Turkish Journal of Veterinary & Animal Sciences

Volume 42 | Number 6 Article 6

1-1-2018

Determination of methicillin resistance and some genotypic characteristics ofstaphylococci isolated from dogs and their owners

ARZU FINDIK

ALPER ÇİFTCİ

TAYLAN ÖNYAY

MERVE GİZEM SEZENER

YAĞMUR KOÇAK

See next page for additional authors

Determination of methicillin resistance and some genotypic characteristics ofstaphylococci isolated from dogs and their owners

Authors
ARZU FINDIK, ALPER ÇİFTCİ, TAYLAN ÖNYAY, MERVE GİZEM SEZENER, YAĞMUR KOÇAK, and TİMUR GÜLHAN

TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2018) 42: 549-555

doi:10.3906/vet-1611-50

Determination of methicillin resistance and some genotypic characteristics of staphylococci isolated from dogs and their owners

Arzu FINDIK[1,*], Alper ÇİFTCİ[1], Taylan ÖNYAY[2], Merve Gizem SEZENER[1], Yağmur KOÇAK[1], Timur GÜLHAN[1]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey

[2]Department of Surgery, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey

Received: 21.11.2016 • **Accepted/Published Online:** 08.03.2017 • **Final Version:** 10.12.2018

Abstract: This study aimed to determine the methicillin resistance and some genotypic characteristics of staphylococci in dogs and their owners. A total of 132 swab samples from 33 healthy dogs and their owners were taken. Isolated staphylococci were identified by PCR. The antibiotic sensitivities of the isolates were determined by disc diffusion method. Determination of *pvl*, *mecA, bla*, and *fem* genes and SCC*mec* types was performed by PCR. Isolates were genotyped according to *coa* and *spa* gene polymorphisms by PCR. Forty-five isolates were identified as *Stapyloccoccus* spp. Among them, 8 isolates were identified as *S. aureus* and 23 isolates were identified as *S. pseudintermedius*. According to the disc diffusion tests, methicillin resistance methicillin-resistant was determined in all *S. aureus* (MRSA). Among the methicillin-resistant strains, 8 were *S. pseudintermedius* (MRSP). However, methicillin resistance was determined genotypically in 10 strains and of these 5 were MRSA and 2 were MRSP. Two *S. aureus* strains and 7 *S. pseudintermedius* strains were *mec A* negative but *bla* positive. No strain carried the *fem* gene. Ten different *coa* types were detected among the strains. All MRSA strains carried *pvl* genes. In conclusion, MRSP should be considered to pose a risk for humans living with dogs or in contact with them.

Key words: *bla*, *fem*, *mecA*, SCC*mec*, staphylococci

1. Introduction

Coagulase-positive *Staphylococcus aureus* and *Staphylococcus pseudintermedius* are clinically important members of staphylococci in veterinary medicine (1). These agents have the ability to develop resistance to antimicrobials such as methicillin. This is particularly important, because methicillin resistance is conferred by different mechanisms and is considered a marker of resistance for other beta lactams. Methicillin resistance is related to the presence of the *mecA* gene encoding an altered penicillin binding protein (PBP2a) with low affinity for all beta lactams. The *mecA* gene is located on a staphylococcal chromosomal cassette (SCC). Other genes regulating *mecA* expression also reside on SCC*mec* (2). *mecA* expression is also regulated by various other *S. aureus* genes such as *fem* and *aux* (3). Methicillin resistance may be mediated by the *blaZ* gene encoding beta lactamase. This gene is located on a transposon, Tn552, and it has regulatory genes that are similar to *mecA* (2).

Methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. pseudintermedius* (MRSP) have become a significant problem both in veterinary and human medicine (1). While humans are considered to be the preferential host for *S. aureus*, this bacterium adapts specifically to different animal hosts by genetically determined mechanisms. As molecular analysis has shown that the MRSA strains isolated from pets and their owners living in the same household were indistinguishable, it has been considered that there is a chance of transmission of the strains between the animals and their owners. Both humans and pets may be considered to act as reservoirs of MRSA circulating in their environment. Unlike *S. aureus*, *S. pseudintermedius* colonization is rare in humans. However, in several cases, zoonotic transmission of MRSP between dogs and humans has been reported (1).

