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Araştırma Makalesi ***Research Article***

Nasal Carriage of Methicillin-Resistant Coagulase Negative Staphylococci (MR-CoNS) Among Veterinarians and Veterinary Students[#]

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Key Words:
Methicillin resistance, *Staphylococcus* spp., veterinarian, veterinary students, nasal carriage, antibiotic susceptibility

Abstract

The aim of this work was to determine the nasal carriage of methicillin resistant coagulase negative staphylococci (MR-CoNS) among veterinarians and veterinary students. In addition, species distribution, antimicrobial susceptibility, resistance genes and staphylococcal chromosomal cassette *mec* (SCC*mec*) type of the isolates were also investigated. MR-CoNS were isolated from 43.8% (39/89) of veterinarians and 44.6% (37/83) of students. While all MR-CoNS isolates were susceptible to quinopristin-dalfopristine, 64.5% of the isolates showed multi-resistance. MR-CoNS strains carried single or various combinations of resistance genes. SCC*mec* type IV and V were the most common in MR-CoNS strains from both veterinarians and students. This study shows that the rate of nasal carriage of multi-resistant MR-CoNS carriage among veterinarians and veterinary students is high.

Özet

Veteriner Hekim ve Veteriner Fakültesi Öğrencilerinde Nazal Metisilin Dirençli Stafilokok (MD-KNS) Taşıyıcılığı

Bu çalışmada veteriner hekimler (n=89) ve veteriner fakültesi öğrencileri (n=83) arasında nazal metisilin dirençli koagulaz negatif stafilokok (MD-KNS) taşıyıcılığının saptanması amaçlandı. Ayrıca, izolatların tür dağılımı, antimikrobiyal duyarlılıkları, antibiyotik direnç genleri ve stafilokokal kaset kromozom *mec* (SCC*mec*) tipleri de araştırıldı. MD-KNS veteriner hekimlerin %43,8'inden (38/89) ve öğrencilerin %44,6'sından (37/83) izole edildi. MD-KNS suşlarının tamamı kinopristin-dalfopristine duyarlı bulunurken; izolatların %64,5'ı çoğul dirençlilik gösterdi. MD-KNS suşları direnç genlerinin tek veya farklı kombinasyonlarını taşıdı. Hem veteriner hekimlerden hem de öğrencilerden izole edilen MD-KNS suşlarında SCC*mec* tip IV ve V en yaygın tipler olarak belirlendi. Bu çalışma, veteriner hekimler ve veteriner fakültesi öğrenciler arasında nazal çoğul dirençli MD-KNS taşıyıcılık oranının yüksek olduğunu göstermektedir.

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Introduction

Coagulase negative staphylococci (CoNS) have been considered as natural inhabitants of skin and mucous membranes of humans and animals (Kloos and Bannerman, 1994; Taponen and Pyörälä, 2009). Although *Staphylococcus aureus* is considered as the most virulent among staphylococci, CoNS are more resistant to antibiotics than *S. aureus* (Diekema et al*.,* 2001; Taponen and Pyörälä, 2009), and may transfer antibiotic resistance genes to *S. aureus* (Hanssen and Ericson Solid, 2006).

Methicillin resistance (MR) in staphylococci is mediated by penicillin binding protein 2a (PBP2a) with a low affinity for ß-lactam antibiotics, which is encoded by the *mec*A gene. The *mec*A gene was located on a large mobile genetic element called as staphylococcal cassette chromosome *mec* (SCC*mec*) (IWG-SCC, 2009). So far, eleven types (I to XI) of SCC*mec* have been described in methicillin-resistant *S. aureus* according to allotypic combinations of *mec* gene and the *ccr* gene complexes (www.sccmec.org/Pages/SCC_TypesEN.html).

It is beleived that coagulase-negative staphylococci (CoNS) may act as a source of potential reservoir to transfer resistance and SCC*mec* genes to *S. aureus*. Gen transfer can take place at anatomic sites such as the nasal mucosa *in vivo*, where CoNS and *S. aureus* can colonize together (Faria et al., 2014). In community-based studies, the prevalence rates of MR-CoNS nasal carriage significantly differed according to geographical areas, and these strains carried different SCC*mec* elements than those previously reported for *S. aureus* (Ruppé et al., 2009).

