking or hands) and stockyard environment (compliance to sheep capacity). Mechanical milking was performed in only two of the 17 different sheep farms in our region. *S. aureus* was found to be more prevalent in hand milked sheep farms.

There are differences between the protocols for treatment of MSSA and MRSA strains in the infections caused by *S.aureus* in the humans and animals. After development of methicillin resistance in the course of time, semi-synthetic penicillins such as oxacillin, nafcillin and cloxacillin have been introduced to clinical practice as the altenative treatment option[33]. Beside that, vancomycin is only one of the few antibiotics that MRSA strains are susceptible. Trimethoprim sulfamethoxazole (SXT), cip-

Figure 1 - PCR image of *mecA* gene of MRSA isolates isolated from the human and sheep specimens.

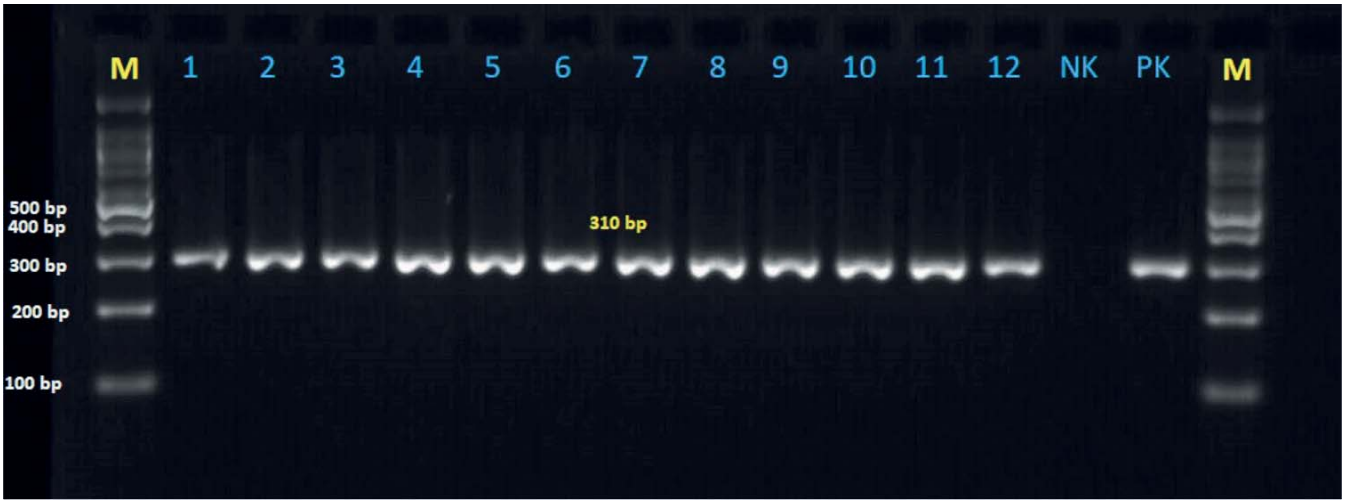


*1-4: nasal MRSA of the sheep caretaker; 5-6: Nasal MRSA of sheep; 7 Milk MRSA with clinical mastitis; 8-12: Milk MRSA with subclinical mastitis; NC: Negative Control; PC: Positive Control, *S. aureus* ATCC 25923; M: 100 bp DNA Marker.

rofloxacin, clindamycin, doxycycline and rifampin are the oldest antibiotics used in the treatment of particularly resistant *S. aureus* isolates[34]. However, it is known that different antibiotic resistance profiles developed by *S. aureus* strains significantly increased due to irregular and excessive use of the antibiotics in humans and animals. Ahmadi et al.[35] have reported in their study that 55 MRSA isolates isolated from human nasal specimens indicated susceptibility to mupirocin, gentamicin and fusidic acid. It has been stated in a study carried out in Italy that 3 MRSA isolates were isolated in the nasal swab specimens from the sheep caretakers. All these isolates were shown to be resistant to amoxicillin-clavulanic acid, cefotaxime, doxycycline, erythromycin, amoxicilline, penicilline and tetracycline. However, all MRSA isolates were detected to be susceptible to vancomycin[8]. Cong et al.[3] have noted that human VRSA strains demonstrate high antimicrobial susceptibility. It was determined in our study that 27 *S. aureus* strains obtained from the sheep caretakers manifested the highest antibiotic resistance to benzylpenicillin (17; 63%), ampicillin (13; 48%) and erythromycin (13; 48%). All the *S. aureus* isolates were found to be susceptible to vancomycin and rifampin. Compared with other studies, differences between the antibiotic resistance rates were considered to be associated with the exposed environment and *S. aureus* colonization.

Different studies have evaluated the antibiotic resistance rates of *S. aureus* isolates isolated from the nasal swabs of the sheep worldwide[36,37]. Abdel-Moein and Zaher[36] have suggested that all the isolates isolated from the nasal specimens of the sheep were resistant to penicilline, oxacilline ve cefoxitin. It has been determined in Tunisia that *S. aureus* strains isolated from the nasal specimens of the sheep exerted resistance to penicillin (8.8%), ciprofloxacin (4.4%), tobramycin (%2.2%) and tetracycline (2.2%)[37]. El-Deeb et al.[38] have demonstrated that antibiotic resistance rate of MRSA strains isolated from nasal specimens of the healthy animals in Saudi Arabia against penicillin, oxacillin and cefoxitin was 100%. It was exhibited in the present study that 16 *S. aureus* strains isolated from the nasal specimens of the sheep manifested the highest antibiotic resistance to benzylpenicillin (14; 87.5%) and ampicillin (11; 69%). All the *S. aureus* isolates were found be susceptible to vancomy-

Figure 2 - PCR image of *nuc* gene of *S. aureus* isolates isolated from the human and sheep specimens.