Several virulence factors are effective in the pathogenesis of *S. aureus* and *S. pseudintermedius* infections. The pathogenesis of *S. pseudintermedius* has recently been investigated and there is scant information about determinants such as coagulase and protein A, which are actually well-known virulence factors of *S. aureus* (4).

In the present study, methicillin resistance and characterization of the genotypic characteristics in staphylococci isolated from dogs and their owners were investigated.

* Correspondence: afindik@omu.edu.tr

2. Materials and methods

2.1. Sampling

A total of 132 swab samples from 33 healthy dogs and their owners (only one person for each dog) were obtained at the Veterinary Hospital of Ondokuz Mayıs University. While swabs from the dogs were taken from the nasal mucosa, dorsum nasi, and perineum, nasal mucosa samples were taken from the owners.

2.2. Isolation and identification of staphylococci

Swabs were inoculated onto blood agar (with 5% sheep blood) and Mueller Hinton agar (with 4% NaCl and 6 mg/L oxacillin) and incubated at 37 °C for 24 h. After incubation suspected colonies were identified as *Staphylococcus* spp. phenotypically (5). Species-specific identifications of isolates were performed by PCR and PCR-RFLP. For confirmation of genus and identification of *S. aureus*, a multiplex PCR was performed for 16S rRNA (*Staphylococcus* spp., 756 bp) and *nuc* genes (*S. aureus*, 279 bp) simultaneously (6). For identification of *S. pseudintermedius* strains, PCR-RFLP was used (7). Briefly, after DNA extraction, a 320 bp *pta* gene fragment was amplified using specific primers (pta_f1, AAAGAC AAA CTT TCA GGTAA and pta_r1, GCA TAA ACAAGC ATT GTA CCG). Amplicons were digested with *Mbo*I and digestion products were electrophoresed on 2% agarose gel. Strains showing two fragments, 213 and 107 bp, of the *pta* gene were confirmed as *S. pseudintermedius.*

2.3. Antibiotic susceptibility tests

The antibiotic sensitivities of the strains were determined and evaluated against eight different antibiotics by Kirby–Bauer agar disc diffusion tests following the protocols described by CLSI (8). Antibiotics used for this purpose were penicillin (10 IU), oxacillin (1 µg), vancomycin (30 µg), oxytetracycline (30 µg), enrofloxacin (5 µg), cefoperazone (75 µg), amoxicillin/clavulanic acid (20/10 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg). Multidrug resistant (MDR) strains were determined according to whether they were resistant against three or more different groups of antibiotics.

2.4. Determination of the *mecA* gene and *SCCmec* types

To determine the *mecA* gene for phenotypically methicillin resistant strains, a PCR protocol was performed as described by Çiftci et al. (6). The strains giving a 320 bp band were evaluated as positive for the *mecA* gene.

To discriminate the SCC*mec* types to which *S. aureus* isolates belong a multiplex PCR protocol was used (9). The primers and expected band sizes used in this multiplex PCR protocol are given in Table 1.

2.5. Determination of *bla* and *fem* genes

All *Staphylococcus* isolates were investigated for the presence of *bla* and *fem* genes as the factors affecting methicillin resistance phenotype. PCR analysis for detecting *bla* and *fem* genes was performed according to the protocol described by Fındık et al. (10). The strains giving 173 bp and 684 bp bands were evaluated as positive for *bla* and *fem* genes, respectively.

2.6. Coagulase (*coa*) and protein A (*spa*) gene typing

coa and *spa* gene polymorphisms in *Staphylococcus* isolates were investigated by PCR (11).

2.7. Determination of the *pvl* gene

To determine the *pvl* gene among *S. aureus* strains PCR analysis was performed as described by Azimian et al. (12). The strains giving a 502 bp band were evaluated as positive for the *pvl* gene.

3. Results

3.1. Isolation and identification of staphylococci

A total of 98 bacteria were isolated from samples. Forty-five (45.91%) of all 98 strains were identified as *Staphylococcus* spp. both phenotypically and genotypically.

Table 1. Oligonucleotide sequences used in multiplex PCR and the resulting band patterns of SCC*mec* types.