Currently, there have been only a few studies on the nasal carriage of MR-CoNS in veterinarians and veterinary students in Turkey (Aslantaş et al., 2012; Bağcıgil et al., 2012). Therefore, we aimed to determine the nasal carriage of MR-CoNS in veterinarians and students and, to characterise on the basis of antibiotic susceptibility, the presence of antibiotic resistance genes and SCC*mec* types.

Materials and Methods

Nasal swab samples

Nasal swab samples were collected from 89 veterinarians and 83 final-year students having close contact with domestic animals between November 2008-June 2009. This study was approved by the Medical Ethics Committee of Medical Faculty at Mustafa Kemal University in Hatay (Protocol Code: 2008/78).

Isolation and identification

Nasal swab samples taken within Stuart Transport Medium were transported at +4°C to the laboratory within the same day. The swabs were placed in 5 ml enrichment broth containing 10 g/l mannitol, 65 g/l sodium chloride, 2.5 g/l yeast extract, 10 g/l tryptone and 2 µg/ml oxacillin at 35°C for 24 h. After overnight incubation, 100 µl of bacterial suspension was plated onto Mannitol Salt Agar containing 2 µg/ml oxacillin, and incubated at 35°C for 24 h. Suspected staphylococcal colonies were subcultured onto Blood Agar and identified according to colony morphology, Gram staining, tube coagulase test and catalase reaction. These isolates were also confirmed by a PCR amplifying sequences specific for *Staphylococcus* 16S rRNA (Strommenger et al., 2003). Methicillin resistant staphylococcus (MRS) isolates were identified to the species level by VITEK®2 Compact System (BioMerieux, France). *mec*A gene was detected as described previously by Choi et al. (2003). Primer pairs (5′-CCTAGTAAAGCTCCGGAA-3′ and 5′-CTAGTCCATTCGGTCCA-3′) was used to amplify a 314 bp product. The PCR amplifications were performed in a reaction volume of 25 µl containing 2.5 µL 10× PCR buffer (750 mM Tris HCl, pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 1.5 µL 25 mM $MgCl_2$, 200 µM of each deoxynucleotide triphosphate, 1 U *Taq* DNA Polymerase (MBI, Fermentas), 25 pmol of each primer and 2 µl of template DNA. After initial denaturation at 95 °C for 2 min, PCR products were amplified by 30 cycles of denaturation at 94 C for 2 min, annealing at 58 °C for 30 sec and extension at 72 °C for 30 sec, with a final extension step at 72 °C for 7 min. The amplified products were detected by staining with ethidium bromide (0.5 µg/mL) after electrophoresis at 100 V for 1h in 1.5% agarose gels.

Antibiotic susceptibility testing

Methicillin resistance was tested phenotypically using disc diffusion method with cefoxitin (30 µg) disks. The isolates showing zone diameter of ≤21mm for *S. aureus* and ≤24 mm for CoNS were considered as methicillin resistant. Antibiotic susceptibility of MRS strains were tested using disk diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI, 2012). The antibiotic disks used were erythromycin (15 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), gentamycin (10 µg), quinopristin-dalfopristin (15 µg), ciprofloxacin (5 µg), mupirocin (200 µg), fusidic acid (10 µg), rifampicin (5 µg), clindamycin (2 µg) and tetracycline (30 µg). Since there

is no standardized CLSI breakpoints for fusidic acid, the results of these antibiotics was interpreted according to Comite de'Antibiogramm de la Societe Francaise de Microbiologie (2001). The isolates resistant to three or more class of antibiotics are considered as multi-resistant.

Determination of antibiotic resistance genes

Antibiotic resistance genes related with macrolide (*erm*A, *erm*B, *erm*C, *msr*A) (Jensen et al., 1999; Martineau et al., 2000), lincosamide (*lun*A) (Lina et al., 1999); aminoglycoside [*aac(6')/aph(2'')*, *aph(3')-IIIa*, *ant(4')-Ia*] (Choi et al., 2003), tetracycline (*tet*K, *tet*M) (Strommenger et al., 2003), mupirocin (*ileS*-2) (Nunes et al., 1999) and fusidic acid (*fus*B, *fus*C) (McLaws et al., 2008) were screened as previously reported.

