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Research article

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF MACROLIDE-LINCOSAMIDE-STREPTOGRAMIN RESISTANCE IN *Staphylococcus aureus* ISOLATES FROM BOVINE AND HUMAN

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In this study, penicillin, oxacillin, and macrolide-lincosamide-streptogramin (MLS) resistance in *S. aureus* strains that were isolated from bovine mastitis cases, and human patients were investigated. Inducible clindamycin resistance (iML) was not found in 30 bovine isolates, while it was detected in 3 (10%) of 30 human isolates. MIC_{90} values of penicillin, oxacillin and macrolide-lincosamides (ML) were 2, 0.19, >256 μg/ml in bovine isolates and were 3, 3 and 0.19-1.5 μg/ml in human isolates, respectively. Streptogramin resistance was not found in both bovine and human isolates. Although the *mecA* gene was detected in all of the oxacillin resistant isolates, *blaZ* gene could not be detected in penicillin resistant isolates. The *erm*(*B*) gene was detected in 5 (38.6%) of 13 ML-resistant bovine isolates, and the *mph*(*C*) gene was detected in 2 (66.66%) of 3 human isolates. As a result, resistance to penicillin and oxacillin was found to be higher in human *S. aureus* isolates, while ML resistance was found to be higher in bovine isolates in this investigation. It was concluded that the presence of genes in extra-chromosomal elements associated to penicillin and macrolide resistance should be investigated. The data obtained from this study will contribute to the studies on antimicrobial susceptibility in the field of human and veterinary medicine.

Keywords: bovine, human, mastitis, MLS resistance, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus (*S. aureus*) causes mastitis in cattle, sheep and goats; botryomycosis and impetigo in pigs; septicemia and ulcerative pododermatitis in poultry; and purulent wound infections in cats and dogs [1]. In humans, the agent is responsible

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for infections such as folliculitis, impetigo, wound infections, scalded skin syndrome, pneumonia, endocarditis, joint infections, toxic shock syndrome, and food poisoning [2]. *S. aureus* is part of the natural microbiota of the nasal cavity of both humans and animals, and 20% of humans are nasal carriers of this bacteria. *S. aureus* strains are mostly transmitted in humans and animals by direct contact with contaminated materials and indirectly consuming contaminated food [1].

Following the discovery of penicillin, Kirby [3] reported that antimicrobial resistance began to develop in staphylococcal isolates, and resistance to penicillin reached a very high level in *S. aureus* strains isolated from humans all over the world in the 1960s [4]. β-lactamase enzyme encoded by the *blaZ* gene causes penicillin resistance in *S. aureus* strains. This gene is transmitted by transposons and can be found in the plasmids or chromosomes [5]. On the other hand, methicillin is an antibiotic that is resistant to the penicillinase enzyme synthesized by penicillin-resistant *S. aureus* strains. It shows its effect by blocking penicillin binding proteins (PBP) which are necessary for cell wall synthesis in bacteria [2]. It has been reported that methicillin-resistant *S. aureus* (MRSA) isolates can synthesize PBP2a, for which β-lactam antibiotics have less affinity, and thus methicillin resistance develops in these isolates [6]. It has been also reported that the *mecA* gene which encodes PBP2a may have been horizontally transferred to *S. aureus* from *S. sciuri* and *S. fleuretti* isolates [7]. Also, *S. aureus* genes such as *fem*, *aux* and *hmt* can cause resistance by blocking cell wall synthesis in the presence of methicillin or other β-lactam antibiotics [8]. In addition, point mutations occurring in *PBP2a* are one of the other mechanisms responsible for methicillin resistance [9]. MRSA has been an important health problem in human medicine for many years. Grundmann et al. [10] have predicted that between 2 million and 53 million people are carriers of MRSA worldwide. It has been also reported that 80,000 people in the USA and 150,000 people in Europe are infected with MRSA every year [11,12]. In animals, the MRSA isolate was first isolated from a milk sample originating from cattle with clinical mastitits on a farm in Belgium [13].