Target	Primer	Sequence	PCR product (bp)	SCC*mec* type				
				I	II	III	IV	V
ccrA2-B	β	**F:** ATTGCCTTGATAATAGCCYTCT	937		X		X	
	α3	**R:** TAAAGGCATCAATGCACAAACACT						
ccrC	ccrCF	**F:** CGTCTATTACAAGATGTTAAGGATAAT	518			X		X
	ccrCR	**R:** CCTTTATAGACTGGATTATTCAAAATAT						
IS1272	1272F1	**F:** GCCACTCATAACATATGGAA	415	X			X	
	1272R1	**R:** CATCCGAGTGAAACCCAAA						
mecA–IS431	5RmecA	**F:** TATACCAAACCCGACAACTAC	359					X
	5R431	**R:** CGGCTACAGTGATAACATCC						

Among the *Staphylococcus* strains 8 (17.77%) were identified as *S. aureus*. Among the 37 *Staphylococcus* spp. strains other than *S. aureus*, 23 (82.23%) were identified as *S. pesudintermedius* by PCR-RFLP (Figure).

3.2. Antibiotic susceptibility tests

According to Kirby–Bauer disc diffusion tests, methicillin resistance was determined in 24 strains (53%) of all identified staphylococci. Eight (17%) of the MRS strains were *S. aureus* (MRSA) and 8 (17%) were *S. pseudintermedius* (MRSP).

The resistance rates to the other 7 antibiotics were as follows: penicillin (95%), vancomycin (7%), oxytetracycline (37%), enrofloxacin (4%), cefoperazone (4%), amoxicillin/clavulanic acid (9%), and trimethoprim/sulfamethoxazole (4%). Multidrug resistance (MDR) among the *S. aureus* strains was 75% (6/8 *S. aureus* strains). MDR among MRSA strains (MDR-MRSA) was 80% (4/5 MRSA strains) and of these strains one was resistant to 5 antibiotics, one was resistant to 4 antibiotics, and 2 were resistant against 3 antibiotics. Of all 23 *S. pseudintermedius* strains only 3 (13.04%) were MDR. No MDR-MRSP was detected.

3.3. Determination of *bla* and *fem* genes

The results of PCR analysis targeting the *bla* gene showed that 21 (46%) *Staphylococcus* isolates were *bla*-positive (7 *S.aureus* and 8 *S.pseudintermedius*) and among them 12 (%57) strains were *mecA* negative. Among *S. aureus* isolates, 2 strains (25%) were *mecA* negative-*bla* positive, the number of *mecA* negative-*bla* positive *S. pseudintermedius* strains was 7 (30.43%). While both *mecA* negative-*bla* positive *S. aureus* showed oxacillin resistance phenotypically, only one of *mecA* negative-*bla* positive *S. pseudintermedius* strains was resistant against oxacillin.

The results of *fem*-PCR showed that no isolate carried the *fem* gene.

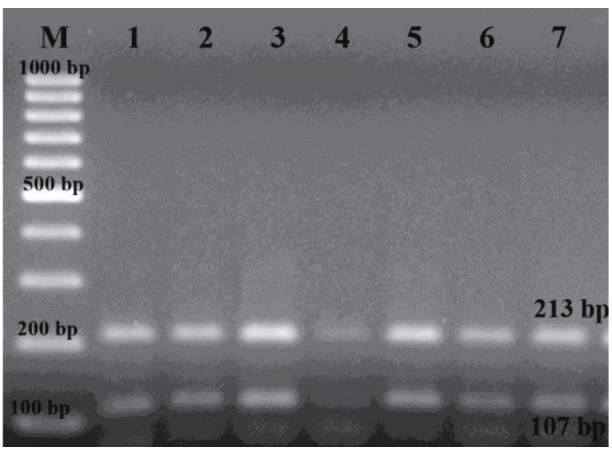

Figure. The RFLP results of *Staphylococcus pseudintermedius*. M: Marker (100–1000 bp); Lanes 1–7: *Staphylococcus pseudintermedius*.