SCC*mec* typing

SCC*mec* types of the isolates were determined using method and primers described by Kondo et al. (2007). SCC*mec* type assignment of the isolates were carried out according to *ccr* and *mec* gene complexes. The following reference strains were used: *S. aureus* HPV107 for SCC*mec* type I, *S. aureus* BK2464 for SCC*mec* type II, *S. aureus* HUSA304 for SCC*mec* type III and *S. aureus* GRE14 for SCC*mec* type IV. The reference strains were kindly provided by Dr. Zeynep Ceren KARAHAN (Microbiology Department, Faculty of Medicine, Ankara University, Ankara, Turkey).

Statistical analysis

Associations between students and veterinarians, antibiotic susceptibility profile and nasal carriage rate among veterinarians were investigated using the Pearson and Likelihood ratio chi-square tests. A *P* value less than of 0.05 was considered statistically significant.

Results

Isolation and identification

MR-CoNS were isolated from 39 veterinarians (43.8%) and 37 students (44.6%). There were no statistically significant difference between veterinarians and students in terms of nasal carriage rate (*P*=0.920) and among veterinarians according to their working places (*P*=0.525). MR-CoNS strains isolated from veterinarians were identified as *S. epidermidis* (n=33), *S. haemolyticus* (n=3), *S. hominis subsp. hominis* (n=2), *S. lentus* (n=1). MR-CoNS strains isolated from veterinary students were identified as *S. epidermidis* (n=26), *S. haemolyticus* (n=5), *S. cohnii* (n=4) and *S. hominis subsp. hominis* (n=2). Distribution of staphylococcus species between veterinarians and students did not differ significantly (*P*=0.316).

Antibiotic susceptibility testing

Antibiotic susceptibility testing displayed a wide variety of susceptibility patterns. Resistance rates for tetracycline, erythromycin and gentamicin were found statistically significant between veterinarians and students (Table 1). While all MR-CoNS isolates were susceptible to quinopristin-dalfopristine, 49 (64.5%) of the isolates were resistant to three or more antibiotics. Resistance to one and two classes of antibiotics were detected in 9 (11.8%) and 18 (23.7%) isolates, respectively.

PCR detection of antibiotic resistance genes

Of the 51 tetracycline-resistant isolates, forty five were positive for *tet*K and 6 isolates were positive for *tet*K and *tet*M (Figure 1). Among the erythromycin-resistant isolates (n=50), *erm*A, *erm*B, *erm*C and *msr*A genes were detected in 9 (18%), 18 (36%), 45 (90%) and 43 (86%) isolates, respectively (Figure 2). Gentamicin resistant isolates (n=40) carried *aac(6')/aph(2'')* (60%), *aph(3')-IIIa* (47,5%) and *ant(4')-Ia* (55%) genes. Twenty three isolates carried *ileS*-2 gene among 24 mupirocin resistant isolates. Of the 14 fusidic acid resistant isolates, *fus*B in three isolates, *fus*B and *fus*C genes in 10 isolates were detected. All clindamycin resistant isolates harboured *lnu*A gene.

SCC*mec* typing

SCC*mec* type IV (46.1%, 35/76) was the most prevalent, followed by SCC*mec* type V (42.1%, 32/76) and SCC*mec* type II (7.9%, 6/76) among MR-CoNS isolates (Figure 3-4). One isolate from veterinarians and two isolates from students were not typeable. Distribution of SCC*mec* types, antibiotic resistance phenotypes and genotypes among MR-CoNS from veterinarians and students are given in Tables 2-3.

Discussion

MR-CoNS have been increasingly reported as the cause of serious infections both in animals and humans by leading the failure in antibiotic treatment and high mortality rates around the world. But, data about nasal carriage of MR-CoNS in community are currently very limited. In this study, nasal MR-CoNS carriage rate was 51% (39/89) in veterinarians and 44.6% (37/83) in students. Recently, higher rates of nasal MR-CoNS carriage among clinic personnel in Turkey have been reported as 72.2% (13/18) (Bağcıgil et al., 2012) and 92.3% (12/13) (Aslantaş et al., 2012). Similarly, Huber et al. (2011) reported a higher MR-CoNS carriage rate (60.2%, 80/133) among veterinarians participating in a course in Switzerland.