In Türkiye, the most widely used macrolide antibiotics in human and veterinary medicine are erythromycin, roxithromycin, azithromycin, clarithromycin, spiramycin and tylosin [14]. These antimicrobials are preferred for the treatment of infections caused by β-lactam resistant staphylococci [2]. In bacterial agents, three mechanisms play a role in the macrolide resistance [15,16]. The most basic mechanism is methylation of the 23s rRNA region encoded by *erm* genes. This resistance is observed as inducible (iML) and constitutive (cML) resistance, phenotypically [17]. Another mechanism that causes macrolide resistance is the active pump (efflux pump) system. The efflux pump is encoded by the *msr*(*A*) and *msr*(*B*) genes and is involved in the removal of erythromycin and streptogramin B from the bacterial cell [18]. The third mechanism that causes macrolide resistance is inactivation of the antibiotic, and more than one gene play a role in this mechanism. Lincosamide nucleotidyltransferase, which causes lincosamide resistance, is encoded by the *lnu*(*A*) and *lnu*(*B*) genes. These genes have been reported to be determined in coagulase negative staphylococci (CNS), *S. aureus*

and *Enterococcus faecium* isolates [19]. It is known that the esterase enzyme, encoded by the *ere*(*A*) and *ere*(*B*) genes, hydrolyzes the lactone ring in macrolide antibiotics [20].

Streptogramin A (quinupristin) and streptogramin B (dalfopristin), that have synergistic effects, inhibit protein synthesis by binding to the 50S ribosomal subunit in bacterial cells. These antimicrobial agents have been recommended for use in the treatment of infections caused by MRSA isolates since 1999. The development of resistance to streptogramin A in *S. aureus* isolates is considered as a phenotypic indicator of resistance to both active substances [2]. However, it has been reported that the *vga*(*A*) and *vga*(*B*) genes play a role in streptogramin A resistance [20].

Macrolide, lincosamide and streptogramin (MLS) group antibiotics are frequently used in the treatment of infections caused by β-lactam-resistant staphylococcal isolates [21]. High-level of β-lactam resistance has been found in staphylococcal strains in studies conducted in Türkiye [22-24]. However, there are a limited number of studies investigating the MIC values of penicillin, oxacillin (methicillin) and macrolide-lincosamide-streptogramin group antimicrobial agents and resistance genes in *S. aureus* strains isolated from bovine mastitis. In this context, in a study, conducted in Türkiye, investigating both phenotypic and genotypic characterization of ML resistance, it was reported that macrolide-lincosamide (ML) resistance was detected in 25% of *S. aureus* strains isolated from milk samples of cows with subclinical mastitis [21]. In this study, it was aimed to investigate penicillin, oxacillin, and MLS resistance in *S. aureus* isolates isolated from bovine mastitis and human by phenotypic and genotypic methods.

MATERIAL AND METHODS

Material

Between 2009 and 2020, 30 *S. aureus* strains were isolated from milk samples of cattle with mastitis and were brought to Van Yüzüncü Yıl University Faculty of Veterinary Medicine Department of Microbiology in Van, Türkiye for routine bacteriological examination. Also, between 2018-2020, 30 *S. aureus* strains were isolated from routine microbiological examination of clinical samples (wound= 12, blood= 9, nasal swab= 7, abscess= 1, urine sample= 1) taken from patients with clinical symptoms of septicemia, respiratory tract disease, urinary tract infection and wound infection at Prof. Dr. Cemil Taşcıoğlu City Hospital Department of Microbiology in İstanbul, Türkiye. In the study, the isolates that were isolated from the samples which were taken before antimicrobial treatment were chosen. The isolates were identified according to Gram stain, microscopic morphology, catalase and coagulase (Staphy Latex Test, Microgen, Englan) reaction and stored in brain heart infusion broth with 20% glycerin at -80°C until examined.

Method

DNA extraction: A commercial DNA extraction kit (GeneAll, Exgene^{TM} Clinic SV Mini, 108.101, Korea) was used to obtain genomic DNA from the isolates. Genomic DNA samples were stored at -20°C until used as templates for the identification of the isolates and determination of antimicrobial resistance genes by PCR.

Identification by PCR: The identification of the isolates was performed by PCR using *Coa* gene specific primers of *S. aureus* reported by Schmitz et al. [25] (Table 1).

Amplification: Commercial mastermix (Abm® 2X PCR Taq Plus Mastermix) was used for the preparation of PCR mix which was used for the identification of the isolates and determination of resistance genes in the isolates by PCR. PCR mixture consisted of 12.5 µl of mastermix, 5 µl of genomic DNA and 1.5 µl of primers. Total volume was completed to 25 µl with PCR water. The applied amplification protocol is shown in Table 1. PCR conditions were modified according to the recommendations of manufacturers of mastermix and primers.

Electrophoresis: Amplicons were electrophoresed in a 1.5% agarose gel at 80 V for 1.5 h and visualized in a gel imaging system.

