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Characterization of methicillin-resistant *Staphylococcus* spp. isolated from dogs in Korea

Yunho Jang¹⁾, Dong hwa Bae²⁾, Jae-Keun Cho³⁾, Gyung Jin Bahk⁴⁾,
Suk-Kyung Lim¹⁾ and Young Ju Lee^{2, *)}

¹⁾Bacterial Disease Division, Animal and Plant Quarantine Agency, Anyang 430–757, Republic of Korea

²⁾College of Veterinary Medicine, Kyungpook National University, Daegu 702–701, Republic of Korea

³⁾Daegu Metropolitan City Research Institute of Health & Environment, Daegu 706–732, Republic of Korea

⁴⁾Department of Food Nutrition, Kunsan National University, Gunsan, Jeonbuk 573–701, Republic of Korea

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Abstract

Staphylococci were isolated from dogs in animal hospitals, animal shelters, and the Daegu PET EXPO to investigate the characteristics of circulating methicillin-resistant Staphylococcal (MRS) strains in companion animals in Korea. A total of 36/157 isolates were classified as MRS, and subdivided as follows: 1 methicillin-resistant *Staphylococcus aureus* (MRSA), 4 methicillin-resistant *Staphylococcus epidermidis*, 2 methicillin-resistant *Staphylococcus haemolyticus*, and 29 MRS spp. Among the 36 MRS isolates tested, 100% were resistant to oxacillin and penicillin, and at least 50% were resistant to sulfamethoxazole/trimethoprim (69.4%), erythromycin (63.9%), tetracycline (58.3%), cefoxitin (55.6%), clindamycin (50.0%) or pirlimycin (50.0%). Additionally, 34/36 MRS isolates (94.4%) were *mecA* positive, 15 of which were further classified as SCC*mec* type V, 6 isolates as type I, 4 isolates as type IIb, 1 isolate as type IVa, 1 isolate as type IV, with 7 isolates being non-classifiable. The results of multilocus sequence typing and *spa* typing for the one MRSA strain were ST 72 (1-4-1-8-4-4-3) and *spa* t148. Our results provide evidence that companion animals like dogs may be MRS carriers, and that continued surveillance of MRS in companion animals is required to prevent increased incidences in humans.

Key Words: antimicrobial resistance, companion animals, methicillin-resistant Staphylococci, MLST, SCC*mec*

*Corresponding author: Young Ju Lee, College of Veterinary Medicine, Kyungpook National University, Daegu 702–701, Republic of Korea
E-mail: youngju@knu.ac.kr
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Introduction

Staphylococcus spp. are gram-positive bacteria that form part of the normal flora on the skin and mucous membranes of humans and other organisms. *S. aureus*, *S. intermedius*, *S. epidermidis*, and *S. haemolyticus* are recognized as some of the most common nosocomial and environmental pathogens, and are associated with numerous diseases^{1,18,27}.

Penicillin was first introduced to treat staphylococcal infections, and was followed by the introduction of penicillinase-stable penicillins, methicillin, nafcillin, oxacillin, and cloxacillin. However, the introduction of these antimicrobial agents has resulted in the emergence of methicillin-resistant *Staphylococcus*²⁴. The increased prevalence of multi-drug resistant *Staphylococcus*, such as methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming a serious problem throughout the world. In Korea, Kwak *et al.* reported that the isolation rate of MRSA from patients in intensive care unit is 88.9%¹³.

In previous years, Korean epidemiological studies on methicillin-resistant Staphylococci (MRS) commonly focused on hospital associated-MRS (HA-MRS), although recent studies are more frequently focused on transmission and infection of community associated-MRS (CA-MRS). Moreover, domestic studies on MRS originating from companion animals like dogs are relatively rare. Presently, there are about 10 million people raising companion animals in Korea, thus MRS derived from companion animals may be significantly associated with community-associated transmission. In this study, we investigated the antimicrobial and genetic characteristics of Staphylococci isolated from dogs to address the possibility that companion animals may be carriers of MRS.

Materials and Methods

Sample collection: To know the distribution of MRS in common companion animal, between January–June, 2011, samples were collected from 47 dogs presented to animal hospitals for diagnostic examination or surgery, and from 96 and 161 dogs in animal shelters and the Daegu PET EXPO, respectively. Ear canals, skin surfaces or ocular exudates from dogs in animal hospitals, and ear canals from dogs in animal shelters and Daegu PET EXPO were swabbed using a sterile cotton wool swab, which was transported to lab same day.