*1-4: nasal MRSA of the sheep caretaker; 5-6: Nasal MRSA of sheep; 7 Milk MRSA with clinical mastitis; 8-12: Milk MRSA with subclinical mastitis; 13-14; Nasal *S. aureus* of the sheep caretaker; 15-16: Milk *S. aureus* of the sheep with mastitis; NC: Negative Control; PC: Positive Control, *S. aureus* ATCC 29213; M: 100 bp DNA Marker.

cin, tetracycline, tigecycline and rifampin. It was concluded that performing screening tests widely is important since nasal *S. aureus* strains isolated from the sheep exhibited different antibiotic resistance rates. It has been reported that MSSA strains isolated from sheep milk specimens have shown high tetracycline resistance rates in Turkey[39]. We have monitored in our study that 5 *S. aureus* isolates isolated from milk of the sheep with clinical mastitis indicated the highest antibiotic resistance to benzylpenicillin (5; 100%). It was detected that those 5 *S. aureus* isolates demonstrated high susceptibility to other antibiotics. On the other side, 30 *S. aureus* isolates isolated from the milk of the sheep with subclinical mastitis presented the highest antibiotic resistance to benzylpenicillin (26; 87%) and gentamicin (19; 63%). All those 30 *S. aureus* isolates were susceptible to vancomycin, tigecycline, nitrofurantoin and rifampin. It was concluded that antibiotics (particularly penicillin) used for the infections developing in the sheep and exposed resistant *S. aureus* colonization in the environment played an important role in the increased antibiotic resistance in our region.

Methicillin has initially emerged as a great therapeutic medication invention in 1957[3]. It was reported that prevalence of methicillin resistance raised over 40% in the invasive infections and asymptomatic colonized patients in the continent Europe. It is estimated that 30% and 1.5% of the population was colonized with MSSA and MRSA in the USA, respectively[40]. Macori et al.[25] have declared that 5 (41.7%) of 12 *S. aureus* isolates isolated from the owners and the employers of the sheep farms. Carfora et al.[26] have announced that they isolated 2 MRSA isolates in the nasal swab specimens from sheep caretakers. In the present study, 4 (2%) MRSA strains were isolated from the nasal specimens of the 204 sheep caretakers in 17 different sheep farms in our region. Nasal MRSA was not found very prevalent in the sheep caretakers. It was concluded that risk for contamination of MRSA with humans who live in the rural or urban area through sheep caretakers was very low.

A study has analyzed the public health risk for MRSA infection resulting from the contact between animals and humans[36, 40]. It was reported in a study carried out in Egypt that 2 (3.8%) MRSA isolates were isolated from nasal specimens of 52 sheep[36]. Researches have demonstrated that MRSA strains were common in the milk specimens of the sheep with mastitis although methicillin was not used in the treatment[8]. In our study, 2% MRSA (8/408) was detected in the nasal and milk specimens taken from the sheep. The discussed studies have used *mecA* gene as the target site for the identification of MRSA. In also our study, all the MRSA isolates were *mecA* positive. It was determined that impact of MRSA was low in the sheep in our region. While MRSA strains isolated from sheep with mastitis are resistant to many antibiotics, MSSA strains show sensitivity to many antibiotics[41]. In our region, it was predicted that the risk of MRSA will be low in antibiotic applications for the treatment of mastitis infections in sheep.

Increased rates of vancomycin resistance is encountered in *S. aureus* strains. Since vancomycin-resistant *S. aureus* (VRSA) was initially reported in 2002, 52 isolates have been declared worldwide[3]. In our study, vancomycin resistance was enountered in none of the 78 *S. aureus* strains isolated in the specimens of the sheep caretakers and sheep. It was determined that humans carry no risk for VRSA through sheep farms in our region. However, it is concluded that taking the prevalence of vancomycin resistant Enterococcus (VRE) into account before administration of vancomycin for the human and animal infections.

Hirotaki et al.[42] have stated that multiplex PCR method targeting nuc gene is 100% sensitive and 100% specific in the diagnosis of *Staphylococcus* species isolated from humans and various animals. It was noted in a study from Egypt that 2 MRSA strains isolated in the nasal specimens of 52 sheep were positive for carriage of *nuc* gene[36]. In our study, *nuc* gene analysis of 78 *S. aureus* strains was performed by sPCR ile yapıldı. Gene analysis result indicated that all the 12 MRSA and 66 MSSA isolates were positive for the carriage of *nuc* gene. We have determined that our study method was 100% sensitive in the identification of MRSA and MSSA isolates. However, *nuc* gene region encoded a weak DNA band in the MRSA under the same PCR conditions.

CONCLUSIONS

As a consequence, *S. aureus* was found to have a low colonization prevalence between the sheep caretakers and sheep in our high-capacity rural corporations dealing with sheep breed. It was monitored that isolated and identified *S. aureus* strains indicated a low MRSA and no VRSA prevalence. It has been suggested that monitoring potential risk of MRSA strains would be important even though they were encountered with low levels. MRSA and MSSA strains were found to present a high antibiotic resistance to penicillin in particularly conventional treatment protocols. Therefore, it was determined that administration of the antibiotics in the treatment of *S. aureus*-associated infections in either humans and animals after performing antibiotic susceptibility tests would be critical. We have concluded that our findings obtained in our study would contribute to the antibiotic treatment protocol in the treatment of the hospital-, community and animal-acquired infections caused by *S. aureus* in our region.

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