Determination of Antimicrobial Susceptibility

Phenotypic characterization of macrolide-lincosamide-streptogramin resistance

Double-disk diffusion test was applied for the phenotypic characterization of MLS resistance [26]. Erthromycine (15 µg) (Oxoid, England) and clindamycin (2 µg) (Oxoid, England) discs were used in the test. The discs were placed at a distance of approximately 15-26 mm between each other on Mueller Hinton Agar (1.05437, Merck, Darmstadt, Germany) that was inoculated with the bacterial suspension. After an incubation period at 37°C for 16-18 hours, isolates that were resistant to erthromycin (zone diameter ≤13 mm), susceptible to clindamycin (zone diameter ≥21 mm) and formed a D shaped inhibition zone on the erthromycin facing side of the clindamycin disc, were characterized as iML. On the other hand, isolates resistant to erthromycin and clindamycin were characterized as cML, while isolates resistant to erthromycin, susceptible to clindamycin but did not form a D zone were considered resistant to macrolide antibiotics.

Determination of MIC values

MIC values of penicillin, oxacilin, erythromycin, azithromycin, spiramycin, clarithromycin, tilmicosin, clindamycin and quinupristin/dalfopristine (Liofilchem, Italy) were analyzed by E-test method. For this purpose, the evaluation criteria reported by CLSI [26] for penicillin, oxacilin, erythromycin, azithromycin, clarithromycin, clindamycin and pirlimycin, and by CLSI [27] for quinupristin/dalfopristin were used. The criteria, reported by Li et al. [28] (value for tylosin), were taken into account for determining the MIC values of spiramycin and tilmicosin.

Table 1. Primer sequences used for the detection of *S. aureus* isolates and antimicrobial resistance genes by PCR

Genes	Oligonucleotide sequences (5'-3')	Amplicon Size (bp)	Amplification (35 cycles) (Denaturation/ Annealing /Elongation)	Reference
Coa	**F:** GCTTCTCAATATGGTCCGAG **R:** CTTGTTGAATCTTGGTCTCTCGC	131	94°C -1 min/53°C - 1.5 min / 72°C - 1 min	[25]
Beta lactam				
blaZ	**F:** TAAGAGATTTGCCTATGCTT **R:** TTAAAGTCTTACCGAAAGCAG	377	94°C- 1 min /53°C'-1 min / 72°C -1 min	[29]
Methicillin				
mecA	**F:** GTGAAGATATACCAAGTGATT **R:** ATGCGCTATAGATTGAAAGGAT	147	94°C - 1 min /53°C -1 min / 72°C -1 min	[30]
Macrolide				
erm(A)	**F:** GTTCAAGAACAATCAATACAGAG **R:** GGATCAGGAAAAGGACATTTTAC	421	94°C - 1 min /56°C - 1 min / 72°C -1 min	[31]
erm(B)	**F:** CCGTTTACGAAATTGGAACAGGTAAAGGGC **R:** GAATCGAGACTTGAGTGTGC	359	94°C - 1 min /62°C - 1 min / 72°C -1 min	[31]
erm(C)	**F:** ATCTTTGAAATCGGCTCAGG **R:** CAAACCCGTATTCCACGATT	295	94°C - 1 min /55°C - 1 min / 72°C - 1 min	[32]
mph(A)	**F:** AACTGTACGCACTTGC **R:** GGTACTCTTCGTTACC	837	94°C - 1 min /50°C - 1 min / 72° - 1 min	[33]
mph(C)	**F:** ATGACTCGACATAATGAAAT **R:** CTACTCTTTCATACCTAACTC	900	94°C - 1 min /52°C - 1 min / 72°C - 1 min	[34]
ere(A)	**F:** AACACCCTGAACCCAAGGGACG **R:** CTTCACATCCGGATTCGCTCGA	420	94°C - 1 min /63°C - 1.5 min / 72°C - 1 min	[33]
ere(B)	**F:** AGAAATGGAGGTTCATACTTACCA **R:** CATATAATCATCACCAATGGCA	546	94°C - 1 min /57°C - 1 min / 72°C - 1 min	[33]
msr(A)	**F:** GGCACAATAAGAGTGTTTAAAGG **R:** AAGTTATATCATGAATAGATTGTCCTGTT	940	94°C - 1 min /57°C - 2 min / 72°C - 1 min	[31]
msr(B)	**F:** TATGATATCCATAATAATTATCCAATC **R:** AAGTTATATCATGAATAGATTGTCCTGTT	595	94°C - 1 min /57°C - 2 min / 72°C - 1 min	[31]
Lincosamide				
lnu(A)	**F:** GGTGGCTGGGGGGTAGATGTATTAACTGG **R:** CTTCTTTTGAAATACATGGTATTTTTCGATC	323	94°C - 1 min /66°C - 1 min / 72°C - 1 min	[31]
lnu(B)	**F:** CCTACCTATTGTTTGTGGAA **R:** ATAACGTTACTCTCCTATTC	944	94°C - 1 min /52°C - 1 min / 72°C - 1 min	[35]