Sample analysis: Samples were inoculated into tryptic soy broth (TSB; Difco, USA) with 10% NaCl and incubated at 37°C for 18–24 h. Subsequently, one loopful of each TSB inoculate was streaked on Baird-Parker agar plates (Oxoid, UK) supplemented with egg yolk-tellurite emulsion (Oxoid, UK). After a 24 h incubation period at 37°C, suspected colonies were transferred to blood agar plates with 5% sheep blood. Isolated colonies were then Gram stained and tested for catalase and oxidase production, and presumptively identified by the BBL Crystal Gram-Positive Identification Kit (Becton Dickinson, USA). For identification of staphylococcal species, PCR was done as described previously^{2,20,26}.

Characteristics of MRS isolates: Confirmation of MRS was done by PCR amplifying sequences specific for *mecA*, as described previously by Oliveira *et al.*²². MRS isolates were tested for additional resistance using the disc diffusion test with Rosco Neo-Sensitabs (Rosco Diagnostics, Denmark), and a panel of sixteen antimicrobials, including amoxicillin/clavulanic acid (20/10 µg, AMC), cefoxitin (30 µg, FOX), ceftiofur (30 µg, TIO), cephalothin (30 µg, CEF), chloramphenicol (30 µg, CHL), clindamycin (2 µg, CLI), erythromycin (30 µg, ERY), enrofloxacin (5 µg, EFX), gentamicin (10 µg, GEN), novobiocin (10 µg, NOV), oxacillin (1 µg, OXA), penicillin (10 U, PEN), pirlimycin (2 µg,

Table 1. Frequency of 36 methicillin-resistant *Staphylococci* isolates, according to origin

Isolates	Origin of <i>Staphylococci</i> isolate ^a		
	Animal hospital patient (n = 30)	Animal shelter (n = 38)	Daegu Pet EXPO (n = 89)
Methicillin-sensitive <i>Staphylococcus</i> spp. (n = 121)	20 (66.7) ^b	34 (89.5)	67 (75.3)
Methicillin-resistant <i>Staphylococcus</i> spp. (n = 36)	9 (27.3)	5 (13.2)	22 (24.7)
MRSI ^c	0 (0)	0 (0)	0 (0)
MRSE ^d	0 (0)	0 (0)	4 (4.5)
MRSH ^e	0 (0)	0 (0)	2 (2.2)
MRSA ^f	0 (0)	1 (2.6)	0 (0)
Other MRS ^g	9 (27.3)	4 (10.5)	16 (18.0)

^aA total of 49, 96 and 161 samples were collected from animal hospitals, animal shelters, and the Daegu PET EXPO, respectively.

^bNumber of isolates included (%).

^cMRSI, methicillin-resistant *Staphylococcus intermedius*.

^dMRSE, methicillin-resistant *Staphylococcus epidermidis*.

^eMRSH, methicillin-resistant *Staphylococcus haemolyticus*.

^fMRSA, methicillin-resistant *Staphylococcus aureus*.

^gMRS, methicillin-resistant *Staphylococci*.

PIR), tetracycline (30 µg, TET), sulfamethoxazole/trimethoprim (1.25/23.75 µg, SXT) and vancomycin (30 µg, VAN). MRS isolates were also tested with OXA, using a minimal inhibitory concentration (MIC) recommended in the guidelines of the Clinical and Laboratory Standard Institute^{6,7)}. An *S. aureus* strain (ATCC 25923) was included as quality control. The SCCmec cassette was typed by PCR, as previously described^{5,22)}. One MRSA strain was additionally characterized by *spa*-typing⁹⁾ and multilocus sequence typing (MLST)⁸⁾.

Statistical analysis: A differentiation was made between dogs suffering from an infectious process in hospital and in other locations. The χ^2 -test (with a significance level of 5%) was used to compare carriage of MRS between three groups of animals.

Results

A total of 157 *Staphylococcus* spp. isolates, derived from 30, 38, and 89 swab samples taken

from dogs in animal hospitals, animal shelters, and the Daegu PET EXPO, respectively, were tested for methicillin susceptibility by the disc diffusion test using the OXA disc. Thirty-six *Staphylococcus* isolates (22.9%) were identified as MRS, and further characterized according to species as follows: 1 MRSA isolate, 4 methicillin resistance *S. epidermidis* (MRSE) isolates, 2 methicillin resistance *S. haemolyticus* (MRSH) isolates, and 29 isolates that could not be classified beyond MRS (Table 1).

The resistance rates of the 36 MRS isolates to 16 antimicrobial agents is shown in Table 2. All (100%) of the MRS isolates were resistant to both OXA and PEN, and over 50% of the MRS isolates were resistant to STX (69.4%), ERY (63.9%), TET (58.3%), FOX (55.6%), CLI (50.0%), and PIR (50.0%). MRS from animal hospital patients and EXPO showed relatively high resistance to CLI, ERY, SXT, TET (55.6%, hospital) and SXT (72.7%, EXPO), ERY (68.2%, EXPO), all MRS from animal shelter showed resistance to FOX.