3.4. *coa* and *spa* gene typing

Among all the *Staphylococcus* isolates 23 (51.1%) had the *coa* gene. However, 3 *S. aureus* isolates and 8 *S. pseudintermedius* isolates were negative for the *coa* gene. Ten different *coa* types (C1–C10) were detected among the staphylococci. *coa* positive isolates gave 13 different bands of sizes ranging from 190 to 990 bp. Ten strains of all *coa* positive strains had only one (300 bp) band and these strains belonged to C4 *coa* type. The results of *coa* PCR typing are given in Table 2.

Among the *Staphylococcus* strains 22 (48%) were negative for the *spa* gene. Among the other 23 (51%) strains only two *spa* types were detected (S1 and S2). All *spa* positive strains produced 120 bp *spa* gene product and belonged to S1 *spa* type, except one strain. S2 included only one strain, producing a 210 bp band.

3.5. *mecA* gene determination and *SCCmec* typing

The results of genotypical determination of methicillin resistance by *mecA* gene targeting PCR showed that 10 strains (22.22%) were MRS. Five of them (11.11%) were MRSA and 2 (4.44%) were MRSP. One of the MRSP isolates was of human origin. The prevalence of MRSP found in dogs and humans was the same, 3.03%.

Ten strains that were *mecA* positive staphylococci were tested for their SCC*mec* types by PCR. Of these strains 5 were MRSA, 2 were MRSP, and 3 were other Staphylococci. All strains except one (one MRSP strain) showed a clear band pattern. However, only 4 strains that showed predicted band patterns could be typed by SCC*mec PCR* protocol. Of these 4 strains 2 MRSA strains showed different SCC*mec* types: one MRSA was SCC*mec* type III and the other was SCC*mec* type IV. Five strains (3 were MRSA and 2 were other Staphylococci) with

Table 2. *coa* types and band sizes of staphylococci.

coa types	Band sizes (bp)	n	(%)
C1	510–610	1	2.22
C2	300–510	4	8.89
C3	190	2	4.45
C4	300	10	22.22
C5	750	1	2.22
C6	370–560	1	2.22
C7	370	1	2.22
C8	580	1	2.22
C9	300–400–510–990	1	2.22
C10	300–400–490–510–990	1	2.22

a band pattern that was not compatible with one of the five predicted band patterns and one strain that showed no clear band were named nontypeable. One MRSP strain that could be typed as SCC*mec* type IV was of human origin.

3.6. Determination of the *pvl* gene

Of all 8 *S. aureus* strains 7 (87.50%) were *pvl* gene positive. One *S. aureus* strain was *pvl* negative MSSA (*mecA* negative). All 5 MRSA strains were *pvl* positive.

The total results of the study are presented in Table 3.

4. Discussion

S. aureus is a major inhabitant of the skin and mucosae of humans. This organism occasionally lives on domestic animals. However, animals are usually colonized with other species of Staphylococci such as *S. pseudintermedius*. *S. pseudintermedius* is part of the normal flora of the skin and mucosae of pets such as dogs and cats and is an important opportunistic pathogen. *S. pseudintermedius* colonization is rare in humans and its occurrence in humans usually depends on the state (frequency and proximity) of contact with dogs and cats (13). *S. aureus* has the characteristic ability to rapidly develop antibiotic resistance (1). Methicillin-resistant staphylococci become resistant to the beta-lactam class of antibiotics. Like MRSA, methicillin resistance in *S. pseudintermedius* is also mediated by *mecA* acquisition. Recently MRSA and MRSP have emerged as important problems for both public and animal health (1). In people, generally two types of MRSA infection that are genetically, epidemiologically, and clinically different from each other have been identified. One is healthcare-associated MRSA (HA-MRSA) infection and the other is community-associated MRSA (CA-MRSA) infection. After the recognition of MRSA infections in companion animals at the end of the 1990s, researchers' attention turned to the investigation of MRSA carriage in many companion animals and its possible transmission to pet owners (1,14). The emergence of CA-MRSA in recent times and awareness of the importance of antibiotic resistance characteristics of this organism in the community have also led many researchers to study MRSA in pets (14). Researchers have reported that MRSA carriage in pets can be a potential health risk for their owners but they have also noted that pets can become a reservoir of MRSA through exposure to infected humans. Although the zoonotic importance of MRSP has been reported to be less than that of MRSA, several cases related to zoonotic transmission have been reported. Baptiste et al. (15) did not isolate any MRSA from healthy dogs but they isolated 3 (5.45%) MRSA strains from dogs with clinical infections. The dog with the joint infection was also found positive for nasal and fecal carriage of MRSA and a student who treated the dog had an MRSA-positive nasal swab. MRSA was found at the 17.9% and 9% percentages in veterinary staff and their dogs, respectively, by Loeffler and Lloyd (13). Hanselman et al. (16) isolated MRSA and MRSP at very low percentages, i.e. 0.5% and 2.1%, respectively. Among 45 *Staphylococcus* isolates, 17.77% were *S. aureus* and in all staphylococci 5 (11.11%) including 3 (6.66%) human and 2 (4.44%) dog isolates were MRSA. Variable results were obtained in various studies as seen above and in the present study the prevalence of MRSA in dogs and humans was partly similar to those of others. Among *S. aureus* isolates the prevalence of MRSA was 62.5%. Although the rate was very high, the low numbers of *S. aureus* should be taken into account. The results of typing studies have supported the hypothesis that MRSA in household pets has emerged as a direct result of MRSA in humans (1). Moreover, it is known that *S. aureus* colonization is transient and dogs do not become colonized naturally with *S. aureus*. Humans seem to be the ultimate source of *S. aureus*. In the present study the humans colonized with MRSA may be considered as one of the potential risk factors for MRSA infection in household pets.