Detection of genes associated with antimicrobial resistance

The presence of *blaZ* [29] and *mecA* [30] genes were investigated in penicillin and oxacillin resistant isolates, respectively, by PCR. In addition, *erm*(*A*) and *erm*(*B*) [31], *erm*(*C*) [32], *mph*(*A*) [33], *mph*(*C*) [34], *lnu*(*A*) [31], *lnu*(*B*) [35], *ere*(*A*) and *ere*(*B*) [33], *msr*(*A*) and *msr*(*B*) [31] genes were investigated for the genotypic characterization of MLS resistance by PCR using gene specific primers (Table 1). PCR mix was prepared as described in the identification process. The applied amplification protocol is shown in Table 1. PCR conditions were modified according to the recommendations of the manufacturers of mastermix and primers.

Statistical analysis

The relationship between *S. aureus* isolates isolated from human and bovine samples was determined by the Chi-Square statistical test (Minitab, Demo ver 16) method.

RESULTS

Determination of Antimicrobial Susceptibility

Phenotypic characterization of macrolide-lincosamide resistance

Thirteen (43.33%) of 30 *S. aureus* isolates obtained from cattle were found to be resistant to both erythromycin and clindamycin (cML). None of the isolates were characterized as iML by the double disc diffusion method (Table 2).

Table 2. Distribution of macrolide-lincosamide resistance in *S. aureus* isolates isolated from cattle and humans

Phenotip	Bovine isolates n (%)	Human isolates n (%)
iML (D-Zone$^+$)	0 (0)	3 (10)
cML	13 (43.33)	0 (0)
M (D-Zone$^-$)	0 (0)	2 (6.66)
E ve DA Susceptible	17 (56.66)	25 (83.33)
Total	30 (100)	30 (100)

iML: İnducible clindamycin (lincosamide) resistance
cML: Constitutive erythromycin (macrolide) ve clindamycin (lincosamide) resistance
M: Erythromycin (macrolide) resistance
E: Erythromycin DA: Clindamycin

On the other hand, only erythromycin (macrolide) resistance was detected in 2 (6.66%) of human isolates, while 25 (83.33%) isolates were found to be susceptible to both of erythromycin and clindamycin (Table 2). Also, iML resistance was detected in 3 (10%) of them (Figure 1).

Statistically, there was no significant relationship between human and animal isolates in terms of iML resistance ($p>0.05$).