Table 1. Antibiotic susceptibility profiles of MR-CoNS isolates.
Tablo 1. MD-KNS izolatlarının antibiyotik duyarlılık profilleri.

Antibiotic	No (%) of resistant MR-CoNS isolates						*P* value
	S. epidermidis		*S. haemolyticus*		Others*		
	Veterinarian (n=33)	Student (n=26)	Veterinarian (n=3)	Student (n=5)	Veterinarian (n=3)	Student (n=6)	
Tetracycline	29 (87.9)	13 (50.0)	3 (100)	1 (20)	1 (33.3)	4 (66.7)	0.001
Erythromycin	27 (81.8)	14 (53.8)	2 (66.7)	0 (0)	2 (66.7)	5 (83.3)	0.010
Gentamicin	21 (63.6)	12 (46.2)	3 (100)	1 (20)	1 (33.3)	2 (33.3)	0.040
Mupirocin	15 (45.4)	6 (23.1)	0 (0)	1 (20)	1 (33.3)	1 (16.7)	0.069
Ciprofloxacin	13 (39.4)	3 (11.5)	3 (100)	4 (80)	1 (33.3)	2 (33.3)	0.077
Trimethoprim/ sulfamethoxazole	10 (30.3)	7 (26.9)	3 (100)	4 (80)	0 (0)	5 (83.3)	0.374
Clindamycin	8 (24.2)	4 (15.4)	2 (66.7)	0 (0)	1 (33.3)	0 (0)	0.057
Fusidic acid	6 (18.2)	4 (15.4)	0 (0)	2 (40)	0 (0)	2 (33.3)	0.483
Rifampicin	2 (6.1)	3 (11.5)	1 (33.3)	3 (60)	0 (0)	1 (16.7)	0.148
Quinopristin-dalfopristine	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	-

**S. hominis subsp. hominis* (n=3), *S. hominis* (n=1), *S. cohnii* (n=3), *S. cohnii* subsp. *cohnii* (n=1), *S. lentus* (n=1)

Of the MR-CoNS species isolated *S. epidermidis* (n=59) was the most common, followed by *S. haemolyticus* (n=8). The importance of *S. epidermidis* has been emphasized as a major actor for the dissemination of methicillin resistance among staphylococci in community (Lebeaux et al., 2012). These two species have been frequently isolated from clinical cases of humans in Turkey (Koksal et al., 2009; Mert et al., 2011). Similarly, Huber et al. (2011) reported that *S. epidermidis* and *S. haemolyticus* were frequently isolated MR-CoNS species among veterinarians. However, *S. hominis* (Bağcıgil et al., 2012) and *S. lentus* (Aslantaş et al., 2012) have been reported to be the most common MR-CoNS species isolated from clinic personnel.

High level of multi-drug resistance was determined among MR-CoNS isolates in this study. This is explained by extensive use and selective pressure of antibiotics. Similar results have been reported among both clinical (Koksal et al., 2009; Mert et al., 2011) and commensal MR-CoNS isolates (Higuchi et al., 2007). It has been proposed that antibiotic resistant CoNS isolates may serve as a pool of resistance genes that could be possibly transferred to other Gram positive bacteria, including *S. aureus* and thus pose great public health problem (Hanssen and Ericson Sollid, 2006).

Different types of SCC*mec* in MR-CoNS have been reported depending on the host and geographical locations (Fessler et al., 2010; Zhang et al., 2009). It has been suggested that type IV is often associated with *S. epidermidis* (Fessler et al., 2010; Ibrahem et al., 2009), type V is predominant in *S. haemolyticus* (Ruppé et al., 2009). In this study, SCC*mec* type IV was the most common type in *S. epidermidis* (32/59), while in *S. haemolyticus* the dominant type was type V (7/8). *S. epidermidis* is known to be an important reservoir of SCC*mec* elements and carry SCC*mec* type IV at a high frequency. It has been speculated that *S. epidermidis* horizontally transferred SCC*mec* type IV to *S. aureus* by homolog recombination (Hanssen and Ericson Sollid, 2006). Also, the relatively smaller size of SCC*mec* type IV compared with other SCC*mec* types facilitate its mobility and transfer among staphylococci (Oliveria and de Lencastre, 2002).