Several researchers (16,17) have obtained various rates between 1.5% and 2.1% relating to the prevalence of MRSP colonization in healthy dogs. It was reported that the prevalence varied from 0% to 7% in dogs with skin diseases (17). Moreover, in another study, the prevalence of MRSP in inpatient and outpatient dogs was 30% (18). In this study, one of the MRSP isolates was of human origin and another was from a dog. The prevalence of MRSP in healthy dogs in this study was 3.03% and this rate was similar to the results of other studies. Guardabassi et al. (14) reported that *S. pseudintermedius* occurred frequently in the owners of dogs with deep pyoderma. However, in medical laboratories, *S. pseudintermedius* was reported to be misidentified as *S. aureus*; thus the exact incidence in humans may have been underestimated. Similar to MSSP, colonization of humans with MRSP is known to be uncommon and transient. However, in other studies (18,19) transmission of MRSP from pets (dogs and cats) to their owners was investigated and the prevalence of MRSP in humans (owners, veterinarians, veterinary personnel) was reported at various rates, between 0.4% and 7.9%. Only one (3.03%) MRSP strain was isolated from a healthy human who was the owner of a healthy dog. Moreover, an MSSP strain was isolated from this dog. It is unclear how the owner became colonized with MRSP. However, it is possible that the MRSP strains may transmit to humans from another dog that has already been colonized/infected with this strain or directly from other sources.

Beside *mecA* and *bla* genes, some chromosomal genes that are not linked to *mecA* and SCC*mec* such as *fem* (factors essential for methicillin resistance), *aux* (auxiliary factors), and *hmt* (high methicillin resistance) are considered to contribute to methicillin resistance.

Table 3. The total isolation and identification results of staphylococci and their genotypic characteristics.