The antimicrobial and genetic characteristics

Table 2. Antimicrobial resistance rates of 36 methicillin-resistant staphylococci isolates in this study

Antimicrobial agent ^a	Number (%) of resistant isolates	
	Resistant ^b	Intermediate & Susceptible ^b
OXA	36 (100)	0 (0)
PEN	36 (100)	0 (0)
SXT	25 (69.4)	11 (30.6)
ERY	23 (63.9)	13 (36.1)
TET	21 (58.3)	15 (41.7)
FOX	20 (55.6)	16 (44.4)
CLI	18 (50.0)	18 (50.0)
PIR	18 (50.0)	18 (50.0)
EFX	17 (47.2)	19 (52.8)
TIO	14 (38.9)	22 (61.1)
CHL	12 (33.3)	24 (66.7)
GEN	11 (30.6)	25 (69.4)
CEF	8 (22.2)	28 (77.8)
AMC	6 (16.7)	30 (83.3)
VAN	2 (5.6)	34 (94.4)
NOV	1 (2.8)	35 (97.2)

^aAMC, Amoxicillin/Clavulanic acid; CHL, Chloramphenicol; CLI, Clindamycin; ERY, Erythromycin; TIO, Ceftiofur; EFX, Enrofloxacin; FOX, Cefoxitin; GEN, Gentamicin; CEF, Cephalothin; NOV, Novobiocin; OXA, Oxacillin; PEN, Penicillin; PIR, Pirlimycin; SXT, Trimethoprim-Sulfamethoxazole; TET, Tetracycline; VAN, Vancomycin.

^bfollowed by guideline of Clinical and Laboratory Standard Institute

of the 36 MRS isolates are shown in Table 3. All of the 36 MRS showed MICs over 4 µg/mL to OXA. Also, one MRSA isolate showed a multiple drug resistance phenotype with resistance to 6 drugs (AMC, TIO, FOX, CEF, OXA, and PEN), and 31 isolates (86.1%) exhibited simultaneous resistance to four or more different antimicrobials. Thirty-four isolates (94.4%) tested positive for *mecA* by PCR, indicating that they were *mecA*-mediated MRS isolates. SCCmec typing with the 34 *mecA* positive isolates was performed by PCR, as described previously²². Fifteen isolates (41.7%) were SCCmec type V, followed by 6 isolates (16.7%) that were type I, 4 isolates (11.1%) that were type IIb, 1 isolate (2.8%) that was type IV, 1 isolate (2.8%) that was type IVa, and 7 isolates (19.4%) that were non-classifiable. From all origin,

SCCmec type V was most prevalent. SCCmec type V (n = 14) showed relatively high resistance to TET, ERY (78.6%), EFX (71.4%) and CLI, PIR, SXT (64.3%) and all type I (n = 6) and type IIb (n = 4) isolates showed resistance to FOX (type I) and SXT (type IIb). MLST and *spa* typing were performed with one MRSA strain and the results are ST 72 (1-4-1-8-4-4-3) and *spa* t148.

Discussion

Staphylococci are well known as nosocomial and opportunistic pathogens, and concerns regarding the emergence of methicillin-resistant Staphylococci (MRS) have been raised as an important universal problem in human medicine since the 1960s. In veterinary medicine, there has also been an increase in the number of reports detailing the isolation of mainly MRSA and MRSI from veterinarians and companion animals worldwide since the 2000s^{10,16,17,28}. Loeffler *et al.* reported that the prevalence of MRSA among dog patients and veterinary staff members owning healthy dogs was 8.9%¹⁶. In Korea, Pak reported that rate of MRS isolated from dogs and cats was 31.6%²³, and Moon *et al.* reported that rate of MRS isolated from dogs, cats, hospital staffs and hospital environment was 36.9%²¹. In this study, the isolation rate of MRS from dogs in animal shelters, animal hospitals, and the Daegu PET EXPO was 22.9%.