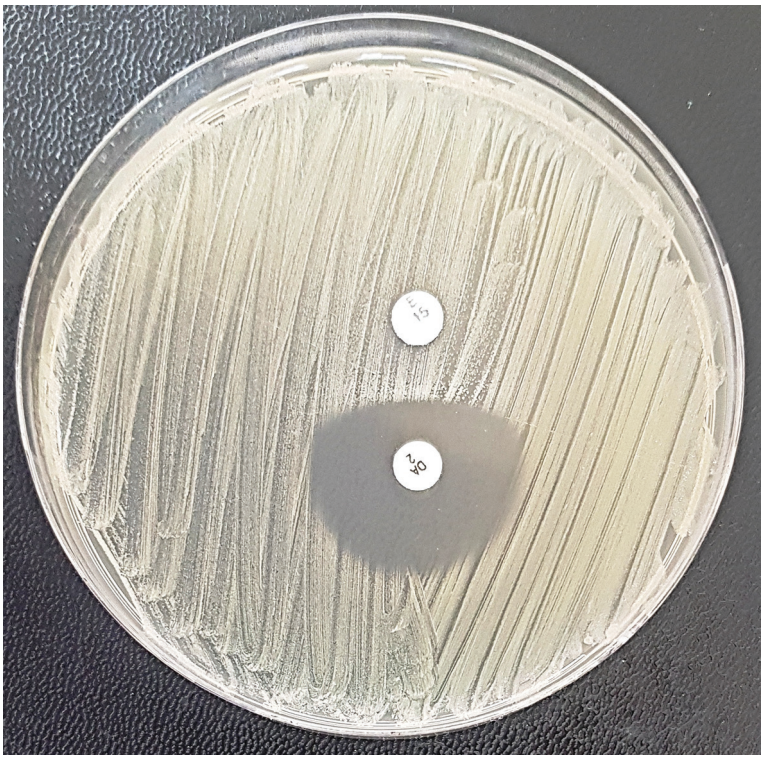

Figure 1. Determination of inducible clindamycin (iML) resistance by double disc method (D-Zone formation, E: erythromycin, DA: clindamycin).

Determination of MIC values

MIC values of penicillin, oxacillin, erythromycin, azithromycin, spiramycin, clarithromycin, tilmicosin, clindamycin, and quinupristin/dalfopristin were determined as 0.012-2, 0.047-0.19, 0.19->256, 0.38->256, 0.38->256, 0.125->256, 0.50-24, 0.064->256, 0.125-0.38 μg/ml, respectively in bovine isolates (Table 3). These values were 0.016->32, 0.047->256, 0.094-32, 0.25->256, 0.19-2, 0.064-32, 0.50-3, 0.125-0.19, 0.094-0.50 μg/ml in human isolates (Table 4).

Of the bovine isolates, 5 (16.66%) were resistant to penicillin whereas 13 (43.33%) of them were found to be resistant to erythromycin, azithromycin, spiramycin, clarithromycin and clindamycin. Only, one isolate was resistant to tilmicosin. Oxacillin and quinupristin/dalfopristine resistance was not detected in the isolates (Table 5). While macrolide-lincosamide resistance was detected in 13 (43.33%) of the isolates, penicillin and/or oxacillin resistance was not detected in these isolates.

Penicillin, oxacillin, clarithromycin, erythromycin and azithromycin resistance was found in 23 (76.66%), 2 (6.66%), 2 (6.66%), 3 (10%) and 3 (10%) human isolates, respectively. Spiramycin, tilmicosin, clindamycin, and quinupristin/dalfopristin

Table 3. Distribution of MIC values of antimicrobial agents in bovine *S. aureus* isolates (n=30)

Antibiotic	MIC (µg/ml)																														
	0.012	**0.016**	**0.023**	**0.032**	**0.047**	**0.064**	**0.094**	**0.125**	**0.19**	**0.25**	**0.38**	**0.50**	**0.75**	**1**	**1.5**	**2**	**3**	**4**	**6**	**8**	**12**	**16**	**20**	**24**	**32**	**48**	**64**	**96**	**128**	**192**	**>256**
P	5	18	-	-	-	-	1	1	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
OX	-	-	-	-	4	3	12	7	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
E	-	-	-	-	-	-	-	-	2	1	6	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**13**
AZM	-	-	-	-	-	-	-	-	-	-	1	2	4	7	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	**13**
SP	-	-	-	-	-	-	-	-	-	-	1	2	6	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**13**
CLR	-	-	-	-	-	-	-	2	2	12	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**13**
TIL	-	-	-	-	-	-	-	-	-	-	-	1	2	7	6	-	1	-	2	4	5	1	-	1	-	-	-	-	-	-	**-**
DA	-	-	-	-	-	1	3	11	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**13**
QDA	-	-	-	-	-	-	-	1	11	**16**	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**

P: Penicillin, OX: Oxacillin, E: Erythromycin, AZM: Azithromycin, SP: Spiramycin, CLR: Clarithromycin, TIL: Tilmicosin, DA: Clindamycin, QDA: Quinupristin/Dalfopristine

Underlined Value: MIC_{50} Value in Bold: MIC_{90}

Table 4. Distribution of MIC values of antimicrobial agents in human *S. aureus* isolates (n=30)