Table 2. Distribution of SCC*mec* types, antibiotic resistance phenotypes and genotypes among MR-CoNS from veterinarians.
Tablo 2. Veteriner hekimlerden izole edilen MD-KNS izolatlarının SCC*mec* tipleri, antibiyotik direnç fenotip ve genotiplerine göre dağılımı.

Code	Species	SCC*mec* Type	Resistance phenotype	Resistance genotype
V1	*S. epidermidis*	IV	TE, E, CN, MUP	*tet*K, *erm*B, *erm*C, *msr*A, *ant(4')-Ia*, *ileS-2*
V4	*S. epidermidis*	II	TE, E, CN, DA, RA, CIP, SXT	*tet*K, *erm*C, *msr*A, *lnu*A, *aac(6')/aph(2'')*, *ant(4')-Ia*, *ileS-2*
V5	*S. epidermidis*	V	TE, E, CN, MUP	*tet*K, *tet*M, *erm*B, *erm*C, *msr*A, *aac(6')/aph(2''), ant(4')-Ia, ileS*-2
V8	*S. epidermidis*	V	CN	*aac(6')/aph(2''), ant(4')-Ia*
V20	*S. epidermidis*	V	TE, E, CIP, SXT, MUP	*tet*K, *erm*C, *msr*A
V22	*S. epidermidis*	IV	TE, E, CN, DA, SXT	*tet*K, *erm*C, *aac(6')/aph(2''), ant(*4')-Ia, *lnu*A
V23	*S. epidermidis*	IV	TE, E, CN	*tet*K, *aac(6')/aph*(2''), *aph*(3')-*IIIa*, *erm*B, *erm*C, *msr*A
V24	*S. epidermidis*	IV	TE, E, CN, CIP	*tet*K, *aac(6')/aph(2''), aph(3')-IIIa*, *erm*C, *msr*A
V25	*S. epidermidis*	IV	TE, E, FD, MUP	*tet*K, *aac(6')/aph(2''), aph(3')-IIIa*, *erm*B, *erm*C, *msr*A, *ile*S-2, *fus*B, *fus*C
V29	*S. epidermidis*	IV	TE, E, MUP	*tet*K, *erm*C, *msr*A, *ileS-2*
V30	*S. epidermidis*	V	TE, E, CIP, MUP	*tet*K, *erm*B, *erm*C, *msr*A, *ileS-2*
V31	*S. epidermidis*	V	TE, E, CN, MUP	*tet*K, *tet*M, *erm*A, *erm*B, *ant(4')-Ia*, *erm*C, *msr*A, *aac(6')/aph(2''*), *aph*(3')-*IIIa*, *ile*S-2
V33	*S. epidermidis*	IV	TE, E, CN, DA, CIP	*tet*K, *erm*C, *msr*A, *ant(4')-Ia, aac(6')/aph(2''*), *aph*(3')-*IIIa*
V36	*S. epidermidis*	IV	TE, E, CIP	*tet*K, *erm*C, *msr*A
V37	*S. epidermidis*	IV	TE, E, CN, CIP, FD, MUP	*tet*K, *tet*M, *erm*A, *erm*B, *erm*C,*msr*A, *aac(6')/aph(2''), aph(3')- IIIa*,
V39	*S. epidermidis*	IV	TE, E, CN, CIP, MUP	*tet*K, *erm*B, *erm*C, *msr*A, *aac(6')/aph*(2''), *aph*(3')- *IIIa*, *tet*K,
V41	*S. epidermidis*	V	TE, CN, FD	*ant(4')-Ia*, *fus*B, *fus*C
V43	*S. epidermidis*	V	TE	*tet*K
V46	*S. epidermidis*	IV	TE, E, DA, SXT, FD, MUP	*tet*K, *erm*A, *ile*S-2
V48	*S. epidermidis*	IV	TE, E, CN, DA, CIP, SXT, FD	*tet*K, *erm*A, *erm*C, *aac(6')/aph*(2''), *lnu*A, *fus*B, *fus*C
V49	*S. epidermidis*	IV	SXT, TE	*tet*K
V51	*S. epidermidis*	IV	E, DA	*erm*A, *lnu*A
V53	*S. epidermidis*	V	TE, E, CN, SXT, CIP, DA, RA, FD, MUP	*tet*K, *erm*C, *msr*A, *aac(6')/aph*(2''), *aph(3')- IIIa, ant(4')-Ia*, *lnu*A, *ile*S-2, *fus*B, *fus*C
V54	*S. epidermidis*	IV	TE, E, CN, MUP	*tet*K, *erm*C, *aac(6')/aph*(2''), *ile*S-2
V56	*S. epidermidis*	V	TE, E, CN, SXT	*tet*K, *erm*C, *ant*(4')-Ia
V64	*S. epidermidis*	V	CN, E	*erm*C, *msr*A, *aac(6')/aph*(2''), *aph*(3')- *IIIa*, *ant(4')-Ia*, *tet*K