In this study, MRS isolates were universally resistant (100%) to OXA and PEN, and 86.1% of MRS isolates exhibiting simultaneous resistance to four or more different antimicrobials. Park *et al.* reported that MRSA from human patients were resistant to GEN and ERY²⁴, and Peck *et al.* reported that MRS isolates had high resistance to PEN, GEN, ERY, CLI, and TET²⁵. Also, Kim *et al.* reported that the rate of multiple drug resistance in MRSA from human patients was 64.0%¹². Youn *et al.* reported that multi-drug resistance in MRSI occurred in 94.2% of isolates obtained from animal hospital staff and

Table 3. Characteristics of 36 methicillin-resistant staphylococci present in this study

Sample	Origin	Species	Antimicrobial-associated co-resistance pattern ^a	MIC ($\mu\text{g/mL}$)	<i>mecA</i>	SCCmec type	<i>spa</i>	MLST
11-3	A ⁱ	spp.	OXA, PEN	4	+	V	NT ^e	NT
11-23	B ^g	aureus	AMC, TIO, FOX, CEF, OXA, PEN	32	+	IVa	t148	ST72
11-39	A	spp.	AMC, TIO, EFX, FOX, OXA, PEN, TET	256	+	V	NT	NT
11-40	B	spp.	AMC, TIO, FOX, CEF, EFX, OXA, PEN, TET, VAN	256	+	V	NT	NT
11-41	A	spp.	CLI, ERY, EFX, GEN, OXA, PEN, PIR, SXT, TET, VAN	4	+	V	NT	NT
11-42	B	spp.	AMC, CHL, CLI, ERY, TIO, EFX, FOX, CEF, GEN, OXA, PEN, PIR, SXT, TET	128	+	V	NT	NT
11-45	A	spp.	CLI, ERY, EFX, GEN, OXA, PEN, PIR, SXT, TET	16	+	NC ^b	NT	NT
11-47	A	spp.	AMC, TIO, CHL, CLI, ERY, OXA, PEN, SXT, TET	128	+	IIIb	NT	NT
11-48	A	spp.	AMC, TIO, CHL, CLI, ERY, OXA, PEN, PIR, SXT, TET	128	+	IIIb	NT	NT
11-50	A	spp.	CHL, CLI, ERY, EFX, GEN, OXA, PEN, PIR, SXT	16	+	V	NT	NT
11-58	A	spp.	TIO, FOX, OXA, PEN	4	+	V	NT	NT
11-70	B	spp.	CHL, CLI, ERY, TIO, FOX, GEN, OXA, PEN, PIR, SXT, TET	4	+	V	NT	NT
11-84	A	spp.	EFX, OXA, PEN, TET	4	+	NC ^b	NT	NT
11-116	B	spp.	ERY, FOX, OXA, PEN, PIR, SXT	4	+	I	NT	NT
4	C ^h	spp.	CLI, ERY, EFX, OXA, PEN, PIR, SXT, TET	8	+	V	NT	NT
24	C	spp.	ERY, GEN, OXA, PEN, PIR, SXT, TET	32	+	V	NT	NT
24-16	C	haemolyticus	TIO, EFX, FOX, GEN, OXA, PEN, SXT, TET	4	+	I	NT	NT
24-23	C	spp.	CHL, ERY, EFX, OXA, PEN, SXT, TET	32	+	V	NT	NT
24-24	C	spp.	CHL, CLI, ERY, EFX, FOX, OXA, PEN, PIR, SXT, TET	32	+	V	NT	NT
24-45	C	spp.	CHL, CLI, ERY, TIO, EFX, OXA, PEN, PIR, SXT	256	+	NC ^c	NT	NT
24-47	C	spp.	CHL, CLI, ERY, TIO, EFX, FOX, CEF, OXA, PEN, PIR, SXT	256	+	NC ^c	NT	NT
25-53	C	spp.	CHL, CLI, ERY, TIO, EFX, CEF, OXA, PEN, PIR, SXT	256	+	NC ^c	NT	NT
24-89	C	spp.	CLI, ERY, FOX, CEF, GEN, NOV, OXA, PEN, PIR, SXT, TET	4	+	IIIb	NT	NT
24-102	C	spp.	FOX, OXA, PEN, SXT	4	+	IIIb	NT	NT
24-115	C	haemolyticus	ERY, TIO, EFX, FOX, CEF, GEN, OXA, PEN, SXT	512	+	I	NT	NT
24-170	C	spp.	CLI, ERY, EFX, GEN, OXA, PEN	16	+	V	NT	NT
24-204	C	epidermidis	FOX, OXA, PEN	4	+	IV	NT	NT
24-212	C	spp.	ERY, FOX, OXA, PEN, TET, SXT	4	+	NB ^d	NT	NT
24-224	C	epidermidis	FOX, OXA, PEN	8	+	I	NT	NT
24-245	C	spp.	TIO, FOX, CEF, OXA, PEN, SXT	32	-	NT	NT	NT
24-252	C	spp.	CLI, ERY, EFX, OXA, PEN, PIR, SXT, TET	4	+	V	NT	NT
24-255	C	spp.	CHL, CLI, ERY, GEN, OXA, PEN, PIR, SXT, TET	4	-	NT	NT	NT
37	C	spp.	CHL, CLI, ERY, EFX, FOX, OXA, PEN, SXT, TET	16	+	NC ^b	NT	NT
68	C	epidermidis	FOX, OXA, PEN	4	+	I	NT	NT
69	C	epidermidis	FOX, OXA, PEN	4	+	I	NT	NT
401-2	C	spp.	CLI, ERY, OXA, PEN, PIR, TET	4	+	V	NT	NT