Antibiotic	MIC (µg/ml)																														
	0.012	**0.016**	**0.023**	**0.032**	**0.047**	**0.064**	**0.094**	**0.125**	**0.19**	**0.25**	**0.38**	**0.50**	**0.75**	**1**	**1.5**	**2**	**3**	**4**	**6**	**8**	**12**	**16**	**20**	**24**	**32**	**>32**	**48**	**64**	**96**	**128**	**192**	**>256**
P	-	3	-	1	-	-	-	1	2	3	5	2	3	4	2	-	**1**	1	-	-	-	1	-	-	-	1	-	-	-	-	-	-
OX	-	-	-	-	1	-	2	4	3	4	7	1	2	1	1	-	**2**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
E	-	-	-	-	-	-	1	2	6	8	9	**1**	-	-	-	-	-	-	-	1	-	-	-	1	1	-	-	-	-	-	-	-
AZM	-	-	-	-	-	-	-	-	-	1	-	5	10	6	**5**	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	2
SP	-	-	-	-	-	-	-	-	1	-	1	1	9	13	**4**	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CLR	-	-	-	-	-	1	-	1	9	13	**3**	-	-	-	-	-	-	-	1	-	-	-	-	1	1	-	-	-	-	-	-	-
TIL	-	-	-	-	-	-	-	-	-	-	-	1	2	8	**16**	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DA	-	-	-	-	-	-	-	9	**21**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
QDA	-	-	-	-	-	-	2	3	9	**13**	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

P: Penicillin, OX: Oxacillin, E: Erythromycin, AZM: Azithromycin, SP: Spiramycin, CLR: Clarithromycin, TIL: Tilmicosin, DA: Clindamycin, QDA: Quinupristin/Dalfopristine

Underlined Value: MIC50 Value in Bold: MIC90

resistance was not observed in any of the isolates (Table 5). Penicillin and macrolide resistance were determined in 3 of the examined isolates. Furthermore, one isolate was found to be resistant to both penicillin and oxacillin.

Table 5. Antimicrobial susceptibility of *S. aureus* isolates isolated from cattle and humans.

Antibiotic	Bovine isolates n (%)			Human isolates n (%)		
	S	I	R	S	I	R
P	25 (83.33)	0	5 (16.66)	5 (16.66)	2 (6.66)	23 (76.66)
OX	30 (100)	0	0	26 (86.66)	2 (6.66)	2 (6.66)
E	17 (56.66)	0	13 (43.33)	27 (90)	0	3 (10)
AZM	16 (53.33)	1 (3.33)	13 (43.33)	27 (90)	0	3 (10)
SP	17 (56.66)	0	13 (43.33)	30 (100)	0	0
CLR	17 (56.66)	0	13 (43.33)	27 (90)	1 (3.33)	2 (6.66)
TIL	29 (96.66)	0	1 (3.33)	30 (100)	0	0
DA	17 (56.66)	0	13 (43.33)	30 (100)	0	0
QDA	30 (100)	0	0	30 (100)	0	0

P: Penicillin, OX: Oxacillin, E: Erythromycin, AZM: Azithromycin, SP: Spiramycin, CLR: Clarithromycin, TIL: Tilmicosin, DA: Clindamycin, QDA: Quinupristin/Dalfopristine
S: Susceptible I: Intermediate R: Resistant

Penicillin resistance was found to be higher in *S. aureus* strains isolated from humans than in strains of bovine origin ($p<0.05$). But, the level of macrolide resistance was higher in bovine isolates in comparison with the level of the human isolates ($p<0.05$).

Determination of antimicrobial resistance genes by PCR

The blaZ gene was not detected in 5 bovine and 23 human isolates that were resistant to penicillin. The *mecA* gene associated with methicillin resistance was found in two oxacillin resistant and two intermediate resistant human *S. aureus* isolates.

None of the ML resistant isolates harbored *erm(A)*, *mph(A)*, *ere(A)*, *ere(B)*, *msr(A)*, *msr(B)*, *lnu(A)* and *lnu(B)* genes while 5 (%38.46) of 13 ML resistant bovine isolates were found to be positive for *erm(B)*. *Mph*(*C*) gene was detected in 2 (66.66%) of 3 macrolide resistant human isolates. While *erm*(*C*) gene was detected in 1 human isolate with a positive D zone reaction, MIC value of erythromycin was found to be low (0.19 μg/ml) in this isolate.

DISCUSSION

Mastitis causes significant economic losses in dairy cattle farms all over the world. *Staphylococcus* species are reported to be the most frequently isolated bacterial agents from mastitis cases [36-38]. *S. aureus* causes gangrenous and contagious mastitis and

can also infect humans through contaminated food products or direct contact [1,2,39]. For this reason, antimicrobial agents are used in the treatment of infections caused by *S. aureus* in order to protect both animal and public health and to prevent economic losses in cattle breeding. In this respect, it is critical to monitor the resistance profile that develops in isolates with up-to-date data, in both national and international areas.