Table 2. Distribution of SCC*mec* types, antibiotic resistance phenotypes and genotypes among MR-CoNS from veterinarians – Continued.
Tablo 2. Veteriner hekimlerden izole edilen MD-KNS izolatlarının SCC*mec* tipleri, antibiyotik direnç fenotip ve genotiplerine göre dağılımı – Devamı.

Code	Species	SCC*mec* Type	Resistance phenotype	Resistance genotype
V65	*S. epidermidis*	V	TE	*tet*K
V81	*S. epidermidis*	V	TE, E, CN, MUP	*tet*K, *tet*M,*erm*A, *erm*B, *erm*C, *msr*A, *ant(4')-Ia*, *aac(6')/aph*(2''), *lnu*A, *ile*S-2
V83	*S. epidermidis*	IV	TE, E, CIP	*tet*K, *erm*C, *msr*A, *ant(4')-Ia*, *ile*S-2
V85	*S. epidermidis*	IV	CN, SXT	*ant(4')-Ia*
V101	*S. epidermidis*	IV	TE, E, CN, DA, CIP	*tet*K, *erm*C, *msr*A, *aac(6')/aph*(2''), *lnu*A
V102	*S. epidermidis*	IV	TE, E, SXT, MUP	*tet*K, *erm*B, *erm*C, *msr*A, *ile*S-2
V109	*S. epidermidis*	IV	TE, E, CN, CIP, SXT, MUP	*tet*K, *erm*C, *msr*A, *ant(*4')-Ia, *ile*S-2
V6	*S. haemolyticus*	V	TE, E, CN, RA, CIP, SXT	*tet*K, *tet*M, *erm*C, *msr*A, *aac(6')/aph*(2''), *aph(3')-IIIa*
V17	*S. haemolyticus*	N.T.	TE, CN, DA, CIP, SXT	*tet*K, *aac(6')/aph(2''), aph(3')-IIIa*, *erm*A, *erm*C, *msr*A, *lnu*A
V34	*S. haemolyticus*	V	TE, E, CN, DA, CIP, SXT	*tet*K, *erm*A, *erm*B, *erm*C, *msr*A, *aac*(6')/*aph*(2''), *aph*(3')-*IIIa*, *lnu*A
V86	*S. hominis subsp. hominis*	IV	CIP	-
V88	*S. hominis subsp. hominis*	IV	E	*erm*A, *msr*A
V80	*S. lentus*	II	TE, E, CN, DA, MUP	*tet*K, *tet*M, *erm*A, *erm*B, *erm*C, *msr*A, *ant(4')-Ia*, aac(6')/aph(2''), *lnu*A, *ileS-2*

Table 3. Distribution of SCC*mec* types, antibiotic resistance phenotypes and genotypes among MR-CoNS from students.

Tablo 3. Öğrencilerden izole edilen MD-KNS izolatlarının SCC*mec* tipleri, antibiyotik direnç fenotip ve genotiplerine göre dağılımı.