^aAMC, Amoxicillin; CHL, Chloramphenicol; CLI, Clindamycin; ERY, Erythromycin; TIO, Cefotiofur; EFX, Enrofloxacin; FOX, Cefoxitin; GEN, Gentamicin; CEF, Cephalothin; NOV, Novobiocin; OXA, Oxacillin; PEN, Penicillin; PIR, Pirlimycin; SXT, Trimethoprim-Sulfamethoxazole; TET, Tetracycline; VAN, Vancomycin.

^bNC, Non-classifiable; locus F band pattern only.

^cNC, Non-classifiable; loci C and D multiplex band pattern.

^dNB, No band.

^eNT, Not tested.

Aⁱ, Animal hospital

B^g, Animal shelter

C^h, Pet EXPO

from the environment in veterinary hospitals³¹. Collectively, these studies indicate that MRS isolated from companion animals is closely associated with humans and the community at large. Therefore, resistance monitoring to widely used drugs used for treating companion animals is needed to assess the possibility that companion animals are potential carriers of MRS.

Methicillin resistance in Staphylococci is usually caused by the acquisition of a mobile gene element, termed a staphylococcal cassette chromosome *mec* (SCC*mec*). The *mecA* gene encodes the penicillin binding proteins PBP2a or PBP2b, which have a low affinity for related β -lactams such as methicillin, and can be used as a biomarker to confirm methicillin resistance with antimicrobial susceptibility tests. Several studies have reported that the rate of *mecA* gene positive MRS isolates varies from about 80% to 100%^{3,15,19,29}. In this study, we observed that 34/36 (94.4%) of MRS isolates were *mecA* positive, consistent with previous studies.

SCC*mec* typing has become an essential tool for investigating MRS epidemiology, and has led to the classifications of hospital-associated MRS (HA-MRS) and community-associated MRS (CA-MRS). In Korea, there are a limited number of reports relating SCC*mec* types MRS to veterinary medicine. Kwon *et al.* reported that 3 MRSA strains from hospitalized dogs were SCC*mec* type II¹⁴ and Cho *et al.* reported 1 MRSA strain from a hospitalized cat was SCC*mec* type IIIB¹¹. Other studies have reported that MRSI and MRSA isolated from animal hospitals were SCC*mec* type IVs and V^{21,29,30}. In this study, 17 isolates were identified as SCC*mec* type IV, IVA and V, which are classified as CA-MRS and 10 isolates were types I and IIIB, which are classified as HA-MRS. Although there are some difference of resistance pattern among SCC*mec* type and origins of sample, it is not enough to explain specific tendency yet.

As suggested in previous studies, the emergence of HA-MRS and CA-MRS in veterinary medicine may become a growing problem to

public health in the near future.

MLST and *spaA* typing are discriminatory methods for characterizing bacterial isolates. Kwon *et al.* and Moon *et al.* reported that MLST from MRSA originating from animals were ST5-SCC*mec* type II and ST72-SCC*mec* type IV, respectively^{14,21}. We found that a MRSA isolate was ST72-SCC*mec* type IVA and *spa* t148. Kim *et al.* reported that ST72-SCC*mec* type IVA was the most common type of CA-MRSA found in Korean isolates¹². Therefore, the MRSA strain identified in this study is also potentially transmittable between companion animals and humans.

The emergence of HA-MRS in animals is associated with multiple factors, including the widespread use of antimicrobials, complex treatments, prolonged hospitalizations of critically ill animal patients, the development of emergency medicine, the development of large intensive care units in veterinary clinics sharing many similarities with human hospitals⁴, and the emergence of CA-MRS associated with human MRSA carriers. A large proportion of the human population is in contact with dogs on a daily basis; thus, the potential exists for transferring MRS or resistance genes between companion animals and humans. Therefore, future epidemiological studies should include pet owners, as well as veterinary hospitals and their staff, to trace the routes of colonization and infection and to facilitate the eradication of potential reservoirs of highly epidemic MRS clones in companion animals that represent a continuous threat for owners and animals alike.

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