Strain no.	Origin	PCR results								
		nuc	*mecA*	*pta*	RFLP	*coa* (bp)	*spa* (bp)	*bla*	*fem*	*pvl*
7	human	-	-	-	-	-	-	+	-	
8	dog	-	-	+	-	-	120	+	-	
11	human	-	+	+	-	-	120	+	-	+
15	dog	-	-	+	-	-	120	-	-	+
17	human	+	-	+	-	-	120	+	-	+
19	dog	+	+	-	-	-	120	+	-	+
21	dog	-	-	+	-	-	120	-	-	
26	dog	+	-	+	-	-	120	+	-	
33	dog	-	-	-	-	510, 610	120	-	-	
38	dog	-	-	+	+	300, 510	-	-	-	
41	dog	-	-	+	-	300, 510	120	-	-	
42	dog	-	-	+	+	-	-	-	-	
44	human	+	+	+	-	190	120	+	-	+
45	human	+	+	+	-	190	120	+	-	+
46	dog	-	-	+	+	300	-	-	-	
47	dog	-	-	+	+	-	120	-	-	
48	human	-	+	+	+	-	120	+	-	+
49	dog	-	-	+	+	-	-	+	-	
50	dog	-	-	-	-	-	120	-	-	+
55	dog	-	-	+	+	300	-	-	-	
56	human	+	+	+	-	750	210	+	-	+
58	dog	-	-	+	+	300	-	-	-	
59	dog	-	-	+	+	300	-	-	-	
62	dog	-	-	+	+	300	-	+	-	
63	dog	-	-	+	+	300, 510	-	-	-	
65	dog	-	-	+	+	300	-	+	-	
66	dog	-	-	+	+	300	-	+	-	
67	dog	-	-	+	+	-	120	+	-	+
68	dog	-	-	+	+	-	120	+	-	
69	dog	-	-	-	-	-	-	-	-	
71	human	-	+	+	-	-	-	+	-	
72	dog	-	+	+	+	300	120	-	-	
74	dog	+	-	+	-	370, 560	120	-	-	+
75	dog	-	-	+	-	-	120	-	-	+
76	dog	-	-	+	+	300	-	-	-	
78	dog	+	+	+	-	370	120	+	-	+
79	dog	-	-	+	+	300, 510	-	+	-	
87	dog	-	-	-	-	580	-	-	-	+
89	dog	-	-	+	-	-	120	+	-	+
91	human	-	+	-	-	-	-	+	-	
93	dog	-	-	+	+	300 bp	-	-	-	
94	dog	-	-	+	+	300, 400, 490, 510, 990	120	-	-	
96	dog	-	-	+	+	300, 400, 510, 990	-	-	-	+
97	dog	-	-	+	+	-	-	-	-	
98	dog	-	-	+	+	-	-	-	-	

mecA gene-negative but *bla* gene-positive *S. aureus* strains showing oxacillin resistance in the agar disc diffusion test were detected in this study. Moreover, all strains were *fem* gene-negative. The *femA* gene and the *nuc* gene, which are constitutively expressed, have been used as molecular targets for the identification of *S. aureus*. However, absence (or polymorphism) of the *fem A* gene in *S. aureus* strains has been reported (20). Fındık et al. (10) have also reported that MRSA isolates from healthy dogs were *fem* gene-negative. In our study *fem* gene-negative MRSA may be explained as some mutation or variations in the sequences around *fem A*, but further investigations, especially sequencing studies, should be performed to understand the genomic variation.

Among all the Staphylococci strains, 10 different *coa* types were detected and the predominant *coa* type, C4, included 10 strains. Among all 5 MRSA strains, 3 different *coa* types (C3, C5, and C7) were found. Among all the *S. aureus* isolates, 3 (37.50%) were coagulase-negative (one was MRSA isolated from a dog and two were MSSA). In another study, coagulase gene-negative variant *S. aureus* isolates from animal and human sources were also reported (21). Among *S. pseudintermedius* strains 9 (39.13%) were coagulase negative. Ten (43. 48%) of *S. pseudintermedius* were in the same *coa* type, C4 showing one 300 bp band. According to *spa*-PCR typing results, the strains were divided into two *spa* types (S1 and S2) and all of the isolates except for one, which was assigned to S1. All MRSA, except for one, were assigned to S1. Findik et al. (10) found three *spa* types among *S. aureus* isolates and reported that MRSA strains were of the less common *spa* type in their study. In this study most MRSA were of the common *spa* type. Recently one of the most commonly used molecular typing methods for *S. pseudintermedius* strains is *spa* typing. There was no polymorphism in the *spa* gene in *S. pseudintermedius* strains in this study. All strains were assigned to S1 type. Resistance to β-lactams in MRSA is conferred by the acquisition of a mobile genetic element, SCC*mec* carrying the *mecA* gene. Moreover, some SCC*mec* types carry various additional genetic elements (*Tn554*, *pT181*), which have been found in HA-MRSA strains. CA-MRSA is different from HA-MRSA in terms of some characteristics. Namely, most of CA-MRSA strains are SCC*mec* type IV or V and they show rare multiresistance to antimicrobials. Moreover, it is reported that *SCCmec* type IV, which is the smallest cassette containing the *mecA* gene, is found in some CA-MRSA clones that are becoming endemic in many parts of the world (22). Gene encoding for Panton–Valentine leukocidin (PVL) has been demonstrated primarily among CA-MRSA strains. There was a high probability that all MRSA strains isolated from humans and dogs in this study were CA-MRSA because all had *pvl* genes. Of these strains 80% were also MDR-MRSA. However, only one human strain was determined as SCC*mec* type IV; the remaining two could not be typed with the primers we used. Of the two MRSA strains isolated from dogs, one was SCC*mec* type III and the other could not be typed. PVL-producing CA-MRSA have rarely been reported in animals and they have been found in companion animals such as dogs, cats, and birds (23). Transmission of PVL-producing MRSA between humans and dogs has been reported in a family (22). In this study, this type of transmission did not occur. However, these PVL-producing strains were considered to pose a risk for transmission between humans and dogs living together in the same household. On the other hand, SCC*mec* typing results were not so satisfying because there were strains that could not be typed.