Various studies have been conducted for the determination of penicillin, oxacillin (methicillin) and MLS resistance by the disc diffusion method and the investigation of antimicrobial resistance genes in *S. aureus* strains isolated from bovine samples by PCR [41-44]. There have been a limited number of studies investigating the MIC values of penicillin, oxacillin (methicillin) and MLS group antimicrobial agents, as well as resistance genes in *S. aureus* strains isolated from clinical and subclinical bovine mastitis in Türkiye and elsewhere.

In this study, 13 (43.33%) of the 30 *S. aureus* strains isolated from bovine mastitis were found to be resistant to erythromycin and clindamycin by the disc diffusion method. While no iML phenotype was found in isolates, it was determined that all of them had the cML phenotype. There have been few studies investigating the iML phenotype in *S. aureus* isolates obtained from cattle. In these studies, the prevalence of iML resistance in the isolates was determined as very low (0.39-2.9%) [21,40]. However, some researchers indicated the iML phenotype in the isolates was present at a higher rate (52.8%-95%) [28,45].

Penicillins are frequently used in the treatment of infections caused by *S. aureus* in animals. For this reason, the use of methicillin, which was developed as an alternative to penicillin, has increased in recent years, and this induces resistance in bacterial agents. In the present study, penicillin resistance was observed in 5 (16.66%) of the bovine isolates, but oxacillin (methicillin) resistance was not detected. Although penicillin resistance has been found at varying rates (62.96%-84.09%) in *S. aureus* isolates in previous studies, it has been reported that the *blaZ* gene is detected in most of the penicillin resistant isolates [42,43]. In this study, while penicillin resistance was determined in a limited number of isolates, *blaZ* gene could not be found in the chromosomal DNA. Genes discovered in extra-chromosomal elements might cause the resistance mechanism in the isolates. Methicillin resistance in *S. aureus* isolates obtained from various clinical cases in cattle was found at low rates (0.68-14.2%) in previous studies [40-44]. On the other hand, Wang et al. (2008) did not detect oxacillin (methicillin) resistance in 72 *S. aureus*. Also, they found the MIC_{50} and MIC_{90} values of oxacillin as 2 μg/ml. Similarly, in the present study, oxacillin resistance was not detected in 30 *S. aureus* strains isolated from bovine mastitis. MIC_{50} and MIC_{90} values were found to be 0.094 μg/ml and 0.19 μg/ml, respectively. It was thought that the limited use of oxacillin in cattle breeding in the region may cause low MIC_{50} and MIC_{90} values.

Macrolides display a bacteriostatic effect by inhibiting protein synthesis in bacteria. The most commonly used antibiotics from this group are erythromycin, azithromycin,

clarithromycin and spiramycin. In the present study, 13 (43.33%) of the bovine isolates were found to be resistant to erythromycin, azithromycin, spiramycin clarithromycin and clindamycin. MIC_{90} values of erythromycin, azithromycin, spiramycin clarithromycin and clindamycine were found to be greater than 256 μg/ml in the isolates. On the other hand, one isolate was found to be resistant to tilmicosin and the MIC_{90} value of tilmicosin was 12 μg/ml. MIC values for the ML group antibiotics were found to be similar to those reported by Aslantaş et al. [21] and Li et al. [28]. No quinupristin/dalfopristine resistant strains were detected in the study, and the MIC_{90} value of quinupristin/dalfopristine was similar to the MIC_{90} values obtained by Li et al. [28].

Macrolide resistance is encoded by various genes in *S. aureus*. Accordingly, *erm(A)*, *erm(B)*, *erm(C)*, *mph(C)*, *ere(A)*, *msr(A)*, *msr(B)* and *lnu(A)* genes have been determined with a ratio of 21%, 11.11-52%, 22.22-95%, 1.49-67.5%, 35%, 1.49-78.94%, 15-100% and 2.98-100% in ML resistant isolates, respectively [21,28,38,41,42,44]. However *erm*(*A*), *mph*(*A*), *ere*(*A*), *ere*(*B*), *msr*(*A*), *msr*(*B*), *lnu*(*A*) and *lnu*(*B*) genes were not detected in 13 bovine isolates resistant to ML antibiotics, in this study. The *erm*(*B*) gene was found to be positive in only 5 (38.46%) of the bovine isolates. The *erm*(*B*) rate detected in the isolates was found to be consistent with the data reported by Li et al. [28].

Although there are various studies investigating the resistance profile against penicillin, oxacillin (methicillin) and MLS group antimicrobial agents in *S. aureus* strains isolated from various infections in human, a limited number of research in which the MIC values of antibacterial agents have been determined.