Code	Species	SCC*mec*	Resistance phenotype	Resistance genotype
S3	*S. epidermidis*	IV	TE, CN, RA, MUP	*tet*K, *aac(6')/aph(2'')*, *ileS-2*
S4	*S. epidermidis*	V	CN, DA	*aph(3')-IIIa*, *lnu*A
S6	*S. epidermidis*	V	TE, E, CN, SXT	*tet*K, *erm*C, *msr*A, *aac(6')/aph(2'')*, *aph(3')-IIIa*, *ant(4')-I*
S7	*S. epidermidis*	V	TE, E, SXT	*tet*K, *erm*B, *erm*C, *msr*A
S8	*S. epiermidis*	V	TE	*tet*K
S14	*S. epidermidis*	-	E, CN, RA	*erm*C, *msr*A, *aac(6')/aph(2'')*
S17	*S. epidermidis*	IV	TE, SXT	*tet*K
S33	*S. epidermidis*	V	E, DA, RA, CIP, SXT, FD	*erm*C, *msr*A, *lnu*A, *fus*B
S37	*S. epidermidis*	IV	TE, CN, FD	*aac(6')/aph(2'')*, *fus*B, *fus*C
S39	*S. epidermidis*	IV	E, CN, SXT, FD	ant(4')-Ia, *erm*C, *msr*A, *fus*B, *fus*C
S40	*S. epidermidis*	V	E	*erm*C, *msr*A
S41	*S. epidermidis*	V	E, MUP	*erm*C,*msr*A
S43	*S. epidermidis*	IV	E, MUP	*erm*C,*msr*A, *ileS-2*
S48	*S. epidermidis*	V	CN, E, MUP	*erm*C, *msr*A, *aph(3')-IIIa* , *ileS-2*
S55	*S. epidermidis*	V	CN, E	*erm*B, *erm*C, *msr*A, *aph(3')-IIIa*, *ant(4')-Ia*
S57	*S. epidermidis*	V	TE, MUP	*tet*K, *ileS-2*
S58	*S. epidermidis*	V	CN, SXT	*aac(6')/aph(2'')*, *aph(3')-IIIa*
S64	*S. epidermidis*	IV	TE	*tet*K
S66	*S. epidermidis*	IV	TE, CN, CIP	*tet*K, *aph(3')-IIIa*
S74	*S. epidermidis*	IV	CN, MUP	*ant(4')-Ia*, *ileS-2*
S79	*S. epidermidis*	V	CN, E	*aac(6')/aph(2'')*, *erm*B, *msr*A
S80	*S. epidermidis*	IV	TE, E,	tetK, *erm*B, *erm*C, *msr*A
S82	*S. epidermidis*	II	E	-
S83	*S. epidermidis*	IV	TE, CIP,	*tet*K
S76	*S. epidermidis*	IV	E, DA, SXT	*tet*K, *erm*C, *msr*A, *lnu*A
S70	*S. epidermidis*	IV	TE, FD	*tet*K, *fus*B
S25	*S. haemolyticus*	V	TE, CN	*tet*K, *aac(6')/aph(2'')*
S50	*S. haemolyticus*	V	CIP, SXT	*tet*K
S52	*S. haemolyticus*	V	RA, CIP, SXT, MUP	*ileS-2*
S75	*S. haemolyticus*	V	RA, CIP, SXT, FD	*fus*B
S77	*S. haemolyticus*	V	RA, CIP, SXT, FD	*fus*B, *fus*C
S59	*S. hominis subsp. hominis*	V	TE, E, CN, RA, CIP, FD, SXT	*tet*K, *erm*B, *erm*C, *msr*A, *aac(6')/aph(2'')*, *aph(3')-IIIa*, *fus*B, *fus*C
S62	*S. cohnii subsp cohnii*	V	E, CIP, SXT	*erm*C, *msr*A, *ileS-2*
S65	*S. hominis*	-	TE, CN, SXT, FD	*tet*K, *aac(6')/aph(2'')*, *ant(4')-Ia*, *fus*B, *fus*C
S49	*S. cohnii*	II	TE, E, SXT	*tet*K, *erm*C, *msr*A
S78	*S. cohnii*	II	E, SXT, MUP	*tet*K, *erm*B, *erm*C, *msr*A, *lnu*A, *ileS-2*
S15	*S. cohnii*	IV	TE, E, DA	*tet*K, *erm*C, *msr*A

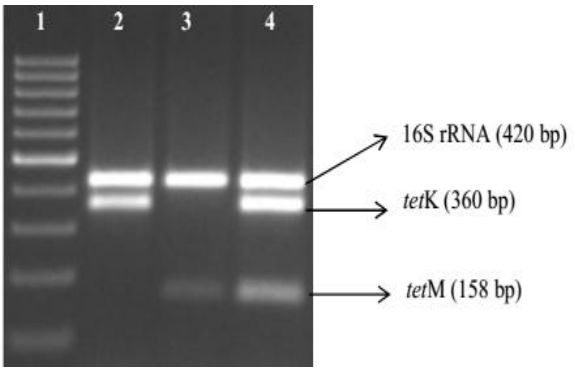

Figure 1. Agarose gel electrophoresis of *tet*K, *tet*M and 16S rRNA genes.