Multidrug resistance among *S. pseudintermedius* strains isolated from healthy dogs and cats has been explored and it has been reported that 25.42% of *S. pseudintermedius* isolates from nostrils were MDR-*S. pseudintermedius* (24). However, Loeffler et al. (25) reported that 23% of all *S. pseudintermedius* isolates from a veterinary dermatology clinic in Germany were MDR. Moreover, the emergence of MDR-MRSP in Europe has been reported to become an alarming problem. We also detected that 13.04% of all *S. pseudintermedius* in this study were MDR. Similar to the result reported by Gandolfi-Decristophoris et al. (24), we did not find any MDR-MRSP. Although methicillin resistance is of particular interest, other resistances against different antimicrobials are also important and infections caused by MDR-SP may be a critical problem for clinicians because they limit the choice of active antibiotic treatments. On the other hand, no MDR-MRSP was detected. That result was gratifying because it might have forced veterinarians to use antimicrobials in human use for challenging infections, which is also an ethical problem.

One of the two MRSP strains isolated in this study was SCC*mec* type IV but the other strains could not be typed. The different types of SCC*mec* elements reported to be found in MRSP are SCC*mec* II-III, SCC*mec* III, SCC*mec* IV, SCC*mec* V, SCC*mec* VII, and nontypeable cassettes (26).

In conclusion, although it was found at a low percentage, MRSP was isolated in humans and *S. pseudintermedius*; especially MRSP was considered to pose a risk for people living with dogs or in contact with them. Similarly MRSA isolates that were all found to be CA-MRSA and MDR-MRSA were considered to pose a risk for transmission to household pets. Generally there was no polymorphism for the *spa* gene among all isolates. However, *coa* gene polymorphism and variant coagulase negatives were detected among isolates. Further studies, especially in which different primer sets are used in SCC*mec* typing, would be useful to characterize both MRSA and MRSP strains.