In this study, iML resistance was determined in 10% of *S. aureus* isolates from human samples. While all of these isolates were found to be resistant to penicillin, methicillin resistance was not detected in any of them. In a review which analyzed the data of the studies conducted on prevalence of MLS resistance in *S. aureus* strains isolated from humans in Iran, it was reported that the prevalence of iML resistance was 10% in *S. aureus* strains isolated from clinical samples taken from humans [46]. Çetin et al. [47] reported that iML resistance was found in 15% of *S. aureus* isolates. However, iML resistance was determined at a higher rate in other investigations [32,39,48,49]. Although methicillin resistance was limited (6.66%) in the isolates examined in this study, the methicillin-resistant isolates were found to carry *mecA* gene in agreement with other studies [50]. In the study, MIC_{90} values of the ML group antimicrobial agents in human isolates ranged from 0.19 to 3 μg/ml and only three isolates were resistant to erythromycin. The increased use of β-lactam antibiotics was assumed to be the reason for the higher rate of penicillin resistance in human isolates. In other studies [32,48,50-52], *erm* group resistance genes were detected in macrolide-resistant isolates but, in this study only the *mph*(*C*) gene was found in 66.66% of the isolates.

In this study, the macrolide resistance was determined to be higher in *S. aureus* strains isolated from cattle than those isolated from humans. On the other hand, penicillin resistance was found to be high in human isolates. The genes involved in penicillin and macrolide antibiotic resistance could only be detected in the chromosomal DNA of a

limited number of isolates. Statistical relationship was not found between methicillin and macrolide resistance in both bovine and human isolates.

It is suggested that antimicrobial susceptibility of the isolates should be determined before antimicrobial treatment. The antimicrobial resistance profiles of many isolates that will be obtained from new cases and the presence of resistance-related genes in extra-chromosomal elements should be investigated in future studies to clarify the resistance mechanism.

Ethical Approval

This study was approved by Van Yüzüncü Yıl University Animal Researches Ethic Committee with the decision number of 2020/11-03.

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Author's contributions

OG, IHE and CA have carried out the all lab work. KG and ZI have been involved in drafting the manuscript or revising it critically for important intellectual content. All authors read and approved the final manuscript.

Declaration of conflicting of interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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FENOTIPSKA I GENOTIPSKA KARAKTERIZACIJA MAKROLID-LINKOZAMID-STREPTOGRAMIN REZISTENCIJE *Streptococcus aureus* IZOLATA POREKLOM OD GOVEDA I ČOVEKA

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U okviru studije obavljeno je ispitivanje rezistencije na makrolid-linkozamid-streptogramin (MLS) sojeva bakterije *S. aureus*, izolovanih iz slučajeva mastitisa kod krava ili izolovanih u humanoj populaciji. Inducibilna rezistencija na klindamicin (iML) nije uočena kod 30 izolata poreklom od krava dok je detektovana kod 3 (10%) od ukupno 30 izolata poreklom od ljudi. MIC_{90} vrednosti kod izolata poreklom od krava bila je za penicilin 2, oxacilin 0,19 i za makrolid-linkozamid > 256 μg/ml (ML). U slučaju izolata iz ljudi, vrednosti su bile za penicilin 3, oxacillin 3 i za macrolide-lincosamide 0,19 - 1,5 μg/ml. Streptograminska rezistencija nije ustanovljena kako kod izolata poreklom od krava tako ni izolata poreklom od ljudi. Iako su *mec*A geni ustanovljeni kod svih izolata rezistentnih na oxacilin, *bla*Z nije bilo moguće da se dokaže u izolatima koji su bili rezistentni na penicilin. Gen *erm*(B) detektovan je u 5 (38,6%), od ukupno 13 ML rezistentnih izolata iz krava, a *mph*(C) gen je detektovan u 2 (66,66%) od ukupno 3 izolata iz ljudi. Kao rezultat ovog ispitivanja, ustnovljeno je da je rezistencija na penicilin i oxacilin veća u *S. aureus* izolatima poreklom od ljudi, dok je ML rezistencija bila veća u slučaju izolata poreklom od krava. Zaključujemo je da je potrebno da se ispita prisustvo gena u ekstra-hromozomskim elementima povezano sa rezistencijom na penicilin i makrolide. Rezultati dobijeni u okviru ove studije, treba da doprinesu studijama antimikrobne osetljivosti u humanoj i veterinarskoj medicini.