Şekil 1. 16S rRNA, *tetK* ve *tet*M genlerinin agaroz jel elektroforez görüntüsü.
1: 100 bp molecular marker, 2: 16S rRNA and *tet*K, 3: 16S rRNA and *tet*M, 3: 16S rRNA, *tet*K and *tet*M

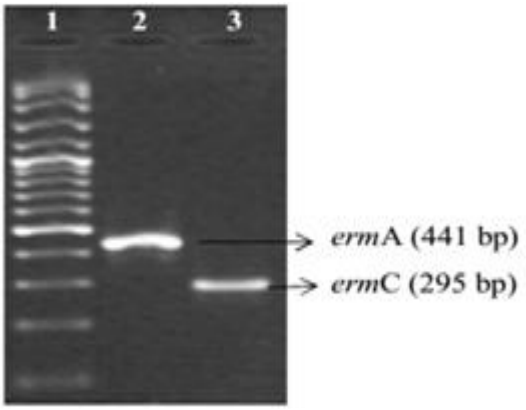

Figure 2. Agarose gel electrophoresis of *erm*A and *erm*C genes.

Şekil 2. *erm*A ve *erm*C genlerinin agaroz jel elektroforez görüntüsü.
1: 100 bp molecular marker, 2: *erm*A, 3: *erm*C

Horizontal transfer of resistance genes between CoNS and *S. aureus* may results in evolution of *S. aureus* with higher resistance potential. This situation may represent health challenges in both human and veterinary medicine. For that reason, infection control programmes similar to those used for MRSA should be implemented to prevent further dissemination of multi-resistant MR-CoNS and there is a need to monitor resistance properties of CoNS.

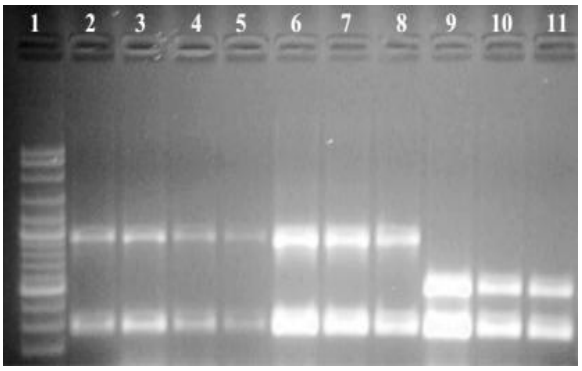

Figure 3. mPCR 1 for the assignment of the type of *ccr* gene complex.

Şekil 3. mPZR1 ile *ccr* gen kompleksinin tipinin belirlenmesi.
1: 100 bp molecular marker, Lane 1-4: *ccr*AB2 (SCC*mec* type II); Lane 5-7: *ccr*AB2 (SCC*mec* type IV); Lane 8-10: *ccr*C (SCC*mec* type V)

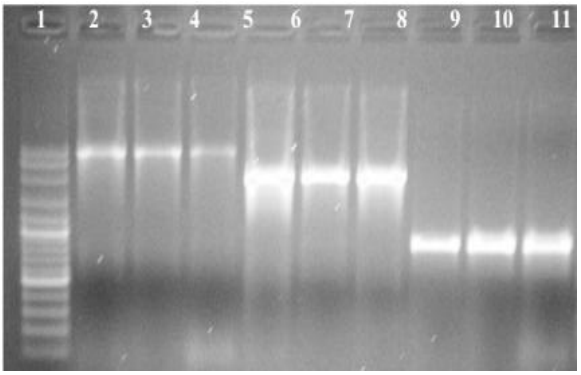

Figure 4. mPCR 2 for assignment of the *mec* gene complex.

Şekil 4. mPZR2 ile *mec* gen kompleks tipinin belirlenmesi.
1: 100 bp molecular marker, 2-4: *mec* complex B (SCC*mec* type IV); 5-7: *mec* complex A (SCC*mec* type II); 8-10: *mec* complex C (SCC*mec* type V)

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