References

1. Weese JS, van Duijkeren E. Methicillin resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol 2010; 140: 418-429.
2. Pereira IA, da Motta CC, Rojas ACCM, Coelho IS, Coelho SMO, de Souza MMS. Challanges in predicting *Staphylococcus* spp. β-lactamic resistance in pet animals. Afr J Microbiol Res 2014; 8: 3662-3669.
3. deLencastre H, Tomasz A. Reassessment of the number of auxiliary genes essential for expression of high level methicillin resistance in *Staphylococcus aureus*. Antimicrob Agent Chemother 1994; 38: 2590-2598.
4. Fitzgerald DB. The *Staphylococcus intermedius* group of bacterial pathogens: species reclassification, pathogenesis and emergence of methicillin resistance. Vet Dermatol 2009; 20: 490-495.
5. Markey B, Leonard F, Archambault M, Cullianane A, Maguire D. *Staphylococcus*. In: Clinical Veterinary Microbiology, 2nd ed. Amsterdam, Netherlands: Mosby Elsevier; 2013. pp. 105-119.
6. Çiftci A, Fındık A, Onuk EE, Savasan S. Detection of methicillin resistance and slime factor production of *Staphylococcus aureus* in bovine mastitis. Braz J Microbiol 2009; 40: 254-261.
7. Bannoehr J, Franco A, Luresca M, Battisti A, Fitzgerald JR. Molecular diagnostic identification of *Staphylococcus pseudintermedius*. J Clin Microbiol 2009; 47: 469-471.
8. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty-first informational supplement approved standard M100-S21, v. 31, Wayne, PA, USA; 2011. pp. 76-79.
9. Boye K, Bartels MD, Andersen IS, Møller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I–V. Clin Microbiol Infect 2007; 13: 725-727.
10. Fındık A, Akan N, Onuk EE, Çakıroğlu D, Çiftci A. Methicillin resistance profile and molecular typing of *Staphylococcus aureus* strains isolated from the noses of healthy dogs. Kafkas Univ Vet Fak 2009; 15: 925-930.
11. Montesinos I, Salido E, Delgado T, Cuervo M, Sierra A. Epidemiologic genotyping of methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis at a University Hospital and comparison with antibiotyping and protein A and coagulase gene polymorphisms. J Clin Microbiol 2002; 40: 2119-2125.
12. Azimian A, Havaei SA, Fazeli H, Naderi M, Ghazvini K, Samiee SM, Soleimani M, Peerayeh SN. Genetic characterization of a vancomycin-resistant *Staphylococcus aureus* isolate from the respiratory tract of a patient in a university hospital in northeastern Iran. J Clin Microbiol 2012; 50: 3581-3585.
13. Loeffler A, Lloyd DH. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiol Infect 2010; 138: 595-605.
14. Guardabassi L, Loeber M, Jacobson A. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. Vet Microbiol 2004; 98: 23-27.
15. Baptiste KE, Williams K, Willams NJ, Wattret A, Clegg PD, Dawson S, Corkill JE, O'Neill T, Hart CA. Methicillin-resistant staphylococci in companion animals. Emerg Infect Dis 2005; 11: 1942-1944.
16. Hanselman BA, Kruth S, Weese JS. Methicillin-resistant staphylococcal colonization in dogs entering a veterinary teaching hospital. Vet Microbiol 2008; 26: 277-281.
17. Griffeth GC, Morris DO, Abraham JL, Shofer FS, Rankin SC. Screening for skin carriage of methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* in dogs with healthy and inflamed skin. Vet Dermatol 2008; 19: 142-149.
18. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. J Clin Microbiol 2007; 45: 1118-1125.
19. Morris DO, Boston RC, O'Shea K, Rankin SC. The prevalence of carriage of methicillin-resistant staphylococci by veterinary dermatology practice staff and their respective pets. Vet Dermatol 2010; 21: 400-407.
20. Chikkala R, George NO, Ratnakar KS, Iyer RN, Sritharan V. Heterogeneity in *femA* in the Indian isolates of *Staphylococcus aureus* limits its usefulness as a species specific marker. Adv Infect Dis 2012; 2: 82-88.
21. Akineden O, Hassan AA, Schneider E, Usleber E. A coagulase-negative variant of *Staphylococcus aureus* from bovine mastitis milk. J Dairy Res 2011; 78: 38-42.
22. Van Duijkeren E, Wolfhagen MJ, Heck MEOC, Wannet WJB. Transmission of a Panton–Valentine Leucocidin positive methicillin-resistant *Staphylococcus aureus* strain between humans and a dog. J Clin Microbiol 2005; 43: 6209-6211.
23. Rankin S, Roberts S, O'Shea K, Maloney D, Lorenzo M, Benson CE. Panton–Valentine Leukocidin (PVL) toxin positive MRSA strains isolated from companion animals. Vet Microbiol 2005; 108: 145-148.
24. Gandolfi-Decristophoris P, Regula G, Petrini O, Zinsstag J, Schelling E. Prevalence and risk factors for carriage of multi-drug resistant staphylococci in healthy cats and dogs. J Vet Sci 2013; 14: 449-456.
25. Loeffler A, Linek M, Moodley A, Guardabassi L, Sung JM, Winkler M, Weiss R, Lloyd DH. First report of multiresistant, mecA-positive *Staphylococcus intermedius* in Europe: 12 cases from a veterinary dermatology referral clinic in Germany. Vet Dermatol 2007; 18: 412-421.
26. van Duijkeren E, Catry B, Greko C, Moreno MA, Pomba MC, Pyörälä S, Ružauskas M, Sanders P, Threlfall EJ, Torren-Edo J et al. Review on methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother 2011; 66: 2705-2714.